

Inamuddin  
Mohd Imran Ahamed  
Abdullah M. Asiri *Editors*

# Applications of Ion Exchange Materials in the Environment

 Springer

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Editors

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# Preface

The chemical species present in water streams have turned into a noteworthy worry for the innovative scientific world since their harmful impacts have officially settled on human well-being, aquatic vegetation and animals.

The range of chemical species found in water sources including heavy metals, pesticides, dyes and uncharged species is increasing exponentially owing to industrialization, urbanization and uncontrolled use of chemicals without proper legislation. To safeguard the natural environment such as water, soil and land, it is desirable to find a solution to the unregulated discharge of chemicals into these environmental entities. The government bodies may frame strict guidelines and take stern actions against individuals not complying with the guidelines; however, at the same time, the discharge should be regulated and treated to remove the harmful chemicals to protect people and the environment. An assortment of methodologies is documented in the literature for removal of unsafe contaminants from water such as ion exchange, chemical precipitation, adsorption, membrane filtration, electrochemical treatment technologies and thermal treatment. Among these strategies, ion exchange is one of the more efficient and cheaper techniques for the removal of toxic species from water streams. This book provides an overview of the use of this technique for the treatment of wastewater containing harmful impurities.

*Applications of Ion Exchange Materials in the Environment* will cover applications of ion exchange materials in the area of environmental analysis and treatment. The book presents the applications of organic, inorganic and composite ion exchange materials in various fields including chemical and biochemical separations, water purification and removal of harmful impurities such as dyes and cationic and anionic complexes.

It provides an in-depth knowledge of ion exchange materials and their applications suitable for postgraduate students and researchers as well as industrial research and development specialists working in environmental science, chemistry and chemical and biochemical technology. Additionally, this book presents an overview of ion exchange columns and operation suitable for engineers and industrialists. This book is divided into the following ten chapters.

Chapter 1 discusses the microbial removal of organic dyes and metal pollutants along with the mechanisms involved. Innovations to enhance the removal of dyes and metal ions are also presented.

Chapter 2 introduces heavy metals like chromium, nickel, copper, zinc, cadmium, mercury and lead. The physical and chemical methods of heavy metal treatment are summarized. Finally, challenges and future perspectives of wastewater treatment by ion exchange membranes are highlighted.

Chapter 3 provides a systematic and comparative presentation of the available information on the separation of heavy metals and dyes, vitamins, haemoglobin owing to their application in various fields of biotechnology.

Chapter 4 aims to present the developments in the field of geopolymers used for the removal of alkali and alkaline earth metals, ammonium ion and heavy metals from wastewater.

Chapter 5 presents the study for the microwave-assisted hydrothermal synthesis of zirconium phosphate which is further used for the removal of  $\text{Cs}^+$  and  $\text{Sr}^{2+}$  ions from aqueous solutions.

Chapter 6 discusses the ion exchange capabilities of metal hexacyanoferrates in areas such as potentiometric ion sensors and recovery of metal ions. The electrochemically diffusion-driven processes with meaningful examples, ranging from ESIX-based depletion of ions to battery systems, are discussed.

Chapter 7 highlights the low-cost methods with different degrees of effectiveness for heavy metal ions removal using agro-based biosorbents and biopolymers based on cellulose, chitosan and alginate. Factors influencing the efficiency of nanofibre membranes and packed bed adsorbents are discussed. Different types of composite ion exchangers used for water treatment are also discussed.

Chapter 8 introduces the characteristics, occurrence, properties, applications, production and consumption of rare earth elements. It explores the methods used for the rare earth elements recovery from nickel metal hydride batteries and permanent magnets using ion exchange materials. A detailed overview of the separation of high purity rare earth elements using cation exchangers is discussed.

Chapter 9 demonstrates various materials (inorganic to nanocomposite) recently developed for the removal of heavy metals from wastewater. Their mechanisms and treatment performance are also mentioned.

Chapter 10 discusses the applications of organic ion exchange resins in water treatment. The emphasis is given for the removal of heavy metals, organics, salinity, boron, anions and cations.

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# Chapter 1

## Green Approach: Microbes for Removal of Dyes and Metals via Ion Binding



Adeline Su Yien Ting

**Abstract** Metal ions and organic dyes are common pollutants in the environment. These are toxic to living organisms and the environment. Removal strategies using biological agents have been attempted, with many of these successfully performed by a variety of micro-organisms. The use of micro-organisms is aligned to the green approach, as micro-organisms are capable of biosorbing, bioaccumulating and/or biodegrading the pollutants. This approach is environmentally friendly and sustainable, as readily available biological resources are utilized to convert harmful pollutants into less hazardous forms. In this chapter, the microbes for removal of organic dyes and metal pollutants are discussed, along with their mechanisms involved. Innovations to enhance the removal of dyes and metal ions are also presented.

### 1.1 Introduction

Pollution in the environment due to metal ions and organic dyes is mainly attributed to rampant, anthropogenic factors. Through industrialization (mining, electroplating and welding) and urbanization, metals such as aluminium ( $\text{Al}^{3+}$ ), copper ( $\text{Cu}^{2+}$ ), lead ( $\text{Pb}^{2+}$ ), zinc ( $\text{Zn}^{2+}$ ) and cadmium ( $\text{Cd}^{2+}$ ); and dyes (azo, triphenylmethane and anthraquinone dyes) are released into the environment [129]. Metals typically contaminate wastewaters from fertilizer and pesticide manufacturing. Metals are potent with carcinogenic and mutagenic potentials [12, 74]. The prolonged accumulation of toxic metals in the environment may lead to biomagnification, impacting living organisms via biomagnification of toxic metals in the food chain [1, 8]. For dyes, they typically originate from residual dyes in wastewaters from the various industries. There are over 10,000 different synthetic dyes available in the market, with the collective production of 700,000–1,000,000 ton annually [29].

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Common dyes found in the environment include azo, triphenylmethane and anthraquinone, used in various industries: in the dyeing of textiles and paper; manufacturing of printing ink, plastic, cosmetic; pharmaceuticals and food production [48]. Dyes are toxic to many living organisms with similar potency to metal toxicity. Low concentrations (<1 ppm) of synthetic dyes also impart colour to water bodies, affecting aesthetic value, impeding photosynthesis and disrupting aquatic organisms [43].

Removal of pollutants is therefore pertinent to ensure the biosafety of the environment. The typical methods employed are the conventional physicochemical (bioremediation), which include ion exchange, reverse osmosis and chemical precipitation. Nevertheless, these conventional approaches are limited by the formation of toxic by-products (sludge) and the high cost involved in their disposal [24, 91, 127]. This led to the development of bioremediation as a greener and more environmentally friendly alternative for the removal of pollutants. Bioremediation adopts the use of biological origins such as plants and micro-organisms (bacteria, fungi and algae) to remove pollutants from the environment. Of these, micro-organisms are the most useful as they have been shown to tolerate these pollutants, withstand adverse environmental conditions, are amenable to innovations and upscaling. The microbes have also been found useful for a broader range of application [16–18, 30, 41, 66, 103].

In this chapter, the role, potential and importance of microbes for removal of dyes and metallic species are discussed. The type of microbes, their mechanisms of removal and innovations to further improve their efficacy are presented. Attempts are made to highlight the emerging role of microbes as a green approach in mitigating dye and metal pollution in the environment.

## 1.2 Pollutants in the Environment

Pollutants in the environment are broadly categorized as the organic and inorganic pollutants. The organic pollutants comprise primarily of compounds such as synthetic dyes, pharmaceutical products, pesticides and phenol-based compounds, while metals are the main inorganic pollutants found in the environment [56]. This chapter is devoted to emphasizing the role of dyes and metal ions in the environment, which include an array of triphenylmethane (TPM) dyes (crystal violet, methyl violet, cotton blue, malachite green) and metal cations ( $\text{Pb}^{2+}$ ,  $\text{Al}^{3+}$ ,  $\text{Zn}^{2+}$ ,  $\text{Cu}^{2+}$  and  $\text{Cd}^{2+}$ ). Both TPM dyes and metal cations are often found in excess in wastewaters of the food, pharmaceutical, fertilizer and electroplating industries [23, 54]. These pollutants in the environment present a serious health threat to living organisms. Exposure (absorbing, inhaling or ingesting) to high concentrations of these pollutants typically result in poisoning, irritation and/or damages to the vital organs and body systems [13, 110].

### 1.2.1 Toxic Metals

Metals in the environment are classified into three main groups; toxic metals ( $\text{Cu}^{2+}$ ,  $\text{Pb}^{2+}$  and  $\text{Zn}^{2+}$ ), valuable elements ( $\text{Pt}^{2+}$  or  $\text{Pt}^{4+}$ ,  $\text{Ag}^+$  or  $\text{Ag}^{2+}$  and  $\text{Au}^+$  or  $\text{Au}^{3+}$ ) and radionuclides (U, Ra) [128]. These are often discharged into the environment through natural occurrences (weathering, erosion, volcanic activities) and anthropogenic activities, with the latter overwhelming the former (Table 1.1). Metals have been found in waterlogged areas such as lakes, estuaries and peat swamps, where residual metal accumulates from effluent discharged into the water environment [27, 28]. In the environment, metals are typically found in various oxidative forms [77]. The various oxidative forms give rise to either +1 and +2 (example for Cu) or +3 and +6 (example for Cr), each conferring different degrees of toxicity. For example, the hexavalent chromium ( $\text{Cr}^{6+}$ ) has been revealed to be more toxic than the trivalent state ( $\text{Cr}^{3+}$ ) [80]. The oxidative forms of metals are relatively water-soluble. In water-soluble forms, metals are easily transported in the wastewaters and enter the water and food cycles easily. Of the various types of metals, some are not required by living organisms ( $\text{Al}^{3+}$ ,  $\text{Pb}^{2+}$  and  $\text{Cd}^{2+}$ ), but some are essential metals ( $\text{Cu}^{2+}$ ,  $\text{Zn}^{2+}$ ,  $\text{Fe}^{3+}$  and  $\text{Mn}^{2+}$ ) which are critical to support physiological and enzymatic activities in living organisms. Essential metals are required in trace amounts, but high concentrations lead to toxicity and other health threats (Table 1.1). The World Health Organization (WHO) (2011) has proposed threshold levels for specific metals as a guideline on the permissible levels of common metals in drinking water (Table 1.1). This guideline serves as a reference to various water management practices.

**Table 1.1** Summary of common metals in the environment

Metal ion	Toxicity	Concentrations in drinking water (mg/L)	Threshold concentrations (mg/L)	Pollution source	Health risk
<sup>e</sup> $\text{Cu}^{2+}$	Average	$\leq 0.005$ to $> 30$	2.0	Metal finishing and plating	Damage to brain, liver and gastrointestinal
<sup>e</sup> $\text{Zn}^{2+}$	Average	$<0.1$	3.0	Metal finishing and plating	Affects the nerve systems
<sup>ne</sup> $\text{Pb}^{2+}$	High	$<0.005$	0.01	Mining, welding, plumbing; petrol discharge	Affects cardiovascular, nerve, immunity and reproductive systems
<sup>ne</sup> $\text{Cd}^{2+}$	High	$<0.001$	0.003	Steel industries, batteries	Carcinogenic affects renal function, destroys red blood cells

<sup>e</sup> Essential metals; <sup>ne</sup> Non-essential metals

Source Cheremisinoff [21], Davis et al. [31], Järup [58], Thakur [120] and World Health Organization [131]

### 1.2.2 Triphenylmethane Dyes

Triphenylmethane (TPM) dyes are common dyes used as colourants in many industries. TPM dyes are relatively cheaper than other dyes and have intense colours and high tinctorial strength. The TPM dyes consist of hydrocarbon (triphenylmethane), tertiary alcohol (triphenylcarbinol) and the quinonoid and amino/hydroxyl groups to give rise to the chromophore and auxochromes, respectively [47]. TPM dyes are considered basic dyes with cationic properties (positively charged) and can easily react with materials that are negatively charged. TPM dyes are variable, with colours ranging from shades of violet, blue, green and red. These shades are identified by the following names: crystal violet, ethyl violet, methyl violet, bromophenol blue, brilliant blue FCF, Coomassie brilliant blue, methyl blue, patent blue V, brilliant green, malachite green, methyl green and cresol red [16, 70, 73, 81, 89, 138]. TPM dyes are typically water-soluble, hence are easy to use but also persistent in water environments. Of available TPM dyes, this chapter is aimed to focus on the following four TPM dyes: crystal violet, methyl violet, methyl blue and malachite green. These are the main dyes used for the dyeing of textile items (e.g. wool, silk, cotton, leather, nylon and polyacrylonitrile), paper, plastic, printing inks, food, cosmetic, and for manufacturing of drugs and therapeutic agents (Table 1.2). The persistence of dyes or their metabolites (e.g. leucomalachite green, leucocrystal violet) in the environment is toxic to living organisms: animals, plants and human [6, 11, 43, 100]. Common symptoms include irritation and sensitization of the eyes, skin, respiratory and the gastrointestinal tract [81, 84, 125] (Table 1.2). Aggravated symptoms in the cells and organs may occur as a result of prolonged exposure to the dyes, including carcinogenic and mutagenic effects [73].

**Table 1.2** Summary of the four selected triphenylmethane dyes, their applications in various industries and health hazards

TPM dye	Other names	Industrial applications	Health hazards	References
Crystal violet	Basic violet 3, gentian violet and methyl violet 10b	Textile industry; printing; biological stain; pharmaceutical (antimicrobial, skin disinfectant)	Irritant (to the eyes, skin and digestive tract); can cause respiratory and kidney failures	[44, 84]
Methyl violet	Methyl violet 2b	Textile industry; printing; dyeing of leather and rubber; pH indicator and cell viability assay	Irritant (to the eyes, skin, respiratory and gastrointestinal systems)	[78]
Malachite green	Basic green 4, aniline green and diamond green B	Textile industry; pharmaceutical (antifungal and antiparasitic)	Irritant (to the eyes); failure of the reproductive system	[68, 125]
Methyl blue	Acid blue 93 and cotton blue	Textile industry; biological stain and dyeing of leather	Irritant (to the eyes, skin, respiratory and gastrointestinal systems)	[4]

### 1.3 Bioremediation Approaches in Removing Pollutants

The bioremediation approach to removing pollutants is achieved through the use of organisms, utilizing live or dead cells. The live cells include a variety of micro-organisms and plants [44, 100, 103, 111]. Micro-organisms are primarily studied for their potential in removing pollutants, as these are the natural decomposers in the environment. A variety of bacteria, fungi, yeast and actinomycetes have been explored, and some have demonstrated potential to degrade recalcitrant pollutant molecules such as synthetic dyes, toxic metals, polycyclic aromatic hydrocarbons, pesticides, crude oil and petroleum-based products [37, 59, 134]. The dead organisms (i.e. organic in nature) may also aid bioremediation. This includes dead microbial cells and plant tissues (agricultural waste). Both dead and live cells perform biosorption as their first uptake mechanism. This process binds metal or dye ions to the negatively charged surfaces of the biomass. In live cells, this ionic binding of pollutant ions to the functional groups of the cells precedes the bioaccumulation or biodegradation processes. Biosorption also occurs more rapidly, with uptake often occurring within hours rather than days compared to bioaccumulation [96]. While both live and dead cells can be used for pollutant removal, the living organisms are potentially more effective to remove pollutants by biodegrading or biotransforming the pollutants into simpler, less toxic molecules. With biodegradation, it is possible to achieve complete mineralization of pollutants into carbon dioxide and water [29]. This is a desirable pathway to achieve complete removal of the toxic pollutants. The dead cells typically remove pollutants as such, without biotransformation or biodegradation.

The living and dead cells remove pollutants via two main mechanisms: biosorption and/or biodegradation [86, 109]. Biosorption involves the binding of pollutants onto surfaces of the cells used. On the contrary, biodegradation relies on not just the binding of pollutants to cells, but the bioaccumulation of pollutants into the cells or biodegradation via extracellular molecules produced. Biosorption capitalizes on the cell wall of cells, which are rich in various functional groups. These groups (carboxyl, hydroxyl, amine and phosphoryl) are negatively charged, therefore binds strongly to the positively charged pollutants (dyes, metal cations) [69]. Hence, the primary mode of removal of pollutants is via the ionic binding to the live or dead cells.

#### 1.3.1 Non-microbial Strategies

The non-microbial strategies involve the use of sorbents of non-microbial origin. The most common sorbents used are agricultural wastes, which include the activated charcoal. Activated charcoal is popularly produced commercially in granular or powder forms, and has high sorption capacity, attributed to their microporous nature and large surface area (500–1500 m<sup>2</sup>/g) [42, 51, 63]. Activated charcoal has



been found to remove TPM dyes and metals successfully. Commercial activated charcoal could adsorb as much as 151.52 and 200 mg/g of crystal violet and basic green 4 (malachite green), respectively [68, 94]. Toxic metals adsorbed on activated charcoal include  $\text{Cd}^{2+}$ ,  $\text{Cr}^{6+}$ ,  $\text{Cu}^{2+}$ ,  $\text{Ni}^{2+}$ ,  $\text{Pb}^{2+}$  and  $\text{Zn}^{2+}$  [42, 50, 51, 63, 98]. The sorption capacity of activated charcoal is evident, but their application is challenged by the demands in producing activated charcoal (high-cost, energy-consuming processes). Activated charcoal also has poor reusability due to poor amenability to sorption–desorption processes [50].

Other agricultural wastes used include leaves, bagasse, fruit peels, among others. These are effective biosorbents to remove various pollutants (e.g. synthetic dyes, toxic metals, phenolic compounds and radionuclides) [97, 122, 123]. Adsorption of ionic pollutants to these wastes depends on the cell wall composition of the biosorbents, which consists primarily of cellulose, hemicellulose and lignin [75]. These lignocellulosic materials are rich in functional groups, particularly the negatively charged hydroxyl groups that are able to attract and bind the cationic metals and other pollutants, especially when present in low concentrations [68, 86]. Several types of the wastes have been found to remove TPM dyes successfully. This includes raw forms of sugarcane bagasse [86], biochar of *Miscanthus sacchariflorus* (grass) [65], cones of *Pinus brutia* (Calabrian pine) [89], walnut shells [118], leaves of *Artocarpus odoratissimus* (tarap) [78], leaves of *Ananas comosus* (pineapple) [97], leaves of *Psidium guajava* (Guava) [104], rice husk [75], spent tea leaves [3], spent ground coffee [61], olive pomace [68], orange peel [107], oak charcoal [53] and stems and leaves of potatoes [52]. Modified (via esterification, etherification and oxidizing agents) or converted (into activated carbon) forms of the agricultural wastes have also been found suitable to remove pollutants successfully [3, 123]. Modifications via chemical processes resulted in additional functional groups on the sorbents, such as carboxylic groups, which increased their biosorption capacity [86]. Despite the efficacy of agricultural wastes as sorbents, their large-scale application is, however, limited by the resulting toxic sludge generated and potential clogging problems in bioreactors.

### 1.3.2 Microbial-Based Strategies

Micro-organisms have also been explored for their potential in removing toxic pollutant ions. Bacteria, fungi and algae are increasingly popular as environmentally friendly alternatives to conventional sorbents. Out of these, bacteria are the most commonly studied. Their ability to rapidly grow and increase in biomass and amenability to upscaling are attractive traits for use as biosorbents. Bacteria are also adaptable to harsh conditions, whilst demonstrating the efficient removal of dyes and metals [79, 119, 137]. Bacteria that are well known as efficient removers of toxic pollutants include species of *Bacillus*, *Pseudomonas*, *Burkholderia*, *Klebsiella*, *Shewanella* and *Sphingomonas* [4, 6, 79, 96, 132, 135–137]. Species of *Escherichia*, *Proteus*, *Bacillus* and *Salmonella* have also shown dye-degrading

potential [32, 57]. Bacteria are capable of removing pollutants via biosorption or/and biodegradation [36, 96]. The biosorption process in bacteria is dependent on the negatively charged carboxyl, amine, hydroxyl and phosphonate groups [4, 11]. The degradation of dyes by bacteria is regulated by a variety of enzymes such as laccase, lignin peroxidase, manganese peroxidase and NADH-DCIP reductase [15, 37]. Removal of metals by bacteria involves both active and passive removal mechanisms, which includes precipitation, as well as intracellular and extracellular accumulation of metals [67].

Fungi are also capable of removing toxic pollutants, which include various metals, synthetic dyes, polycyclic aromatic hydrocarbons, pesticides and hydrocarbon [2, 56, 72]. These pollutants bind to the anionic surfaces of fungi. Common fungal species known to remove metals and dyes include species of *Phanerochaete*, *Corioloropsis*, *Irpex*, *Trichoderma*, *Aspergillus*, *Penicillium*, *Rhizopus*, *Pleurotus ostreatus*, *Polyporus picipes*, *Gloeophyllum odoratum*, *Colletotrichum gloeosporioides* and *Diaporthe* sp. [16, 17, 19, 22, 45, 56, 100, 103, 108, 121]. Fungi produce non-specific, extracellular enzymes to catalyse the breakdown of pollutants [17, 103]. These enzymes could also actively exclude, adsorb, compartmentalize or sequester metals as means to detoxify metals [105]. These are typically achieved in live fungal cells. For dead fungal cells, pollutants are removed via biosorption, aided by the ionic binding to functional groups on the surface of the biomass [22].

According to the literature, the single-celled fungi, yeasts, have also been studied. Yeasts for the treatment of pollutants were typically sourced from industrial waste where they were available in abundance especially from fermentation, brewing and distillation processes [117, 134, 138]. Metal cations ( $\text{Cd}^{2+}$ ,  $\text{Cr}^{3+}$ ,  $\text{Cu}^{2+}$ ,  $\text{Hg}^{2+}$  and  $\text{Pb}^{2+}$ ) bind easily to the negative charges found on the surface of a variety of yeast species, which include *Candida tropicalis*, *Saccharomyces cerevisiae*, *Pichia acacia*, *Kluyveromyces lactis*, *Cyberlindnera fabianii*, *Wickerhamomyces anomalus* and *Cryptococcus* sp. [7, 55, 71, 76, 134]. The potential of yeast in removing dyes, however, has not been extensively studied. To date, only *S. cerevisiae* and *Yarrowia lipolytica* have been reported as positive for decolourization of malachite green, brilliant green, methyl violet, aniline blue and crystal violet [5, 48, 106, 117]. The extensive use of yeasts as bioagents for metal or dye removal is, however, limited due to their poor reusability and the generation of toxic sludge [134].

Algae have also demonstrated potential for removing pollutants. Algae are photosynthetic and range from single-celled microscopic algae to as large as kelps [20]. In most cases, the effective metal removal has been documented for brown, green and red algae [14, 24]. These include *Aphanocapsa roseana* de Bary [95], *Chlamydomonas* sp., *Euglena* sp., *Cystoseira compressa* [10], *Durvillaea antarctica* [24], *Chlorella vulgaris* [20], *Gracilaria edulis* [60], *Cosmarium* sp. [29], *Scenedesmus quadricauda* [69], *Pterocladia capillacea* [39], *Kappaphycus alvarezii* [39] and *Sargassum muticum* [14]. Algae are present as an attractive alternative, as they are abundant in nature, fast-growing, have a large surface area-to-volume ratio and known to have a high tolerance and uptake capacities for pollutants [24, 29]. The efficient removal of pollutants by algae is attributed to the functional

groups (primarily carboxylic) found in the alginate polymers of algae, which perform the ionic bonding with metal and dye ions [69]. In addition, algae are fast-growing, able to achieve sufficient biomass for application within a short period of time; hence, this process is extremely cost-effective. Nevertheless, further studies are required to better understand the response of algae to pollutants and environmental parameters, particularly pertaining to the removal of TPM dyes [60, 69].

## 1.4 Mechanisms for Removal of Pollutant Ions

Micro-organisms typically remove metal and dye pollutants via biosorption and/or bioaccumulation. The biosorption process involves the binding of the pollutant ions to the surface of the cell. This is usually the result of chemical and/or physical interactions between the pollutant ions with the functional groups found on the surface of the microbial cells. Biosorption is performed by both live and dead cells, through the binding of ions to the cells as the primary process. This takes place even when the cells are no longer viable (dead cells). On the contrary, bioaccumulation only occurs in live cells. In live cells, bioaccumulation is an extended process where upon binding of ionic pollutants to the cell surface, the pollutant ions may be further transported across the cell membrane into the cells and compartmentalized or immobilized within the cells. For some pollutants such as dyes, the dye molecules may be further degraded enzymatically by live cells. In both bioaccumulation and biodegradation processes, biosorption has to take place and the ionic bonding here is important to ensure the effectiveness of the processes.

### 1.4.1 Mechanisms for Removal of Metal Ions

The metal ions are typically adsorbed to microbial cells via the physical and/or chemical interactions between the metal cations and the negatively charged functional groups in the cell walls and cell membranes [64]. The successful binding of metal cations to these functional groups has been confirmed through changes in peaks of the functional groups, detected via Fourier transform infrared spectroscopy (FTIR). The functional groups in the cell walls are critical for metal binding. In bacteria, these functional groups are found in the peptidoglycan of their cell wall, rich with *N*-acetylglucosamine (NAG) and *N*-acetylmuramic acid (NAM) [30]. Bacteria cells also bind metals through the numerous functional groups found in the capsule or slime layer produced [33, 90]. The slime and capsules also contribute to the metal complexation process [38]. A wide variety of fungal species have been documented to adsorb metals, and these include *Penicillium lilacinum*, *Microsphaeropsis* sp. and *Trichoderma asperellum* [40, 113, 133]. *T. asperellum* showed preference for removal of  $\text{Zn}^{2+}$  (18 mg/g),  $\text{Cu}^{2+}$  (17.26 mg/g),  $\text{Pb}^{2+}$  (19.24 mg/g),  $\text{Cd}^{2+}$  (19.78 mg/g) and  $\text{Cr}^{3+}$  (16.75 mg/g) [113]. For

*Microsphaeropsis* sp. LSE10, the isolate was capable to adsorb significantly higher amount of  $\text{Cd}^{2+}$  at 247.5 mg/g [133]. The isolate *P. lilacinum* reportedly removed  $\text{Cu}^{2+}$  (85.4%) and  $\text{Cd}^{2+}$  (31.43%) [40].

Bioaccumulation of metals also occurs in some microbial species. This involves the movement of metal cations across cellular structures (cell membrane) of the live cells, to be further compartmentalized or detoxified. The movement of metal cations across the cell membrane demands energy from the cells and is therefore exclusive to only live cells. The energy is also required for cells to perform metal precipitation, redox reactions, crystallization and/or covalent bonding with cellular structures [83, 130]. The bioaccumulation process occurs at a slower rate than biosorption, presumably due to the adaptive stage required by live cells in response to metal toxicity [19]. Nevertheless, bioaccumulation often results in the higher removal of metals than biosorption. The superiority of live cells in removing metals was observed in *Lasiodiplodia* sp. MXSF31, capable of bioaccumulating  $\text{Pb}^{2+}$  ( $5.6 \times 10^5$  mg  $\text{kg}^{-1}$ ),  $\text{Cd}^{2+}$  ( $4.6 \times 10^4$  mg  $\text{kg}^{-1}$ ) and  $\text{Zn}^{2+}$  ( $7.0 \times 10^4$  mg  $\text{kg}^{-1}$ ) [35]; and *Mucor* sp. for  $\text{Cd}^{2+}$  removal (173 mg  $\text{g}^{-1}$ ) [34].

The removal mechanism of metal ions can be enhanced by optimizing several factors: pH, temperature, adsorbent dosage/amount and initial metal concentrations. Of these, the initial pH of the metal solution is critical as it influences metal precipitation, availability of functional groups, and the interaction between metal cations and hydrogen protons with the functional groups [9]. At low pH (acidic), more hydrogen ions are available to compete with metal cations for binding onto the functional groups [62]. As hydrogen ions bind to the functional groups, less binding sites are available for metal cations. This leads to poorer metal removal activities under low pH conditions. Typically, the pH range of 4 to 8 is the optimum for metal biosorption [26, 128]. The other factors, particularly adsorbent dosage and initial metal concentrations, vary depending on the type of microbial cells used [18].

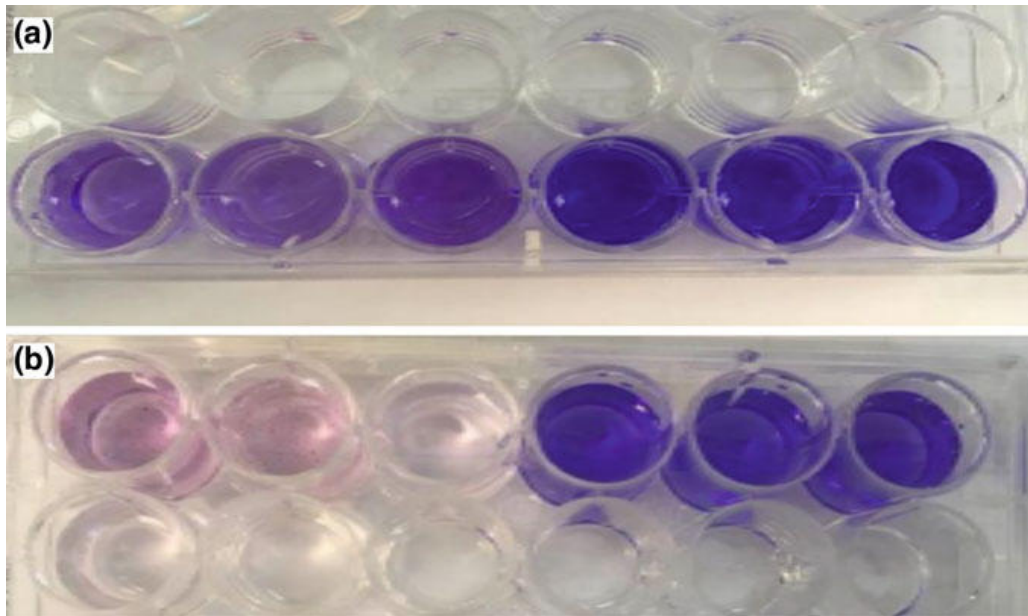
#### 1.4.2 Mechanisms for Removal of Dyes

Removal of dyes by microbial cells involves two mechanisms: the biosorption or/ and biodegradation [100, 121]. While the former occurs most commonly, the latter is a more effective mechanism as it often leads to the breakdown of toxic dye molecules into less hazardous molecules. The dead and live cells can both perform biosorption, but the biodegradation process is exclusive to live cells [46, 100, 102, 109, 121]. In biosorption, dye ions bind to the functional groups present in the cell wall (i.e. carboxyl, hydroxyl, amino and phosphate groups), specifically the lipid, chitin, glucan and chitosan components of the cell wall [99]. Dye biosorption is detected visually, (a) when microbial biomass is stained with the dye (dyes binding to the cells), (b) with shifts detected in spectra peaks via FTIR and (c) decolourization of the dye solution [85, 100, 121].

However, the biodegradation of dyes only occurs in live cells. This process is dependent on the enzymatic breakdown of the dye molecules. Biodegradation of dyes is most effectively performed by lignolytic enzymes, as these enzymes are responsible for the catalytic breakdown of dyes, particularly their chromophoric centres, into less harmful compounds [100]. Live cells typically bind dye ions onto their cell wall, subsequently secreting extracellular enzymes to catalyse the degradation of the dye molecules [85, 109, 121]. In some microbial cells, the dye molecules are transported across the membrane into the cells, and further degraded by the intracellular enzymes. The lignolytic enzymes are well known for their ability to degrade dye molecules. This includes the enzymes laccase (Lac), manganese peroxidase (MnP), lignin peroxidase (LiP), tyrosinase and the reductase enzymes [99, 116, 124]. Among the enzymes, laccase (EC 1.10.3.2) is the most studied enzyme for its role in biodegradation of dyes. This enzyme is found in most micro-organisms [111]. The oxidative activity takes place in the presence of oxygen, and the oxidation of dye molecules by Lac releases free radicals for further dye degradation. Laccase production in several isolates has demonstrated effective decolourization of aniline blue, methylene blue, malachite green and Bismarck brown [109, 112].

The biodegradation of dyes often results in less toxic compounds, which are validated by toxicity assays of treated dye samples via microbial or seed germination assays [59, 80, 100]. In a study by Chen and Ting [16], the mere presence of dyes was found to induce production of dye-degrading enzymes. Isolate *Coriolopsis* sp. (1c3) produced more LiP and NADH-DCIP reductase in dye solutions, compared to controls (absence of dyes). It was assumed that the enzymes produced and their roles in biodegradation could perhaps be species specific. For *Coriolopsis* sp. (1c3), biodegradation potential was most evidently attributed to LiP and NADH-DCIP. The enzyme laccase was not involved in this process at all, although these three enzymes are typically known to be crucial in degrading TPM dyes. *Coriolopsis* sp. may rely more on LiP and NADH-DCIP reductase for dye biodegradation. The enzymatic breakdown of dyes results in notable changes to dye intensity (reduced intensity) (Fig. 1.1). The biodegradation takes place after ionic bonding, where enzymes secreted aids during the breakdown of dye molecules into simpler fragments or less hazardous molecules. The breakdown of dyes would eventually lead to reducing the toxicity level of the dyes. This can be established via phytotoxicity tests, where treated dye solutions are used to treat seeds, leading to seedling germination (Fig. 1.2).

The dye removal was more efficient when optimized conditions were provided, especially initial dye concentration and incubation conditions (agitation, aeration) [100, 101]. These factors played an important role as dye binding is an ionic process, following monolayer sorption kinetics [18]. Initial dye concentrations are important as high initial dye concentrations often lead to decreased decolourization efficiency. However, the high initial dye concentrations are toxic to living cells and may kill the cells or inhibit production of enzymes crucial for dye degradation [16]. This has been observed in cases of many fungi, including *Phanerochaete chrysosporium*, which has been typically used in degradation studies of most dyes.



**Fig. 1.1** Decolourization of crystal violet (three wells from the left) due to enzymatic biodegradation by **a** free-cell and **b** pseudo-biofilm forms of *Coriolopsis sp.* Three wells from the right are controls (without treatment of *Coriolopsis sp.*)

**Fig. 1.2** Seedling germination and growth for mung beans applied with cotton blue dye solution treated with *Penicillium simplicissimum*



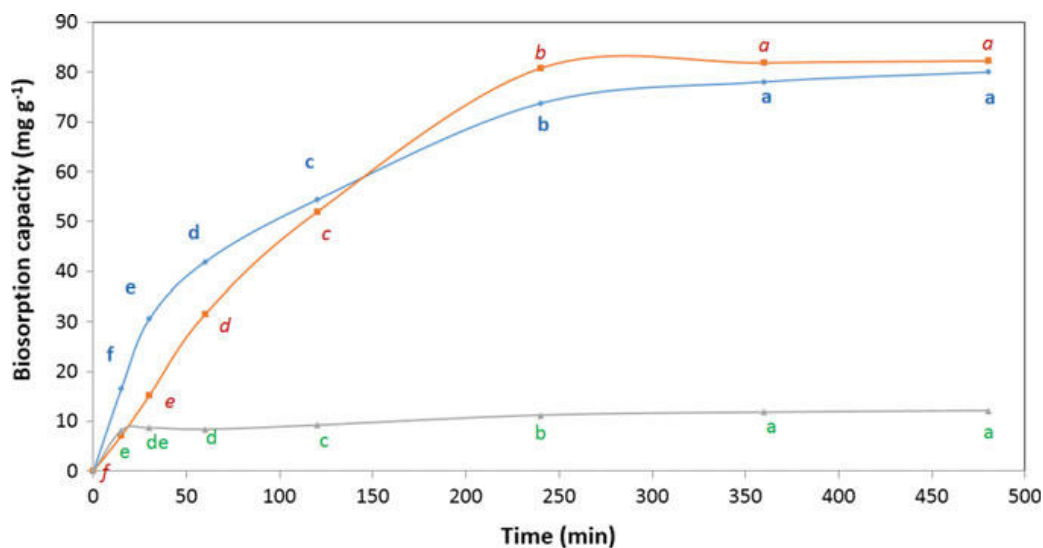
With an initial dye concentration of 0.05 g/L, *P. chrysosporium* decolourized methyl violet at 90%. On the contrary, when the initial dye concentration was increased to 0.4 g/L, decolourization efficiency decreased to 30% [103]. Similar observations were noted for decolourization of crystal violet, methyl violet and malachite green by *Penicillium simplicissimum* and *Coriolopsis sp.* [16, 17].

Another important factor is the incubation condition, i.e. agitation and aeration. Agitation is crucial to allow oxygenation in the removal process. It also promotes better contact of biomass with dye molecules. Studies have demonstrated that decolourization rate and efficiency were enhanced when incubated with agitation [100]. This has been noted for *P. ostreatus* (BWPH and MB strains), *P. picipes* (RWP17) and *G. odoratum* (DCA) for the degradation of brilliant green, with 73.81–95.00% achieved with agitation, as compared to static incubation (56.74–79.30%). The superiority of decolourization under agitated conditions was also evident for *Absidia spinosa* M15 in which degradation of cresol red was 97.1% under 120 rpm agitation, compared to only 44.5% under static conditions [70]. Agitation at 150 rpm was also useful in enhancing degradation (96%) of malachite green by *Aspergillus ochraceus*, compared to without agitation (72%) [108]. This efficiency was attributed to enhance contact between cells and dye compounds for oxidative metabolism. Nevertheless, the rate of agitation, i.e. at 120 or 150 rpm, was dependent on fungal species. Higher decolourization efficiencies for brilliant green were observed for *P. ostreatus* (BWPH and MB strains), *P. picipes* (RWP17) and *G. odoratum* (DCA) when agitated (73.81–95.00%) as compared to static incubation (56.74–79.30%).

## 1.5 Innovations in the Removal of Pollutant Ions

Removal of pollutant ions can be improved by introducing new approaches or modifications to the microbes or to the techniques. The microbial cells can be modified by physical and chemical pre-treatments, grafting, immobilization or genetic engineering, to enhance sorption capacity. All these strategies are based on the enhancement of ionic binding of dye or metal ions to the cells. In addition, the use of microbial consortium, solid-state fermentation (SSF), and testing in single- and multi-metal solutions can also be explored as strategies to improve removal of pollutants. Pre-treatments include both physical and chemical treatments that when applied to increase the binding of ions to the cells. Physical pre-treatments include treatment with heat via boiling or autoclaving, while chemical pre-treatments involve the use of acids, alkalis or detergents. Both physical and chemical pre-treatments liberate functional groups of cell walls so that more binding sites are available for ionic binding of metals or dyes [126]. Of the reagents used, pre-treatment with acids (hydrochloric acid, nitric acid) or alkalis (sodium carbonate) has demonstrated good removal efficacy, especially for metal ions. Treatment with 0.5 M sodium carbonate for fungal isolate STRI: ICBG-Panama: TK1285 enhanced  $\text{Cr}^{3+}$  removal to 91.2% from 27% compared against non-treated cells [64]. Yang et al. (2013) reported the similar effects of citric-acid treated *Fusarium* sp., with enhanced thorium(IV) removal (75.47 mg/g) compared to non-treated cells (11.35 mg/g). Therefore, pre-treatments are beneficial as these generally enhance the performance of biosorbents in removing pollutant ions.

Grafting is the other innovation that enhances the removal of pollutants [82]. This technique involves the polymerization and layer-by-layer grafting of functional groups to the microbial biomass to increase ionic binding. This is achieved by simple cross-linking formations and adding functional groups to the surface, modifying the surface and enhancing surface area for biosorption activities. One common approach is the grafting of poly(allylamine hydrochloride) (PAA) layers onto the bacterial surface using glutaraldehyde as the cross-linking agent [82]. This has resulted in metal removal that was tenfold higher than the non-modified bacteria. In addition to grafting, the surface area and functional groups can be enhanced via immobilization as well. Immobilization is performed using a polymer matrix (e.g. alginate, polyacrylamide and polyurethane) to encapsulate or entrap microbial cells within [88]. The polymer matrix contributed to enhancing biosorption, by providing superior surface binding or functional groups for efficient biosorption of metal ions [22]. The amount of metals adsorbed is significantly increased by immobilization compared to free-cell forms (Fig. 1.3). Improvement in metal removal (i.e.  $\text{Cr}^{3+}$ ) using alginate-immobilized isolate STRI: ICBG-Panama: TK1285 could be as high as 98.5% compared to 27% by free-cell forms [64]. In addition, immobilized microbial cells also have lesser issues with respect to blockage and separation of biosorbents from wastewaters. Application of immobilized forms also allowed for high biomass loading with standardized preferred sizes, reusability and is relatively less expensive [22].



**Fig. 1.3** Biosorption of Pb (II) by free-cells of *Diaporthe* sp. (filled triangle) and alginate-immobilized *Diaporthe* sp. (filled box) as compared to control (plain alginate beads) (filled diamond) at various time intervals. Means with the same letters within the same treatment are not significantly different according to Tukey's comparisons ( $\text{HSD}_{(0.05)}$ ). Biosorption conditions—initial concentration of metal ion: 100 mg/L; pH  $5.0 \pm 0.2$ , temperature:  $30 \pm 2$  °C, agitation rate: 200 rpm

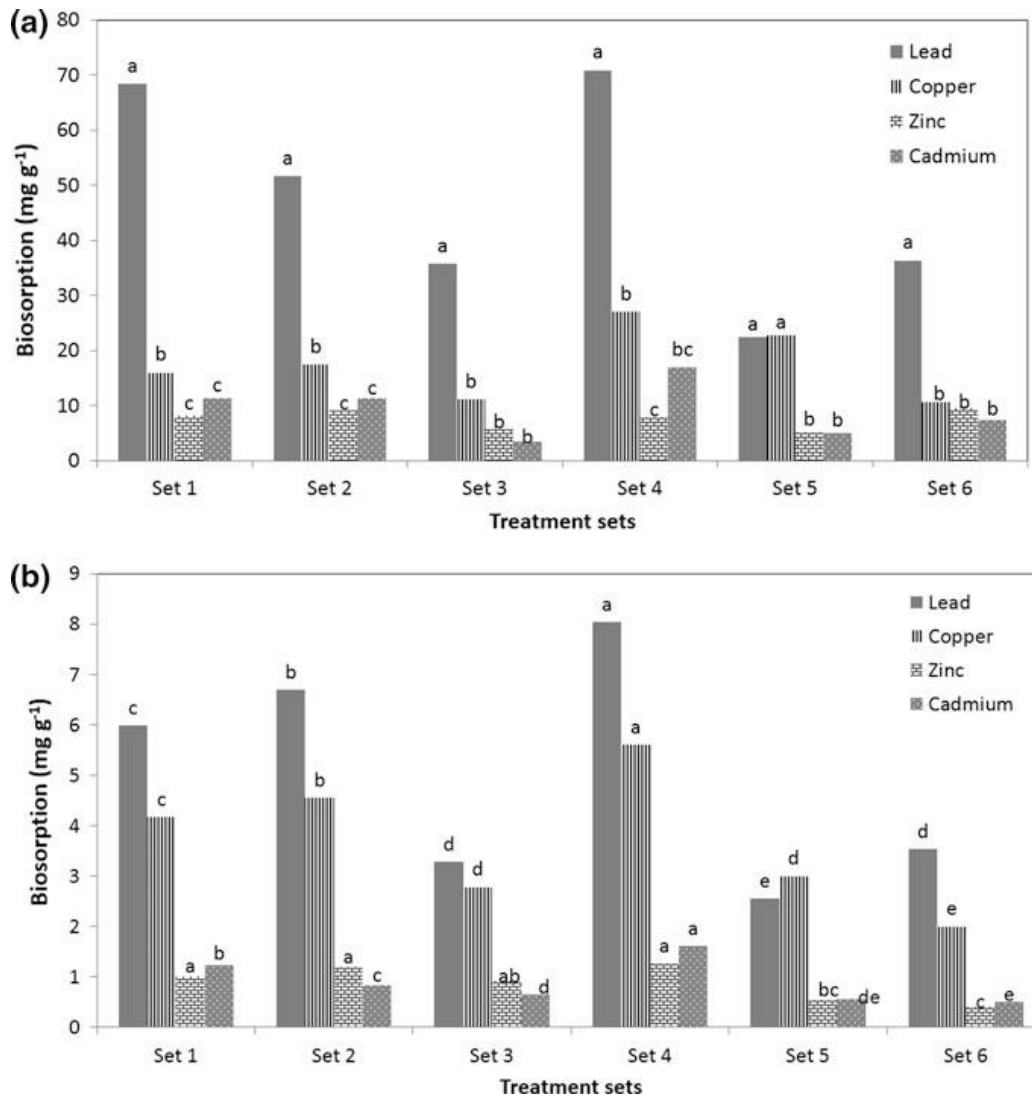


The other alternative to improving microbial innovation is via the genetic engineering approach. This method explores the recombinant approach of introducing dye-degrading enzymes and the over-expression of these enzymes in hosts such as bacteria [111]. As a result, mass production of the stable enzymes (at relatively low costs) is possible. In recent years, the laccase gene (Lac4) from *Pantoea ananatis* Sd-1 was successfully cloned and expressed in *Escherichia coli* by Shi et al. [111]. This recombinant enzyme laccase was stable under various conditions: acidic (pH 1–3), high temperature (30–70 °C) and the presence of metal ions ( $\text{Fe}^{2+}$  and  $\text{Cu}^{2+}$ ). The enzyme is valuable for the biodegradation of aniline blue (47%) as well as the azo dyes Remazol brilliant blue R (35%) and Congo red (89%).

New strategies to examine the efficacy of biosorbents can be adopted. One such strategy is the innovative approach of applying microbial consortium. The use of multiple strains of microbes in a consortium may contribute to better removal rate as various species responsible for the breakdown of different molecules of the pollutants are present to synergistically co-metabolize the pollutants [25]. Sim and Ting [114] adopted the mixed consortia approach and tested the mixed consortia on both single- and multi-metal systems. Results revealed the improved removal of  $\text{Cu}^{2+}$ ,  $\text{Pb}^{2+}$ ,  $\text{Zn}^{2+}$  and  $\text{Cd}^{2+}$  by microbial consortia than the use of a single fungal isolate. Similarly, Mastretta et al. [87] reported the enhanced  $\text{Cd}^{2+}$  accumulation (by three-folds) by bacteria consortium (mixtures of *Pseudomonas* sp., *Sanguibacter* sp., *Stenotrophomonas* sp., *Enterobacter* sp.), compared to single isolates.

The other strategy is to investigate the efficacy of the biosorbents in both single- and multi- or binary-systems. This can be achieved by performing assessments on these solution systems, as the multi- and binary-systems closely mimic the environment. This helps to validate the efficacy of the microbial cells as their interactions with multiple pollutant ions in the environment have been tested [49, 114, 115]. According to these studies, removal of metal ions in multi-metal systems is generally inferior compared to single-metal solutions (Fig. 1.4). This is closely linked to pollutants binding to biosorbents via ionic binding and may be limited by the surface available for binding of metals, and the general competitiveness among metals for binding. In multi-metal solutions, the biosorption of pollutant ions was the rate-limiting chemisorption process, with cationic metals competing for limited binding sites [114, 115].

Other innovations such as the use of solid-state fermentation (SSF) were also found to enhance removal of pollutants. In SSF culture conditions, secretion of enzymes is induced, typically in large quantities, due to the presence of the substrate as support for mycelial growth. With SSF, production of enzymes such as laccase can be increased and subsequently immobilized in 3% sodium alginate to facilitate decolourization of textile dyes [93]. The enzymes can be purified and applied for dye degradation applications. This concept of SSF has been adopted and modified to induce pseudo-biofilm formation in fungi, which improved the degradation of crystal violet and cotton blue [92].



**Fig. 1.4** Biosorption by biosorbents (Set 1—Sm + Sb; Set 2—Sm + Ta; Set 3—Sb + Ta; Set 4—Sm; Set 5—Sb and Set 6—Ta) for the removal of Pb<sup>2+</sup>, Cu<sup>2+</sup>, Zn<sup>2+</sup> and Cd<sup>2+</sup> in **a** single-metal and **b** multi-metal solutions. Means with the same letters within the biosorbent sets are not significantly different (HSD<sub>(0.05)</sub>). Note Sm—*Stenotrophomonas maltophilia*, Sb—*Saccharicola bicolour*, Ta—*Trichoderma asperellum*

## 1.6 Conclusions and Future Prospects

The use of microbial cells, or their derivatives (e.g. enzymes), is the green approach in mitigating pollutants in the environment. Microbial cells are non-toxic and produce biomolecules such as enzymes to remove the metal and dye ions. Removal of metal and dye ions can be improved via several innovations, which include grafting, genetic engineering, using microbial consortia and others. These innovations have significantly enhanced the removal of pollutants by improving biosorption of pollutant ions (e.g. grafting of functional groups) or by

bioaccumulation or biodegradation (e.g. production of laccase enzymes). The alternative of using microbial cells for pollutant removal is good but requires further optimization studies and explorations to further increase the feasibility of the use. Future studies are to include considerations of their applications to a wider range of toxic pollutants, the reuse of the microbial cells and the economic impact of the microbial-based approach.

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# Chapter 2

## Removal of Heavy Metal from Wastewater Using Ion Exchange Membranes



Z. F. Pan and L. An

**Abstract** Clean water supplies are vital for industry, agriculture, and energy production. However, the water pollution issue is becoming more serious due to ever-increasing wastewater discharges from the industries into the environment. As the freshwater resource is limited, it is extremely crucial to reuse the wastewater after it has been treated to remove the heavy metal ions and other organic pollutants, which is believed to be the only way to find the new water resource. In view of the significance of treatment of wastewater contaminants, various remediation technologies are proposed and developed for efficient removal of heavy metal ions, including ultrafiltration, nanofiltration, reverse osmosis, forward osmosis, adsorption, electro dialysis method, and fuel cell method. This chapter starts with a brief introduction of heavy metals, which are chromium, nickel, copper, zinc, cadmium, mercury, and lead. Then both physical treatment and chemical treatment are summarized. Finally, the remaining challenges and future perspectives are highlighted.

### Abbreviations

AAEM	Alkaline anion exchange membrane
AC	Activated carbon
AFM	Atomic force microscope
AMAH	2-acrylamido-2-methylpropane sulfonic acid based hydrogel
APTES	Aminopropyltriethoxysilane
BET	Brunauer–Emmett–Teller
BSA	Bovine serum albumin
CdS	Cadmium sulfide
CEM	Cation exchange membrane
CPANM	Chitosan/poly(ethylene oxide)/activated carbon (AC) nanofibrous membrane

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CPF	Chitosan/PEO fiber
EDA	Ethylenediamine
FTIR	Fourier transform infrared
GO	Graphene oxide
HFO	Hydrous ferric oxide
HMO	Hydrous manganese dioxide
HNT	Halloysite nanotube
HPEI	Hyperbranched polyethylenimine
IEM	Ion exchange membrane
MMM	Mixed matrix membrane
MOF	Metal–organic framework
NP	Nanoparticle
PA	Polyamide
PANI	Polyaniline
PDA	Polydopamine
PEO	Poly(ethylene oxide)
PES	Polyethersulfone
PPy	Polypyrrole
PSf	Polysulfone
PVA	Polyvinyl alcohol
PVC	Polyvinyl chloride
PVDF	Polyvinylidene fluoride
SEM	Scanning electron microscope
TEM	Transmission electron microscope
TFC	Thin-film composite
UCrFC	Urine/Cr(VI) fuel cell
XRD	X-ray diffractor

## 2.1 Introduction

Water is one of the primitive requirements for humans' living and society development, suggesting that water is basically necessary for a variety of fields [1]. On the one hand, the freshwater that human can use directly is limited, resulting in that more than a billion people currently live in water-scarce regions, and as many as 3.5 billion could experience water scarcity by 2025 [2]. On the other hand, the water pollution problem has been ever increasingly serious due to the rapid development of industries, including fertilizer industries, metal plating facilities, tanneries, batteries, paper industries, mining operations, and pesticides [3]. Both the two issues lead to a severe water shortage. Since the exploitation of new freshwater is not easy and expensive, treatment of wastewater that is polluted is acceptable to remove the containments such as heavy metal ions, dyes, biodegradable waste, nitrates and phosphates, sediment, fluoride, hazardous and toxic chemicals, radioactive

pollutants, and pharmaceuticals for obtaining the freshwater [4]. Among the various pollutants, rejection of heavy metal ions is essential and challenging due to the fact that heavy metal ions are not biodegradable and tend to accumulate in living organisms [3]. In addition, excessive inhalation of heavy metal ions may cause serious toxic effects to humans. In general, the heavy metals are defined as the elements with atomic weights between 63.5 and 200.6 as well as a specific gravity greater than 5.0 [5]. In order to dispose of the pollutants in wastewater, both physical and chemical treatments have been adopted, which include ion exchange, supercritical fluid extraction, adsorption [6], filtration, electrodialysis, precipitation [7], and the electrochemical process [8]. Particularly, each treatment has its specific advantages and disadvantages. The advantage of ion exchange is the high transformation of components, but operational cost is high and only limited metal ions can be rejected. The advantages of adsorption are easy operation, less sludge production, and utilization of low-cost adsorbents, but the desorption still needs to be addressed so that the adsorbents can be reused. The advantages of filtration are lower space requirement and high removal of heavy metals, but it is expensive and complex as well as suffers from membrane fouling. The advantage of electrodialysis is the high rejection rate, but it requires high energy input and membrane clogging may occur. The advantages of precipitation are the low-cost and high dewatering qualities, but it uses plenty of chemicals and the precipitate generation rate is not satisfactory. The advantages of the electrochemical process are low chemical usage and efficient removal, but it needs high energy input. Typically, ion exchange membrane (IEM) plays an important role in these treatments, which is made up of specific porous material [1]. This chapter gives an introduction of common heavy metal ions, which are chromium (Cr), nickel (Ni), copper (Cu), zinc (Zn), cadmium (Cd), mercury (Hg), and lead (Pb) followed by both physical and chemical treatments. Finally, the remaining challenges and future perspectives are highlighted.

## 2.2 Heavy Metal

### 2.2.1 Chromium

The general information of Cr, which is the most available seventh element in the earth, can be concluded as follows: CAS number is #7440-47-3, atomic weight is 52, atomic number is 24, density is  $7.2 \text{ g cm}^{-3}$ , melting point is  $1857 \text{ }^\circ\text{C}$ , boiling point is  $2672 \text{ }^\circ\text{C}$ , and oxidation states are +2, +3, and +6. In nature, Cr occurs primarily as ferric chromite, which is the most stable state of oxidation. In spite of the stable state, it also occurs in divalent and hexavalent forms [9]. In general, the principal industrial consumers of Cr are metallurgical, refractory, and chemical industries. Hexavalent Cr(VI) and trivalent Cr(III) forms are produced during these industrial processes. In addition, Cr(VI) is more harmful than the Cr(III) for plants,

animals, and organisms [10, 11]. Particularly, vomiting, liver and kidney damage, skin inflammation, the creation of ulcer, and pulmonary congestion may be caused by excessive inhalation of chromium [12, 13]. Considering the harmful effects, the industrial wastewater must be treated to remove the Cr(VI) or to change the Cr(VI) into less toxic one before it is released to the environment.

### 2.2.2 *Nickel*

The general information of Ni can be concluded as follows: CAS number is #7440-02-0, atomic weight is 58.71, atomic number is 28, density is  $8.9 \text{ g cm}^{-3}$ , the melting point is  $1453 \text{ }^\circ\text{C}$ , the boiling point is  $2732 \text{ }^\circ\text{C}$ , and oxidation states are +1, +2, and +3. In nature, Ni exists primarily in sulfide or oxide ores as an earth element, which is the twenty-fourth most abundant element on earth. The procedures of extraction of Ni are flotation, magnetic separation, roasting, and melting [9]. Ni plays an important role in both industrial use and un-industrial use. The former includes steel and alloy production, nickel–cadmium battery production, electroplating, chemical catalysis, and manufacture of electronic components. The latter is associated with coins, jewelery, watches, and eyeglass frames [9]. A dry cough, chest pain, creates breathing problem, nausea, diarrhea, skin eruption, pulmonary fibrosis, gastrointestinal ache, renal edema may be caused by excessive inhalation of nickel [11, 14–16]. Therefore, to avoid the human health risks, adequate treatment technology is required to recover the copper from the wastewater.

### 2.2.3 *Copper*

The general information of Cu, which is an essential trace element, can be concluded as follows: CAS number is #7440-50-8, atomic weight is 63.54, atomic number is 29, density is  $8.94 \text{ g cm}^{-3}$ , the melting point is  $1083 \text{ }^\circ\text{C}$ , the boiling point is  $2595 \text{ }^\circ\text{C}$ , and oxidation states are +1 and +2. In nature, Cu occurs primarily as sulfidic copper ores (approximately 90%), oxidic copper ores (9%), and metallic copper ores (<1%). The processes of copper production are different based on the type of ores. Both sulfidic copper ores and oxidic copper ores involve extensive and concentration procedures subjected to roasting, converting, and electrolytic refining [9]. The most essential utilization of Cu is the transmission of electricity in wires and cables. Besides, Cu compounds are widely used in wood preservatives, fungicides, as pigments and antifouling agents in paints, as nutritional additives to livestock, and additive to fertilizers [9]. Comparing to Cu(I), Cu(II) is believed to be more toxic in the environment [17]. Hair loss, anemia, kidney damage, and headache may be caused by excessive inhalation of copper [18]. In order to remove the Cu from wastewater, it is urgent to develop an attractive treatment methodology to recover the Cu.

### 2.2.4 Zinc

The general information of Zn, which is an essential trace element, can be encapsulated as follows: CAS number is #7440-66-6, atomic weight is 65.38, atomic number is 30, density is  $7.13 \text{ g cm}^{-3}$ , the melting point is  $419.5 \text{ }^\circ\text{C}$ , the boiling point is  $908 \text{ }^\circ\text{C}$ , and oxidation state is +2. In nature, sphalerite is the most important Zn ore, and zinc oxide is the most common compound in the industry [9]. Zinc can act as a protective coating for other metals, preventing the corrosion of metals. It is also used in dye casting, construction industry, and noncorrosive alloys. Although it is required necessary for the humans in trace level, pain, vomiting, skin inflammation, fever, vomiting, and anemia may be caused by excessive inhalation of zinc [19]. As a result, the removal of zinc from the industrial effluent has recently gained a lot of attention.

### 2.2.5 Cadmium

The general information of Cd, which is silver-white malleable metal, can be summarized as follows: CAS number is #7440-43-9, atomic weight is 112.4, atomic number is 48, density is  $8.6 \text{ g cm}^{-3}$ , the melting point is  $320.9 \text{ }^\circ\text{C}$ , the boiling point is  $765 \text{ }^\circ\text{C}$ , and oxidation state is +2. Cd is a common impurity in zinc and lead ores; thus, it is principally obtained during the refining of zinc and lead as a by-product [9]. Cd has been used in a number of industrial applications. As it is capable to protect iron from rusting, Cd is used to coat iron products via electroplating [9]. In addition, Cd can be used in alloy synthesis, which contributes to improving the mechanical resistance at increased temperature, including copper–cadmium alloy and nickel–cadmium alloy [9]. Cd is also able to serve as an electrode component in a nickel–cadmium battery, which is the most important application of Cd currently [1]. Cadmium sulfide (CdS) and cadmium sulfoselenide are used as color pigments in various plastics and paints [9]. It is worth mentioning that CdS has been introduced into the photocatalysts for ultraviolet light response functioning as quantum dots in photoelectrochemical cells, which expands the absorption spectrum from ultraviolet light to visible light and facilitates the electron–hole pairs separation [20]. Cadmium oxide is used in black and white television phosphors and in the blue and green phosphors of color television cathode ray tubes [21]. However, it is regarded as most toxic heavy metal found in the industrial effluent as well. Both acute poisoning and chronic poisoning may be exerted on human health due to the excessive inhalation of cadmium. Generally, the former one was rare, while the latter has received ever-increasing attention. Lung cancer, hepatic toxicity, and harm to the kidney, liver, respiratory system, and reproductive organs may be caused by excessive inhalation of cadmium [11, 22]. Because of the negative effects, Cd from the wastewater is required to be removed via economically reliable and efficient treatment.

### 2.2.6 *Mercury*

The general information of Hg, which is the only liquid metallic element at standard conditions for temperature and pressure, can be concluded as follows: CAS number is #7439-97-6, atomic weight is 200.6, atomic number is 80, density is  $13.6 \text{ g cm}^{-3}$ , melting point is  $-38.9 \text{ }^\circ\text{C}$ , boiling point is  $356.6 \text{ }^\circ\text{C}$ , and oxidation states are +1 and +2. In nature, up to 70%, Hg is contained in cinnabar, which is a red mercury sulfide and is the main component of mercury ores. In general, half of the Hg is consumed in chlorine–caustic soda manufacture, and half of the Hg is used in dental amalgams, mercury electronic switches, fluorescent lamps, and electronic devices [9]. Since the Hg metal ions, including Hg(I) and Hg(II), can transport in aqueous solution, it can accumulate in ecosystems, which is harmful to human health and the environment. Metallic Hg is rather volatile and the Hg vapor is toxic to human as well [23–25]. The Minamata happened in Japan in 1956 has raised worldwide awareness of the mercury pollution [26]. Kidney, brain, reproductive, and respiratory systems may be damaged due to excessive inhalation of mercury. Hence, it is urgent to develop an efficient methodology for the removal of mercury from the wastewater.

### 2.2.7 *Lead*

The general information of Pb, which is a relatively unreactive post-transition metal, can be concluded as follows: CAS number is #7439-92-1, atomic weight is 207.19, atomic number is 82, density is  $11.3 \text{ g cm}^{-3}$ , melting point is  $327.5 \text{ }^\circ\text{C}$ , boiling point is  $1740 \text{ }^\circ\text{C}$ , and oxidation states are +2 and +4 [9]. In nature, Pb exists in the form of sulfide, cerussite ( $\text{PbCl}_2$ ), and galena [27]. Currently, 71% of Pb was used in batteries mainly for vehicles, which plays a dominant role. Others are used in pigment (12%), ammunition (6%), and cable sheeting (3%) [9]. Kidney and nervous system damage, mental retardation, and cancer may be caused by excessive inhalation of Pb [28, 29]. In view of the risks to the plants and animals, development of a treatment technology for removal of Pb has attracted ever-increasing attention.

In summary, the mentioned heavy metal ions can accumulate in humans and environment, which leads to various negative effects on humans as well as environmental problems based on their toxicity. The toxic effects are concluded in Table 2.1. As clean water is one of the most important elements for all living organisms, the heavy metal ions occurred in water resources should be removed efficiently and cost-effectively.



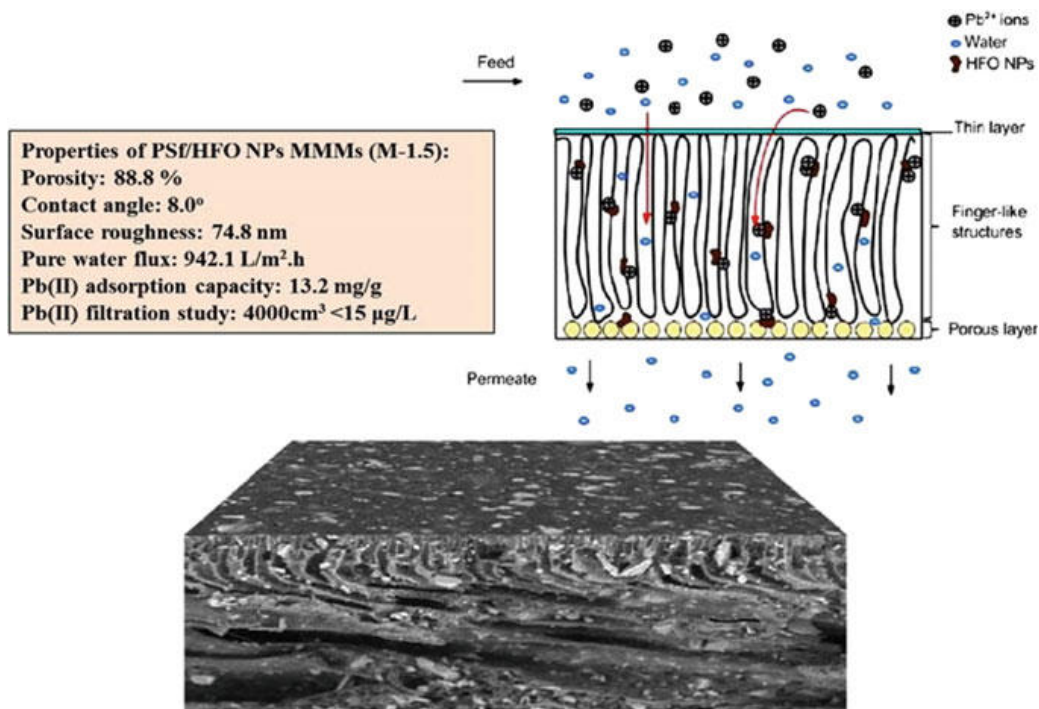
**Table 2.1** Toxic effects of heavy metals

Heavy metal	Toxic effects
Cr	Vomiting, liver and kidney damage, skin inflammation, the creation of ulcer, and pulmonary congestion
Ni	A dry cough, chest pain, creates breathing problem, nausea, diarrhea, skin eruption, pulmonary fibrosis, gastrointestinal ache, renal edema
Cu	Hair loss, anemia, kidney damage, and headache
Zn	Pain, vomiting, skin inflammation, fever, vomiting, and anemia
Cd	Lung cancer, hepatic toxicity, and harm to the kidney, liver, respiratory system, and reproductive organs
Hg	Kidney, brain, reproductive, and respiratory systems
Pb	Kidney and nervous system damage, mental retardation, and cancer

## 2.3 Physical Treatment Methods

### 2.3.1 Ultrafiltration

Ultrafiltration is a separation technique, in which pressure gradient or concentration gradient as a driven force leads to a separation, requiring low energy for the wastewater treatment [30]. This process is capable to remove the particles with the size range of 10–100 nm [1]. Daraei et al. [31] prepared nanocomposite polymeric membrane from polyethersulfone (PES) and polyaniline/iron(II, III) oxide (PANI/Fe<sub>3</sub>O<sub>4</sub>) nanoparticles (NPs) by phase inversion method for Cu(II) removal. It was indicated that the most possible removal mechanism by this membrane was sorption. The results showed that the membrane with 0.1 wt% NPs exhibited the highest ion rejection, which was 85 and 75% for high concentration aqueous solution (20 mg L<sup>-1</sup> Cu(NO<sub>3</sub>)<sub>2</sub>) and low concentration aqueous solution (5 mg L<sup>-1</sup> Cu(NO<sub>3</sub>)<sub>2</sub>), respectively. Similarly, Gohari et al. [32] synthesized PES/hydrous manganese dioxide (HMO) ultrafiltration mixed matrix membranes (MMMs) for removal of Pb(II). The effect of weight ratio of HMO:PES on the membrane pure water flux, hydrophilicity, porosity, and Pb(II) adsorption capacity was investigated. It was demonstrated that a higher ratio resulted in a decreased pore size. In addition, a higher HMO loading resulted in a higher water flux, which was ascribed to the lower contact angle value, increased porosity, and greater surface roughness. The results from Pb(II) adsorption experiments showed that a remarkable uptake capacity of 204.1 mg g<sup>-1</sup> was achieved at pH between 6 and 8, suggesting that the membrane with weight ratio of 2.0 was capable to treat natural water directly. The original adsorption capacity was 97.5% maintained after a simple recovery process, indicating its potential to be applied in industrial applications. Ghaemi et al. [33, 34] prepared the polypyrrole (PPy)-coated alumina (Al<sub>2</sub>O<sub>3</sub>) NPs via an oxidative polymerization reaction. Afterward, the PPy@Al<sub>2</sub>O<sub>3</sub> NPs were added into pristine PES membrane for removal of Cu(II). It was found that the composite membrane



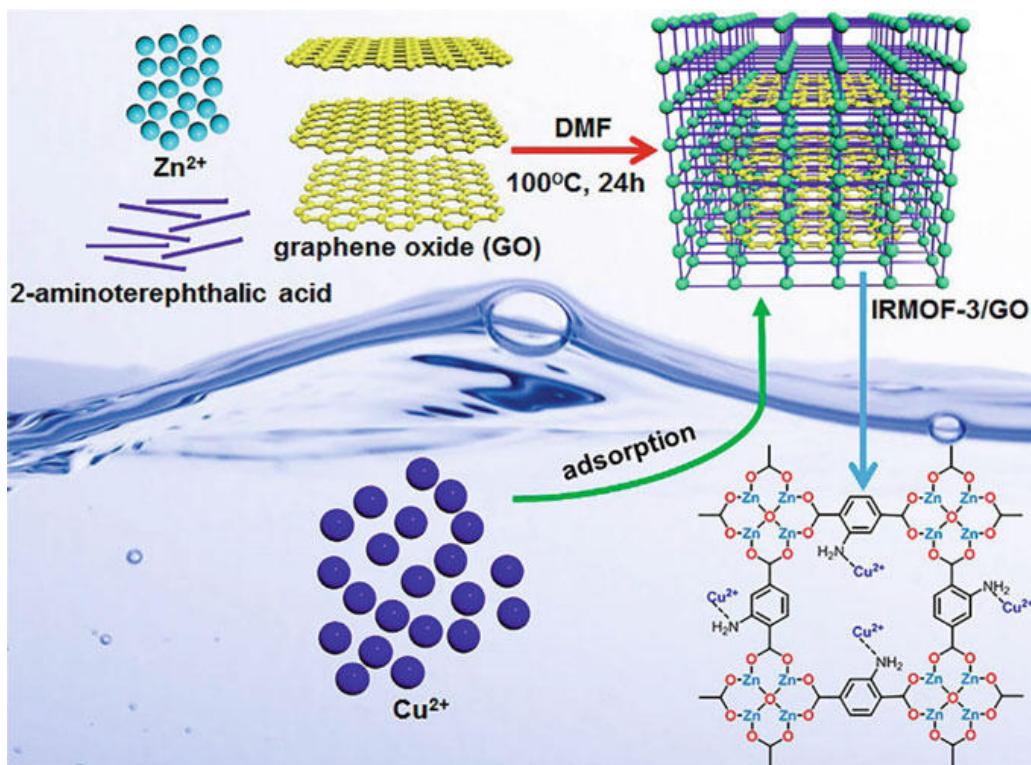
**Fig. 2.1** Schematic of the PSf/HFO NPs ultrafiltration membrane [36]. Reproduced with the permission from Elsevier

with 1.0 wt% PPy@Al<sub>2</sub>O<sub>3</sub> NPs yielded the highest Cu rejection (80%) comparing to other doping amount. Mukherjee et al. [35] integrated graphene oxide (GO) with mixed matrix membranes (MMM) via non-solvent induced phase inversion method. After GO was added into MMM, the membrane became highly permeable, hydrophilic, and charged. It was demonstrated that the optimal amount of GO addition was 0.2 wt%, which was ascribed to adsorption, diffusion, and convection. The results from the rejection tests showed that the adsorption capacities of Pb(II) (pH = 6.7), Cu(II) (pH = 6.5), Cd(II) (pH = 6.4), and Cr(VI) (pH = 3.5) were 79, 75, 68, and 154 mg g<sup>-1</sup>, respectively. Abdullah et al. [36] proposed a polysulfone (PSf)/hydrous ferric oxide nanoparticles (HFO NPs) ultrafiltration MMMs for removal of Pb(II) from aqueous solution as shown in Fig. 2.1. The physiochemical properties of this membrane were characterized via transmission electron microscope (TEM), Brunauer–Emmett–Teller (BET), X-ray diffractor (XRD), Fourier transform infrared (FTIR), scanning electron microscope (SEM), atomic force microscope (AFM), pure water flux analysis, contact angle measurement, and membrane porosity analysis. The results showed that the specific surface area of the HFO NPs was 233.49 m<sup>2</sup> g<sup>-1</sup> and a higher weight ratio of HFO NPs in membrane led to a significant increase in membrane pure water flux from 229.5 L m<sup>-2</sup> h<sup>-1</sup> to 942.1 L m<sup>-2</sup> h<sup>-1</sup>, which was because of the enhanced hydrophilicity and greater surface roughness. It was confirmed that the optimal weight ratio of HFO NPs:PSf was 1.5 and the optimum pH was 6.5–7.0, which resulted in the highest adsorption capacity of Pb(II) of 13.2 mg g<sup>-1</sup>. The pore size of the membrane may be larger

than the size of metal ions, and hence, chemical or polymeric agents are added into the membrane for better removal performance. Moreover, it will be ideal that a universal membrane can be synthesized by removing the majority of heavy metal ions, which should attract more attention in the future.

### 2.3.2 Nanofiltration

Nanofiltration is also a membrane filtration-based method, and the mechanism is similar to ultrafiltration. While comparing to ultrafiltration, nanofiltration membranes have smaller pore sizes (1–10 nm). It is believed that nanofiltration is an intermediate technique between ultrafiltration and reverse osmosis [37]. Zhang et al. [38] synthesized nanometric graphene oxide framework nanofiltration membranes for removal of heavy metal ions. Initially, the GO framework was constructed by the cross-linking of ethylenediamine (EDA). Afterward, the GO framework was modified by hyperbranched polyethyleneimine (HPEI), introducing numerous amine functional groups. It was illustrated that the EDA cross-linking not only provided enhanced structural stability but also enlarged the nanochannels, resulting in promoted water permeability. HPEI 60 K could enhance the surface charge and reduce the transport resistance. The rejections of Pb(II), Ni(II), Zn(II), and Cd(II) were 95.7, 96.0, 97.4, and 90.5%, respectively. Ghaemi et al. [39] modified Fe<sub>3</sub>O<sub>4</sub> nanoparticles with silica, metformine, and amine coating, which were employed for fabrication of polyethersulfone (PES) mixed matrix membranes. The optimal adding of modified Fe<sub>3</sub>O<sub>4</sub> nanoparticles was 0.1 wt%, showing a copper removal as high as 92%. This remarkable removal ability was attributed to the improved hydrophilicity and the presence of nucleophilic functional groups on nanoparticles. Bolisetty et al. [40] developed amyloid–carbon hybrid membranes for universal water purification for removal of heavy metal ions, metal cyanides, and nuclear waste. The amount of heavy metal ions dropped three to five orders of magnitude per passage after filtration, and this membrane was able to reuse for numerous times. The rejected valuable heavy metal contaminants could be recycled easily via a simple thermal reduction process. Zeng et al. [41] synthesized halloysite nanotubes (HNTs) functionalized with 3-aminopropyltriethoxysilane (APTES), and polyvinylidene fluoride (PVDF) nanofiltration membranes for removal of Cu(II), Cd(II), and Cr(VI). The introduction of A-HNTs nanoparticles significantly improved the hydrophilicity of membranes due to the presence of abundant hydrophilic functional groups including hydroxyl and amino, resulting in a higher pure water flux. Particularly, the rejection of Cu(II) reached almost 100% with the pH > 9. Habiba et al. [42] synthesized chitosan/polyvinyl alcohol (PVA)/zeolite nanofibrous composite membrane via electrospinning for removal of Cr(VI), Fe (III), and Ni(II). This membrane showed remarkable stability in neutral, acidic, and basic media for 20 days as well as after five recycling runs. It was indicated that the

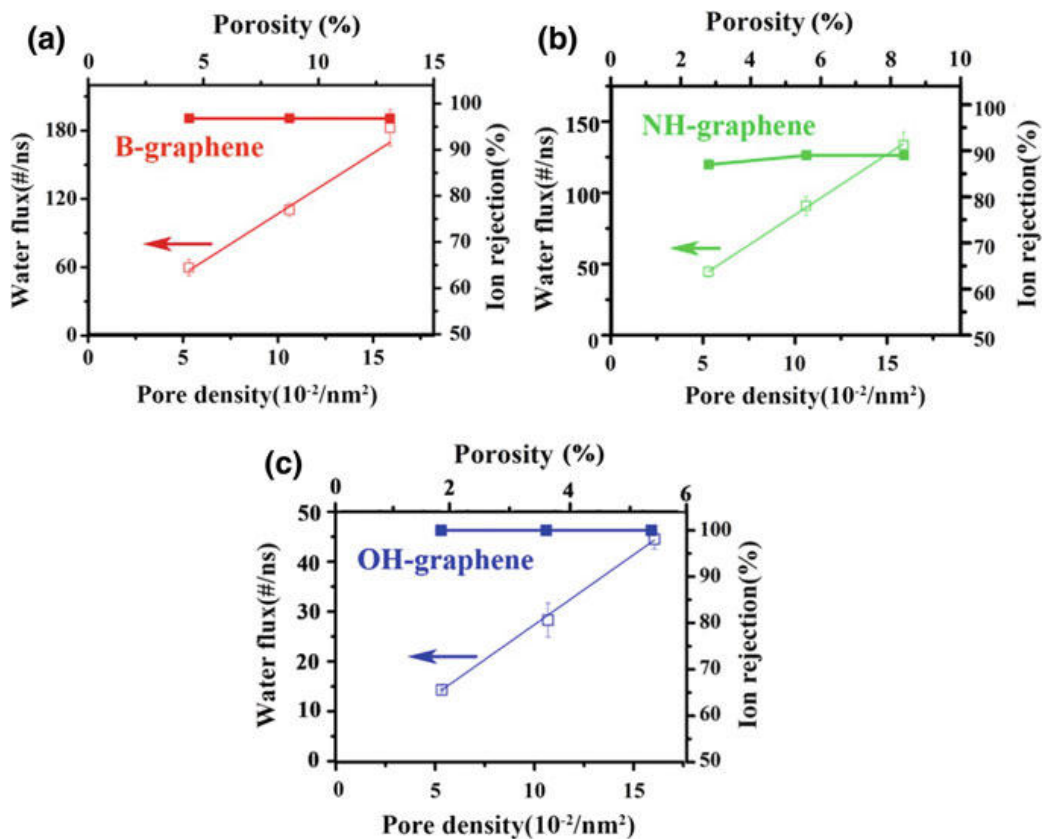


**Fig. 2.2** Schematic diagram of the preparation of IRMOF-3/GO and the adsorption of Cu(II) on IRMOF-3/GO [43]. Reproduced with the permission from the American Chemical Society

Cr(VI) adsorption can be well described by the pseudo-second-order kinetic model. In addition, the adsorption of Fe(III) and Ni(II) was well described by Lagergren first-order model. However, the adsorption capacity at high concentration was not satisfactory. Rao et al. [43] prepared metal–organic framework/graphene oxide composite (IRMOF-3/GO) decorated polydopamine (PDA)-coated PSf membrane for Cu(II) removal as shown in Fig. 2.2. It was reported that this membrane exhibited a rejection of Cu(II) as high as 90% with a high flux of  $31 \text{ L m}^{-2} \text{ h}^{-1}$ , which was attributed to the adsorption effect of IRMOF-3/GO and the enhancement of membrane surface potential. Meschke et al. [44] found that the dominant parameter of divalent cation removal was the size, while the pH exhibited negligible effect on the rejection. For monovalent cations, as the hydrated radius was smaller, the size exclusion is less dominating. They also reported that in acid media, the separation during concentrating was difficult to realize [45]. This was because of the shearing forces and the build-up concentration as well as the electric field gradient forced the permeation of the solutes. Nanofiltration is an effective method for heavy metal removal, which is easily operative and energy saving. Similarly, developing a universal membrane for removing the majority of heavy metal ions can be one of the future research directions.

### 2.3.3 Reverse Osmosis

Reverse osmosis is a water purification technology that employs semi-permeable membranes for removal of contaminants and only allows the water to pass through the membrane [37]. The pore size of the reverse osmosis membranes is in the range of 0.1–1.0 nm. Particularly, to overcome osmotic pressure, an applied pressure is essential in reverse osmosis; thus, the operation requires high energy [1]. Li et al. [46] theoretically evaluated the rejection performance of heavy metal ions utilizing nanoporous graphene surfaces as reverse osmosis membranes with functionalized groups (boron, nitrogen, and hydroxyl groups) as shown in Fig. 2.3. It was demonstrated that water permeability is 2–5 orders of magnitude higher than that of commercial membranes, which was attributed to the lower free energy barrier than ions when passing through graphene pores. The barrier for ion transport was ascribed to the combining contributions from the ion dehydration effect and the surface electrostatic interaction. This characteristic of the membrane showed the possibility that this membrane could be applied for the rejection of heavy metal ions. Petrinic et al. [47] proposed a combination of ultrafiltration and reverse

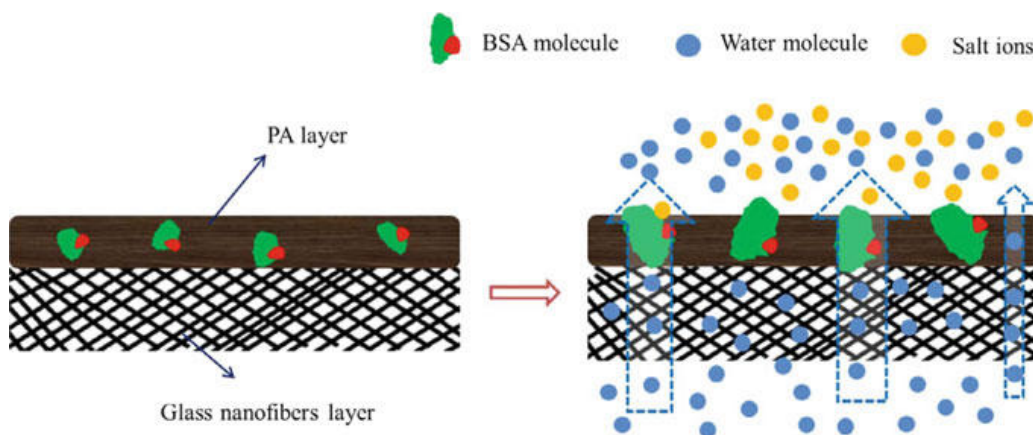


**Fig. 2.3** Effect of pore density (or porosity) on water flux and ion rejection at an applied pressure of 200 MPa: **a** B-graphene, **b** NH-graphene, and **c** OH-graphene membranes [46]. Reproduced with the permission from Elsevier

osmosis for heavy metal removal. In view of the membrane fouling and clogging problem, the ultrafiltration serves as a pre-treatment to remove the contaminants, whose sizes were larger than 0.4  $\mu\text{m}$ , before a final reverse osmosis process. It was demonstrated that almost 90% of suspended solids were first rejected by the ultrafiltration process, and 91.3–99.8% of metal elements and organic/inorganic compounds were removed by the reverse osmosis. It is widely agreed that the major shortcoming of reverse osmosis is the high power consumption and the restoration of the membranes [3]. Therefore, it is favorable to combine reverse osmosis with other wastewater treatment to compensate the high cost.

### 2.3.4 Forward Osmosis

Forward osmosis is an emerging technology for removal of heavy metals. Although it is similar to reverse osmosis, the major difference lies in the driven force. As the driven force of forward osmosis is the osmotic pressure gradient, this process possesses several advantages in terms of efficient water recovery, low energy consumption, less membrane fouling, and easy fouling removal [48]. Cui et al. [49] developed a forward osmosis using a thin-film composite (TFC) made from interfacial polymerization on a macrovoid-free polyimide support as the forward osmosis membrane and a novel bulky hydroacid complex  $\text{Na}_4[\text{Co}(\text{C}_6\text{H}_4\text{O}_7)_2] \cdot 2\text{H}_2\text{O}$  (Na–Co–CA) as the draw solute. It was reported that the process exhibited a heavy metal rejection as high as 99.5% with water fluxes fixed at around  $11 \text{ L m}^{-2} \text{ h}^{-1}$ , draw solution fixed at 1 M, and heavy metal salts concentration fixed at 2000 ppm, which was higher than most of nanofiltration processes. The rejection could be further increased by increasing the concentrations of draw solutions and heavy metal ions, or raising the temperature. Zhao et al. [50] synthesized forward osmosis membrane with the glass nanofiber supporting layer and the bovine serum albumin (BSA)-embedded polyamide (PA) swellable active layer as shown in Fig. 2.4.

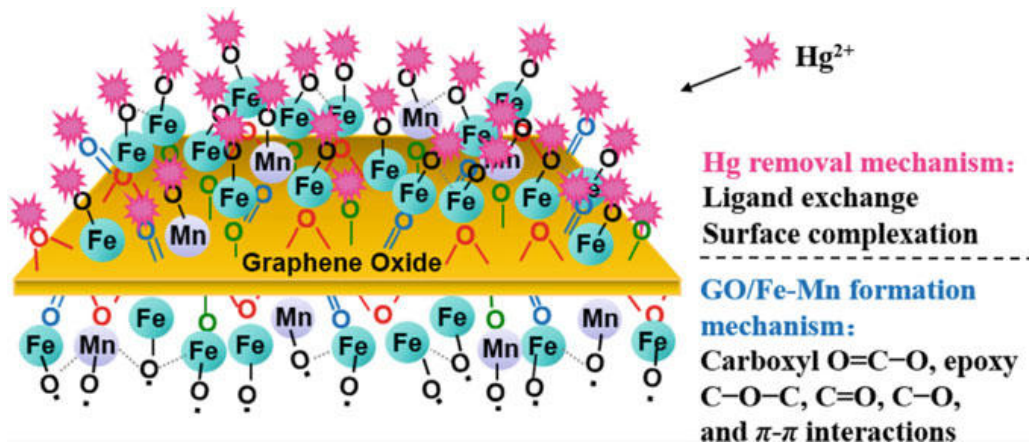


**Fig. 2.4** Schematic diagram of the separation mechanism of the prepared FO membranes [50]. Reproduced with the permission from Elsevier

The glass nanofiber supporting layer provided transport channels for flow process, and active layer bi-functioned as water channels and reinforced barrier. The results showed that all the recovery ratios of Cu(II), Pb(II), and Cd(II) were exceeding 99.4%. The impressive performance was attributed to the double-layer structure, which was beneficial for enhancing the dissolution-diffusion-flow separation process as well as stabilizing the separation selectivity. Several issues associated with forward osmosis still need to be addressed to realize the commercialization. These include (1) the low-performance forward osmosis membranes; (2) the ineffective draw solutes; and (3) high cost in draw solute regeneration.

### 2.3.5 Adsorption

Adsorption is an efficient and admirable process to remove heavy metal ions. In principle, the advantages of this method are low operating cost, simple and flexible design, low fouling problems, and negligible toxic pollutant production [51]. In addition, adsorbents can be recreated by the desorption process after the adsorption process, which means that it is reversible technique. Li et al. [52] synthesized chitosan/sulfhydryl-functionalized graphene oxide composite (CS/GO-SH) via covalent modification and electrostatic self-assembly. This membrane was characterized by FTIR, Raman spectroscopy, SEM, XRD, and thermogravimetric examination. The results showed that this membrane possessed abundant multifunctional groups, including  $-OH$ ,  $-COOH$ ,  $-SH$ , and  $-NH_2$ . In unitary metal ion system, the adsorption capacities of Cu(II) and Pb(II) are similar, while the adsorption capacity of Cd(II) is lower. For ternary metal ion system, the order of adsorption capacity is  $Cd(II) > Cu(II) > Pb(II)$ . Chen et al. [53] prepared 4-aminothiophenol and 3-aminopropyltriethoxysilane functionalized GO via graft modification for Cu(II) removal from aqueous solution. It was illustrated that sulfhydryl ( $-SH$ ) and amino groups ( $-NH_2$ ) were associated with 4-aminothiophenol modified GO (GO-SH) and 3-aminopropyltriethoxysilane modified GO (GO-N). The maximum adsorption capacities of GO-SH and GO-N were 99.17 and 103.28  $mg\ g^{-1}$ , which were much higher than the pristine GO (32.91  $mg\ g^{-1}$ ). The reason for this enhancement was the increase in sorption sites as well as the chelation with heavy metals. Henriques et al. [54] reported that combination of oxygen and nitrogen functional groups effectively improved the adsorption capacity of GO toward Hg(II). This improvement was attributed to shifting of the equilibrium of the reaction derived from the fact that the nitrogen and oxygen functional groups possessed different acidic properties. It was indicated that a small dose of this material (10  $mg\ L^{-1}$ ) showed a high removal rate of 95% after 24 h and a maximum sorption capacity of 35  $mg\ g^{-1}$ . Wan et al. [55] proposed a GO-based sorbent for Pb(II) removal. Aiming at improving the adsorption selectivity and ability in solid-liquid separation of GO, nanosized hydrated manganese oxide (HMO) ( $10.8 \pm 4.1\ nm$ ) was in situ grown on GO. The HMO@GO exhibited splendid adsorption selectivity and capacity toward Pb(II), reaching 500  $mg\ g^{-1}$  in the presence of Ca(II). The outstanding performance



**Fig. 2.5** Schematic of the separation mechanism of GO/Fe-Mn composite [56]. Reproduced with the permission from Elsevier

was mainly ascribed to the abundant oxygen-containing groups on GO that preferably sequestered Pb(II) through specific interaction, and the host GO offering the pre-concentration of Pb(II) for enhanced sequestration through the Donnan membrane effect. Similarly, Tang et al. [56] fabricated GO/Fe-Mn composite with molar ratio of Fe/Mn = 3/1 and mass ratio of Fe/GO = 1/7.5 for Hg(II) removal as shown in Fig. 2.5. The separation of Hg(II) was dominated by ligand exchange and surface complexation. The adsorption capacity was  $32.9 \text{ mg g}^{-1}$ . It was worth mentioning that the bioavailability of mercury also could be reduced, thus promoting the seedling growth. Shariful et al. [57] prepared chitosan/poly(ethylene oxide) (PEO) nanofibrous membrane via electrospinning process. The beadless fibers possessed average diameter, maximum tensile strength, and the specific surface area as  $115 \pm 31 \text{ nm}$ ,  $1.58 \text{ MPa}$ , and  $218 \text{ m}^2 \text{ g}^{-1}$ , respectively. Moreover, higher specific surface area and hydrophilicity also contributed to maximum adsorption capability. Shariful et al. [58] synthesized chitosan/poly(ethylene oxide)/activated carbon (AC) nanofibrous membrane (CPANM) via electrospinning process. The membrane showed that the highest adsorption capacities for Pb(II), Zn(II), Cu(II), Fe(III), and Cr(VI) were 176.9, 186.2, 195.3, 217.4, and 261.1  $\text{mg g}^{-1}$ , respectively. It was explained that not only the  $-\text{NH}_2$  in chitosan/PEO fiber (CPF) acted as active group, but also the  $-\text{COOH}$  in the fiber surface, which was introduced by the AC, contributed to superior adsorption. The adsorption process has become the leading technique in the heavy metal removal due to its superiorities, including low operating cost, simple and flexible design, low fouling problems, and negligible toxic pollutant production. The rejection performance was mainly dominated by the surface area, functional groups, pore size distribution, and the polarity of the adsorbent.



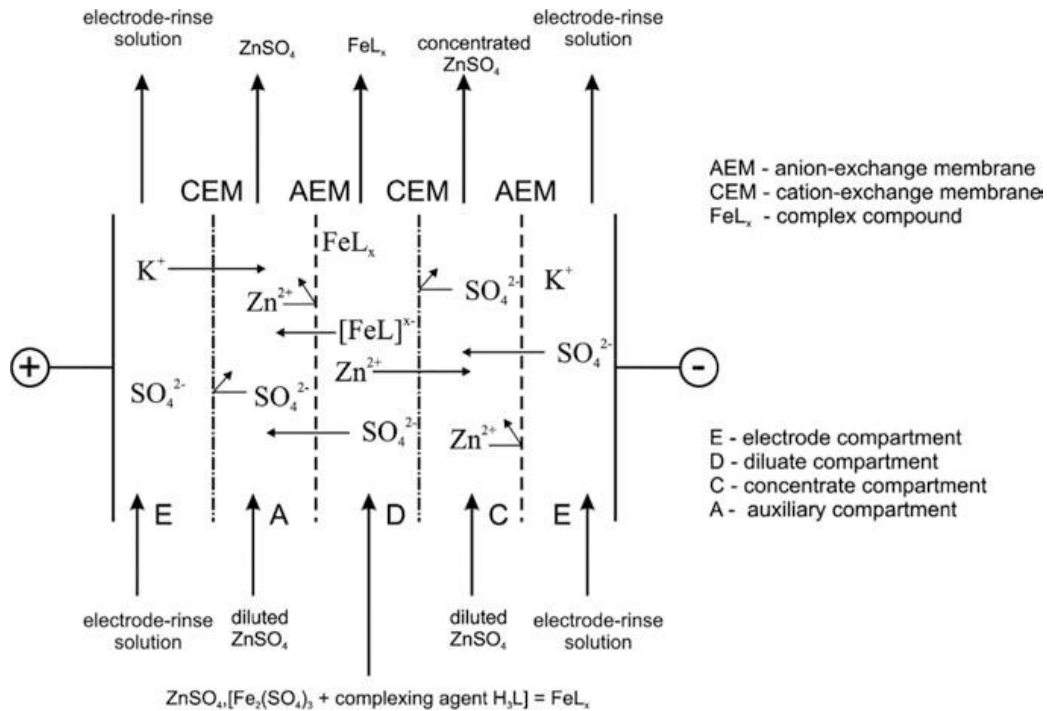
## 2.4 Chemical Treatment Methods

### 2.4.1 *Electrodialysis Method*

Electrodialysis is used to transport ions from one solution through IEMs to another solution under the control of an applied electric field [59]. The advantages of this separation process are as follows: (1) high water recovery; (2) easily scale up; (3) low usage of chemicals; and (4) easy combination with other technologies [60]. Babilas et al. [61] used electrodialysis to selectively recover zinc from simulated electroplating industry wastes, which was enhanced with complex formation method. It was demonstrated that the Zn recovery and current efficiency were 86.6 and 84.95%, respectively. Nemati et al. [60] prepared a heterogeneous cation exchange membrane (CEM) consisting of polyvinyl chloride (PVC) and 2-acrylamido-2-methylpropane sulfonic acid-based hydrogel (AMAH). It was indicated that this membrane showed promising dimensional stability, higher water content, more hydrophilic surface, and more porous structure. The results from rejection experiment showed that the outstanding rejection capacities of Pb(II) and Ni(II) were about 99.9%, suggesting that the membrane possessed the desirable potential for scaling practical applications. Ge et al. [62] used homemade nanofiltration membrane in electrodialysis to replace the conventional CEM so as to separate the multivalent ions and monovalent ions. It was reported that the porous structure of nanofiltration membrane could decrease the transfer resistance of ions, enhance the limiting current density, and improve the ion flux. The separation was feasible because the divalent cations rather than monovalent cations could be rejected effectively due to the presence of the dense layer of nanofiltration membrane. Since the anode releases electrons, where oxidation reactions take place, thus the anode side will be of acidic nature. Therefore, more attention should be paid into the corrosion problem. On the cathode, the electrons are received and reduction reactions take place, and thus cathode side will be alkaline. Hence, the scaling issue should be addressed for long-term durability.

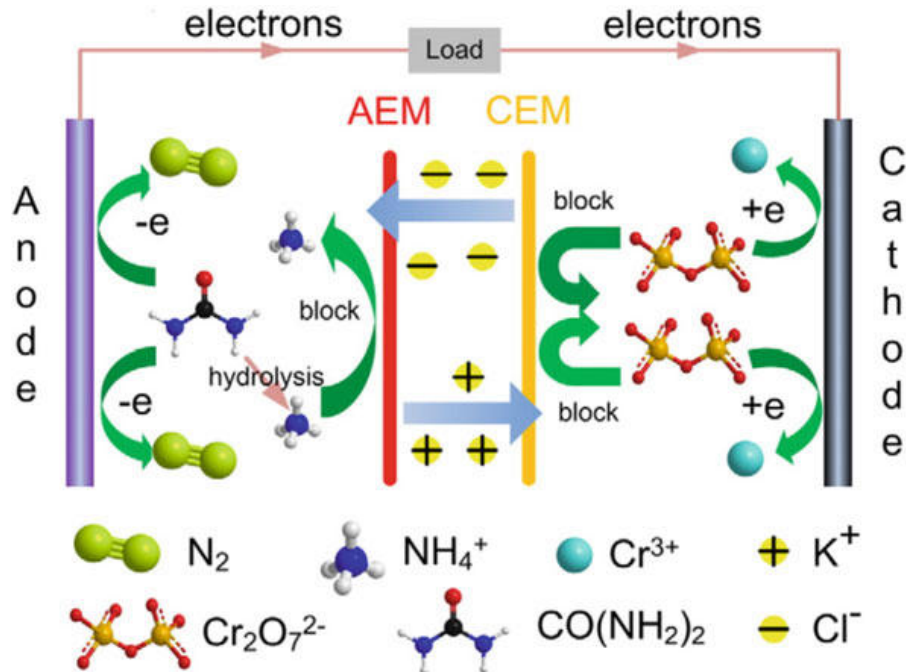
### 2.4.2 *Fuel Cell Method*

Fuel cells are generally considered as an emerging energy conversion technology [63–65]. In principle, there is a reduction reaction at fuel cell cathode, which is basically identical to converting Cr(VI) to Cr(III) [66]. Therefore, Zhang et al. [67–69] proposed the strategy using fuel cell to deal with the Cr(VI)-containing wastewater with cogeneration of electricity. Zhang et al. [67] developed an alkaline fuel cell with ethanol-fed into the anode as fuel and Cr(VI) containing solution fed into the cathode as oxidant to operate the Cr(VI) reduction. The removal efficiency was found to improve with increasing Cr(VI) feeding concentration. The removal efficiency increased from 89.1 to 96.0% as the Cr(VI) concentration increased from



**Fig. 2.6** Schematic representation of electrodesialysis setup [61]. Reproduced with the permission from Elsevier

0.55 to 3.94 mM. When the  $\text{H}^+$  concentration increased from 0.1 to 1.0 M, the removal efficiency increased from 64.6 to 83.3% as well. The cathodic efficiency was promoted from 24.86 to 63.15% as the Cr(VI) concentration increased from 0.5 to 3.94 mM. It should mention that the cathodic efficiency was always lower than 100%, which was attributed to the presence of oxygen that occupied the electrons for Cr(VI) reduction (Fig. 2.6). Xu et al. [68] developed a urine/Cr(VI) fuel cell (UCrFC) for reducing Cr(VI) and generating electricity simultaneously as shown in Fig. 2.7. It could be seen that the configuration of the UCrFC, which was composed of a Ni-based anode, an alkaline anion exchange membrane (AAEM), a CEM, and a cathode made of carbon cloth. It was observed that the removal efficiency of Cr(VI) was 93% after operating for 71 h. Afterward, Zhang et al. [69] proposed that a phenol-Cr(VI) coupled redox fuel cell was able to remove the phenol and Cr(VI) efficiently without energy supply. Phenol was oxidized on Ni/C anode to release electrons, and Cr(VI) was reduced to Cr(III) by receiving the electrons transported through the external circuit. It was indicated that the removal efficiency of phenol was 98.6% within 132 h, and removal efficiency of Cr(VI) was 99.8% within 60 h. Qian et al. [70] studied the dependency of migration and reduction of mixed  $\text{Cr}_2\text{O}_7^{2-}$ , Cu(II), and Cd(II) on electric field, ion exchange membranes (IEMs) and metal concentration in microbial fuel cells. Circuit current directed reduction of more metals on the cathodes with less migration across the IEMs, compared to the more metal transport in the absence of circuit current. A higher metal concentration led to more transport of net metal ions through the IEMs. In summary, employing a



**Fig. 2.7** Schematic of urine/Cr(VI) fuel cell (UCrFC) configuration [68]. Reproduced with the permission from Elsevier

fuel cell to reduce harmful heavy metal ions is a novel strategy in dealing with industrial wastewater. Though the removal efficiency is relatively high, the required operating time is long and the power output is negligible. Hence, future research direction should be focused on shorting the operating time as well as improving the peak power density of this type of reactor.

## 2.5 Remaining Challenges and Perspectives

Nowadays, the heavy metal removal from the wastewater is becoming more serious because several threats are arising to human health and the environment. Although several treatments that use ion exchange membranes have been proposed and improved, challenges are still existing in terms of the above-mentioned treatment technologies. Membrane filtration technology is capable to remove heavy metal ions efficiently, but it suffers from high cost, process complexity, membrane fouling, and low permeate flux. The use of osmosis processes such as reverse osmosis and forward osmosis has been restricted in terms of the high cost for large-scale applications. Adsorption is believed to be an effective method to treat low concentration wastewater. However, since the adsorbent is the most important component in adsorption process, cost-effectiveness and availability are two major concerns in finding the most probable adsorbent for heavy metal removal in wastewater. Electrochemical treatment is considered as an alternative choice for

heavy metal removal, which is rapid and well-controlled as well as requires fewer chemicals and provides promising yields. However, the high initial capital investment and expensive electricity supply still need to be taken into consideration. Especially in fuel cell mode, the power output demands a substantial improvement. More importantly, in the pace of expanding the breakthrough in laboratory scale into pilot scale and industrial scale, we need to take more aspects into consideration so as to select the optimal treatment.

## 2.6 Conclusion

In terms of the various negative effects of heavy metal accumulation on humans as well as environment, the removal of heavy metal ions from industrial wastewater is an essential part of most of the current research in the environmental field. In this chapter, both physical methods (ultrafiltration, nanofiltration, reverse osmosis, forward osmosis, and adsorption) and chemical methods (electrodialysis method and fuel cell method) are discussed. Particularly, in order to select the most suitable method among these methods, some parameters should be taken into consideration, including main target heavy metal ions, initial concentration of the target ions, economic parameter, environmental impact, pH values, and the overall performance. In addition, the advantages and limitations of each method have been introduced. Because the ion exchange membrane is the most critical component in all the processes, more research attention has been paid into this field. There are five directions associated with membranes that can be concluded as follows: (1) the preparation method of membranes should be simplified and easily controlled; (2) it is quite necessary to investigate the effects of coexisting ions in the solution on the removal of target ions; (3) it is desirable to fabricate a universal membrane for removing the majority of heavy metal ions; (4) it is crucial to develop novel and facile methods of regenerating membranes that have been fouled; and (5) special attention should be paid to modeling and simulation of transport phenomena, which can improve our understanding of mechanisms involved in removal of heavy metal ions.

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# Chapter 3

## Separation and Purification of Uncharged Molecules



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**Abstract** The purification and separation of uncharged complexes is a challenging issue in the field of biotechnology. The most common metals required for our biological system are iron and cobalt, which play an important role in all living systems as these coordinate with haemoglobin and vitamin B<sub>12</sub>, respectively. Therefore, careful measurement of vitamin B<sub>12</sub> and haemoglobin is a prerequisite for early detection of several diseases. Since detection of these compounds usually carried out in blood samples, therefore, purification of these compounds is utmost important before their measurement. Furthermore, the separation of uncharged dyes from wastewater sources is important to protect human health through preservation of supply of water which will protect the spread of various life-threatening diseases. Although there are several unit operations applied for the separation of uncharged complexes, however, all operations have their own drawbacks. The aim of this book chapter is the systematic and comparative presentation of the available information on the separation of these compounds owing to their application in various fields of biotechnology.

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### 3.1 Introduction

A complex is defined as a molecular entity which is formed by the weak association of two or more component molecular entities, or the corresponding chemical species. It is obvious that the bonding energy between the components is normally lesser than a covalent bond. The complex indicates an association of the molecular entity where a metal atom or ion will be in the centre and surrounded by ligands. Ligands are usually considered as electron donors; therefore, these are attracted to the metal at the centre of the complex. Additionally, metals are considered as electron acceptors. They are usually transition metals (d-block metals) such as titanium, cobalt, vanadium, manganese, chromium, iron, cobalt, copper, gold, nickel, platinum and palladium. Other metals, such as magnesium, aluminium, calcium and tin also form complexes with suitable ligands. Moreover, the number of electrons on the central metal is crucial for complex formation. Since central metals can exist in different oxidation states—i.e. with different numbers of d electrons; therefore, complexes are either positively charged, uncharged or negatively charged. The various properties of the complex such as stereochemistry, stability, spectroscopic, magnetic and reactivity can be changed by oxidizing or reducing the metal in the centre of a complex with the similar number or type of ligands.

One of the distinguishing features of life is that cells are made of matter which is any substance that occupies space with a certain mass. Elements are unique forms of matter with specific chemical and physical properties that cannot be broken down into smaller substances by ordinary chemical reactions. The iron and cobalt are considered as the most common metals which play an important role for all living organisms. For example, iron complexes are used in the transportation of oxygen in the blood and tissues and cobalt acts as a coordinator for the formation of vitamin B<sub>12</sub>. The haemoglobin content varies, depending upon on age and sex with the average range for an adult male is in the range of 14–18 g/L. However, haemoglobin content in the adult female is in the range from 12 to 16 g/L. The deficiency of haemoglobin level is considered as anaemia which is due to the interference of other molecules with haemoglobin which represents the number of red blood cells present in the body. There are several factors such as huge blood loss, disorder of bone marrow function, nutritional deficiency and inadequate intake of iron responsible for low haemoglobin content in the blood. Vitamin B<sub>12</sub> maintains the routine function of the brain as well as in the nervous system. It also helps for red blood cells formation and to create and regulate DNA. The human body produces millions of red blood cells every minute to maintain the normal function of the biological system. Vitamin B<sub>12</sub> helps to multiple of RBC properly in our body. The reduction of red blood cells occurs when vitamin B<sub>12</sub> levels are low in our body. Therefore, careful measurements of Vitamin B<sub>12</sub> and haemoglobin are required for detection of several diseases.

In the developed countries, the fastest growing industries have originated complexity and variability in their wastewater over the last decades and there are fresh and complex charges present in it. A huge variety of dyes are used in

cosmetics ingredients and hair colour products and the mixture of trichromatic is carried out for production of various shades. The wastewater generated from the fabric dyeing industry or the textile industries is a considerable source of environmental contamination. High levels of chemicals and residual coloured materials are present in these wastewater containing dyes. These dyes are important to be analysed for the quality control. According to some effluent regulations, the industries such as textile, pharmaceutical or even food-processing industries should lower the colour content from their wastewater. It is difficult to treat the wastewater coming from industries using dyes as the chemicals presents are recalcitrant molecules which posses resistant to the aerobic digestion [1]. Dye (colour) separation from the industrial effluents is one of the most recent and serious environmental concerns [2]. Every industry has to build up some efficient and economic techniques to do the same. Adsorption is one of the important processes of dye removal. It is a very cost-effective method of colour removal where activated carbon is the most commonly and extensively used adsorbent [3]. Moreover, reusability of these adsorbed dyes using various desorption processes could be an alternative route for sustainable economic uplift and positive drive with regard to economic concern. Recently, several desorption processes used for separation of adsorbed dyes are mass spectrometry using liquid secondary ion (LSI), fast atom bombardment (FAB) and plasma desorption (PD) mass spectrometry etc. These techniques have facilitated the characterization of various compounds. Thin layer chromatography (TLC) has been widely used for the analysis of volatile organic dyes [4, 5].

Recent advances of analytical chemistry depend on the progress towards the usability of more powerful tools of separation, as well as instrumental methods of determination. The chemical analysis of desired constituents is characterized through their separation followed by analysis. To this point, this book chapter has dealt principally with solutions of separation and purification of uncharged molecules. The aim of this chapter is to examine the characteristics of vitamin B<sub>12</sub>, haemoglobin and several dyes which are uncharged macromolecules and their possible separation and purification.

### 3.2 Separation and Purification of Vitamin B<sub>12</sub>

With regard to food and medicine, vitamin B<sub>12</sub> has an important role in living systems. It is a naturally gifted uncharged complex biomolecule where a carbon bonded with metal. Vitamin B<sub>12</sub> was invented in the 1920s and lots of research was made since then. Various applications with regard to anaemia and other diseases have come into the picture where vitamin B<sub>12</sub> had a pivotal role [6]. A real breakthrough came in 1956 when the structure of vitamin B<sub>12</sub> was recognized. Since then the application of X-ray crystallographic technique was immensely increased in the field of vitamin B<sub>12</sub> research [7]. The hydroxycobalamin (OH-Cbl) and 5- deoxyadenosylcobalamin (Ado-Cbl), methylcobalamin (Me-Cbl) are the

natural forms of vitamin B<sub>12</sub>. However, commercially name of vitamin B<sub>12</sub> is cyanocobalamin (CN-Cbl) which is the stable form of vitamin B<sub>12</sub>, produced by several microorganisms.

It is also noted that the nervous system is deeply affected if the supply of vitamin B<sub>12</sub> is less in a biological system as myelin sheath surrounded in nerve cells in humans required vitamin B<sub>12</sub> for maintenance [8]. Additionally, vitamin B<sub>12</sub> assists in growth and development of the cell. Furthermore, vitamin B<sub>12</sub> helps for metabolism of fat and carbohydrate as well as DNA replication. Additionally, it also helps in the maturation of red blood cell (RBC) in bone marrow [9]. It is also reported that several enzymes such as methionine synthase required vitamin B<sub>12</sub> as a cofactor to maintain the integrity of function. Methyl malonyl coenzyme A (CoA) mutase requires vitamin B<sub>12</sub> as a cofactor for maintaining 3D structure of this enzyme. The deficiency of vitamin B<sub>12</sub> is the cause of megaloblasts which leads to associated with tiredness, listlessness and breathlessness. It is also reported that infection is also the cause of vitamin B<sub>12</sub> deficiency. Deficiency of vitamin B<sub>12</sub> for longer time damage the nervous system and nerve could not regenerate. The poor content of it also causes nutritional imbalance, malabsorption associated with gastrointestinal problems [10]. The daily requirement of vitamin B<sub>12</sub> is comparatively low in the range of 1–2 µg in comparison with other vitamins [11, 12]. Since the amount of requirement is very low, therefore, deficiency may be as low in the range of nanogram to picogram level. Therefore, purification and separation along with the measurement of vitamin B<sub>12</sub> are challenging problems for chemists. It is to be noted that no specific reproducible method exists for detection of vitamin B<sub>12</sub>. Therefore, it is utmost important for all researchers to review existing methods and design the ultrasensitive and specific methods. Furthermore, existing methods used for the determination of vitamin B<sub>12</sub> have their own advantages. In addition, time-consuming, laborious, non-specific and expensive are the main drawbacks.

*L. leichmannii* having ATCC 7830 is normally used for the microbial assay. Therefore, it is also considered as a test organism for the determination of vitamin B<sub>12</sub> [26]. The other method used for determination is isotopic-mediated assay [27] where isotope is labelled. Furthermore, liquid chromatography, i.e. HPLC [28] has been widely used for the measurement of vitamin B<sub>12</sub> in the various samples. In the HPLC system, the sample is injected and quantitative and qualitative measurements could be possible through the proper selection of liquid and solid phases. The other two methods such as chemiluminescence (CL) assay [22] and fluorimetric assay [17] were used for measurement of vitamin B<sub>12</sub> level at lower concentration. In CL, after chemical reaction, emission of light is measured. However, the intensity of fluorescence is measured through fluorimetric assay. The capillary electrophoresis (CE) [21] and MALDI are the latest tools used for the determination of vitamin B<sub>12</sub> in various samples. The components in the mixture are separated according to their molecular masses. These methods are quite expensive for the determination of vitamin B<sub>12</sub>. Moreover, TOFMS [29] and AAS [30] have also been the widely used techniques for the determination of vitamin B<sub>12</sub>. Recently, the application of biosensor has tremendously increased for detection purpose as it has several advantages. It is normally used for the detection of vitamin B<sub>12</sub> which is present in