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Review Paper

Towards sustainable development of microalgal biosorption for treating effluents containing heavy metals

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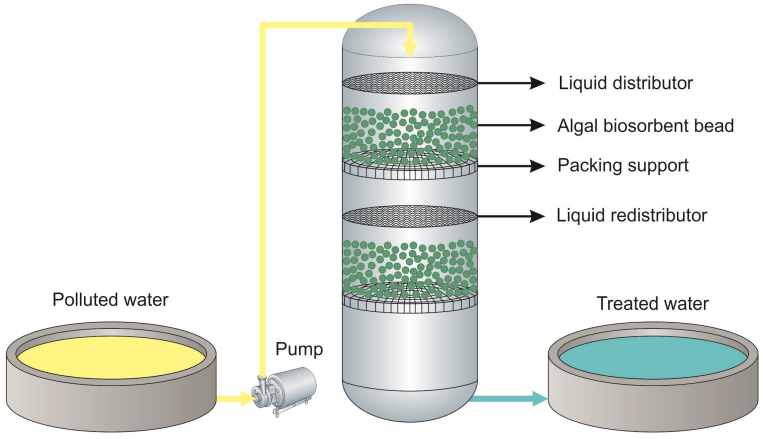
HIGHLIGHTS

- Biosorbents for heavy metals removal were reviewed.
- Microalgae show promising biosorption capacity.
- Microalgae need to be cross-linked for effective biosorption.
- Biomass pretreament prior to cross-linking could improve sorption capacity and rate.

GRAPHICAL ABSTRACT



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ABSTRACT

Effluents containing heavy metals are hazardous to human health and the environment even at low concentrations. It is costly and unsustainable to use conventional methods to remove heavy metals from dilute effluents. Microalgal biomass owing to its high metal biosorption capacity, is a promising alternative biosorbent for treating dilute heavy metal solutions. However, the application of freely suspended algal biomass for metal removal has a number of drawbacks such as small particle size, low chemical resistance, low mechanical strength, and difficulty in separation of biomass and effluent. The present article reviews the techniques used to address these drawbacks. It also discusses the key factors affecting biosorption efficiency including initial concentration of metal ions, contact time, solution pH, solution temperature, biosorbent concentration, agitation rate, and competing ions. Biomass cross-linking with appropriate agents such as polysolfane, formaldehyde, or chlorohydrin could improve mechanical strength, chemical resistance, and separation of the biomass from the effluent. However, cross-linked biomass usually shows low sorption capacity and slow rate of metal uptake. These disadvantages could be minimized by using physical and/or chemical pretreatments prior to biomass cross-linking. Alkaline detergent, sodium hydrogen carbonate without autoclaving, sodium hydroxide or sodium carbonate plus autoclaving, or supercritical carbon dioxide at mild conditions are among the most effective pretreatments. Apart from liberating more latent metal binding sites on the biomass, supercritical CO_2 could also improve the porosity of the biomass thereby improving sorption rate of the cross-linked biomass. High sorption capacity and rapid metal uptake will allow substantial reduction in size of biosorption columns, which will consequently improve the economic and sustainability features of algal-based metal biosorption processes.

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Contents

1. Introduction

Prevalence of heavy metals in the environment occurs due to their massive industrial, domestic, agricultural, medical, and technological applications (Tchounwou et al., 2012). Their adverse effects on human health and the environment have motivated investigations into sustainable ways of treating heavy metal-containing effluents. Based on the level of toxicity, arsenic (As), cadmium (Cd), chromium (Cr), lead (Pb), and mercury (Hg) are ranked among the priority metals that are of public health importance (Tchounwou et al., 2012).

According to the United State Environmental Protection Agency (US EPA, 2009), the maximum contaminant level (MCL) of the priority metals in drinking water is as follows: arsenic, 0.10 ppm; cadmium, 0.005 ppm; lead, 0.015 ppm; chromium, 0.1 ppm; and mercury, 0.002 ppm. Arsenic causes skin damage, problems with circulatory systems, and increases the risk of developing cancer. Cadmium and lead cause kidney damage while the latter is also associated with high blood pressure in adults. Moreover, lead also delays physical and mental development in infants and children. Chromium causes allergic dermatitis while mercury causes kidney problem.

The conventional methods of removing heavy metals from effluents include ion exchange, evaporation, chemical precipitation, membrane separation, chemical oxidation or reduction, electrochemical treatment, and reverse osmosis (Atkinson et al., 1998; Crini, 2006; Sulaymon et al., 2013). These methods have a number of disadvantages such as lack of specificity and being ineffective at low concentrations (Gray, 1999), while membrane separation-based methods are associated with membrane fouling by slightly soluble components (Huang and Koseology, 1993). In addition, these methods need sludge dewatering facilities, highly skilled operators, multiple basin configurations, and are attributed with large footprints (Zhou et al., 1999). Therefore, they are generally not cost-effective (Sulaymon et al., 2013).

Alternatively, passive biosorption, which involves deactivated (dead) biomass can be used and is potentially cost-effective. For instance and based on a 2013 estimate, the price of resin stood at about US$ 30 to 60/kg while the cost of virgin microalgae to make biosorbent was about US$ 1 to 3/kg (Sulaymon et al., 2013). Microorganisms such as fungi (Machado et al., 2009; El-Sayed, 2012), bacteria (Al-Gheethi et al., 2017; Li et al., 2018), and various species of microalgae (Romera et al., 2007; Mata et al., 2008; Yang et al., 2015) have been used with success for removing heavy metals from aqueous solutions.

Microalgae particularly show high metal biosorption capacities, however, the use of freely suspended microalgae as biosorbent has some drawbacks such as their small size, low mechanical strength, and difficulty in separating the biomass and effluent (Gadd, 2009). These disadvantages have been minimized by using cross-linked biomass (Gadd, 2009). Cross-linking improves mechanical strength and separation of biomass from effluent (Holan et al., 1992; Leusch et al., 1995; Bai and Abraham, 2003). It also improves biomass resistance to alkali and acid (Bai et al., 2003). However, it also reduces sorption capacity (Leusch et al., 1995; Bai and Abraham, 2003) and slows down the rate of metal removal (Bai and Abraham, 2003). These disadvantages associated with cross-linked biomass should be minimized to improve the economic features of the process and to ensure the sustainability of microalgal metal biosorption.

This article reviews the application of living and dead biomass as biosorbent, factors affecting biosorption processes, modification of biomass to improve its usage as biosorbent, and drawbacks of cross-linked microalgae for metal biosorption. Solutions on how these challenges could be addressed are also discussed.

2. Biosorption process

The prefix “bio” in biosorption symbolizes a biological entity (living or dead) and the products derived from them. Sorption is a term used to describe both adsorption and absorption. Absorption means to incorporate a substance in one state into another of a different state. Most absorbed substances are thousands of molecular layer thick in the bulk phase of the fluid onto which they absorbed. Adsorption in contrast, is a surface phenomenon. It involves adherence of atoms, ions or molecule to the surface of another substance called adsorbent. The common known examples are gas/solid and liquid/solid systems. Hence, the differences between adsorption and absorption include where it occurs, forces involved, and the thickness of the layer of the sorbate.

Gadd (2009) defined biosorption as removal of a substance from a solution by biological materials to reduce the concentration of the sorbate in the solution and to accumulate the sorbate in the sorbate-sorbent interface. Such biological materials (sorbents) can be organic or inorganic, dead or living, soluble or insoluble while the sorbate includes atoms, molecules, ions, *etc*. Biosorption is broadly classified into active biosorption (“bioaccumulation”) which involves living cells and passive biosorption (“biosorption”) when dead biomass is used.

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2.1. Passive biosorption

Biosorption by dead biomass is called passive biosorption. It occurs through interaction of metal ions in a solution with functional groups on the cell wall of the biomass. Passive biosorption is rapid occurring within 5-10 min of initial contact with the cells (Gipps and Coller, 1980; Geisweid and Urback, 1983). Moreover, it is metabolism-independent (Skowroriski, 1986; Trevors et al., 1986). This was in fact conformed to the observation by Junlian et al. (2010) indicating that the inclusion of glucose (an excellent metabolism accelerant) and sodium azide (a good metabolism inhibitor) had no effect on the Ni^{2+} uptake by dead *Pseudomonas putida*.

2.2. Active biosorption

Biosorption by living cells is called active biosorption and is also known as bio-accumulation. It consists of two consecutive steps (Skowroriski, 1984a and b). The first step is a rapid metabolism-independent binding similar to that of dead biomass. This is followed by the second slow metabolism-dependent phase where metals are transported across the cell membrane (Skowroriski, 1984a and b). This was also confirmed by the findings Junlian et al. (2010) who showed glucose, a good source of carbon and energy for bacteria, improved Ni^{2+} uptake by *P. putida*. While in contrast, sodium azide, a good metabolism inhibitor, slowed down cell growth rate and reduced Ni^{2+} uptake (Junlian et al., 2010).

2.3. Biosorption mechanism

Biosorption mechanism is complex and involves a combination of several independent mechanisms leading to the metal uptake (Brown et al., 2000). Understanding of the underlying mechanism could help to develop an efficient biosorption process. It allows the optimization of the beneficial mechanism and minimization of the interfering one which would otherwise reduce efficiency (Mack et al., 2007). It could also help with tailoring biosorbent and with adjusting the process conditions towards overall increase of process efficiency (Javanbakht et al., 2014). Biosorption mechanisms include chemisorption, complexation, adsorption-complexation on surface and pores, ion exchange, precipitation, heavy metal hydroxide condensation, and surface adsorption (Javanbakht et al., 2014).

Biosorption mechanisms are generally based on physico-chemical interactions between metal ions and the functional groups on the surface of biosorbent. These functional groups are present in biomass components such as polysaccharides, lipids, and proteins. They include carboxyl, imidazole, sulfydryl, amino, phosphate, sulfate, thioether, phenol, carbonyl, amide, and hydroxyl moieties (Javanbakht et al., 2014; Talaro and Chess, 2015). However, some functional groups may not contribute to metal binding due to steric, conformational, or other barriers (Volesky and Holan, 1995).

Other factors, which could contribute to the overall mechanism are whether the cells are living or dead, the type of microorganisms, and the type of metal species (Madrid and Camara, 1997). For instance, exposure of active (living) microalgae to metal ions above its cellular needs may interfere with its regular metabolism. Active cells including microalgae resist against the adverse effects of high metal concentrations through extracellular or intracellular metal binding (Monteiro and Castro, 2012). This intracellular detoxification is achieved by metal binding to specific intracellular compounds such as class III metallothioneins or phytochelatins (Perez-Rama et al., 2001). Active cells are also detoxified by transporting metal ions to cellular compartments such as vacuoles or polyphosphate bodies (Pawlik-Skowronska, 2003). Similarly, active cells efflux metals back into the solution using active transport (Costa and Franca, 2003; Jjembe, 2004; Worms et al., 2006; Levy et al., 2008).

Both living and dead microalgae achieve extracellular metal-binding by physical adsorption, chemisorption, complexation, chelation, and reduction (Greene et al., 1986; Raize et al., 2004; Chojnacka et al., 2005). In addition, Pereira et al. (2011) reported that many cyanobacterial (blue-green algae) including *Gloeothece* sp. produce extracellular polymeric substances (EPS) forming as sheath on the cell surface or liberated into the surroundings as released polysaccharides (RPS). These substances have been shown to contribute to extracellular sorption of Pb^{2+} and Cu^{2+} by algal cells. A schematic representation of microalgal biosorption of metal ions is illustrated in **Figure1**.

It is important to note that all or some of the mechanisms shown in **Figure 1** can occur in an active cell. In contrast, in dead biomass, all mechanisms are possible except active transport and intracellular interactions.

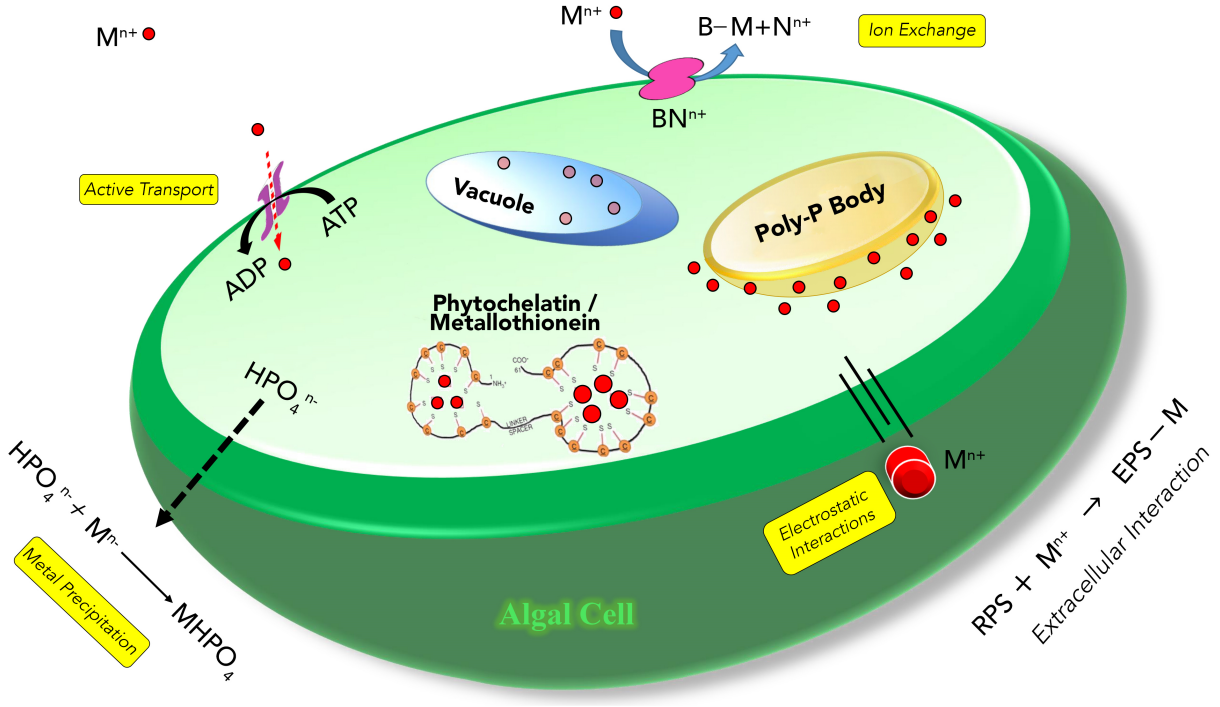

Fig. 1. Schematic representation of microalgal biosorption of metal ions.
M^{n+}: metal ions; Poly–P: polyphosphate; RPS: Released polysaccharides; BN^{n+}: Biomolecule containing exchangeable ions; B–M: Biomolecule containing metal ions; EPS: extracellular polymeric substances. Adapted from Kim and Kang (2006) and Monteiro and Castro (2012).

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Table 1.
Features of passive and active biosorption.*

Features	Passive Biosorption	Active Biosorption
Cost	Usually low. Waste streams or by-products can be used as biosorbents. Costs largely include transportation and other simple processing charges.	Usually high. Living cells are used as biosorbents; thus cell maintenance cost is high.
pH	The solution pH significantly controls sorption capacity. However, the process can be operated under a wide range of pH.	Both the metal sorption capacity and the living cells themselves are strongly affected by extreme solution pH.
Temperature	Temperature has minimal effect as deactivated biomass is used.	Temperature could severely affect the process.
Maintenance/storage	Easy to store and use.	External metabolic energy is required.
Selectivity	Poor but can be improved by biomass modification.	Better than passive biosorption.
Versatility	Reasonably good. The binding sites attract a variety of ions.	Not flexible. Affected by high metal/salt conditions.
Metal uptake	Very high.	Usually low as living cells are sensitive to metal toxicity.
Rate of uptake	Usually rapid. Most passive biosorption kinetics are rapid.	Usually slower than passive biosorption. Intracellular metal accumulation takes longer time.
Regeneration and reuse	Biosorbent regeneration is high with possible reuse over several cycles.	Biosorbent regeneration is low since it involves intracellular metal binding.
Metal recovery	An appropriate eluent can recover significant amounts of bound metal ions. Acid or alkaline solutions are good eluents.	Even if possible, the biomass cannot be utilized for next cycles.

* Source: Adapted from Vijayaraghavan and Yun (2008).

2.4. Features of active and passive biosorption

Passive and active biosorptions are among the alternative methods of treating wastewaters. Both processes have peculiar features making them better alternatives to conventional ways of treating effluents. These include cost effectiveness, chemical and biological sludge minimization, and high efficiency in detoxifying dilute effluents. However, if the effluent contains heavy metals, passive biosorption has compelling features making it a preferred option to an active biosorption process. In that regards, features of both passive and active biosorptions are listed in **Table 1**.

2.5. Application of living and dead biosorbents in removing metal ions

Various living and dead biomass of fungi, bacteria, and microalgae have been used for heavy metal biosorption. An ideal biosorbent should be abundant, non-toxic, re-usable, and possess a high metal binding capacity (Wang and Chen, 2009). Researchers have conducted comparative studies on the biosorption capacity of dead and living biomass. **Table 2** clearly shows that dead biosorbents exhibit higher biosorption efficiencies or capacities than the corresponding living cells at the same selected key experimental parameters. This could be ascribed to the fact that metal sorption by dead biomass involves only extracellular metal binding which is rapid (Gipps and Coller, 1980; Geisweid and Urback, 1983). While, metal removal by living cells involves both extracellular metal binding and slow intracellular metal accumulation (Skowroriski, 1984a and b). Besides, living cells adapt to their environments by accumulating metals below the toxicity level. For instance, bacteria possess genes to limit their metal ions intake below the toxic level (Silver and Phung, 2005). Findings have shown that efflux mechanism exists in *Bacillus cereus* to keep Cd (II) ion accumulation below 20 mg/L (Haung et al., 2014). It worth noting that killing of living cells technically removes the intracellular metal accumulation step. Since this step is inherently slow, its removal in the dead biomass contributes to higher biosorption efficiencies or swifter metal uptake compared to living cells.

Method of deactivation (killing) of living cells also contributes to biosorption efficiency of the resulting dead biomass. Physical means of killing cells such as rapid autoclaving at 121 ºC for 15-20 min (El-Sayed, 2012; Al-Gheethi, 2017), dry heating at low temperatures, i.e., 45-80 ºC (Holan et al., 1992; Machado et al., 2009), or super critical carbon dioxide (SC-CO_2) treatment at mild conditions (31 ºC, 7 MPa for 15 min) have reportedly improved the sorption capacities of the dead cells. However, deactivation by excessive heat could reduce biosorption capacity. Junlian et al. (2010) reported that dry heating of *P. putida* at 100 ºC for 30 min reduced its biosorption capacity and increased biomass loss. It also made the cell structure denser than the living cells or the 0.1 M HCl treated cells. The decrease in biosorption capacity could be attributed to thermo-denaturation of peptidoglycan (PEG), lipids, and proteins due to excessive heat. The thermo-denaturation decreased the number of active metal binding sites on the biomass surface (Costa et al., 1998; Terry and Stone, 2002; Vannela and Verma, 2006; Junlian et al., 2010). It also reduced the accessibility of metal ions to the binding sites (Junlian et al., 2010).

Chemical and/or physical means of cell deactivation can improve the biosorption capacity or increase the mass loss. For instance, low concentration (0.1 M) HCl maintained the biosorption capacity and caused small reduction in the mass of *P. putida* (Junlian et al., 2010). Similarly, Yan and Viraraghavan (2000) reported that detergent without autoclaving improved biosorption capacity of dead *Mucor rouxii* with minor biomass loss. They also observed that NaOH or Na_2CO_3 plus autoclaving and $NaHCO_3$ without autoclaving enhanced the biosorption capacity of the dead *M. rouxii* but with little biomass loss. Autoclaving has been reported to break cell membrane; hence exposing more metal binding functional groups (Machado et al., 2009).

2.6. Factors affecting biosorption processes

Biosorption efficiency depends on several operating parameters. These parameters are sometimes called “environmental factors” of a biosorption process. They affect heavy metal biosorption efficiency by changing the net surface charge of the biomass, metal speciation, and rate of metal uptake or selectivity of the metal bound onto the binding sites. These factors include initial concentration of metal ions, contact time, solution pH, solution temperature, biosorbent concentration, agitation rate, and competing ions.

2.6.1. Initial metal concentration

Biosorption efficiency is decreased as initial metal concentration increases (Bai and Abraham, 2001; Oves et al., 2013; Arivalagan et al., 2014). At low initial metal concentrations, the binding sites are unsaturated but become saturated at high initial concentrations. This explains why the percentage metal adsorbed is higher at low initial concentrations than at high initial concentrations. In contrast, biosorption capacity is increased as initial metal concentration is increased (Bai and Abraham, 2001; Horsfall and Spiff, 2005; Cheng et al., 2016). In better words, for a fixed mass of biosorbent, the biosorption capacity increases as initial metal concentration increases until the maximum biosorbent capacity is reached.

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Table 2.
Results of biosorption of metal ions using living and dead biomass.

Type of biomass	Heavy metal ion	Initial metal conc. (ppm)	Time (min)	pH	Cell killing method	Removal efficiency (% or mg/g biomass)		References
						Dead cell	Living cell	
Chlamydomonas reinhardtii (Green algae)	Pb (II)	0.103	65	6	Live cell was freeze-dried at -100 °C for 35 h	0.286	0.057	Flouty and Estephane (2012)
	Cu (II)	0.032				0.109	0.056	
Chlorella minutissima (Green algae)	Zn (II)	392.4	0.5-180	[a] ,7[b]	Live cells freeze-dried	123.5[a]	33.7[b]	Yang et al. (2015)
	Mn (II)	219.6		[a] ,7[b]		34.5[a]	21.2[b]	
	Cd (II)	67		[a] ,7[b]		303.03[a]	35.4[b]	
	Cu (II)	25.4		[a] ,7[b]		16.2[a]	3.3[b]	
Saccharomyces cerevisiae (Fungus)	Cd (II)	250	[a], 180[b]	6	Live cells autoclaved at 121 °C for 15 min	55[a]	36[b]	El-Sayed (2012)
Saccharomyces cerevisiae (Fungus)	Cu (II)	5-50	60	6	Live cells dried at 45 °C until constant mass	9.91	7.7	Machado et al. (2009)
	Ni (II)	5-200				7.87	0.47	
	Zn (II)	5-50				10.66	0.78	
Curtobacterium sp. (Bacterium)	Cd (II)	20	360	6	Live cells autoclaved at 121 °C for 20 min, then freeze-dried	98%	87%	Li et al. (2018)
Bacillus subtilis (Bacterium)	Zn (II)	8	10	6	Live cells killed by super critical CO_2 at 31 °C, 7 MPa for 15 min	98.5%	96.3%	Al-Gheethi et al. (2017)
					Live cells steam autoclaved at 121 °C for 20 min	99.2%	96.3%	
Pseudomonas putida (Bacterium)	Ni (II)	46	20	6.5	Live cells suspended in 0.1 mol/L HCl for 30 min	46	38	Junlian et al. (2010)
Streptomyces esciscaucasicus (Bacterium)	Zn (II)	1-150	1440	5	Live cells autoclaved at 121°C for 20 min	54	42.8	Li et al. (2010)
Bacillus sphaericus (Bacterium)	Cu (II)	10	2880	7	Live cells dried at 105 °C for 120 min	82%	63%	Al-Daghistani (2012)
	Ni (II)	1.66				59%	52.7%	
	Cr (II)	5				76.5%	66.6%	

At maximum biosorption capacity, an equilibrium is attained. Thus, the adsorption rate of a metal onto the biosorbent equals its desorption rate into the solution. Initial metal concentration represents a driving force required to overcome mass transfer resistance between the solid biosorbent and the liquid phase (Xiao et al., 2010). An increase in the adsorbate concentration increases the driving force required to transfer the ions onto the surface of the biosorbent and consequently, reduces the residual metal ions remaining after sorption.

2.6.2. Contact time

Investigations on the contact time is important in practice and for research purposes. These help with determining the actual size of the contactor and consequently, with minimizing waste of material and energy. The contact time obtained at maximum sorption capacity (equilibrium time) is required for equilibrium or isotherm studies.

Biosorption efficiency or capacity increases with contact time until the equilibrium is attained. Rapid sorption is most desirable in practice because it reduces the size of biosorption column and makes the process more cost-effective. There is a wide variation in the time required to attain the equilibrium. As shown in **Tables 2** and **3**, the equilibrium time ranges from 10-2880 min. Besides, in some biosorption studies, rapid kinetics were observed at the initial contacts. Yang et al. (2015) observed a rapid biosorption of Zn (II), Mn (II), and Cd (II) at pH 6; Cu at pH 4 in the first 1 min. This accounted for 90 to 95% of the metal adsorbed by *Chlorella minutissima* (green algae) but the equilibrium was attained in 20 min. Similarly, Puranik and Paknikar (1999) observed a rapid metal uptake in the first 5 min, accounting for 85% sorption of lead as well as 70% sorption of cadmium and zinc by a *Citrobacter* strain.

Contact time required to remove a specified amount of metal depends on the experimental conditions. Such conditions include type of biosorbent (quantity and quality of functional groups), particle size, type of metals, and temperature of the solution. Experimental conditions enhancing mass transfer of metals from bulk solution to binding sites could increase the rate of metal uptake (Weber, 1985; Puranik and Paknikar, 1999). For instance, favorable mixing can lead to rapid metal uptake because it suppresses kinetic limitations due to both bulk metal transport and diffusion through the boundary layer around the biosorbent surface (Puranik and Paknikar, 1999).

Contact time is also dependent on cells physiology (living or dead) and initial metal concentration. For instance, for actively growing cells, increases in initial metal concentrations beyond the minimum metal inhibitory concentration (MIC) increase the stationary phase and equilibrium time (Haung et al., 2014). Similarly, Arivalagan et al. (2014) reported that metal uptake in *B. cereus* was slowed at lag phase (0-10 h) due to less binding sites as the biomass concentration was very low. They also observed that metal uptake was rapid at mid-log phase (10-20 h) indicating the availability of enough active binding sites and that it remained unchanged at stationary phase (20-24 h) due to the attainment of equilibrium. Hence, if there is a need to use living cells for biosorption of metal ions, it is more appealing to harvest the cells at stationary phase and to maintain the initial metal concentration below the inhibitory concentration. Otherwise, it would be better to deactivate the cells by using appropriate methods as explained in *Section 2.5*.

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Table 3.
Factors affecting biosorption efficiency or capacity.

Type of biomass	Heavy metal ion	Initial metal conc. (ppm)	Time (min)	pH	Temp. (°C)	Agitation (rpm)	Biomass Conc. (g/L)	Max. Eff. (%) or capacity (mg/g)	Isotherm model	References
Chlamydomonas reinhardtii (Green algae)	Pb (II)	0.103	65	6	30	–	0.2	0.286	–	Flouty and Estephane (2012)
	Cu (II)	0.032						0.109		
Chlorella minutissima (Green algae)	Zn (II)	392.4	0.5–180	6	28	140	4	123.5	Langmuir	Yang et al. (2015)
	Mn (II)	219.6		6				34.5		
	Cd (II)	67		6				303.03		
	Cu (II)	25.4		4				16.2		
Chondrus crispus (Red Algae)	Cd (II)	10–150	120	6	–	–	0.5	75.2	Langmuir	Romera et al. (2007)
	Ni (II)			6				37.2		
	Zn (II)			6				45.7		
	Cu (II)			4				40.5		
	Pb (II)			4				204		
Ascophyllum nodosum (Brown algae)	Cd (II)	10–150	120	6	–	–	0.5	87.7	Langmuir	Romera et al. (2007)
	Ni (II)			6				43.3		
	Zn (II)			6				42		
	Cu (II)			4				58.8		
	Pb (II)			3				178.6		
Fucus spiralis (Brown algae)	Cd (II)	10–150	120	6	–	–	0.5	114.9	Langmuir	Romera et al. (2007)
	Ni (II)			6				50		
	Zn (II)			6				53.2		
	Cu (II)			4				70.9		
	Pb (II)			3				204.1		
Fucus vesiculosus (Brown algae)	Cu (II)	50–150	120	6	23	150	0.5	105.4	Langmuir	Mata et al. (2008)
	Cd (II)							108.2		
	Pb (II)							211.3		
Saccharomyces cerevisiae (Fungus)	Cd (II)	250	30	6	28	120	3	55	–	El-sayed et al. (2012)
Saccharomyces cerevisiae (Fungus)	Cu (II)	5–50	60	6	25	150	4	9.91	Langmuir	Machado et al. (2009)
	Ni (II)	5–200						7.87		
	Zn (II)	5–50						10.66		
Curtobacterium sp (Bacterium)	Cd (II)	20	360	6	28	–	1	98%	–	Li et al. (2018)
Bacillus subtilis (Bacterium)	Zn (II)	8	10	6	35	125	0.6-0.8	99.2%	–	Al-Gheethi et al. (2017)
Pseudomonas putida (Bacterium)	Ni (II)	46	20	6.5	–	200	0.5	46	Freundlich	Junlian et al. (2010)
Streptomyces ciscaucasicus (Bacterium)	Zn (II)	1–150	1440	5	28	90	2	54	Langmuir & Freundlich	Li et al. (2010)
Bacillus sphaericus (Bacterium)	Cu (II)	10	2880	7	37-70	100	–	82%	–	Al-Daghistani (2012)
	Ni (II)	1.66						59%		
	Cr (VI)	5						76.5%		
Rhizopus nigricans	Cr (VI)	100	480	2	45	120	5	99.2%	Langmuir & Freundlich	Bai and Abraham (2003)

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2.6.3. pH of the solution

The pH of the sorbate solution is one of the key parameters affecting biosorption. It contributes to metal precipitation, speciation, and its availability for biosorption (Esposito et al., 2002). It also controls the net surface charge of the functional groups (binding sites) on the biomass surface (Fiol et al., 2006). Functional groups commonly found on biosorbent surface include carboxyl, sulfhydryl, hydroxyl, amino groups, *etc.* (Farooq et al., 2010; Abdul-Ghani et al., 2014).

High solution pH deprotonates biosorbent surface function groups resulting in a net negative surface charge, which could in turn, increase the biosorption of the available positively charged metal ions (Farooq et al., 2010). In contrast, low solution pH protonates biosorbent surface functional groups resulting in a net surface positive charge. This consequently increases the biosorption of metal complex existing as anions at that particular pH (Parsons et al., 2003; Mark et al., 2007) but reduces the sorption of positively charged metal ions (Gupta et al., 2010).

An ideal pH can be easily determined by using isoelectric point pH (pH_{IEP}) or point of zero charge (pH_{PZC}). This is the pH of the solution at which the net surface charge on the biomass equals zero. Solution pH above pH_{PZC} makes the net surface charge on the biomass negative while solution pH below pH_{PZC} makes the net surface charge positive (**Equation 1**) (Farooq et al., 2010) Electrokinetic studies estimating the isoelectric point pH (pH_{IEP}) could be found in the literature (Bueno et al., 2008; Cayllahua et al., 2009).

$$-BH_2^+ \xleftarrow{Below\ pHpzc} -BH \xleftarrow{Above\ pHpzc} -B^- \qquad \text{Eq. 1}$$

where – BH represents a biomass having a net zero charge

An alternative approach to narrow down the range of search for optimum pH for metal biosorption is to categorize metal ions into three categories as reported by Darnall et al. (1986). In that regards, class I metals include Al (III), Cu (II), Cr (III), Co (II), Fe (III), Ni (II), Pb (II), Zn (II), and UO^{2+}. They are strongly bound at near neutral pH 7 but are not bound or could be desorbed from the biosorbent at pH < 2. Class II metals include $PtCl_4^{2-}, CrO_4^{2-}, SeO_4^{2-}$ and behave in an opposite manner compared to the Class I metals. They bind strongly at low pH but weakly at pH > 5. Class II metals could be extended to other metals that exist as anionic species at certain pH ranges. For instance, at pH 2-6, the dominant species of Cr (VI) ions in aqueous solution are $HCrO_4^-, Cr_2O_7^{2-}, Cr_4O_{13}^{2-}, Cr_3O_{10}^{2-}$. These Cr (VI) anions are bound maximally at pH 1-2 (Bai et al., 2001; Ucun et al., 2002) when the net surface charge on the biosorbent is positive. Class III metals (Ag^+, Hg^{2+}, and $AuCl_4^-$) are bound most strongly among all and their binding is pH independent.

Effective biosorption of base metal cations occurs in the pH range of 3-7 and is extremely pH dependent (Mack et al., 2007). Base metals fall in Class I metals. Similarly, most of the heavy metals ions listed in **Tables 2** and **3** also fall in the base or Class I metals. The range of optimum pH for the maximum uptake of these metals is pH 3-7. Thus, pH 7 should be regarded as the limit during biosorption of such metals as further increases could cause precipitation of the corresponding metal hydroxides and reduce the metal availability for biosorption.

Yang et al. (2015) reported that 92% of the metals were available within the pH range of 4-8 while the least availability at pH 10 was recorded for Zn, Mn, Cd, and Cu. Non-availability of metals for biosorption is caused by precipitation. Precipitation starts at pH > 5 for lead, pH 6.7 for nickel (Britton, 1943), pH > 6 for copper (Elliot and Huang, 1981; Asmal et al., 1998), pH 7 for chromium (Blazquez et al., 2009), and pH > 8 for cadium (Lodeiro et al., 2006). It should be noted that the formation of such insoluble precipitates might introduce errors into biosorption results as the removal of metal ions is not completely through binding on the surface function groups (Volesky et al., 1995).

2.6.4. Solution temperature

The effect of temperature on biosorption efficiency or capacity varies widely. Some authors observed that metal uptake was independent of temperature. For instance, from 4-55 ºC, no significant changes were recorded in the amounts of lead, cadmium, and zinc sorption by a *Citrobacter* strain (Puranik and Paknikar, 1999). Similarly, no changes in copper, nickel, or chromium uptake by *B. sphaericus* was observed from 37 to 70 ºC (Al-Daghistani et al., 2012). On the contrary, some reports claim that increases in temperature decreased biosorption capacity indicating an exothermic process. For such processes, low temperatures would promote metal sorption on the binding sites. Aksu (2001) observed that Cd (II) uptake by *Chlorella vulgaris* was decreased as temperature increased from 20 to 50 ºC. In fact, the maximum and minimum uptakes were recorded at 20 ºC and 50 ºC, respectively. Ray et al. (2006) observed a decrease in Pb (II) sorption by *B. cereus* from 20 to 40 ºC. They recorded the maximum sorption at 20 ºC and minimum sorption at 40 ºC. Similarly a decrease in Pb (II), Cd (II), and Co (II) sorption by a green algae occurred when the temperature was increased from 10 to 40 ºC (Bulgariu and Bulgariu, 2012). Similar reductions in percentage biosorption was observed with increases in temperature from 20 to 50 ºC (Sari et al., 2007).

Yet more complex temperature profiles have been observed in biosorption process. Al-Gheethi et al. (2017) observed an increase in biosorption efficiency of *B. subtilis* deactivated by SC-CO_2 or autoclaving when temperature was increased from 25 to 35 ºC. Moreover, the maximum efficiency was obtained at 35 ºC but a further increase in temperature to 45 ºC reduced the efficiency suggesting that the biosorption process became exothermic above 35 ºC. Similarly, Yang et al. (2015) observed an increase in biosorption efficiency of *C. minutissima* (green algae) during the biosorption of Zn, Cd, and Cu from 10-28 ºC but further increases in temperature to 37 ºC decreased the efficiency. Ozer et al. (2004) while using *Cladophora crispata* for biosorption of Cu (II) recorded an increased metal uptake from 15-25ºC but further increases in temperature to 50 ºC reduced the metal uptake.

Overall, increase in temperature enhances metal uptake by decreasing the solution viscosity. This consequently increases the diffusion rate of the targeted metal ions across the external boundary layer and internal pores of the biosorbent (Barka et al., 2013). On the other hand, reduction in metal uptake after reaching the equilibrium could be due to the damage to the active binding sites (Ozer and Ozer, 2003) or increasing tendency to metal desorption at very high temperatures (Saltah et al., 2007).

2.6.5. Biomass concentration

Biomass concentration is another important factor affecting biosorption efficiency and the equilibrium sorption capacity. It needs to be adjusted at an optimum level to maximize the amount of metal uptake. Increase in biomass concentration enhances removal efficiency. However, increases in biomass concentration above the equilibrium sorption capacity could usually lead to reductions in biosorption capacity.

Padmavathy et al. (2003) observed that Ni (II) equilibrium sorption capacity reduced from 8 to 1.8 mg/g when *Saccharomyces cerevisiae* concentration was increased from 0.5 to 8 g/L. Similarly, Bai et al. (2001) observed that Cr (VI) equilibrium sorption capacity reduced from 26 to 3 mg/g while removal efficiency was increased from 80 to 99% when *Rhizopus nigricans* concentration was increased from 1 to 10 g/L. The removal efficiency reached its maximum value at the biomass concentration of 8 g/L and then remained constant. Cayllahua et al. (2009) observed increases in Ni (II) removal efficiency from 44 to 53.8% but deceases in equilibrium sorption capacity from 8.6 to 4.2 mg/g as *Rhodococcus opacus* concentration was increased from 2 to 5 g/L. Further increases in the biosorbent concentration above 5 g/L resulted in no increment in removal efficiency.

Excessive increases in biosorbent concentration beyond the optimum point could sometimes reduce the removal efficiency as well. For instance, Bueno et al. (2008) reported increases in the removal efficiency of Pb (II), Cr (III), and Cu (II) with an increase in *R. opacus* concentration from 0.5 to 1 g/L. But further increases in the biosorbent concentration to 2.5 g/L slightly reduced the removal efficiency. Ekmekyapar et al. (2006) investigated the impacts of increasing *Cladonia rangiformis* concentration from 1 to 10 g/L on its removal efficiency of Cu (II). They claimed that increasing biomass concentration increased the removal efficiency of the metal reaching its maximum value at 5 g/L but further increases to 10 g/L decreased the removal efficiency.

Overall, it could be concluded that a high biosorbent concentration increases the specific surface area and consequently, the number of active binding sites leading to increased removal efficiencies (Bai and Abraham,

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2001; Fraile et al., 2005; Cayllahua et al., 2009; Barka et al., 2013). However, excessive increases in the biosorbent concentration above the optimum value could cause reductions in biosorption capacity, which could be attributed to partial biomass aggregation at high concentrations. Biomass aggregations decrease the effective specific surface area and effective active binding sites (Ahuja et al., 1999; Munoz et al., 2006; Gupta et al., 2008; Barka et al., 2013)

The opposite trends observed in removal efficiency and equilibrium sorption capacity in response to increases in biomass concentration can be explained using the **Equations 2** and **3**, respectively

$$Removal\ efficiency\ (\%) = \frac{(C_0 - C_e)}{C_e} \times 100 \quad \text{Eq. 2}$$

$$Equilibrium\ sorption\ capacity\ (mg/g) = \frac{(C_0 - C_e)}{M} V \quad \text{Eq. 3}$$

Where "C_0" is the initial concentration, "C_e" represents the equilibrium concentration, "M" denotes the mass of the adsorbent, and V is the volume of the solution.

2.6.6. Agitation rate

Agitation speed is another factor affecting biosorption efficiency. More specifically, biosorption capacity or efficiency is positively correlated with agitation speed. However, excessively high agitation rates could cause reductions in biosorption capacity or efficiency. Bai and Abraham (2001) varied the agitation rate from 70 to 180 rpm during Cr (VI) removal by *Rhizopus nigricans*. They observed that Cr (VI) removal efficiency of all the agitated samples were significantly greater than that of no agitation sample. Moreover, removal efficiency peaked at 120 rpm and further increases in agitation rate, i.e., above 120 rpm, reduced the efficiency, still higher values compared to the maximum value obtained under no agitation, were recorded though. Ucun et al. (2002) during Cr (VI) biosorption with *Pinus sylvestris* observed increases in removal efficiency in response to elevating the agitation rate from 100 to 150 rpm. Further increases to 240 rpm however, reduced the removal efficiency. Other researchers have also reported similar observations (Ekmekyapar et al., 2006).

Overall, it could be deduced that increasing agitation speed enhances the mass transfer of the metal ions from the bulk fluid to the binding sites (Ekmekyapar et al., 2006) while reduces the surface film resistance and boundary thickness around the biosorbent (Benefield et al., 1982). Consequently, high agitation speeds allow more contact between the metal ions in the solution and the active binding sites, thereby increasing the removal efficiency of the metal ions (Bai and Abraham, 2001). However, excessively high agitation speeds could make the suspension non-homogeneous, thereby resulting in reductions in removal efficiency. Therefore, moderate agitation speeds are advisable leading to increased removal efficiencies during the biosorption of metals.

2.6.7. Competing ions

Industrial effluents hardly contain a single metallic ion. Hence, in practice, the presence of more than one ion in the effluents makes treatment by using biosorbents a rather difficult process. More specifically, the co-existing ions compete with the targeted metal for binding sites if they have the tendency towards the same sites. But if they have a preference for different binding sites, this may lower the specificity of the biosorbent (Mark et al., 2007).

Pearson's metals classification (1963) has been a useful predictive tool to know chemical coordination characteristics of the elements in multiple metallic ionic solutions (Tsezos, 1996). It can also assist with interpreting equilibrium data of such systems. The basis of the Pearson's metal classification can be summarized into a simple general rule: "hard acids bind with hard bases; soft acids bind with soft bases". The borderline acids can form strong bonds with both hard and soft bases while the borderline bases can form strong bonds with both hard and soft acids (Burrows et al., 2009). **Table 4** shows the grouping of ligands (Lewis bases) and metals (Lewis acids) using the hard soft acid and base (HSAB) concept.

Tuzun et al. (2005) observed that in a mixed metal system, the presence of other cations reduced the biosorption of Hg (II), Cd (II), and Pb (II) by 49, 61, and 34%, respectively, relative to a single metal system. They indicated that the metals showed competitive binding due to their preference to the same

Table 4.
Hard, soft, and borderline acids and bases (Adapted from Pearson (1963); Pearson (1966); Alfarraa et al. (2004); and Burrows et al. (2009)).

Hard bases (Ligands)	**Soft bases (Ligands)**	**Borderline bases (Ligands)**
H_2O, HO^-, F^-, CH_3COO^-, , SO_4^{2-}, Cl^-, CO_3^{2-}, NO_3^-, RO^-, RNH_2, ROH, R_2O, NH_3, *etc.*	R_2S, RSH, RS^-, I^-, CO, C_2H_4, C_6H_6, H^-, R^-, SCN^-, CN^-, *etc.*	NO_2^-, SO_3^{2-}, Br^-, N_3^-, NCS^-, *etc.*
Hard acids (Metals)	**Soft acids (Metals)**	**Borderline acids (Metals)**
H^+, L^+, Na^+, K^+, Mg^{2+}, Ca^{2+}, Al^{3+}, Cr^{3+}, Fe^{3+}, Ti^{4+}, Sc^{3+}, Co^{3+}, Cr^{2+}, Mn^{2+}, As^{3+}, VO^{2+}, *etc.*	Cu^+, Ag^+, Pd^{2+}, Pt^{2+}, Hg^{2+}, I_2, Cd^{2+}, Au^+, *etc.*	Fe^{2+}, Co^{2+}, Cu^{2+}, Zn^{2+}, Cd^{2+}, Sn^{2+}, Sb^{3+}, Ni^{2+}, Rh^{3+}, Ir^{3+}, Ru^{3+}, Pb^{2+}, Os^{3+}, *etc.*

binding sites. It is important to note that all these metal ions existed in their simple ionic form at the sorption pH. Consequently, Pearson's metal classification is applicable considering the fact that Pb (II) is soft metal while Hg (II) and Cd (II) are bordeline metals. They could form a stable bond with the same ligands on the algal surface. The decreasing order of sorption reported was $Pb^{2+} > Hg^{2+} > Cd^{2+}$. This followed the same order as Pauling Electronegativity, i.e., Pb^{2+}, 2.33 > Hg^{2+}, 2.00 > Cd^{2+}, 1.69 and ionic radii, i.e., Pb^{2+}, 1.19 A > Hg^{2+}, 1.02 A > Cd^{2+}, 0.95 A.

Sengil and Ozacar (2009) studied the sorption of the pairs of Pb^{2+}, Cu^{2+}, and Zn^{2+} ions onto valonia tannin resin. They observed that in the mixed metal systems, the presence of the other cation reduced the biosorption of Pb^{2+}, Cu^{2+}, and Zn^{2+} ions by 29%, 23%, and 98%, respectively. This indicated the competitive behavior of these metals for the same binding sites. At the optimum sorption pH of 5, all the ions existed in their simple ionic form. Based on the Pearson's reasoning, they all belong to borderline metals (acids); hence, can bind to the same ligands (soft or hard) on the biosorbent surface. According to the experimental results, the decreasing order of sorption reported was $Pb^{2+} > Cu^{2+} > Zn^{2+}$. The ionic radii order is Pb^{2+}, 1.19A > Zn^{2+}, 0.74 A > Cu^{2+}, 0.73A while Pauling electronegativity order is Pb^{2+}, 2.33 > Cu^{2+}, 1.90 > Zn^{2+}, 1.65. Pauling electronegative order followed the experimental sorption order while ionic radii did not. This inconsistency could be avoided if their covalent indexes (CI) were used. Covalent index is Xm^2r (Xm is Pauling electronegativity value, and r the metal ionic radius) (Pereira et al., 2011). The covalent indexes (CI) for the ions are: Pb_{CI}^{2+}, 6.46 > Cu_{CI}^{2+}, 2.64 > Zn_{CI}^{2+}, 2.01. This order was the same as the experimental affinity order obtained.

Sulaymon et al. (2013) studied the competitive binding behavior of Pb (II), Cd (II), Cu (II), and As (III). They used a mixed culture of microalgae including Cyanophyta (92% *Oscillatoria princeps*, 2% *Oscillatoria subrevis*, 1% *Oscillatoria formosa*) and Chlorophyta (3% *Spirogyra aequinoctialis*, 3% *Mougeta* sp.) and 1% others. The sequence of the biosorbent affinity constant for single metal systems was K_{Pb}, 16.55 > K_{Cu}, 15.97 > K_{Cd}, 10.52 > K_{As}, 7.45 but these constants were significantly reduced in the binary, ternary and quaternary systems. This revealed that the investigated metals exhibited competitive binding behaviors due to their preference for the same binding sites.

Pb (II) and Cu (II) belong to borderline metals. Cd (II) is a soft metal while As (III) is hard. Borderline metals can bind to both hard and soft bases (ligands) on the biomass. The ionic radii order is Pb^{2+}, 1.19A > Cd^{2+}, 0.95A > Cu^{2+}, 0.73A > As^{3+}, 0.58A while the Pauling electronegativity order is Pb^{2+}, 2.33 > As^{3+}, 2.18 > Cu^{2+}, 1.90 > Cd^{2+}, 1.69. Their covalent indexes (CI) are Pb_{CI}^{2+}, 6.46 > As_{CI}^{3+}, 2.76 > Cd_{CI}^{2+}, 2.72 > Cu_{CI}^{2+}, 2.64.

In fact, metal affinity follows the same order as the covalent index (CI) (Pereira et al., 2011). This would be so as long as the ions exist in their simple ionic forms at the optimum sorption pH. However, this was contrary to what observed by Sulaymon et al. (2013). They argued that at pH 4 (the optimum sorption pH), As_2O_3 might not exit in its simple ionic form as As (III). This was because the dissolution of As_2O_3 to arsenous acid was thermodynamically possible at low pH (Nordstrom et al., 2014). Such dissolution could be expressed in **Equation 4**:

$$As_3O_3(cr) + H_2O\ (l) \leftrightarrow H_2AsO_3\ (aq) \quad \Delta G\ (-ve) \quad \text{Eq. 4}$$

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At about pK of 9.2, arsenous acid is deprotonated according to **Equation 5** (Nordstrom et al., 2014)

$$H_3AsO_3(aq) \leftrightarrow H_2AsO_3^-(aq) + H^+(aq) \quad \text{Eq. 5}$$

This means at a sorption pH 4 used by Sulaymon et al. (2013), availability of asernic as As (III) would be low. In the extreme case of high pH, it could only exist as anionic species ($H_2AsO_3^-$). Nevertheless, at the pH of 4, the net surface charge on the mixed algae used would be negative. This indicates that the sorption of that anionic species would be low. This also explains why As (III) had the least affinity coefficient, which did not follow the same order as covalent index.

3. Immobilization for enhancing algal biosorbent separation

Immobilization simply means to make catalyst, enzyme, cells, biomass, *etc.* immobile (fixed) within a distinct phase (the biomass phase) which is different from the bulk phase (Robinson, 1998). Compared to the free biomass system, immobilization provides a better stability, increased activity and selectivity, higher resistance, improved separation and purification, and reusability of biomass (Ismail et al., 2015).

Unmodified freely suspended microalgae naturally has a low mechanical strength. When the algal biomass is in contact with water, it becomes softened and swelled up (Holan et al., 1993). This low mechanical strength biomass cannot withstand the harsh operating conditions induced by either fluid turbulence in a packed column or stirring in an agitated tank. Besides, the use of freely suspended biosorbent for metal removal from aqueous solution has additional drawbacks such as small particle size and difficulty in separation of the biomass from the treated solution (Gadd et al., 2009). These drawbacks could be minimized by using immobilized biomass particles in packed or fluidized-bed columns (Holan et al., 1993; Leusch et al., 1995; Volesky, 2001; Bia and Abraham, 2003; Volesky, 2007). Upon immobilization, the biosorbent beads can be reused for several biosorption cycles and can be regenerated when become saturated.

In batch laboratory equilibrium biosorption studies, centrifugation and/or filtration through filter papers are commonly used to separate the treated solution (filtrate) from the algal biosorbent. These separation techniques cannot be used in an industrial scale biosorption because they are not cost effective (Grima et al., 2003).

A fixed-bed biosorption column can be easily run in a continuous mode. Such biosorption columns should resemble an ion exchange resin or granular activated carbon systems. The column should be packed with immobilized algal bead as shown in **Figure 2**. Unlike membrane separation of powdery microalgae from treated water, immobilized microalgae is less prone to clogging (Aksu, 1998). The treated water from the packed column will be less turbid as it contains little or no microalgae.

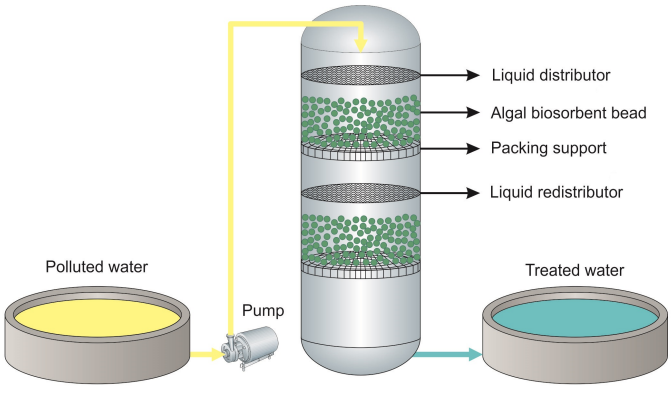

Fig. 2. An immobilized microalgal biosorption column for treating heavy metal polluted water.

3.1. Immobilization techniques

Biomass immobilization is categorized into four categories based on the type of bond involved: i) physical adsorption, ii) encapsulation and entrapment, iii) covalent bonding, and iv) cross-linking. Each of these techniques has advantages and disadvantages. The selection of a suitable technique largely depends on the process operating conditions and whether the biomass is active (living) or dead.

3.1.1. Physical adsorption

In this technique, immobilization of the biomass onto a support is achieved by physical adsorption through hydrophobic interactions, Vander Waals forces, or hydrogen bonding (Ligler and Taitt, 2011). This technique is simple and allows the reuse of expensive support materials. However, it has a low stability and desorption of the biomass from the support is high (Salleh et al., 2006).

3.1.2. Encapsulation and entrapment

Here, the biomass is entrapped or encapsulated in a polymeric matrix. It is primarily used to immobilize living cells. The gels commonly used for entrapment are sodium alginate, agar, agarose, carragenaan, and chitosan (Robinson, 1998). The drawbacks of this technique include instability and/or cell leakage from the polymeric matrix (Robinson, 1998).

3.1.3. Covalent bonding

In this technique, immobilization occurs through the formation of covalent bonds between the biomass and the support materials (Kok et al., 2001a and b). This covalent binding reduces leakage or desorption of the cells from the support during utilization while it also provides high stability. However, it could decrease the activity of the binding sites on the biomass. The support materials are also non-renewable (Ismail et al., 2015).

3.1.4. Cross-linking method

Cross-linking technique combines both covalent bonding and entrapment (Taylor and Schultz, 1996). Its advantages include low biomass leakage, low biomass desorption, and high stability. While the drawbacks associated with this technique include diffusion limitation and reduced activity of binding sites (Ismail et al., 2015). It should be noted that the latter can occur if inappropriate cross-linking agents are used.

3.1.4.1. Capacity and mechanical stability of cross-linked microalgae

An industrial scale biosorption should have properties comparable to commercial ion exchange resins. It should be cost-effective, possess high mechanical strength, controlled size with acceptable hardness, durability, and porosity (Holan et al., 1993). Several approaches could be used to meet those criteria including grafting into synthetic polymers, entrapment into inorganic or organic materials, and cross-linking (Holan et al., 1993). However, cross–linking is more promising (Holan et al., 1993).

A wide range of materials have been used for cross-linking microalgae. They include formaldehyde, glutaraldehyde, glutaraldehyde embedded in polyethylene imine, divinyl sulfone, and epichlorohydrin (Holan et al., 1993; Leusch et al., 1995; Liu et al., 2009). However, most cross-linked biomass have sorption capacities lower than their non-immobilized counterparts due to reduction of effective binding sites after cross-linking. **Table 5** shows the results of previous studies on biosorption of heavy metals using cross-linked microalgae.

One of the controlling factors in cross-linking is particle size. Leusch et al. (1995) showed that large cross-linked particles sizes (0.84-1.00 mm) led to higher metal biosorption than small particle sizes (0.105-0.295).

A summary on biosorption capacities of the cross-linked microalgae are provided as follows:

i) The biosorption capacity of native *Ascophyllum nodosum* was greater than those of all its cross-linked biomass. Out of the cross-linking agents, formaldehyde cross-linked biomass had the highest capacity while formaldehyde-urea mix had the least (Holan et al., 1993).

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Table 5.
Cross-linking for improving biosorbent mechanical strength

Microalgae	Metal	pH	Biomass modification	Metal uptake (mg/g)	References
Laminaria japonica	Cd (II)	4.3–6.5	Cross-linked with epichloro hydrin[a]	187.7	Liu et al. (2009)
			Cross-linked with epichloro hydrin[b]	145	
			Original algae washed with distilled water only	93.3	
			Cross-linked with glutaraldehyde	92.2	
	Cu (II)	4.3–6.5	Cross-linked with epichloro hydrin[a]	102.9	
			Cross-linked with epichloro hydrin[b]	84.5	
			Original algae washed with distilled water only	55.9	
			Cross-linked with glutaraldehyde	34.3	
	Zn (II)	4.3–6.5	Cross-linked with epichloro hydrin[a]	79.8	
			Cross-linked with epichloro hydrin[b]	81.1	
			Original algae washed with distilled water only	45.8	
			Cross-linked with glutaraldehyde	34.7	
	Ni (II)	4.3–6.5	Cross-linked with epichloro hydrin[a]	53.4	
			Cross-linked with epichloro hydrin[b]	52.2	
			Original algae washed with distilled water only	58.1	
			Cross-linked with glutaraldehyde	34	
Ascophyllum nodosum	Cd (II)	4.9	Native A. nodosum	172	Holan et al. (1993)
			Cross-linked with formaldehyde[c]	125	
			Cross-linked with divinyl sulfone	117	
			Cross-linked with formaldehyde[d]	111	
			Cross-linked with glutaraldehyde	109	
			Cross-linked with formaldeyde-urea	104	
Sargassum fluitans (0.8–1.00 mm)	Cd (II)	3.5	Cross-linked with formaldehyde/HCl	83	Leusch et al. (1995)
			Cross-linked with polyethylene imine/glutaraldehyde	77	
			Cross-linked with glutaraldehyde	91	
	Cu (II)	3.5	Cross-linked with formaldehyde/HCl	89	
			Cross-linked with polyethylene imine/glutaraldehyde	75	
			Cross-linked with glutaraldehyde	75	
	Ni (II)	3.5	Cross-linked with formaldehyde/HCl	46	
			Cross-linked with polyethylene imine/glutaraldehyde	46	
			Cross-linked with glutaraldehyde	43	
	Pb (II)	3.5	Cross-linked with formaldehyde/HCl	299	
			Cross-linked with polyethylene imine/glutaraldehyde	250	
			Cross-linked with glutaraldehyde	277	
	Zn (II)	3.5	Cross-linked with formaldehyde/HCl	43	
			Cross-linked with polyethylene imine/glutaraldehyde	45	
			Cross-linked with glutaraldehyde	46	

[a] 70% 2–propanol in water for washing
[b] 20% 2–propanol in water for washing
[c] Sorption of Cadmium from the solution of $3CdSO_4.\ 8H_2O$
[d] Sorption of Cadmium from the solution of $Cd\ (CH3COO)_2$

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Table 5.
Continued.

Microalgae	Metal	pH	Biomass modification	Metal uptake (mg/g)	References
Ascophyllum nodosum (0.84–1.00 mm)	Cd (II)	3.5	Cross-linked with formaldehyde/HCl	80	Leusch et al. (1995)
			Cross-linked with polyethylene imine/glutaraldehyde	75	
			Cross-linked with Glutaraldehyde	33	
	Cu (II)	3.5	Cross-linked with formaldehyde/HCl	75	
			Cross-linked with polyethylene imine/glutaraldehyde	61	
			Cross-linked with Glutaraldehyde	44	
Ascophyllum nodosum (0.84–1.00 mm)	Ni (II)	3.5	Cross-linked with formaldehyde/HCl	52	Liu et al. (2009)
			Cross-linked with polyethylene imine/glutaraldehyde	39	
			Cross-linked with Glutaraldehyde	22	
	Pb (II)	3.5	Cross-linked with formaldehyde/HCl	229	
			Cross-linked with polyethylene imine/glutaraldehyde	194	
			Cross-linked with Glutaraldehyde	134	
	Zn (II)	3.5	Cross-linked with formaldehyde/HCl	34	
			Cross-linked with polyethylene imine/glutaraldehyde	40	
			Cross-linked with Glutaraldehyde	15	

[a] 70% 2–propanol in water for washing [b] 20% 2–propanol in water for washing [c] Sorption of Cadmium from the solution of $3CdSO_4.\ 8H_2O$
[d] Sorption of Cadmium from the solution of $Cd\ (CH3COO)_2$

ii) Application of appropriate cross-linking agents can improve sorption capacity of the cross-linked microalgae. Dimethyl sulphoxide (DMSO) pretreatment prior to cross-linking of biomass with epichloroydrin increased sorption capacity of *Laminaria japonica* to values higher than the native biomass (Liu et al., 2009).
iii) When the size of both cross-linked *Sargassum fluitans* and *Ascophyllum nodosum* were fixed at 0.84-1.00 mm, cross-linking by formaldehyde/HCl resulted in the highest metal biosorption capacities in both microalgae.
iv) *Sargassum fluitans* cross-linked by formaldehyde/HCl led to the highest biosorption capacity followed by glutaraldehyde while polyethylene imine/glutaraldehyde resulted in the least.
v). *Ascophyllum nodosum* cross-linked by formaldehyde/HCl was attributed to the highest biosorption capacity followed by polyethylene imine/glutaraldehyde while glutaraldehyde alone resulted in the least value.

Besides bisorption capacity, formaldehyde cross-linked biomass exhibits more favorable particle swelling characteristics. More specifically, it has the least swollen volume, distention index, and volume of absorbed solvent while on the other hand, glutaraldehyde has the least favorable attributes (Holan et al., 1993; Leusch et al., 1995). Moreover, formaldehyde cross-linked algal biosorbent desorbs readily with 0.2-0.5 M HCl and maintains its sorption capacity for several sorption/desorption cycles (Holan et al., 1993).

3.1.4.2. Chemical stability of cross-linked microalgae

In addition to maintaining high capacity and mechanical strength, a good biosorbent should also exhibit chemical resistance to acidic and alkaline conditions. Moreover, it should maintain its mass to enhance separation from the treated water and to improve the economy of the operation. This is important because cross-linked biomass is exposed to a wide range of pH during sorption and desorption cycles. Systematic screening of several cross-linked agents could help with selecting a suitable one for practical biosorption applications.

Bia and Abraham (2003) evaluated the sorption capacity, mechanical stability, chemical resistance, and mass reduction of immobilized *Rhizopus* for the cross-linked systems, the sorption capacities decreased in the order of free biomasss > polysulfone cross-linked biomass > polyisoprene cross-linked biomass > polyvinyl alcohol (PVA) cross-linked biomass > calcium alginate entrapped biomass > polyacrylamide cross-linked biomass.

The degree of mechanical stability and chemical resistance of the immobilized systems decreased in the order of polysulfone cross-linked biomass > polyisoprene cross-linked biomass > polyvinyl alcohol (PVA) cross-linked biomass > polyacrylamide cross-linked biomass > calcium alginate entrapped biomass.

Polysulfone cross-linked biomass maintained its sorption capacity, mechanical stability, mass and resistant to acid and alkali for 25 consecutive cycles of sorption and desorption (regeneration). The bound Cr (VI) could be eluted successfully using 0.01N NaOH, $NaHCO_3$, and Na_2CO_3. However, the cross-linked biomass exhibited slower Cr (VI) removal rate than the free biomass due to mass transfer limitation in the former (Bia and Abraham, 2003).

4. Concluding remarks

Biosorption of heavy metals from aqueous solutions has been demonstrated to be technically feasible using a range of biomass including microalgae. Biosorption efficiency depends on initial concentration of metal ions, contact time, solution pH, solution temperature, biosorbent concentration, agitation rate, and competing ions. All these parameters have significant effects on biosorption efficiency except agitation rate and solution temperature.

Despite high biosorption capacity of microalgae, freely suspended microalgae is not attractive because of a number of disadvantages, i.e., small particle size, low chemical resistance, low mechanical strength, and difficulty in separation of biomass from the treated effluent. However, these shortcomings can be addressed by using immobilized microalgae in a biosorption packed bed.

Immobilization by cross-linking technique has been shown to improve the mechanical stability, chemical resistance, and separation of microalgae from the effluent. Among the existing cross-linking agents for microalgae, polysulfone, formaldehyde, and epichlorohydrin have been found to be the most promising.

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Cross-linked biomass generally exhibits lower rate of metal removal and sorption capacity than free biomass. Nevertheless, it has been shown that biomass pretreatment by DMSO prior to cross-linking could result in even higher sorption capacities than the free algal biomass.

Other chemicals and/or physical pretreatment prior to cross-linking biomass should be explored for the liberation of latent binding sites on biomass. In this regards, alkaline detergent, sodium hydroxide (NaOH) or sodium carbonate (Na_2CO_3) plus autoclaving, sodium hydrogen carbonate ($NaHCO_3$) without autoclaving could be used as pretreatment agents prior to cross-linking.

In addition, supercritical carbon dioxide (SC-CO_2) at mild conditions can also be used to deactivate the cells prior to cross-linking. Apart from liberating more latent binding sites, this technique could potentially improve the porosity of the biomass; hence reducing the mass transfer limitations in the cross-linked biomass.

Overall, ideal microalgal biosorbent should exhibit high sorption capacity, rapid sorption rate, good mechanical stability, high chemical resistance, easy separability, and reusability to ensure a cost effective and sustainable biosorption process.

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