3

Prospects for Immobilization of Microbial Sorbents on Carbon Nanotubes for Biosorption: Bioremediation of Heavy Metals Polluted Water

E. Fosso-Kankeu[1,*], A.F. Mulaba-Bafubiandi[2] and A.K. Mishra[3]

[1]School of Chemical and Minerals Engineering, North West University,
Potchefstroom, South Africa
[2]Minerals Processing and Technology Research Center, Department of Extraction
Metallurgy, School of Mining, Metallurgy and Chemical Engineering
Faculty of Engineering and the Built Environment, University of
Johannesburg, Doornfontein, South Africa
[3]UJ Nanomaterials Science Research Group, Department of Chemical Technology,
University of Johannesburg, Doornfontein, South Africa

Abstract

There is growing consideration to abandon the conventional water treatment methods because of their high cost and environmental impact. Alternative methods investigated nowadays present the advantages of being cheaper and ecofriendly.

Among the biosorbents used for the bioremediation of heavy metal pollution of water are microorganisms, which have an inherent ability to uptake heavy metals from solutions.

In order to render microbial biosorbents suitable for process applications, immobilization on adequate supports is required. Apart from their large specific surface area and hollow structure, carbon nanotubes (CNTs) present a number of features making them attractive for immobilization of microbial sorbents to be used in biosorption processes.

This chapter elaborates the potential of microorganisms as biosorbents used in the bioremediation of toxic heavy metals pollution of water systems and explores the possibility of enhancing their performance through immobilization on adequate support matrices, namely carbon nanotubes.

**Corresponding author*: 24838616@nwu.ac.za

Ajay Kumar Mishra (ed.) Application of Nanotechnology in Water Research, (37–62) 2014 © Scrivener Publishing LLC

Keywords: Biosorption, bioremediation, microbial sorbents, metal pollutants, water pollution, immobilization, carbon nanotubes

3.1 Dispersion of Metal Pollutants in Water Sources

Metals are inorganic constituents of the soil unevenly distributed around the world. Natural events leading to exposure of the inner part of the ground often contribute to the dispersion of metals in the environment. However, the growing number of inhabitants on earth as well as the exponential increase of industrial activities has mainly resulted in deforestation and erosion of soils, washing away the metals. Direct anthropogenic actions, which are the major sources of toxic heavy metals, consist of the mismanagement of solid and liquid wastes coming from industries such as electroplating, metal finishing, electronics, metallurgy, and mainly mining. For the scope of this study our discussion will mainly focus on metal pollution related to mining activities.

Effluents arising from mining activities may contain potential pollutants. Polluted water effluents may be produced directly during mineral processing or indirectly when heavy rain comes into contact with sulphide-bearing materials in tailings dumps, resulting in acidic effluents following oxidation. Underground and open pit mining operations always channel processed water into the nearby streams or rivers; however, mining-induced fracture in the collapsed strata promotes contamination of underground water by mine effluents. The main types of water pollution from mining operations could be identified as heavy metal contamination (from processed and acidic effluents); processing chemical pollution (from processed effluents) and acidification (from acidic effluents). Processed mine water and acidic water may contain toxic elements such as heavy metals and reagents (cyanide, sulphuric acid and mercury, used to separate valued metals from ores) that could affect the ecosystem when discharged in the environment. Mining pollution has an obvious impact on biodiversity affecting the wildlife; few terrestrial and aquatic species are known to be naturally tolerant of heavy metals, although some have adapted over time. Salmon species, for example, are particularly sensitive to increased concentrations of copper [1].

In 1989, it was estimated that about 19300 km of streams and rivers, and about 72000 ha of lakes and reservoirs worldwide had been seriously affected by mine effluents [2]. It has also been reported [3] that mining activities have led to the contamination of water in the dolomitic regions of Wonderfonteinspruit (South Africa) with excessive sulphate, nitrate, N-compounds, toxic heavy metals, including radioactive elements such as uranium, and occurrence of acid mine drainage.

Although mostly found in any soil, toxic heavy metals are abundant in mining areas, where metal rich ores have been exposed to the atmosphere. Oxidation of the sulphur content of these rocks by rain water leads to acidic water and dispersion of toxic heavy metals in the surface and groundwater systems.

3.2 Removal of Metal by Conventional Methods

Conventional techniques are used industrially to recover valuable metal or to remediate water pollution; numerous processes are currently used to remove dissolved heavy metals, among which are chemical precipitation and sludge separation, ion exchange, reverse osmosis, filtration, chemical oxidation or reduction, adsorption using activated charcoal or coagulation and electrodialysis [4–9].

Precipitation is the most commonly used removal method, particularly when metal recovery is not a consideration. This process is based on the fact that most metal hydroxides are only slightly soluble and that some metal carbonates and sulphides are also only sparingly water soluble [10]. Metal precipitation from contaminated water involves the conversion of soluble heavy metal salts to insoluble salts that will precipitate. The precipitate can then be removed from the treated water by physical methods such as clarification and/or filtration. The typical precipitation process using sodium hydroxide or lime as reactant is generally applicable to copper, zinc, iron or nickel removal with no special modification; well-sized reverse osmosis (RO) can then be used after prefiltration (following precipitation) to ensure that dilute solutions are totally clean.

For metal removal using ion exchange, chelated resin, for example, serves as a matrix for adsorption of target ions from the effluent system. The most efficient uses are for removing lead, copper and nickel. They exchange sodium for the target metal and pass the hardness ions, calcium and magnesium. They are effective on industrial water rinse without any preconditioning and are very effective in the direct replacement of sludge generating systems. In hydrometallurgy, after leaching process, ion exchange is used for the purification and concentration of dissolved values. Regeneration is very efficient and the target metal is usually recovered in relatively small volumes of regenerant [11].

Recovery of heavy metals by electrodialytic method consists of placing electrodes in separate compartments, where electrolyte solutions are circulated; electrolyte solutions ensure that a good contact between the electrode surface and the surroundings is maintained [12]. Electrolysis (electro-winning or electro-refining) is used for the recovery of valuable metals after purification in hydrometallurgical plants. Ion exchange membranes determine the passage of anions or cations in the direction of anode or cathode electrodes respectively; this prevents the waste of current [12]. When an electrical potential is applied across the ion-exchange membranes, cations in solution begin to migrate toward the cathode, anions toward the anode. Metal removal in this process occurs through electroosmosis mechanism due to electrical, temperature, concentration and mechanical pressure gradients that exist across the membrane [13].

The first report of laboratory experimentation of electrodialytic remediation was done by Jakobsen *et al.* [12], who reported the removal of cadmium from wastewater sludge; the remediation process allowed the reduction of two-thirds of the total cadmium from the wastewater sludge. The recovery of valuable metals by electrolysis is technically challenging and probably not cost effective at a low production rate [14]. Furthermore, these techniques are likely to generate toxic sludge or other waste products that require careful disposal [15].

3.3 Microbial Sorbents for Removal of Toxic Heavy Metals from Water

3.3.1 Biouptake of Metal

The limitations of conventional methods present an opportunity to explore the possibility of considering a biological approach for the recovery or removal of metal from solutions. Biological methods are more effective for large volume and diluted solutions; they are cheaper and ecofriendly. Various types of biomasses such as seaweeds, molds, yeasts, crabshells and bacteria have been tested for metal biosorption with very encouraging results. However, microorganisms, especially bacteria with high growth rates, are favorable for biosorption purposes. The bacteria biosorption process involves a solid phase (biosorbents or cells) and a liquid phase (solvent, normally water) containing a dissolved species to be sorbed (sorbate, a metal ion). The affinity of the sorbent for the sorbate species promotes the attraction of the latter and binding with different mechanisms. Mechanisms involved in metal ions uptake by bacteria mainly depend on non-metabolic and metabolic phenomena [16]. Two major mechanisms could therefore be identified as biosorption (passive binding of metal on cell surface) and accumulation (active sequestration of metal ions inside the cells) [17].

3.3.2 Factors Affecting Microbial Adsorption Capacity

3.3.2.1 Cell Age

According to Alcamo [18], bacteria growth can be separated in different stages, mainly as the lag phase, the log phase and the stationary phase. At each of these phases bacteria exhibit different morphology and the intensity of metabolic activities may vary. As demonstrated in the previous sections, heavy metal uptake by bacteria is dependent on bacterial morphology (surface availability and cell wall structure) and metabolism. It could therefore be predicted that bacteria adsorption capacity will be affected by cell age. Younger cells (24 h old) of an industrial strain of *S. cerevisiae* have been reported [19] to exhibit Ag^+ uptake capacity twice that of older cells (96 h old).

3.3.2.2 Physicochemical Effect

Conditions of the microenvironment such as pH, temperature and initial metal concentration are likely to influence metal removal by microorganisms.

The pH value of the solution is related to the amount of hydrogen ion (H^+) or hydroxyl group present and therefore influences metal uptake through hydrogen ion competition at the cell surface and changes of the solution chemistry of the heavy metals: hydrolysis, complexation by organic and/or inorganic ligands, redox reactions, precipitation, the speciation and the biosorption availability of the heavy metals [20, 21]. In fact, at acidic pH there are hydrogen ions in solution which compete with metals for anionic functional group on cell surface, therefore reducing biosorptive capacity of metal cations [22, 23]. On the other hand, there is dissociation of metal complexes with both organic and inorganic ligands at low pH, promoting the release of aquo ion [24, 25]. Optimal pH for metal biosorption varies for almost each biosorbent; it is reported that the optimal pH value is 5–9 for copper biosorption by *S. cerevisiae,* and 4–5 for uranium [26]. In the study done by Mapolelo and Torto [27], they found that the optimal pH value for Cd and Zn biosorption is 5.8, while for Cr(III) and Pb it is 5.2. This implies that the influence of pH value on metal biosorption varies among metal species and biosorbent, therefore requiring further studies to classify specificity.

The function and cell structure of microbial sorbents can be affected by temperature changes [28]; high temperature to a certain extent may lead to the increase of metabolic activity and energy of the system (metal solution), which could promote the active uptake or attachment of metal to cell surface respectively [29, 30]. Goyal *et al.* [29] found that the metal biosorption of Cr(VI) by *S. cerevisiae* increases with increasing temperature in the range of 25–45°C, which they explained was as a result of the increased affinity of cell surface sites for metal at higher temperature. The decrease of biosorption capacity at higher temperature may be due to the damage of active binding sites in the biomass [31]. Most of the researchers have concluded that the change of temperature in the range of 30–40°C has a small influence on metal adsorption.

3.3.2.3 Cell Biomass

Metal adsorption capacity is also a function of surface availability, which is related to the concentration of biomass. High concentration of biomass provides more surfaces available for metal binding, reducing the adsorption time as well as promoting passive adsorption. However at low concentration of biomass, metal ions are likely to be sequestered inside the cells.

3.3.2.4 Initial Concentration of Metal

Initial metal concentration in solution may affect metal biosorption in two ways: firstly on a physiological aspect and then on a physical aspect. High initial concentration of metal is likely to inhibit metabolic activities and hinder active accumulation of metal; while the surface available for metal binding on bacterial cell could be quickly saturated at higher concentration of metal. Wang and Chen [32] reported that the uptake rate of the metal ion will increase along with increasing its initial concentration if the amount of biomass is kept constant; implying that biosorptive capacity of metal ions is related to the ratio of the concentration of initial metal ions to the concentration of biomass.

3.3.2.5 Metals Competition

The presence of multi-metals in solution could also affect the adsorption of the metal ion of interest. In fact, the degree of removal of heavy metal ions from wastewater by biosorption depends on the multi-metal competition for the binding sites on the surface of sorbent [33]. When various metals are present in solution, the tendency is that the surface available on bacterial cell for binding of specific metal ions will be reduced for each metal ion, as co-ions are likely to interact with functional groups on the surface. Goksungur *et al.* [34] observed that the competitive biosorption capacities of the ethanol-treated yeast for all metal ions of Pb, Cu and Cd were lower than that under the non-competitive conditions. There was a decrease of metal uptake in competitive conditions between same charged species for binding sites of the ethanol-treated yeast cells.

3.3.2.6 Exposure Time

When exposed to heavy metals in solution, bacterial cells can immediately interact with metals and in a few minutes metal uptake can result through the passive mechanism. Kefala *et al.* [30] reported maximum removal of cadmium ions by biomass of *Actinomycetes* after 5 minutes exposure time, which was in agreement with the findings by Sadowski *et al.* [35]. However, metabolic uptake of heavy metals by microbial biomass required longer exposure time. For example, Kim *et al.* [36] observed an increase of lead uptake by *Bacillus* sp over 48 hours exposure. Metal uptake is thus influenced by the exposure time and could allow determination of the mechanism involved in metal removal by bacteria.

3.3.3 Isothermic and Kinetic Equilibrium of Biosorption

Most of the experiments on heavy metals biouptake by microorganisms have only been performed at laboratory scale and very few have been implemented at an industrial level due to limited understanding of the interaction between microbial sorbent and heavy metals. For this reason recent works have focused on investigating the affinity of biomass for metal through the determination of biosorbent adsorption capacity, study of isotherm and kinetic equilibrium of the biosorption process.

The affinity of a microorganism for a given metal determines its ability to uptake a certain amount of the metal from solution. That ability is recognized as metal adsorption capacity, which is defined as the amount of heavy metal adsorbed onto the unit amount of the dry or wet biomass as expressed in mg/g.

Isotherm refers to a relation holding true at a constant temperature. The study of isotherm is important for the fact that adsorption isotherm can be used to describe how metals interact with biosorbent, and therefore is critical in establishing optimal use of biosorbent as well as the design of the bioremediation plant [37]. Various isotherm models are available but the Langmuir and Freundlich models are the most widely accepted surface adsorption models for the single-solute systems. The Langmuir isotherm model assumes that uptake occurs on a homogenous surface by monolayer sorption without interaction between adsorbed molecules. The model illustrates a nonlinear and saturated adsorption process that can allow the determination of the equilibrium constant, hence estimation of biosorption affinity [38].

The Freundlich isotherm model establishes the relationship between the concentration of a solute on the surface of an adsorbent, to the concentration of the solute in the liquid with which it is in contact. The Freundlich model is based on sorption on a heterogenous surface [39, 40].

The rate equation for the biosorption process usually determines the rate of metal adsorption by biosorbent. Various kinetic models have been used to test experimental data, including pseudo-first, pseudo-second order and intraparticle diffusion, to examine the mechanism involved in the biosorption [38]. Among the kinetic models, pseudo-first order and pseudo-second order kinetics are the most widely used models to describe the adsorption process [41, 42]. Important parameters considered for pseudo-second order kinetics are: i) the equilibrium rate constant; ii) the equilibrium adsorption amount, and iii) the adsorption amount at a given time.

3.3.4 Drawbacks Due to Inhibition

Microorganisms use some metals as substrate required for metabolic functions; but most of the metals are likely to be toxic, especially at high concentrations. Metal removal through both passive (biosorption) and active (bioaccumulation) mechanisms can be affected by the toxicity of metal species, which affect microbial biological functions and morphology (reduces cell surface availability) [43].

The cell wall of bacteria contains some functional groups (phosphoryl, carboxyl and amino groups) responsible for binding metals present in the environment. These groups give the cell surface a negative charge density [44], therefore conferring them the ability to bind metal cations [45–48]. It is suggested that the binding of cations by the walls of many Gram-positive bacteria is a function of peptidoglycan-associated teichoic acid [49]. It therefore ensues that the Gram-negative bacteria cell wall could not have the same binding ability as with Gram-positive bacteria, owing to the fact that they possess a much thinner peptidoglycan shielded by an outer membrane [50, 51]. Nevertheless, Gram-negative bacteria possess anionic lipopolysaccharides (LPS) extended beyond the outer membrane proteins and facilitate attachment of metal to cell wall [52–54]. In some cases metals can be sequestered into bacterial cells through two mechanisms: the first is quick and unspecific, driven by chemiosmotic gradient across the cell membrane. The second one is through an active transport requiring the hydrolysis of ATP and is slower than the previous one. Various heavy metals can easily cross the cell membrane through the first mechanism and become toxic once present in high concentrations inside the cell [55]. Most of the light metals are used in bacterial metabolic functions, but the heavy metals are essentially toxic, especially at high concentrations.

The inhibitory effect of metal, whether bacteriostatic or bactericidal, affects the morphology or the metabolic function of bacteria (Figure 3.1). For example, copper can change bacterial rods into spherical form [56]. Toxicity of metal is reported to be related to the strength of the covalent binding of the metal ion to various ionic groups on the cell surface. However, the strength of binding increases with electronegativity of each metal [57, 58]. Metal may affect oxidative phosphorylation and membrane permeability, as seen with vanadate and mercury [59]. In fact, mercury and silver ions are reported to act on cell membrane through precipitation of cellular proteins [60]. Heavy metal ions can strongly bind sulfhydryl groups of proteins and so doing change the structure and enzymatic activities of proteins and cause malfunctioning of metabolic pathways [61, 62]. For example, silver ions denature enzyme of the target cell or organism by binding to reactive groups, resulting in their precipitation

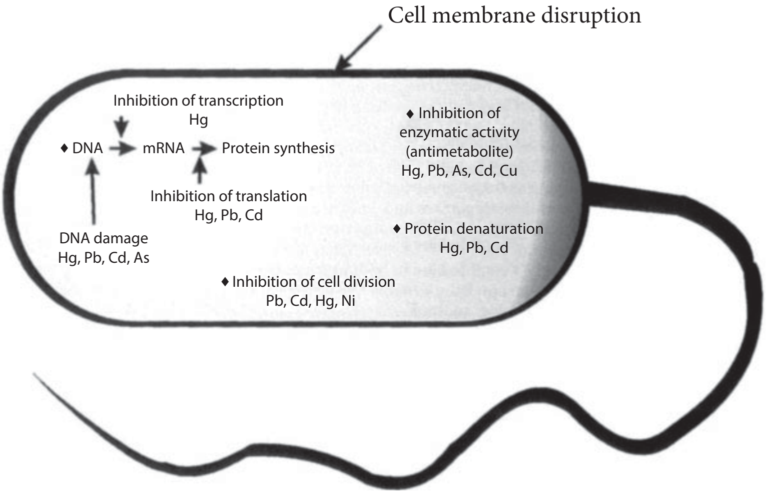

Figure 3.1 Summary of the various toxic influences of metals on the microbial cell demonstrating the ubiquity of metal toxicity (From [59]).

and inactivation; by reacting with the thiol groups on the enzymes and proteins, silver significantly inhibits the growth of bacteria [63]. However, silver also reacts with the amino-, carboxyl-, phosphate-, and imidazole-groups and diminishes the activities of lactate dehydrogenase and glutathione peroxidase.

Heavy metal cations may bind to glutathione and the resulting bis-glutathiono-complexes tend to react with molecular oxygen to oxidize bis-glutathione GS-SG [64], the metal cation and hydrogen peroxide H_2O_2. The metal cations will then catch two glutathione molecules once the oxidized bis-glutathione is reduced again in a NADPH-dependent reaction; this process causes a considerable oxidative stress [62]. Cell mutation can also arise as an interaction of the bacteria gene with heavy metals. Khan and coworkers [65] studied the nuclease activity of nickel (II), cobalt (II) and copper (II) complexes and found that they were responsible for DNA cleavage. According to the explanation of Aruoma *et al.* [66], copper ions bound to DNA react with peroxide and ascorbic acid to generate hydroxyl radicals, which then react with DNA bases in a site-specific manner causing DNA strand breakage.

Bacteria metabolism could also be disturbed as a result of heavy metal competition with cofactor for the binding sites on enzymes. Studying the effect of metal ions (inserted at sulphur-rich sites) on the activity of sulfite oxidase, Neumann and Leimkuhler [67] found that heavy metal caused the inhibition of the enzyme and through this mechanism could become toxic to *Escherichia coli*.

The performances of microbial sorbents in some instances are unsatisfactory and pretreatment of biomasses are undertaken to increase the surface area and/or the number of active groups or to overcome challenges of inhibition, therefore improving the metal binding capacity. Pretreatment methods are either chemical or physical.

3.3.5 Metal Tolerance Mechanisms of Microbial Sorbents

Microorganisms living in an environment contaminated with a high concentration of heavy metals generally develop mechanisms allowing them to adapt to the milieu. These indigenous microorganisms can tolerate relatively high concentrations of heavy metals and are therefore suitable for use as biosorbents for metal uptake processes. Various types of metal resistance mechanisms are developed by microorganisms; these mechanisms include the efflux of metal ions outside the cell, accumulation and complexation of the metal ions inside the cell, and reduction of the heavy metal ions to a less toxic state [62]. In resistant bacteria these mechanisms can occur singly or in combination [68, 62]. Genes conferring these resistance mechanisms mostly reside on plasmids [69–71] but also on the chromosomes [72, 73]. Efflux pumps are the major currently-known groups of plasmid resistance systems supported by proteins acting as metal-transporters (ATPases) or the chemiosmotic system across cell membrane.

Cloning of genes and expression in bacteria allows for the providing of bacteria with traits such as gene resistance, making them improved biosorbents for the biosorption process. The genetically modified bacterium can then tolerate high concentrations of a given metal, increasing its ability to uptake the metal from solutions. For example Siddiqui *et al.* [74] cloned the pMOL28-encoded nickel resistance genes in *Pseudomonas* spp and observed an increase of the ability of the conjugated species to resist higher concentrations from 0.5 to 1.5 mM $NiCl_2$.

3.3.6 Pretreatment of Microbial Sorbent

Various methods have been used by researchers to pretreat biomass in order to modify the functional groups on the cell surface, therefore enhancing the metal binding capacity. Modifications can be introduced either during the growth of a microorganism or in the pregrown biomass. Modifications done during the growth generally consist of changing the composition of the culture medium. *Aspergillus niger* biomass grown in the presence of large amounts of potassium hexacyanoferate from citric acid fermentation plant was reported [75] to exhibit very high biosorption due to change in cell wall composition.

Pretreatment of pregrown cells, consists to affect through a physical or chemical method the number of sites in the biosorbent material, the accessibility of the sites and the chemical state of the sites (i.e., binding strength) [76]. Physical methods include vacuum and freezing/thawing, boiling/heating, autoclaving and mechanical disruption. The various chemical methods used for biomass modification include treatment with various organic and inorganic reagents, such as acid and caustic, methanol and formaldehyde among others. Alkali pretreatment of *Mucor rouxii* biomass contributed to a significant increase in its bioadsorption capacity when compared to autoclaving, while pretreatment of biomass with acid resulted in decreased bioadsorption of heavy metals [77, 78].

However, microbial biosorbents in planktonic form are dispersed and could not ensure optimum mass transfer; furthermore, they may be degraded when exposed to harsh environmental conditions. Immobilization of microbial biosorbents is therefore needed for effective application of biosorption.

3.4 Immobilization of Microbial Sorbents on CNTs

The immobilization of microorganisms on carbon nanotube (CNT) is an approach that is beneficial in the implementation of various techniques. The stability of the complex microorganism-CNTs is crucial for the success of each application and is often dependent on the binding strength or features of both microorganism and CNT. In such an interaction, the nature of microbial cell membranes and the physical characteristics of CNTs play very important roles.

3.4.1 Possible Interaction between Microorganisms and CNTs

3.4.1.1 Microbial Cell Membranes and Functional Groups

Microorganisms are mainly divided into eukaryotic and prokaryotic cells. Eukaryotic microorganisms encompass algae, fungi and protozoa, while only two groups, namely bacteria and archaea, constitute prokaryotes. As opposed to eukaryotes, prokaryotes do not have a membrane-enclosed nucleus as well as few membranous organelles. The cytoplasmic content of these microorganisms is protected by a cell wall or cell membrane, which is the point of contact with the environment. There exist chemical differences between eukaryotic and prokaryotic membranes. Cell wall structure is also the basis of division of bacteria into Gram-positive and Gram-negative groups. The cell wall of algae is mainly composed of a network of cellulose supplemented with a variety of polysaccharides; the cell of fungi is as rigid as for the algae but mainly contains polysaccharides. Gram-positive bacteria cell wall is comprised of a thick layer of peptidoglycan which is responsible for the negative charge of the wall. The overall negative charge of the Gram-negative cell wall is due to the dominant presence of lypopolysaccharides, which are highly charged in nature.

The components of the cell surface of the microorganism also include ligands such as OH^-, HPO_4^{2-}, CO_3^{2+}, $R\text{-}COO^-$, $=C=O$, CN^-, $R\text{-}S^-$, $\text{-}SH^-$ and NH_2-, which are active groups involved in the interaction with the environment.

3.4.1.2 Characteristics of CNTs

Carbon nanotubes have unique physical properties, making them attractive material for various applications. The CNTs can be divided into single-walled carbon nanotubes (SWCNTs) and multi-walled carbon nanotubes (MWCNTs) depending on the number of carbon atom layers. Each type of CNT is based on the principle of hybridized carbon atom layers in the walls of CNT and represents a hollow, concentric cylindrical structure that is closed at both ends [79]. The SWCNTs consist of one layer with diameters ranging from 0.3–3 nm, whereas MWCNTs are composed of more than one layer that form concentric tubes and can reach diameters of up to 100 nm or more. Depending on the adsorbate present, the features of CNTs influencing their adsorption properties include their high porous and hollow structure, light mass density, affinity to adsorbates and large specific surface area [80, 81] The size of surface area is dependent on the extent of pore aggregation due to the entanglement of a certain number of individual tubes [82–85]. Adsorption on CNTs mainly occurs at four regions: at hollow interiors of nanotubes they are open ended, at interstitial pore spaces between the tube bundles, at groves present at the boundary of nanotube bundles or at the external surface of the outermost CNTs [86, 87]. According to Upadhyayula *et al.* [87], the interior space of CNTs cannot serve as adsorption because CNTs have closed caps, and secondly, the smaller diameter of the tubes does not accommodate a typical macromolecular-sized contaminant; while only a few low-molecular-weight, small-sized adsorbates can be adsorbed in the interstitial spacing formed between the bundles of nanotubes [82]. The groves at the boundary of nanotube bundles and the external surface of the outermost CNTs are therefore the most suitable regions for adsorption, providing large pore spaces for microorganisms.

The adsorption of microorganisms on carbon nanotubes results from physical-chemical mechanisms. The structures of microorganisms and CNTs as described above are susceptible to the promotion of chemical interactions such as ionic and covalent binding as well as physical binding, including electrostatic or London-van der Waals forces. During an exercise aiming to systematically compare the adsorptive interactions between CNTs and organic pollutants with significantly different physical-chemical properties, Chen *et al.* [88] found that the adsorption affinity correlated poorly with hydrophobicity but increased in the order of nonpolar aliphatic < nonpolar aromatics < nitroaromatics for CNTs, and the adsorption affinity increased with the number of nitrofunctional groups within the group of nitroaromatics. According to Arepalli *et al.* [89], SWNTs generally have strong van der Waals forces that may cause them to aggregate into microscopic bundles or ropes, which in turn agglomerate loosely into small clumps. The van der Waals forces of MWCNTs are said to be less effective than those of SWCNTs [90], leading to less tight interactions between cells and MWCNTs within the aggregates. In a separate study, Akasaka and Watari [91] found that apart from the effect of van der Waals forces, the diameter of CNTs affect the way in which they precipitate and capture cells;

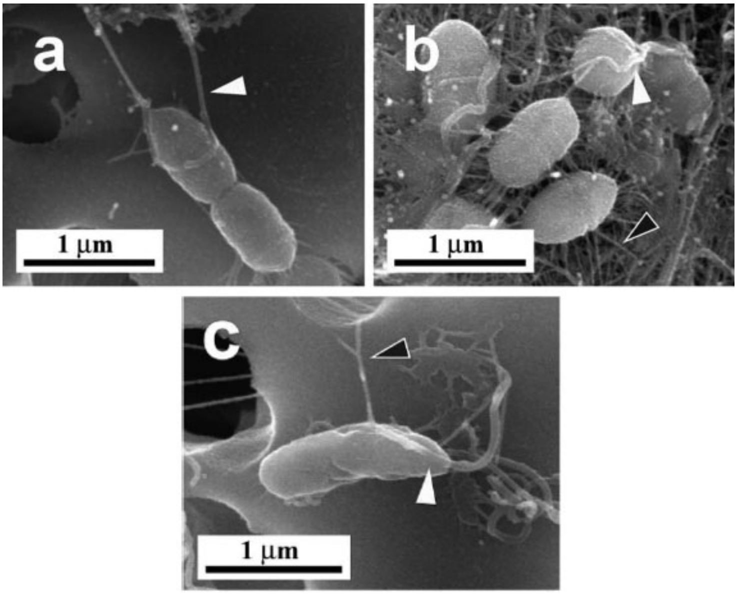

Figure 3.2 SEM images of S. mutans adhered to CNTs: (a) bundles of CNTs wound around S. mutans ; (b) the bacteria adhered to the meshwork comprising bundles of SWCNTs; (c) 30-MWCNTs wound around bacteria (from ref. [91]).

CNTs that captured pathogens by winging ensured stronger adhesion and inhibited the release and budding of captured bacteria (Figure 3.2).

Arias and Yang [90] reported that surface groups on the CNTs coupled with different buffer conditions would affect the interactions between CNTs and cells as well as formation of cell-CNTs aggregates due to either charge-associated effects or reaction environments.

Overall, according to Ren *et al.* [80] a number of factors likely to influence adsorptive interactions between CNTs and organic matters include: CNT properties (size, shape, surface areas, large average pore diameter and volume, morphology, functional groups, impurity), organic matter (hydrophobicity, electron polarizability, polarity, size, functional groups) and environmental conditions (pH, ionic strength).

3.4.2 Adsorption of Microorganisms on CNTs for Bioremediation

The biosorption process has been attractive and considered as an alternative to conventional methods because of the lower cost of the technique. However, the need to culture biosorbent after each cycle of biosorption may render the process costly, hence the need to immobilize the biosorbent to diminish dispersion, loss and degradation which impact on the adsorption capacity. A couple of support materials have been used for the immobilization of biosorbents in bioremediation processes; these include alginate, polyacrylamide, polyvinyl alcohol, polysulfone, silica gel, cellulose and glutaraldehyde [21]. A few basic features have to be considered when selecting the support material; material used as a carrier should have chemical, physical and biological stability during processing as well as in the reaction conditions, sufficient mechanical strength and should have adequate function groups for binding biosorbent and high loading capacity [92]. Binding of microbial sorbent on support matrix can be done using reversible (adsorption, disulphide bonding, chelation) or irreversible (covalent bonding, entrapment, encapsulation, crosslinking) methods.

The discovery of carbon nanotubes in the 90s has led to development of several applications. A few of these applications include: the role of sensors in the detection of pathogens after surface modification of CNTs [93, 94], pristine and modified CNTs have shown antimicrobial activities [95, 96], CNTs have been used for the detection of toxic contaminants upfront in a wastewater treatment plant [97, 98], CNTs have been used for the concentration of bacterial pathogens from contaminated water systems [99, 100], CNTs can be used as filters for seawater pretreatment to remove bacteria from raw seawater, thereby reducing the biofouling problems of reverse osmosis membranes [101], and recently CNTs have been used as support for microbial sorbents for the removal of toxic heavy metals from solutions [102].

The potential of CNTs to adsorb microorganisms has been thoroughly studied, and it has been found that microbial adsorption capacities on CNTs are higher than any other commercially available adsorbent media; CNTs express selective adsorption of bacteria and the adsorption kinetics of bacteria on CNTs is almost instantaneous [87]. Akasaka and Watari [91] showed that both the SWCNTs and MWCNTs can effectively adsorb microorganisms, although the better performance was recorded with the MWCNTs; they also found that the diameter of CNTs played a role in microbial adsorption efficiency.

Bacteria in biofilm state are more bioavailable, protected from adverse conditions in the environment, and the lipopolysaccharides and EPS produced at the surface of biofilm may serve as metal chelators. Therefore, materials that facilitate a higher degree of bacterial colonization are suitable for metal biosorption processes. According to Salah *et al.* [103], desirable qualities of such materials include: a highly porous structure on which microorganisms can easily colonize, the ability to provide modulating effect by adsorbing high concentrations of the toxicant from the bulk but regulate its availability to the microbes, and the ability to contribute to the buffering capacity of biofilm consortium.

Some researchers [94, 104, 105] have functionalized CNTs using carbohydrate ligands in order to modify their adsorption capacities.

Using *P. aeruginosa* immobilized multiwalled carbon nanotubes for biosorption of heavy metals, Tuzen *et al.* [102] showed that such biosorbent could be used for at least 50 cycles without losing its adsorption capacity; furthermore, the biosorbent was successfully used to reduce the level of toxic heavy metals in tap water and spring water below the recommended limit. These results are encouraging for the continued investigation of the use of CNTs as support matrices for microbial sorbents in bioremediation of heavy metals polluted effluents.

3.5 Conclusion

The potential of microorganisms for removal of metals has been convincingly demonstrated by researchers over the years; however, the biosorption process can not yet be considered as a total replacement for the conventional methods for several reasons. Issues related to the availability of biosorbent at low cost, the sustainability of the process and the recovery of toxic metals following adsorption, are still to be effectively addressed. Immobilization of microbial sorbents that allows control and reuse of microbial sorbents may be a solution to these problems. Support matrices previously used for immobilization are not resistant; the carbon nanotubes could therefore be a good alternative given their attractive features. However, the major concern when using CNTs in the bioremediation of water could be their relative toxicity to humans.

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