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Review Article

The Role of Microbes in Bioremediation of Radioactive Wastes

Nada ElShaarawy*

Microbiology Department, Faculty of Science, Ain Shams University, Giza, Egypt.

***Corresponding Author:** Nada ElShaarawy, Microbiology Department, Faculty of Science, Ain Shams University, Giza, Egypt.*

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Abstract

The world is currently undergoing rapid development in industrialization in various industries such as chemical industry, textile industry, nuclear power plant. They release effluents that contain harmful substances (U235, Cs137, and other compounds) that are released in the environment and cause contamination. Also, due to nuclear disaster such as Chernobyl and Fukushima large amount of radioactive substances was spread and cause harmful effects on living organisms and environment, so it is necessary to maintain and manage the disposal of radioactive waste by treating it with physical, chemical, biological methods for the management of radioactive waste. Microbial remediation is the best method and carried out by different mechanisms such as bioaccumulation, biosorption, biotransformation, biosolubilization, bioprecipitation, chelation, and complexation.

Keywords: *Bioremediation, Bioaccumulation, Biosorption, Biotransformation, Biosolubilization, Bioprecipitation, Chelation, Complexation*

1. Introduction

The world is currently undergoing rapid development in industrialization and development of new technology in various industries such as chemical industry, textile industry, and nuclear power plant (Natarajan, 2020). Although these industries are helpful in the development of the nation's economy, they are responsible for the release of effluents that contain harmful substances which affect the environment. The effluent released from the nuclear industry is more harmful to the environment as well as to humans directly. The effluent from nuclear industry consists of radioactive elements such as U235, U238, Np237, Cs137, and other compounds (Natarajan, 2020).

Nuclear energy is widely used nowadays as it is a pollution free energy as it helps to generate and supply energy to entire area when compared with other energy sources, however, it also causes harmful effect to the environment from its waste which is highly radioactive (Natarajan, 2020). Although nuclear energy is a major source for power generation as well as pollution-free energy source, its inclusion in the renewable energy list is a subject of major debate (Chowdhury, 2012). Still in near future, most of the countries have planned to build several nuclear plants for the generation of electricity as a result of their growth in economy and development (Pioro and Kirillov, 2013).

Nuclear energy will not cause any pollution to the environment, but its waste known as radioactive waste causes harmful effects on living organisms and the environment. There was a large scale of contamination over past 60 years of nuclear activity, so it is necessary to maintain and manage the disposal of radioactive waste by treating it with physical, chemical, and biological methods for the management of radioactive waste (Chang, 2016).

Instead of treating, radioactive waste is buried deeply underground or deep in the ocean. Even though they are buried carefully, the radioactive substances are still penetrating deep into the ground and contaminate the ground water and emit radiation which may be due to the imperfect sealing and high radiation exposure of radioactive waste. Examples of these techniques used for radioactive waste management are incineration, wet oxidation, acid digestion, etc. These methods have their own advantages and disadvantages (Valdovinos et al. 2014).

Nowadays, scientists are concentrating on disposal of radioactive waste as the nuclear energy is going to play a major role in near future, improving the existing and introducing novel methods using newer technology to treat and dispose the wastes, since there is no proper method is available for treating radioactive waste (Natarajan, 2020).

Based on the technological development and knowledge about microbes, radioactive wastes can be disposed by bioremediation. The management of radioactive waste using microbes and plants is playing a significant role and may play a major role in the near future. The major methods in the literature are of high cost and difficult in maintenance when compared with bioremediation and plant remediation (Chang, 2016).

2. What're Radioactive Materials (Radionuclides)?

Radionuclides are class of chemicals where the nucleus of the atom is unstable. They achieve stability through changes in the nucleus (spontaneous fission, emission of alpha particles, or conversion of neutrons to protons or the reverse). This process is called radioactive decay or transformation (Fig. 1), and often is followed by the release of ionizing radiation (beta particles, neutrons, or gamma rays) (ATSDR, 2008).

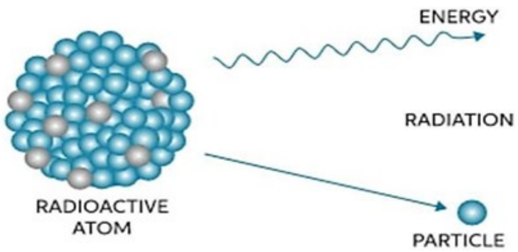


Figure 1. *Radioactive atoms (Radionuclides)*

3. Sources of Radioactive Wastes

3.1 Man-Made Source

Most radioactive waste comes from nuclear electricity production and military activities. However, it is also generated in hospitals from the use of radioactive material to diagnose and treat the sick and sterilize medical products, in universities in conducting vital research in biology, chemistry and engineering, and in agriculture, where nuclear applications have helped produce crops that are more drought and disease resistant, as well as crops with shorter growing periods or increased yield--a practice that has been especially beneficial for some developing countries (IAEA, 2008).

3.2 Nuclear Disaster

Chernobyl Nuclear Accident

Due to the rupture in the nuclear reactor core, a large amount of radioactive substances was released over ten days. The radioactive substances were deposited and later contaminated the sewage. Agriculture was ruined due to the contamination due to iodine and, hence affecting dairy farming. Even for decades the food crops and milk will remain contaminated with the cesium-137 due to the disaster (Gaurav, 2022).

Fukushima Nuclear Accident

Due to the natural earthquakes and tsunamis in 2011, an accident occurred in Fukushima's nuclear reactor. Through the air, amounts of radioactive iodine, strontium and cesium were deposited on the ground and in the water (Gaurav, 2022). Land life will deteriorate because of the Cesium-137 isotope. It won't decay half the way before thirty years since then. This affected and will affect the stocks, agriculture, and people's health for an unforeseeable future (Gaurav, 2022).

3.3 Natural Contamination

Some radioactivity is generated naturally. The sources are gases and minerals that are present in the land. Though they cannot escape unless there is mining or extraction done (Gaurav, 2022). An example of natural radioactive contaminant is radon gas. A naturally occurring gas, radon disperses rapidly in the environment. Cosmic radiation is one more cause of radioactive pollution, entering the earth's atmosphere from space.

4. Effect of Radioactive Waste

Radioactive waste majorly affects the environment and human beings in a direct and indirect way.

4.1 Effects on Environment:

Effects on Wildlife

Different levels in the animal system suffer differently. The higher-level organisms get more affected than insects and flies. Herbivores, especially cattle, graze the contaminated land. That's how the deposition of Ce-13 and I-131 accumulates on the animal tissues in a tragically large amount. These radionuclides enter their metabolic cycles and affect their DNA. This ends up having a mutated animal generation with a higher risk of health issues by just a small amount of radionuclides (Gaurav, 2022).

Effects on Vegetation

Effects of radiation on the protectors of nature are worse too. The damage is mostly done due to the increased ultraviolet waves (the short lengthened) and is directly proportional to the amount of exposure the plant gets. Different parts get affected differently. The stomata stop to stop the evaporation during the increase of radiation. When the chromosomes are hit, the reproduction is disturbed, resulting plants in altered shapes, size and health. Exposure in high amounts simply means deletion of the affected plants (Gaurav, 2022).

Contaminated plants are harmful for the organism who consumes, therefore, radioactive contamination of plants - being the major partner in food production - will lead to severe damage in the food chain (Gaurav, 2022).

Effects on Sea Life

Great sources of nuclear energy and chemical processing i.e., the power plants have been releasing radioisotopes into the water for decades. Few are of cesium, radon, crypton, ruthenium, zinc, and copper. Though the waste is released in a "permissible" amount but permissible does not mean it is safe. These radionuclides can be detected in the soft tissues or on the bones of the fishes (Gaurav, 2022).

4.2 Effects on human beings:

The impact on human beings may range from mild to fatal (figure 2), the magnitude of the effects depends on the level and duration of exposure to radiation. Low level of effects causes mild skin irritation, but if the time duration of exposure is long with low intensity, it will cause nausea, diarrhea, vomiting, loss of hair, etc. Long-term exposure of high radiation will lead to far more severe effects on the human body. The radioactive rays will cause irreparable damage to DNA which leads to mutation and other harmful effects. Skin, lung, and thyroid cancers are some of the diseases caused by radiation (Kautsky et al., 2013; IAEA, 2002).

The effects of mutation lead to the passing of mutated genetic information to generations. If the parents are exposed to radiation, their child could have severe congenital disorder during birth, in both physical and mental aspects (Kautsky et al., 2013; IAEA, 2002).

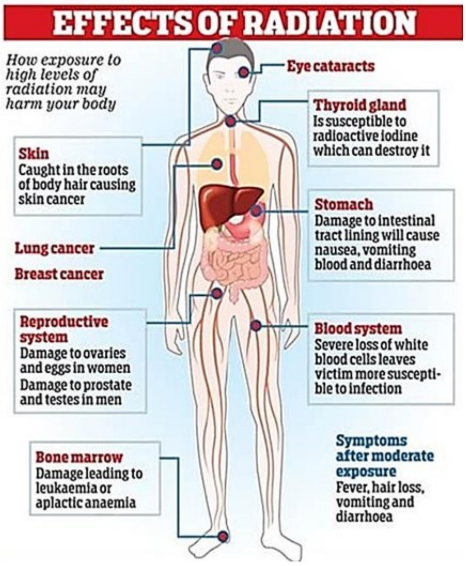


Figure 2. *Effect of radiation on human body.*

A famous example was the 1986 accident at the Chernobyl nuclear plant in Ukraine was the largest uncontrolled radioactive release in history. In the three most-affected countries – Belarus, the Russian Federation and Ukraine (Canada, 2022). Workers and the public were exposed to three main types of radionuclides: iodine- 131, cesium-134 and cesium-137 (Canada, 2022). When iodine-131 is released into the environment, it is quickly transferred to humans and taken up by the thyroid gland. However, I-131 has a short half-life (8 days). Children exposed to radioactive iodine usually receive higher doses than adults, because their thyroid gland is smaller, and they have a higher metabolism (Canada, 2022). Cesium isotopes have longer half-lives (approximately 2 years for cesium-134 and 30 years for cesium-137), increasing the chance of long-term exposure through ingestion of contaminated food and water, inhalation of contaminated air, or from radionuclides deposited in soil (Canada, 2022).

Worker Health Impacts

134 worker suffered acute radiation sickness, 28 of whom died in the first three months. Increased incidences of leukemia and cataracts were recorded for those exposed to higher doses of radiation; otherwise, there has been no increase in the incidence of solid cancers or leukemia among the rest of the exposed workers. There is no evidence of increases in other non-cancerous diseases from ionizing radiation (Canada, 2022).

Public Health Impacts

Among the residents of Belarus, the Russian Federation and Ukraine, as of 2015 there had been almost 20,000 cases of thyroid cancer reported in children and adolescents who were exposed at the time of the accident (Canada, 2022).

Psychological or Mental Health Problems

According to several international studies, people exposed to radiation from Chernobyl have high anxiety levels and are more likely to report unexplained physical symptoms and poor mental health (Canada, 2022).

5. Treatment of Radioactive Wastes

As with all radioactive sources, radioactive waste is potentially hazardous to health. Therefore, it must be managed in a safe way (figure 3) to protect people and the environment (IAEA,2008).

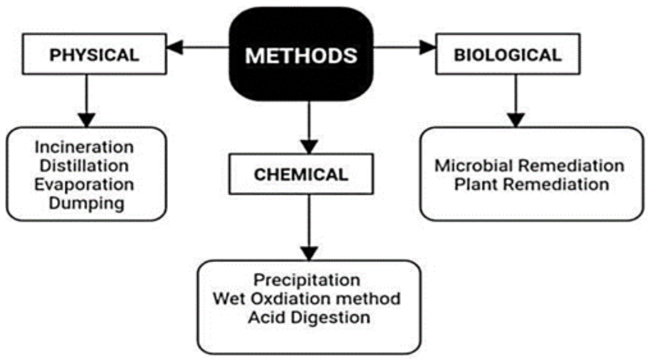


Figure 3. *Methods of treatment of radioactive wastes.*

5.1 Physical Methods

Incineration

This waste treatment process involves combustion of solid radioactive waste at high temperature releasing CO2, H2O, S, and hydrochloric acid as by-products. This method requires gas-filtering systems to control radioactive discharges. (Adenot et al. 2005; IAEA 1992; NEA, 1999).

Distillation

The method of waste treatment process involves reduction of volume of radioactive waste in solid. This is a pretreatment technique of incineration. The disadvantage of this method is that it requires high energy and slow output (NEA, 1999).

Evaporation

It is a kind of unit operation which involves removal of salts, heavy metals, and other hazardous waste materials such as radioactive waste which is present in the effluent wastes. It helps in the reduction of volume of radioactive waste and other toxic materials from the low and intermediate level wastes. The disadvantage of evaporation is that it is highly expensive due to its high-energy requirement, and also, the presence of high amount of inactive salts leads to slow down the process of evaporation with some organic salts during which evaporation explosion may occur (Natarajan, 2020).

Dumping

The solid or mixed radioactive waste is dumped either underground or deep into the ocean. Before dumping into the ocean, the radioactive waste is treated, in chronological order as follows, by incineration, evaporation, and compaction to avoid contamination. Most of the radioactive waste produced until now was stored by dumping. The radioactive waste will remain forever in the buried place. The disadvantage of this method is that there are a lot of possibilities of leakage and contamination of radioactive waste into the ground water or into the ocean (Beaver, 2010).

The other physical methods used are cutting, decontamination, sedimentation, land fill, and many others. These methods are not effective in the treatment of radioactive waste. So, the researchers were looking forward to novel methods to treat radioactive waste (Edwin, 1990).

5.2 Chemical Methods

Chemical Precipitation

Aqueous radioactive waste can be treated using chemical precipitation. This method can be regularly used to manage and remove radioactive substances from low-level and intermediate-level radioactive waste, which are obtained from nuclear power stations and research laboratories. The radioactive components are removed by the process of precipitation and sorption of the particulates which are present in waste (IAEA 2002, 2008).

Wet Oxidation

The principle of this process is based on oxidation of dissolved or suspended components of waste substances. In this method, organic liquid radioactive waste can be treated (The wastes present in organic liquids). Oxygen is used as an oxidizer which transforms/degrades the waste by oxidation process. The other oxidizing agents used for wet oxidation are hydrogen peroxide, ozone, etc. The decomposition of carbon-based waste by the chemical oxidizers results in the production of carbon dioxide, water, and non-toxic elements (IAEA, 1992; NEA, 1999).

Acid Digestion

In this method, radioactive waste is decomposed by strong acid in mixtures like nitric acid and phosphoric acid. The treatments are carried out at high temperature and adequate atmosphere pressure which leads to breakdown of bonds of the waste components. The break-down products consist of inorganic liquids and gases such as O2, CO2, etc. It is an oxidative destruction technology; it oxidizes the organic liquid wastes Such as cellulose, latex rubber, polyethylene, plastic, oils, and other organic materials can be treated (IAEA, 1992; NEA, 1999).

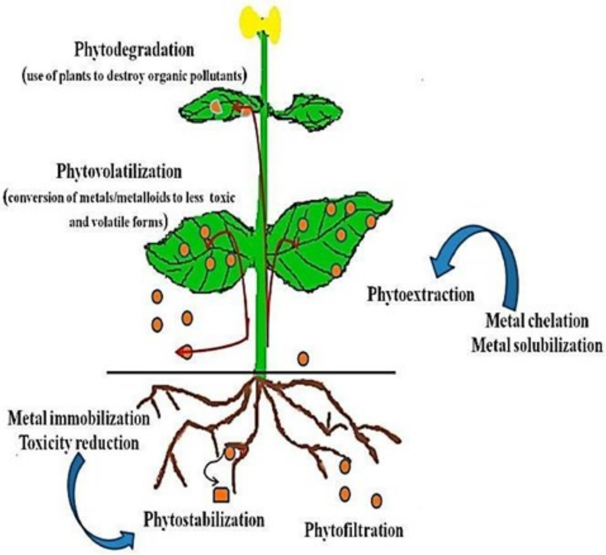
5.3 Biological Methods

5.3.1 Plant Remediation (Phytoremediation/ Green remediation)

Group of remediation techniques that employ plants to clean or partially clean contaminated sites or reduce the danger of toxins. Plants have the capability to uptake pollutants in the environment through the root system that provides a larger surface area, ease mobilization, and detoxification of contaminants within plants by using a variety of mechanisms. Such plant characteristics have been employed to effectively remove radioactive waste (De Filippis, 2015).

Plants use various processes to absorb organic and inorganic pollutants (Figure 4), including….

1) Phytoextraction.
2) Rhizofiltration.
3) Phytovolatilization.
4) Phytostabilization.
5) Phytodegradation



1) Phytoextraction

Phytoextraction is a process in which radioactive nuclide concentrates in the shoots of the plants. The radionuclides are transferred from the roots to the shoots through the vascular bundles, forming a complex with the biomass of the shoots, converting the radionuclides to a less toxic form. The benefit of this process is the easy removal of radionuclides without disturbing the soil structure and its fertility (De Filippis, 2015).

2) Rhizofiltration

In rhizofiltration, radionuclides are adsorbed and precipitated in the roots of plants, but their effectiveness depends entirely on the pH. Cseium-137 and Stroncium-90 both are significantly adsorbed in the roots of some aquatic plants and algae species such as Cladophora and Elodea. Sunflower is the most efficient plant used for rhizofiltration because it can adsorb up to 95% radioactive nuclide from waste stream within two days (De Filippis, 2015).

3) Phytovolatilization

Phytovolatilization is a process involving the volatilization of radioactive nuclide in the form of less toxic substances. Phytovolatilization process proceeds with transpiration of radioactive nuclide into atmosphere. Radionuclides are not removed during this process, but the process effectively releases radionuclides in the form of volatile substances that are less toxic to the environment. The process is cost-effective compared to other bioremediation processes, and its main advantage lies in its non-disruption of soil structure and fertility (De Filippis, 2015).

4) Phytostabilization

During phytostabilization, the roots of plants are involved in the immobilization of radioactive nuclide (De Filippis, 2015). The basic mechanism of the plant stabilization process is the adsorption and precipitation of radionuclides in the roots of plants (Sharma, 2015). The main benefit of this process is that radioactive nuclides are immobilized in roots and can be easily removed if the waste stream of radioactive nuclide is not affected by leaching and soil erosion.

5) Phytodegradation

Phytodegradation is the use of plants to degrade toxic components. The plant produces enzymes which help in the degradation of pollutants from contaminated places. It is an uptake and degradation of the contaminants within the plant by degradation of contaminants by enzymes (Natarajan, 2020).

5.3.2 Microbial Bioremediation.

The treatment process of radioactive waste with the help of microorganisms or microbes like bacteria or fungi depends on the mechanism of the microbes. Certain species of microbes live in extreme habitats such as deep oceans, deserts, volcanoes, and high radiation areas; their purification processes (i.e., oxidation, reduction, adsorption, and precipitation) are significant to decrease the toxicity or produce an insoluble form of the waste. For radiophilic microbes, the most well -known species is *Deinococcus radiodurans* which has a rapid and robust DNA repair systems for both single and double-strand breaks to withstand the high-dose conditions. Several proteins from *D. radiodurans* are responsible in the DNA repairing process, they also have antioxidant enzymes to promote scavengers of ROS activity (Jeong and Choi, 2020).

6. Mechanisms of Interaction Between Microbes and Radioactive Wastes

The different microbial radionuclide interaction mechanisms (figure 5) include:

6.1 Bioaccumulation.
6.2 Biosorption.
6.3 Biotransformation.
6.4 Biosolubilization.
6.5 Bioprecipitation/ Biomineralization.
6.6 Miscellaneous (Chelation / Complexation).

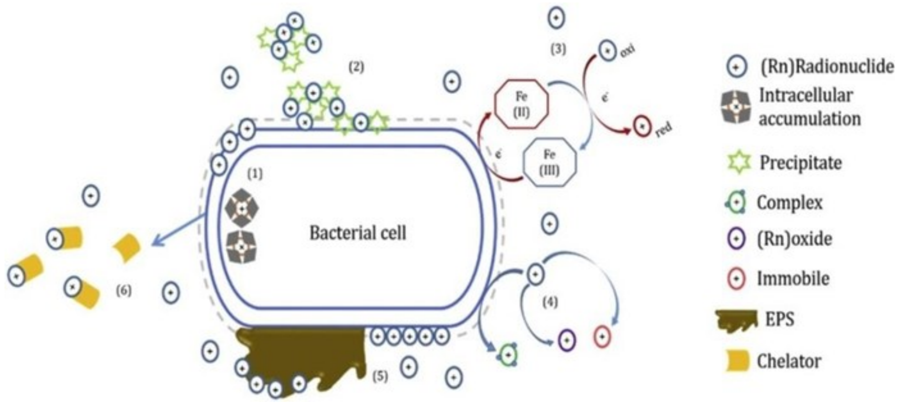


Figure 5. *Microbial mechanisms of interaction with radioactive wastes.*

6.1 Bioaccumulation:

Bioaccumulation is the "retention and concentration of a substance within an organism" (Tabak et al., 2005) or "an enrichment of contaminants in organisms relative to that environment (Jorgensen, 2010). It relies on adsorption of radionuclides on to the cell surface due to electrostatic attraction forces existing between the metal cations and the negatively charged cell surface, resulting in an electrostatic binding (figure 6).

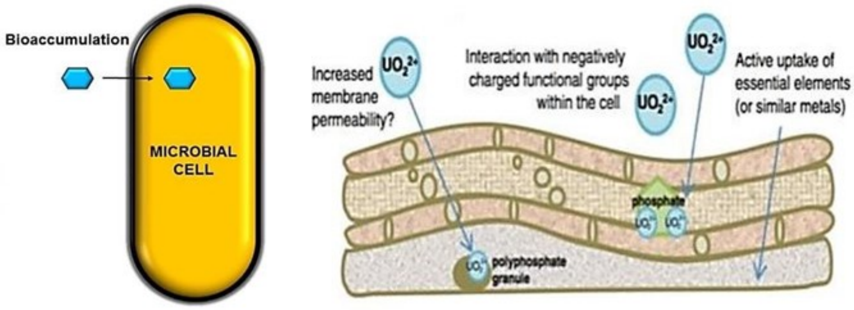


Figure 6. *Bioaccumulation of radioactive wastes by microbes.*

Accumulation could be either intracellular or extracellular. Intracellular accumulation of radionuclides could be attributed directly to the presence or absence of the respective radionuclide transporter, effect of nuclide on permeability of the cell membrane (Merroun and Selenska-Pobell, 2008). Since bioaccumulation involves rapid interactions with anionic groups present in components of cell surface, it is significantly affected by molecular properties of the nuclide, properties, and characteristics of the bacteria present. Size and lipid content also influences it, with a decrease in size leading to less surface area for accumulation (Jorgensen, 2010). The presence of capsule or slime or S-layers dramatically influences the bioaccumulation process. Once accumulated, the nuclides are being acted up on by polyphosphate bodies. Such intracellular chelation mechanisms have suggested giving rise to metal and subsequent radionuclide tolerance (Keasling and Hupf, 1996).

6.2 Biosorption:

Biosorption refers to the passive deposition of the soluble substances at the cell surface (figure 7). The presence of various ionizable groups such as phosphate, carboxyl, hydroxyl, amine, and sulfhydryl at the cell surface generates electronegative attractions for metal cations as results the metal ions get deposited at the cell surface (Lopez-Fernandez et al., 2019).

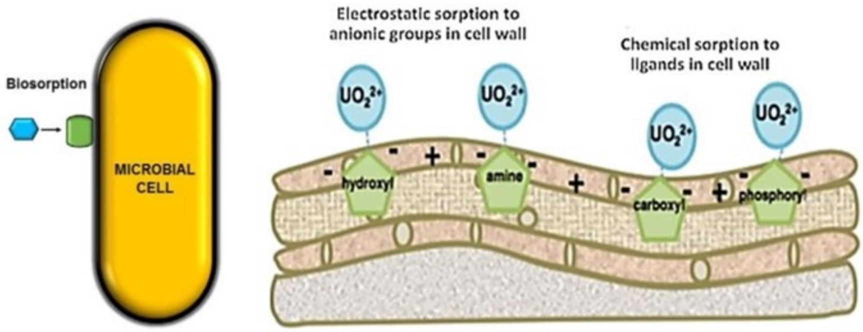


Figure 7. *Biosorption of radioactive wastes by microbes.*

Biosorption is considered as right method for treating low concentration metallic wastes as the process of binding is faster than accumulation process, and also it is easy to remove bound pollutants from the cell surface to regenerate the biosorbent for further use (Newsome et al., 2014; Oyewole et al., 2019).

But there are some problems in biosorption like

- Sometimes problems may arise in bioremediation when other non-targeted cations compete and bind with cell surface as a result the rate of bioremediation decreases drastically (Schiewer and Volesky, 2000).
- Sometimes the cell surface becomes saturated as a result further binding of cations do not takes place (Newsome et al., 2014).
- If the sorbed cell dies, rapid desorption of cation takes place which may alter in bioremediation process (Knopp et al., 2003).

6.3 Biotransformation:

Microorganisms inactivate some radionuclides by adding electrons to them and converting the radionuclides from a soluble to insoluble state (figure 8). The precipitated radionuclide is still radioactive, but it is not transported into solutions, i.e., ground water, as easily as its soluble counterpart (ASM, 2023).

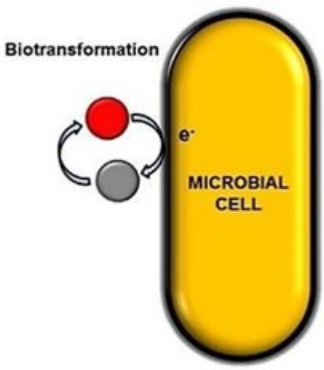


Figure 8. *Biotransformation of radioactive wastes by microbes.*

In oxic environments, oxygen is used as the electron acceptor in microbial respiration, generating H_2O. However, microbes use alternative electron acceptors, including radioactive elements, for respiration when growing in an anoxic environment. This type of respiration uses energetically favorable electron acceptors in a sequence dictated by the redox potential in their environment. This is also how different radioactive elements are used by bacteria. Oxidized forms of a radioactive element are reduced by becoming electron acceptors for microbes using anaerobic respiration (ASM, 2023). Two categories of reduction (direct, indirect reduction):

Direct Reduction

Adding electrons by direct reduction occurs when a microorganism uses the oxidized form directly as an electron acceptor in anaerobic respiration. For example, the microbes *Geobacter metallireducens* and *Shewanella oneidensis* reduce oxidized soluble plutonium Pu(VI/V) to the reduced insoluble form Pu(IV) (ASM, 2023).

Indirect Reduction

Indirect reduction occurs when a microorganism uses a non-radioactive element as an electron acceptor, but the reduced element resulting from this anaerobic respiration provides electrons for the subsequent reduction of a radioactive element within that micro niche. For example, ferric Iron [Fe (III)]-reducing bacteria *G. metallireducens* and *S. oneidensis* reduce uranium U(VI) indirectly via anaerobic growth. Insoluble forms of radionuclides are then easier to process for chemical and physical waste disposal technologies as they reduce the total volume of the waste (ASM, 2023).

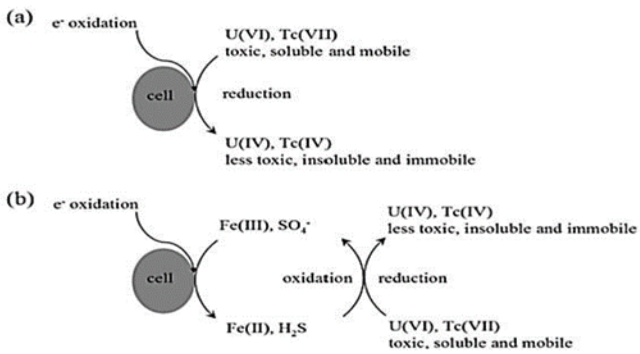


Figure 9. *direct and indirect reduction of radioactive wastes by microbes. (a) Direct reduction of radioactive waste. (b) Indirect reduction of radioactive waste.*

6.4 Biosolubilization

Biosolubilization is a result of autotrophic metabolism during which radionuclides are leached out from their solid matrices (Gadd, 2002). It is an indirect method of solubilization as the autotrophic bacteria obtain energy from reduced Fe or S compounds whilst solubilizing the nuclides and metals. It requires acidic pH, moisture, and oxygen to oxidize Fe, S and leach out metals as sulfides. Such bacteria are mesophilic and acidophilic in nature (Ghauri and Johnson, 1991).

It is affected by the oxidation state of the nuclide, pH surrounding the bacteria, moisture, and inorganic content as substrate for the bacteria. The presence of citrate, a crucial bacterial metabolite, leads to an increase in solubility of nuclides for an extended period of time. However, uranyl citrate complex dissociates in conditions with greater salinity and presence of phosphate-leading to precipitation of uranium and citric acid. Thus, citric acid acts as a Crucial effector in the process of Biosolubilization (Francis et al., 1992).

6.5 Bioprecipitation/ Biomineralization

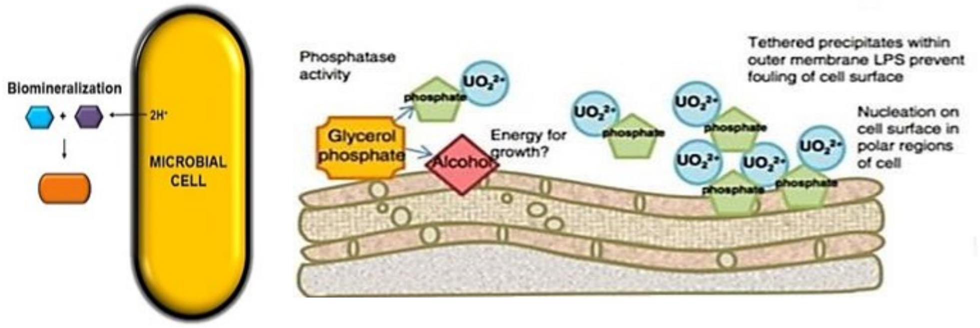


Figure 10. *Bioprecipitation/ Biomineralization of radioactive wastes by microbes.*

Biomineralization refers to the insoluble crystallization of metals (figure 9), a process of metal precipitation with ligands, such as sulfides, carbonates, phosphates, and hydroxides, that are enzymatically generated within the microbial cell wall (ASM, 2023). In one study, a *Salmonella enterica* gene encodes a nonspecific acid phosphatase, which liberates inorganic phosphate from complexed phosphates on the cell surface. The liberated phosphates can subsequently mineralize uranium (ASM, 2023). Gram-negative facultative anaerobes, such as *Serratia spp.*, and other microbial genera isolated from natural environments can also biomineralize and precipitate uranium (ASM, 2023).

6.6 Miscellaneous:

A. Chelation

Microbial production of chelating biopolymers, such as pyomelanin, melanin etc. have been shown to modify the mobilization of radionuclides significantly (Tamponnet et al., 2008). Exposure of such melanin to ionizing radiation has also demonstrated an increase in growth and biomass in fungi; *Cryptococcus neoformans*, *Wangiella dematitidis* and *Cladosporium sphaerospermum* (Dadachova et al., 2007). *P. grimontii* producea fluorescent siderophore pyoverdin (metabolic byproduct) which able to mobilize radionuclides in groundwater.

B. Complexation

Complexation results when organic phosphate groups and teichoic acid and radionuclides interact with each other. U(VI), for example, can be complexed on to the cell wall of Gram-positive bacteria since they are relatively rich in teichoic acid content, in contrast to Gram-negative bacteria. (Gadd, 2002) states the complexation of Co2þ by citric acid, a bacterial metabolite. Nutrient transporter-analogue of K was reported to transport Cs137 into the cell (Shaw, 2005). Speciation and binding of radionuclides to fungal cells influences its precipitation capabilities. Thus, retention of Cs in forest soil had been accredited to mobilization, uptake and translocation of radionuclides and nutrient by fungi (Steiner et al., 2002). Sequestration of Sr in *Serattia sp.* produced hydroxyapatite was reported. Complexing agents include ethylene diamine tetra acetate (EDTA), diethylene triamine penta acetic acid (DTPA), oxalate, citrate, humic acid, fulvic acids, bicarbonates etc.

7. Genetic Engineering: Bioremediation of Radioactive Wastes

Due to adverse environmental conditions, it seems impossible for microbes to survive and remediate pollutants. But there are still some microbes which can resist extreme environmental conditions but fail to remediate the contaminants (Katarína et al., 2018). In this case, genetic engineering provides a new insight in the field of bioremediation as many microbes can be designed in such a way that can remediate contaminants which are not done by normal microbes. In this case by altering gene sequences of desired microbes and enhancing its ability to degrade, digest, and accumulate contaminants or sometimes reconstructing a microbe by inserting a gene which has an extraordinary ability to remediate the specific contamination. Thus, Reconstruction of microbes for bioremediation is done specifically (Jaiswal et al., 2019).

Deinococcus radiodurans is a well-known radio-resistant bacteria that has the ability to reduce radioactive wastes like Cr (VI), U(VI) and Tc (VII) (Fredrickson et al., 2000). Attempts were made to reconstruct *Deinococcus radiodurans* that has the ability to reduce the radionuclides along with other contaminants like other metals and organic pollutants. Incorporation of an *E. coli* (merA) gene provides carbon assimilation property for energy generation generated from toluene and mercury catabolism. Thus, genetically modified *Deinococcus radiodurans* can be a promising tool for bioremediation of radionuclides along with other pollutants (Watanabe, 2001). Similarly, expressing the PhoN gene in *Deinococcus radiodurans* through rDNA technologies uranium bioprecipitation along with cobalt (Misra, et al. 2012).

8. Significant Advantages in Using Microbes for Remediation of Radioactive Waste

a) High specificity.

b) High reusability.

c) High efficiency.

d) Complete removal of pollutant.

e) Low expense.

f) No secondary pollution. (Xu and Zhou 2017)

Table 1. List of bacteria involved in bioremediation of radioactive wastes and their basic mechanisms.

Radioactive waste	Microbes	Result	Mechanism	Reference
Uranium	*Deinococcus radiodurans*	Due to strong DNA repair and antioxidant defense mechanisms, they showed tolerance against high ionizing radiation.	Bioprecipitation	Misra et al. 2012
Technetium	*Desulfovibrio desulfuricans*	The bacteria along with the oxidation of é donor reduced the Tc (VII). The optimum biotreduction was observed when hydrogen act as é donor. When formate or pyruvate was supplied to cell, they enhance bioreduction. Rate was decreased when lactate and ethanol act as é donor.	Bioreduction	Lloyed et al. 1999
Uranium & Technetium	*Anaeromyxobacter dehalogenans*	It reduces Ur (IV) and Tc (VII) to uraninite and Tc (IV) and H_2 acts an é donor.	Bioreduction & Bioaccumulation	Marshall et al., 2009
Plutonium	*Geobacter metallireducens Shewanella oneidensis*	Both the strains have the ability to reduce Pu (VI/V) to insoluble form Pu (IV). But in the presence of EDTA as an é acceptor, rapid reduction of Pu (IV) to Pu (III) was observed.	Bioreduction	Boukhalfa et al., 2007
Strontium	*Halomonas sp.*	80% Strontium bioremediation was observed when it treated with strontium resistant urease producing *bacteria Halomonas sp.*	Biomineralization	Achal et al., 2012
Cobalt (^{60}Co)	*Deinococcus radiodurans*	Some bacterial strains have the ability to uptake Co through NiCoT gene. Expressing that gene into high radiation resistant *Deinococcus radiodurans* through genetic engineering showed increased uptake of radioactive ^{60}Co isotope.	Bioreduction	Gogada et al., 2015
Neptunium	*Shewanella putrefaciens Citrobacter sp.*	*Citrobacter sp.* has the ability to precipitate tetravalent ions such as Np (V), Th (IV), Pu (IV) through enzymatic action. While *Shewanella putrefaciens* can reduce pentavalent Np(V) to tetravalent Np(iV). Therefore, the bacterial consortia treatment showed efficient bioremediation of radioactive 237Np isotope.	Bioreduction & Bioprecipitation	Lloyed et al. 2000

9. Factors Affecting Bioremediation of Radioactive Wastes

Microbes have the ability to adapt themselves with the changing environments and showed a promising approach towards radioactive waste bioremediation. But there are some biotic and abiotic factors which alter the biological processes of microbes by altering the behavior and growth. (Boopathy, 2000; Varjani and Upasani, 2017).

Factors that affect the microbial processes are classified into three groups:

1. Physicochemical factors or abiotic factors.
2. Biological factors or biotic factors.
3. Climatic factors.

9.1 Physicochemical Factors or Abiotic Factors:

The physicochemical factors that affect bioremediation by altering microbial behavior and growth are mainly pH, solubility, presence and absence of electron donor and acceptor, and the ionic strength. In the process of microbial biosorption, pH plays the key role in absorbing pollutants like radionuclides (Srivastava et al., 2014). A slight change in pH may alter the rate of bioremediation.

PH value changes cell surface charge by altering the isoelectric points. The ionic strength of various ligands like carboxylic group, phosphate groups, sulphur and amino groups directly depends on pH (Boopathy, 2000). Changes in pH value bring changes in ionic strength of such ligands and alter the rate of biosorption. The solubility of metal ions is also pH dependent as with decrease in pH the solubility of the metal ion increases which alters the adsorption by microbial cells (Varjani and Upasani, 2017). For example, at pH 3 *Mucor miehei* sorbs 70–80 mg uranium/g dry weight of fungi, and at pH 4 and 5, the biosorption increases 2–3 times, respectively (Gadd and Fomina, 2011).

9.2 Biological Factors or Biotic Factors:

There are some biological factors that have great influence in bioremediation. The Specificity of microbes towards the substrates has a great role in bioremediation, and it has shown that microbes have a wide range of specificity for different types of substrates which may alter the remediation of target pollutants (Boopathy, 2000; Abatenh et al., 2017). Complete bioremediation cannot be achieved by single microbial species; therefore, a microbial consortium is required for complete bioremediation. In microbial consortia, the interaction of microbes is the key factor for bioremediation (Alami et al., 2014). Proper design of microbial consortia is an important step of bioremediation (Boopathy, 2000).

9.3 Climatic Factors:

Climatic conditions greatly influence the microbial extracellular enzyme Productions which may help/alter in bioremediation process (Abatenh et al., 2017).

10. Conclusion

Radioactive materials are extensively used in industrial and research activities in medical, agricultural, and environmental applications, and in various other areas (Valdovinos, 2014). Radiation exposed can cause the death and radioactive contamination to groundwater, ocean, etc. may indirectly effect the food chains, so wastes must be treated with physical, chemical, or biological methods before disposal to serve the human and environment safety regulations.

Bioremediation of radionuclides is a fundamental significance to the advancement of new strategies and innovations to secure the earth. Radionuclide bioremediation to a great extent depends upon the capability of the microorganisms to survive under highly radioactive situations. But there are some biotic and abiotic factors which alter the biological processes of microbes by altering the behavior and growth. Therefore, it is necessary to understand the mechanism how the factors affecting bioremediation will help to find the permanent solution. In this case, genetic engineering can help to overcome the factors which can affect bioremediation by engineering new pathways or by evaluating regulatory factors that are participating in bioremediation (Panpatte, 2021).

Conflict of Interest

The author declare no conflict of interest.

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