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Effect of pH on biosorption of Copper by marine microalgae: A Review

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ABSTRACT

Heavy metals which are released from the municipal waste are recalcitrant in environment, cause pollution and enter the food chain leading to biomagnification and bioaccumulation across trophic levels. Copper beyond certain limits accumulates in the tissues, liver, etc. causing multiple problems. Methods that will not harm the environment and are sustainable are required as conventional methods are expensive and release noxious chemicals into the environment. Biosorption is a sustainable and cost-effective method, it is being studied in recent times as a favourable method for the removal of effluents. Several studies have been conducted using microalgae for filtration and removal of heavy metals using biosorption as they have excellent affinity to heavy metals. It is a rapid and irreversible process and the amount of sludge generated is relatively lower. As microalgae are photosynthetic, they fix CO_2 from the environment and release oxygen, thus being extremely beneficial to the environment. Factors like temperature, light, pH, etc. are important factors for microalgal growth. Low pH levels in association with copper increase the toxicity of the metal. Changes in pH also affect the biosorption efficiency by the marine microalgae, however depending on the species the optimum pH ranges differ.

Keywords: Marine microalgae, heavy metal pollution, copper pollution, biosorption, pH effects.

INTRODUCTION

The contamination of water by heavy metals leads to heavy metal pollution; Arsenic, Lead, Mercury, Copper, Zinc, Cadmium, etc. are the common pollutants. Heavy metal refers to metals or metalloids having atomic density greater than $4 g/cm^3$ and are toxic (Duruibe *et al.* 2007). Water contamination with heavy metals is a rising global concern today as they persist in the environment (Duruibe *et al.*2007; Salama *et al.* 2019).

Smaller organisms consume these heavy metals and it passes through the food chain to higher organisms (Kanamarlapudi *et al.* 2018). The sources of this heavy metal contamination arise from natural and anthropogenic activities. Urbanisation, industrialisation (pollution occurs at both the ends i.e., industrial production and the end products), land runoff, direct or indirect disposal of wastes, accidental spillage, and mining are the sources of pollution. These pollutants include arsenic, lead, mercury, chromium, cobalt, cadmium, zinc, copper, and iron (Kanamarlapudi *et al.* 2018; Verma and Dwivedi, 2013). Heavy metals exhibit bio-toxic effects, which are harmful to the human body when present over the recommended limits. Individual metal toxicity is diagnosed with specific signs although general symptoms have also been reported such as gastrointestinal disorders, haemoglobinuria (red-rust colour to stool), diarrhoea stomatitis, ataxia, tremors, convulsion, etc. (Verma and Dwivedi, 2013). The nature of the toxicity can be mutagenic, neurotoxic, carcinogenic or teratogenic (Kanamarlapudi *et al.* 2018).

The sources of copper are usually natural or anthropogenic, the latter being a major source. Elements like Fe, Zn, Cu and Ni above certain limits have affinity towards sulphur atoms of the enzymes and have the ability to form bonds with amine and carboxylic groups of proteins and inhibit the regular biological functioning. Some are able to link to the cell walls and disturb the transport processes (Fraile *et al.* 2005). Generally, toxicity of copper correlates with free metal ion concentration (Cu^{2+}) (Franklin *et al.* 2000). Excessive intake of copper leads to accumulation in the liver and produces intestinal catarrh (Gupta *et al.* 2005). Acute copper toxicity causes anaemia, acute liver failure, acute renal failure with tubular damage, brain damage, liver cirrhosis, stomach and intestinal irritation, coma, shock also, death whereas mild toxicity may result in nausea, diarrhoea and vomiting (Ali *et al.* 2014; Salama *et al.* 2019). In microalgal cultures, excess of copper has shown inhibition of growth by extending the lag phase of the cultures and therefore, interferes in several cellular processes and photosynthesis, enzyme activity, cell division, respiration and pigment synthesis (Franklin *et al.* 2000).

Many conventional methods for treatment of contaminated wastewater are used. These methods do not substantially decrease heavy metal concentration and also require high energy input, operational cost, high capital investment, release noxious chemical reagents in the environment (Chan *et al.* 2014; Fraile *et al.* 2005; Maznah *et al.* 2012; Yu and Kaewsarn, 1999).

In recent years biosorption/bioaccumulation processes have been considered as economic, eco-friendly and effective alternative technologies for removal of heavy metals from water bodies contaminated from industrial and domestic wastes. This phenomenon is described in a wide range of fungi, bacteria, yeast, moss, aquatic plants and algae (living or dead and dry) (Fraile *et al.* 2005; Gupta *et al.* 2006; Kanamarlapudi *et al.* 2018). Investigations show biosorption of heavy metal cations by microorganisms is rapid, irreversible and necessarily not mediated by metabolic processes (Gupta *et al.* 2006). The process involves two steps: first step is the quick adsorption of metal ions onto the cells and the second step is slower, the transport of metal inside the cells by active transport. Biosorption is a process involving binding of metal ions to the surface of the cell. The process of bioaccumulation involves cultivating the biomass in the vicinity of the metal to be accumulated. The biomass concentration plays an important role (Fraile *et al.* 2005; Kanamarlapudi *et al.* 2018). Biosorption can remove contaminants even when present in dilute concentrations. This process requires low operational cost, is highly efficient, requires no additional nutrient, low quantity of sludge is generated and there is no increase in COD (chemical oxygen demand) as opposed to conventional methods (Kanamarlapudi *et al.* 2018).

Microalgae are floating microscopic, primitive, unicellular phytoplankton which are generally free living and pelagic. Microalgae form the source of the food chain for more than 70% of the world's biomass (Singh and Saxena, 2015). The size ranges from 2 to 20μm. Microalgae are classified as – diatoms; having siliceous cell walls, dinoflagellates; with two dissimilar flagella, blue-green algae (cyanobacteria), green algae, and coccolithophores. In the aquatic system, microalgae are primary producers of oxygen, recycling CO_2 by photosynthesis. Marine microalgae contribute to about half of the global O_2 production. Microalgae have immense value because they are rich sources of essential fatty acid, amino acids, vitamins and pigments (Matsunaga *et al.* 2005;

Pachiappan *et al.* 2015). Microalgae are being cultured for different uses; firstly using them as feed in Aquaculture because of its nutritional properties, to fulfil the nutritional requirements of larval stages of molluscs, echinoderms, crustaceans and fish; and secondly in bioremediation (also known as ‘phycoremediation’) for removal of heavy metals from wastewater as microalgae have an excellent capacity of biosorption of heavy metal (Salama *et al.* 2019) due to their excellent affinity to these heavy metal pollutants, hence they play a vital role in filtering and removing these harmful pollutants.

Most studies show that the sorption of metal is a function of pH, it can affect the toxicity and solubility of heavy metals. Abiotic factors such as pH, temperature, light and salinity are important to establish appropriate conditions for optimum growth of microalgae (Sushant and Madaiah, 2014). Most of the algal species prefer pH roughly between 7-8 and precisely between 8.2-8.7 for optimum growth (Pachiappan *et al.* 2015).

Marine Copper Pollution:

Most commonly copper is used for electrical equipment, it is also used in construction, plumbing, industrial machinery, pesticides, ceramics, automotive industries, it is widely used in alloy forms. Natural sources can be Wind-blown dust, forest fires, decaying vegetation and sea spray (Shrivastava, 2009; www.lenntech.com). Due to constant increasing demand of copper, there is an increase of copper in the environment and depositing sludge on the river bank by disposal of copper contaminated waste (www.lenntech.com). These metals are leached out and in the sloppy areas they are carried by acid water to the downstream or run-off to the sea (Garbarino *et al.* 1995; INECAR, 2000). The solid waste from mines, effluents from the water treatment plants sulphur containing gas, electro-refining plants releasing electrolyte, the acid spillage from sulphuric acid plants are the sources of copper pollution directly into the water bodies (Shrivastava, 2009). The metals are then taken up by the plants and accumulate in them, now this polluted soil and water is taken up by animals grazing on the plants and drinking the polluted water and also the fishes that breed in the polluted environments and eventually they deposit these metals in their tissues and also milk if lactating, Humans being on the top of the food chain are exposed to copper by consuming such animals and plants (www.lenntech.com; Duruibe *et al.* 2007). Infants are even more susceptible to the dangerous effects of heavy metal exposure.

Brage, 1985 studied the effect of the copper sediment on the benthic fauna in fjord areas at 71 stations based on the chemical analysis, varying degrees of polluted areas were selected and analysed for metals in the sample, and found that the higher copper concentrations were toxic to a number of species and the diversity was reduced. The areas with least diversity were the areas receiving a significant amount of copper. High concentrations of copper along with low pH levels are believed to be fatal to the fishes (Khayatzadeh and Abbasi, 2010). In common carp exposure to copper inhibited the skeletal ossification (Stominska and Jezierska, 2000).

Vinodhini and Narayanan, 2009 reported exposing Common Carp fish to heavy metals resulted in elevation in the RBCs, blood glucose and total cholesterol concentration. When the level of serum iron and copper was increased there was decreased activity of vitamin C, this indicates the presence of reactive oxygen species-induced peroxidation. In the aquatic system, some metals are available for uptake into the organisms only as free ions from the solution, others can be transported through the biological membranes as inorganic complexes. In experiments with Cu, the toxicity is correlated with the free metal ion concentration. Studies show *Meretrix meretrix* is able to accumulate Cu in the natural environment and maybe used as biomonitoring organisms (Valavanidis and Vlachogianni, 2014).

Microalgae as an ecofriendly option for sorption of copper from polluted water

Microalgae are primary producers of oxygen in aquatic environments, about half of the global photosynthetic activity is found in euphotic niches that is accomplished by marine microalgae (Anderson, 1996 and Matsunaga *et al.* 2005). Algae are the microorganisms that independently acquire chloroplast, which means they have their own photosynthesis mechanism (Gibbs, 1992). Algae are classified into various major groups, based on their pigment composition, storage products and ultrastructural features (Singh and Saxena, 2015). They can be easily cultivated in the presence of sunlight and CO_2 (Matsunaga *et al.*, 2005; Singh and Saxena, 2015). The suitable regions for algae farming are those experiencing warmer climates preferably not below 15°C throughout the year, Singh and Saxena, 2015 also studied that few microalgae grow at limits of the photic zone, 200-300 meters below the water surface; also, on the seafloor in the wave swept beaches, detritus-laden backwater lagoons and also salt marshes and submerged aquatic vegetation beds, intertidal regions and subtidal illuminated areas (MacIntyre *et al.* 1996).

It is economically and environmentally favourable to culture microalgae, also it is easier to genetically modify them to get the desired products and also increase the production. Microalgae can also be modified accordingly to get antiviral, antibiotic and even anticancer properties (Matsunaga *et al.* 2005).

For mass culturing of microalgae, the simplest method is the open culture system, it has low construction cost and ease of operation is also an advantage. Contamination by *Chlorella*, *Dunaliella* and *Spirulina* are a major disadvantage since these species are tolerant to extreme conditions. Closed systems such as photobioreactors are advantageous for maintaining monoalgal cultures since contamination can be avoided, water loss can be reduced and also will give high productivity with greater cell densities and reduce the overall harvesting cost (Matsunaga *et al.* 2005).

Biosorption involves the binding of the metal ions (bisorbate) to the surface of a biosorbent. Microorganisms, agricultural or industrial wastes, biopolymers and plant derived materials can be used as biosorbent. Biosorbents can 'sequester' metal ions from ppm to ppb levels. It is simple in operation, cost effective, doesn't require nutrient always, does not have the danger of toxic effects mostly, the sludge generated is very less and there no increase in the Chemical Oxygen Demand (COD) of water (Basci *et al.* 2003; Kanamarlapudi *et al.* 2018).

The microalgae require scarce amount of nutrients and this has led to a lot of focus on them as biosorbents, they have high sorption capacity, they are available in almost every condition, the sludge to be disposed is very low and not toxic as well, have a potential of metal regeneration and recovery. They also fix the CO_2 from the environment and produce oxygen; microalgae are economic and eco-friendly solution for the treatment of waste water. Their cell walls contain cellulose, alginic acid and the sulphated polysaccharides, high amount of carboxyl groups; these components are involved in the biosorption processes (Gupta *et al.* 2006; Kanamarlapudi *et al.* 2018; Salama *et al.* 2019).

The heavy metals bind on the functional groups on the cell surface by ion exchange, chelation, complexation and micro-precipitation. The viability depends on the metal selectivity and the regeneration potential. However, the metal selectivity can be increased by chemical modification of the biomass (cross linking with epichlorohydrin or oxidation by potassium permanganate) (Kanamarlapudi *et al.* 2018; Salama *et al.* 2019).

The metal binding groups are present on the cell surface and inside the cytoplasm and especially in the vacuoles. Studies show algal cells carry a net negative charge on their surface because of the presence of COO^-, PO_4^{3-} and other groups used for the bonding of metals by ion exchange (Salama *et al.* 2019). Maznah *et al.* (2011) found the immobilized (in sodium or calcium alginate solution) form of the biomass gave better results than the free-living biomass. The examination of the cells after exposing them to heavy metals to observe the active sorptive areas, EDX analysis confirmed the metals on the surface of the algal cells, hence this confirmed the attachment of metal ion to the functional groups of the cell surface. The immobilisation also made the separation and reuse of algal beads easy (Kanamarlapudi *et al.* 2018).

Algal species are reported to biosorb high rates of Cu and other heavy metals, which is higher than other biosorbents. Living and dead algal cells can remove heavy metals from contaminated water. Perhaps, the live cells are more efficient in the metal removal than the dried dead biomass, they can remove and retain more quantity of metals by biosorption and bioaccumulation for longer period. The microalgae are efficient in heavy metal removal from municipal, petrochemical, dairy and electroplating waste waters (Salama *et al.* 2019).

Chlorella minutissima can remove up to 84% of Cu from municipal waste water. In the acid mine drainages

Oedogonium sp. has removal efficacy of 46% of Cu. *Ditylum brightwellii* can secrete special Cu ligands. Microalgae also produce amino acid like Proline, other binding compounds like metallothioneins and glutathione, these are involved in the mechanism that prevents the damage caused due to metal (Gupta *et al.* 2006; Kanamarlapudi *et al.* 2018; Salama *et al.* 2019).

Microalgal biosorption is also influenced by temperature, ionic strength, presence of counter ions, contact time and pH (Homaidan *et al.* 2014; Kanamarlapudi *et al.* 2018; Sibi, 2019). Homaidan *et al.* (2014) studied the biosorption of copper ions from the aqueous solution using grounded and dried dead algal biomass, the study showed that the parameters mentioned influenced the biosorption capacity. When the *Chlorella* biomass was introduced to high Cu concentration, the shape of the cell became irregular and started to rupture and shrink, also they got attached to each other (Al-Fawwaz, 2010). Ouyang *et al.* (2012) studied the effect of heavy metals on the growth and photosynthesis of *Chlorella vulgaris* with different concentrations of the heavy metals, and concluded that effect of the metals was dose dependent and time dependent.

The microalgae have produced an efficient defense system to the toxic effects of metals and eventually increase their survival in elevated metal concentrations (Sibi, 2019). The sorption isotherms have been used to quantify the sorption of metal, biosorption using algae fits well in the Langmuir equation:

$$q_e = q_{\max} \frac{bC_e}{1 + bC_e}$$

Here q_e and C_e are sorption uptake and metal concentration at equilibrium, respectively. b represents the equilibrium constant of the reaction between the metal solution and algal cell wall. The q_{max} is maximum metal uptake by the biomass (Romera *et al.* 2006).

Effects of pH on biosorption:

In general, for any type of biosorbent used, pH is an important factor because the availability of metal binding sites on the algal cells depends on pH of the solution. Most of the studies have shown that the biosorption of metals is a function of pH of the solution. The pH of the solution can affect the toxicity as well as the solubility of the metal in the water. It also affects the speciation of the metals and algal tolerance and influences the metal chemistry in water and the metal binding sites on the cell surfaces. At a medium range of pH, there is an affiliation to CO_2 concentration, the pH increases steadily as the CO_2 is consumed. The pH influences the availability of nutrients (iron and organic acids) too (Gatamaneni *et al.* 2018). Peterson *et al.* (1984), in their report proved pH dependent metal toxicity. Sunda and Guillard, 1976 reported the effect of reduction in pH resulted in higher metal toxicity to marine microalgae due to shift in chemical equilibrium towards more of the metal in the form of cupric ion.

Since most of the metal binding groups of algae are acidic, their availability depends on pH. Cation biosorption increases as the pH of the solution increases and the biosorption increases. Gupta *et al.* (2006) reported to observe maximum biosorption at pH 5 for *Spirogyra*, also with different concentrations of copper they reported 71, 78, 75 and 70% copper uptake at 100, 150, 200 and 250 mg/L. The reason to this was; at lower pH the cell wall would become positively charged due to an increase in the H^+ ions and that eventually reduced the biosorption capacity. The increasing pH increases the negative charge on the surface of the cell wall and ionization of functional groups leading to increased metal sorption (Fraile *et al.* 2014; Gatamaneni *et al.* 2018; Homaidan *et al.* 2014; Kanamarlapudi *et al.* 2018; Maznah *et al.* 2012; Romera *et al.* 2006; Sheng *et al.* 2007). They could not study the effects of pH above 5 due to the precipitation of copper hydrates which was insoluble, this was also reported by Kanamarlapudi *et al.* (2018) and Romera *et al.* (2006). Fraile *et al.* 2014 subjected the biomass to an acid pretreatment at pH 3, to avoid the precipitation after adding the copper solution that ensured previous fixation of protons on the biomass.

Homaidan *et al.* (2014) studied the effects of pH on biosorption of copper ions, they adjusted the desired (2, 3,4,5,6,7,8,9 and 10) initial pH with different concentrations of NaOH and HCl. They reported the optimum pH to be 7; However, it is different for different species (Salama *et al.* 2019) but high pH also reduced the adsorption capacity, due to low number of H^+ ions, the number of active sites of the functional groups are freely exposed which creates net negative charge and attracts positively charged metal ions (Kanamarlapudi *et al.* 2018; Sheng *et al.* 2007).

Cyanobacteria like *Chroococcus turgidus, Lyngbya confervoides* and *Nostoc commune;* diatoms like *Chaetoceros calcitrans* and *Skeletonema costatum* showed maximum growth at pH 7.5, and the planktonic green alga, *Nannochloropsis oceanica* showed maximum growth at the pH 8.4. *Nannochloropsis salina* shows maximum growth at pH 8. *Chlorella vulgaris* showed reduced growth in both acidic range as well as alkaline range of pH (3.0-6.2 and 8.3-9.0). Some isolates showed low growth at pH 6.3. High pH adversely affects the growth of the microalgae and it was found to be significantly reduced at pH 10.6. At extremely high or low pH the microalgal cell would have to spend more energy for maintaining internal pH for the cell function (Bartley *et al.* 2014; Gatamaneni *et al.* 2018; Rai and Rajashekhar, 2014;).

Karyn *et al.* (2005) studied the effect of pH on the uptake and toxicity of Copper and Zinc in *Chlorella sp.* They suggested physicochemical parameters affect both metal speciation and binding of metals. The standard Langmuir adsorption model was used for the correlation of isotherm data at constant pH values. Yu and Kaewsarn, 1999 gave a modified equation for varying pH values.

$$q\text{max} = \frac{q_{max1} + q_{max2}\,10^{pH-pKa}}{1 + 10^{pH-pKa}}$$

Here pKa = -logKa and Ka is equilibrium constant. This equation gives pH dependent q_{max} values that can be submitted in the equation of Langmuir adsorption.

CONCLUSION

Biosorption is a much more reliable option for wastewater treatment. Using marine microalgae is beneficial for efficient and ecofriendly method of heavy metal removal, since these marine microalgae are photosynthetic, they release O_2 in the environment too. pH plays a crucial role in the efficiency of the biosorption as it influences the growth of the microalgae. It affects the ionic composition of the aquatic bodies which affects the attachment of metal ion onto the microalgal cell wall. This property of marine microalgae of biosorption of heavy metals can be used by industries before directly releasing the waste into the water bodies. This method of treatment can reduce the heavy metal pollution and its effects. Companies culturing microalgae on a large scale for the useful products can also use the dead biomass for the treatment of effluents. Further research with respect to pH will provide a better idea of the optimum pH for different species for maximum efficiency of biosorption, as different microalgal species have different levels of affinities towards a particular heavy metal and accordingly they can be used for efficient biosorption of a specific heavy metal.

Conflicts of Interest: The authors declare that there is no conflict of interest with respect to this review paper.

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