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# Nanotechnology for Bioremediation of Heavy Metals

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## 1. Introduction

Nanotechnology, a highly promising discipline in science and technology is the emerging and novel trend that will redesign the future of several existing know-how, which will change every aspect of our lives and lead to the generation of uniqueness in all the streams of technology. The current revolution in nanoscience is brought about by the concomitant development of several advances in technology. Nanotechnology applies the techniques and processes of microfabrication to build devices for studying bio-systems and has a wide range of applications in variety of fields from space science to deep oceanic research (Vincent 2003). Noria Taniguchi used the term ‘Nanotechnology’ while measuring precise machining tolerances of materials in the range of 0.1-100 nanometer (Bhat 2003).

Biological synthesis of metal nanoparticles using microbes, such as bacteria, yeasts, algae, actinnomycetes and fungi, is gaining momentum due to the eco-friendly nature of the organisms which reduce toxic chemicals (Muralisastry et al. 2003). Metal-microbe interaction is very important in several biotechnological applications, including in the fields of biomineralization, bioremediation, bioleaching, and microbial corrosion (Joerger et al. 2001). Nano materials, besides providing new research challenges, form the basis of a new class of atomically engineered materials. Confluence of environmental biotechnology and nanotechnology will lead to the most exciting progress in the development of nano-devices having bio-capabilities in novel metal remediation strategies.

## 2. Nanotechnology - A New Scientific Frontier

The parentage of the modern subject of nanoparticles derives from the work of Michel Faraday, who carried out studies on nanoscale gold particles in aqueous solution and established the first scientific basis (Thomas and Kulkarni 2003). Nanotechnology is an *enabling technology* that leads to generation of new capabilities, new products and new markets. Multiple events have converged to provide a persuasive argument for supporting a focus in nanotechnology: i) historical trends and a projected end of this trend in the absence of new scientific principles ii) new research trends to explain relatively unknown frontiers iii) discovery of new phenomena iv) superior products designed by nature v) advanced computational methods coupled with massive computational capabilities and vi) possibility of new high-performance products (Tolles and Rath 2003). Nanomaterials research is now concentrating on the development of materials that can be designed to have desired properties by manipulating and attaching atoms in different ways.

## 3. Unique Properties of Nanoparticles

Nanocrystals cover a size range 1-100 nm and are intermediate to the molecular size regime on one hand and the macroscopic bulk on the other. The significance of nanophase particle is that the behavior is completely different from the commonly accepted and familiar properties of the macro particles. The physical, chemical and electronic properties of nanoparticles depend strongly on the number, kind of atoms that make up the particle, interaction of crystal atoms and atoms in the grain boundaries. Laws relating to physical, chemical, biological, electrical, magnetic and other properties at the nano-scale are different from those that apply to macro matter. Van der Waal's forces, electron resistance and magnetism are the more important governing forces of nanoparticles instead of forces, such as gravity or inertia (Bhat 2003). The unusual physicochemical and optoelectronic properties of nanoparticles are due to confinement of electrons within particles of dimensions smaller than the bulk electron delocalization length, termed quantum confinement. Because of the special properties of the nanophase materials, there is great deal of interest in the cost-effective synthesis.

## 4. Synthesis of Nanophase Materials

Many important nanostructures are composed of the group IV elements Si or Ge, type III-V semiconducting compounds, such as GaAs or type II-VI semiconducting materials such as CdS (Poole and Owens 2003). The materials used to form various types of nanostructures generally have bulk properties. However, it is modified when their sizes are reduced to nanorange. Mechanical, ferroelectric and ferromagnetic properties of materials change when measurements are made in micrometer or nanometer range.

Nanophase materials can be synthesized by low temperature and high temperature methods (Komarneni 1995). Low temperature method includes precipitation of solutions from room temperature to 100°C, hydrothermal synthesis (>100°C and > 1 atmosphere pressure), inverse micelle method and sol gel synthesis. The high temperature nanophase material synthesis includes gas condensation, wire explosion and liquid aerosol thermolysis. Hydrothermal, microwave-hydrothermal and microwave solvothermal are the conventional techniques used for the preparation of nanophase materials of different sizes and shapes (Komarneni 2003).

Fabrication of nanopowder/colloidal particles includes i) extensive ball milling ii) condensation or precipitation iii) drawing glassy materials iv) self assembly that includes biological fabrication v) forming materials around/within templates, and vi) growth of a second material on a crystalline lattice in which the lattice parameters don't match.

Widely used method for the fabrication of nanostructures is lithography, which makes use of a radiation-sensitive layer to form well-defined pattern on a surface. Molecular-beam epitaxy and the growth of one crystalline material on the surface of another, is a second technique that has been perfected. There are also chemical methods: the utilization of self-assembly and the spontaneous aggregation of molecular groups (Poole and Owens 2003). Gedanken (2003) reported that 20 kHz, ultra sound radiation could rupture chemical bonds and explained the role of few parameters in determining the yield of reaction and the unique products that were obtained in the form of amorphous nanoparticles in material science. These methods are cheaper because of less energy consumption and are ideally suited for precise control of size and shape of nanophases. However the main drawback with these techniques is the cost and chemical contamination.

## 5. Instrumentation for Nanotechnology

Nanotechnology revolution is due to the improvement of old and the introduction of new instrumentation systems for evaluating and characterizing nanostructures. Research in this vast area has been possible only because of the development of tools and instruments that are effective at nano levels. Many of the systems are very large and expensive, often requiring specialists to operate them. Whan (1986), in his review, described the instruments for determining the position of atoms in materials, instruments for observing and characterizing the surface of the structures, and various spectroscopic devices for obtaining information of the properties of nanostructures. Electron beams provide crystallographic information about nanoparticle surfaces and also produce images of the surface.

In a transmission electron microscope (TEM), the electrons from source, such as electron gun, enter the sample, are scattered as they pass through it, are focused by an objective lens, are amplified by a magnifying (projector) lens, and finally produce the desired image. Field ion microscopy is another technique in which the resolution approach is interatomic. The scanning transmission electron microscope (SEM), the scanning tunneling microscope (STM) and the atomic force microscope (AFM) are the efficient instrumentation systems to obtain images of the surface of a specimen by scanning the surface with an electron beam in a raster pattern.

Nanomaterials can be investigated and characterized using spectroscopic techniques in the infrared and Raman region of the spectrum (frequencies from $10^{12}$ to $4 \times 10^{14}$ Hz, wavelength λ from 300 to 1μm), as well as visible and ultraviolet spectroscopy (frequencies from $4 \times 10^{14}$ to $1.5 \times 10^{15}$, λ from 0.8 to 0.2 μm). Emission spectroscopy can be studied by varying the frequency of the incident light, by studying the frequency distribution of the emitted light, or by combining both techniques (Poole and Owen 2003).

Photoluminescence excitation (PLE) is a standard one for obtaining information on the nature of nanostructures, such as quantum dot. This technique involves scanning the frequency of the excitation signal, and recording the emission within a very narrow spectral range. Thermoluminescence is another spectral technique that can provide information on surface states, detrapping, and other processes involved in light emission from nanoparticles. In this technique, the emission of light is brought about by heating.

## 6. Application and Current Status of Nanotechnology

Nanotechnology is concerned with materials and systems whose structure and components exhibit significantly improved physical, chemical and biological properties and that enable the exploitation of novel phenomenon and processes due to their nanoscale size. The unique chemical, electrical, magnetic, optical and other properties of nanoscale particles have already led to their evaluation and use in a broad range of industries, including biotechnology, catalysis, data storage, energy storage, microelectronics and others. The possibility to modify existing materials through technology has become a recipe for the preparation of advanced materials (Komarneni 2003). The domain of this technology is not restricted to only the realm of materials and applications, but also extends to life sciences.

## 7. Metal Pollution and its Impact

Contamination of heavy metals in the environment is a major global concern because of their toxicity and threat to human life and environment (Ceribasi 2001). Urbanization, industrialization and modern agriculture activities are the main reasons for heavy metal pollution. The group of heavy metals are about 65 and are defined in a number of criteria, such as their cationic-hydroxide formation, specific gravity greater than 5 g/ml, complex formation, hard-soft acids and bases, and, more recently, association with eutrophication and environmental toxicity. Metal concentration has been linked to birth defects, cancer, skin lesions, retardation leading to disabilities, liver and kidney damage and a host of other maladies (ATSOR 2001). Wastewater from various industries, such as electroplating, cement, paint etc., discharge heavy metals, such as cadmium, copper, lead, mercury, nickel, zinc and arsenic which are highly toxic to living systems. Persistence and non-biodegradability of toxic heavy metals with their hazardous effect cause serious threat to living organisms. Changes in trace element profile of the soil cause physiological and genetic changes in various life, such as plants, aquatic and benthic fauna, insects, earthworms, fish, birds and mammals as evidenced by recent research work (Mudakavi et al. 1998).

## 8. Current Strategies for Metal Remediation

Technologies involving physical, chemical or biological agents are available for the remediation of heavy metal contaminated effluents and sludge (Table 1). Microbe based technology presents an economic alternative for today’s mining, mineral and waste water treatment industries. In the past few decades, new metal treatment and recovery techniques, based on biosorption, have been explored using both dead and living microbial biomass with remarkable efficiency. Biological approach for metal detoxification offers high potential for selective removal of toxic metals. It has an advantage of operation flexibility and easy adaptability for *in-situ* and *ex-situ* application in a range of bioreactors (Lloyd and Lovley 2001).

## 9. Bioremediation through Nanotechnology

Researchers in the field of nanoparticle synthesis and assembly have turned to biological systems, since they have potential to control the shape, which is not possible in conventional chemical synthesis. Muralisastry et al. (2004) reported that an amalgamation of curiosity, environmental compulsions, and conviction, that nature has evolved the best process for synthesis of inorganic materials on nano and macro-length scales, has contributed to the development of a relatively new and largely unexplored area of research based on the use of microbes in the biosynthesis of nanomaterials. Organisms, synthesizing inorganic materials, include magnetotactic bacteria, sillceous material synthesizing diatoms and S- layer bacteria which produce gypsum and calcium carbonate layers (Joerger et al. 2001). Advancement in nanoscience will achieve the control of matter via controlled molecular assembly.

**Table 1.** Comparison of conventional and bioremediation metal clean up strategies

| Strategy | Methods | Disadvantage | Remarks |
|---|---|---|---|
| **Conventional:** | | | |
| Evaporation | Single/multi stage or vapor compression evaporator | Scaling or fouling | High/ commercial |
| Distillation | Packed column with heating and concentration device | Scaling or fouling | Medium/ commercial |
| Solvent extraction | Standard process | Required for the processing | Moderately high/ commercial |
| Adsorption | Batch or continuous Adsorption beds | Limited to low concentration | Medium/ commercial |
| Ion exchange | Synthetic product | Require pretreatment | High/ commercial |
| Membrane process | Standard manufacture units | Separation is imperfect | Medium/ commercial |
| Electrochemical process | DC power and plating apparatus | Impurity upsets the process | Medium/ commercial |
| Starch xanthate process | Synthetic process | Preparation is tedious | Medium/ experimental |
| **Bioremediation:** | | | |
| Bioaccumulation | Live microbes/ideal for genetic manipulations. | Emerging technology | Lab level |
| Biosorption | Live or dead microorganism | Emerging technology | Low cost/ commercial |
| Phytoremediation | Live or dead plant biomass | Emerging technology | Low cost/*ex-situ* / *in-situ* remediation |
| Plant microbe interaction | Plant and microorganisms. | Emerging technology | Low cost/*ex-situ* remediation |

Material scientists are viewing the uses of microbes in toxic heavy metal bioremediation with interest for nanofabrication of environmentally useful submicron scale particles. If we could build it in microbes, it is possible to use them as eco-friendly and effective nanofactories for heavy metal remediation. Formation of inorganic particles within microorganisms might become a central discipline in biometric and bioengineering applications. Biological systems provide many examples of specifically tailored, nanostructured molecules with highly optimized properties and characteristics. Thus biological materials are considered as a nanophase system in its own right and as the starting point for producing other novel nanophase systems (Table 2). The fungal and actinomycete-mediated green chemistry approach towards the synthesis of nanoparticles has many advantages, such as ease with which the process can be scaled up, economic viability and possibility of easily covering large surface areas by suitable growth of the mycelia, etc (Muralisastry et al. 2003).

**Table 2.** Microorganisms in nanoparticles synthesis

| Organism | Nanoparticle | Mechanism | Size (nm) | Reference |
|---|---|---|---|---|
| *Pseudomonas stutzeri* AG259 | Silver | Intracellular | 200 | Joerger et al. (2001) |
| *Verticillium* sp | Gold / Silver | Intracellular | 2-20 | Muralisastry et al. (2003) |
| *Thermomonospora* sp | Gold / Silver | Extracellular | - | Muralisastry et al. (2003) |
| *Lactobacillus* | Gold / Silver | Intracellular | - | Nair & Pradeep (2002) |
| *Torulla* sp | Lead | Intracellular | - | Kowshick et al. (2002) |
| *Schizosaccharomyces pombe* | Cadmium | Intracellular | - | Kowshick et al. (2002) |
| *Fusarium oxysporium* | Gold / Silver | Extracellular | 2-50 | Mukherjee (2001) |
| Magnetotactic bacterium | Magneite / Greigite | Intracellular/ Extracellular | 35-120 | Joerger et al. (2001) |
| Diatoms | Siliceous | Intracellular/ Extracellular | - | Joerger et al. (2001) |
| *Rhodococcus* sp | Gold | Intracellular | 5-15 | Ahmad et al. (2003) |

## 10. Case Studies

Joerger et al. (2001) have shown that the bacteria *Pseudomonas stutzeri* AG259 isolated from silver mine, when placed in a concentrated aqueous solution of $AgNo_3$, resulted in the reduction of the $Ag^+$ ions and the formation of silver nanoparticles of well defined size and distinct morphology within the periplasmic space of bacteria. Ahmad et al. (2003) reported an alkalotolerent actinnomycetes (*Rhodococcus* sp) capable of synthesizing gold nanoparticles of the dimension 5-15 nm with good monodispersity formed on the cell wall as well as on the cytoplasmic membrane. However, the particles are more concentrated on the cytoplasmic membrane than on the cell wall, possibly due to reduction of the metal ions by the enzymes present in the cell wall and on the cytoplasmic membrane. An acidophilus fungus, *Verticillium* sp isolated from the *Taxus* plant when challenged with $Ag^+$ and $AuCl^-_4$ ions, led to their reduction and accumulation as silver and gold nanoparticles. The growth of the silver nanoparticles occurred within the fungal biomass and the possible mechanism could be the extracellular reduction of the $Ag^+$ ions in the solution, followed by precipitation onto the cells (Muralisastry et al. 2003). A novel alkalothermophilic (extremophilic) actinomycete, *Thermomonospora* sp., isolated from self-heating compost exposed to $AuCl_2$, completely reduced it to $AuCl_4$ ions producing gold nanoparticles, indicating that it secretes four distinct proteins of molecular masses between 80 and 10 kDa.

## 11. Magnetotactic Bacteria

Alivisatos (2001) reported the presence of inorganic crystals in magnetotactic (magnetic sensing) bacteria. The bacterium has fixed within it a chain of about 20 magnetic crystals with the size between 35 and 120 nm diameter. The chain of magnetic crystals (magnetosomes) is visible in electron microscope and imparts the bacterium with a magnetic dipole movement along its length. These crystals constitute a miniature compass and it is a marvel of natural nanoscale engineering. It is madeup of the perfect material-either magnetite or greigite, both highly magnetic iron materials. The crystals align the bacteria with the external magnetic field. In nature, this enables the bacteria to navigate with respect to the earth's magnetic field towards their ideal environment in the upper micro-aerobic sediments of ponds and streams (magnetotaxis). The magnetic separation of heavy metals and radionuclides in conjugation with microbial accumulation by magnetotactic bacteria, can be applied to mineral processing and environmental management of wastes. Magnetotactic bacteria immobilize heavy metals from a surrounding solution and applying a low intensity, focusing magnetic field and can easily separate them. This principle can be extended to develop a treatment process for the removal of metals from wastewater.

## 12. Comparison of Current Strategies with Nanotechnology

Material scientists have been viewing microbes as an eco-friendly nanofactories for metal remediation though biotechnological applications employing microbes, such as bacteria, yeast, algae, diatoms and actinnomycetes. However, compared to bacteria, fungi and actinnomycetes are known to secrete much higher amounts of proteins, thereby significantly increasing nanoparticles by biosynthetic approach. Nanomaterial *in vivo* biosynthesis is the best option for metal bioremediation, since biologically controlled mineralization process produces materials with well-defined characteristics. The biominerals are composite materials and consist of an inorganic component and a special organic matrix; the organic matrix has a vital influence on the morphology of the inorganic compound.

Metal nanoparticles bring about halocarbon mineralization efficiently, economically and eco-friendly. The reaction, studied with silver and gold nanoparticles, results in the catalytic destruction of halocarbons forming silver halide (silver chloride) and amorphous carbon.The reaction is more efficient with silver nanoparticles in the size range of 2-150 nm (Nair and Pradeep 2003). Many hydrocarbons are toxic, mutagenic and resistant to microbial degradation. However, they can be catalytically destroyed by metal nanoparticles. Application of this reaction in detection, extraction, and degradation of environmentally significant halocarbons in general and pesticides in particular, will be a promising and novel technology.

## 13. Future Prospects

The impact from advances emerging from nanotechnology developed over the next 15-20 years has been estimated by National Science Foundation to be approximately $ 1 trillion. In anticipation of this economic impact, nanotechnology research programme in several countries has increased substantially in recent years (Tolles and Rath 2003). Technological merits of nanoparticles provide a vision for transmitting new discoveries into products. It is possible to produce synthetic macroscopic ‘living-like’ organisms made of nanoparticles that would remediate hazardous heavy metals from contaminated environment. Attempts are being made to develop nano-thick particulate coatings onto macroscopic and microscopic structures using a novel pulse laser deposition technique. There have been other concerted efforts of integrating microelectronics and molecular biology into a platform technology with a number of potential commercial applications (Bhat 2003). Surface study of the biogenic nanoparticles (i.e. nature of capping surfactants/peptides/proteins) would lead to the possibility of genetically engineered microbes to overexpress specific reducing molecules and capping agents and there by, control the size. The rational use of constrained environment within cells, such as periplasmic space and cytoplasmic vesicular compartments (e.g. magnetosomes) to modulate nanoparticles size and shape, is an exciting possibility yet to be explored (Muralisastry et al. 2003). Traditional metallurgical research, organic matter, optical property optimization, biological materials and function are the vital areas in nanotechnology that could be the inspiration to make eco-friendly nanomaterials to remediate heavy metal pollution in the environment.

## 14. Conclusion

In future, modification and adaptation of nanotechnology will extend the quality and length of life. The breath of anticipated opportunities, cross-disciplinary nature, potential for innovation, historical track records and the impact of the potential gains of nanotechnology research have led to the recognization of this area with special emphasis. The social benefits are significant from nanomaterials and the new products are applicable to information technology, medicine, energy, and environment. An important challenge in nanotechnology is to tailor optical, electric and electronic properties of nanoparticles by controlling the size and shape. Utilization of microbe for intracellular/extracellular synthesis of nanoparticles with different chemical composition, size/shapes and controlled monodispersity can be a novel, economically viable and eco-friendly strategy that can reduce toxic chemicals in the conventional protocol.

## Reference

Ahmad A, Senapati S, Khan MI, Rajivkumar, Ramani R, Srinivas V, Muralisastry (2003) Intracellular synthesis of gold nanoparticles by a novel alkalotolerent actinomycete, *Rhodococcous* species. Nanotechnology 14:824-828

Alivisatos AP (2001) Less is more in medicine. Scientific American 9:59-65

ATSDR (2001) Agency for toxic substances and disease registry. CERLLA Comprehensive environmental response, compensation and liability act, Priority list of hazardous substances. http:// www.atsdr.cdc.gov/clist.html

Bhat JSA (2003) Hearlding a new future-Nanobiotechnology? Curr Sci 85(2):147-154

Ceribasi IH, Yetis U (2001) Biosorption of Ni (ii) and Pb (ii) by *Phanaerochaete chysosporium* from a binary metal system- kinetics. Water SA 27(1):15-19

Gedanken A (2003) Sonochemistry and its application to nanochemistry. Curr Sci 85(12):1730-1734

Joerger K, Joerger R, Olsson E, Granqvist CG (2001) Bacteria as workers in the living factory: Metal accumulating-bacteria and their potential for material science. Tibtech 19:15-20

Komarnei S (1995) Nanophase materials In: McGraw-Hill Year book of Science and Technology, McGraw-Hill, New York, pp 285-288

Komarnei S (2003) Nanophase materials by hydrothermal. Microwave-hydrothermal and microwave-solvothermal methods. Curr Sci 85(12):1730-1734

Kowshik M, Vogel W, Urban J, Kulkarni SK, Paknikar KM (2002) Microbial synthesis of semiconductor CdS nanoparticles, their characterization, and their use in the fabrication of an ideal diode. Biotechnol Bioeng 78:583-588

Lloyd JR, Lovley DR (2001) Microbial detoxification of metals and radionuclides. Curr Opi Biotech 12:248-253

Mudakavi JR, Narayana (1998) Toxic heavy metal contamination of the soil and biota: Part II Environmental implications. IJEP 18(2):101-108

Mukherjee P, Ahmad A, Mandal D, Senapati S, Sainkar SR, Khan MI, Parischa R, Ajayakumar PV, Alam M, Kumar R, Muralisatry (2001) Fungus-mediated synthesis of silver nanoparticles and their immobilization in the mycelial matrix: A novel biological approach to nanoparticle synthesis. Nano Lett 1:515-519

Muralisastry, Ahmad A, Khan MI, Rajivkumar (2003) Biosynthesis of metal nanoparticles using fungi and actinnomycetes. Curr Sci 85(2):162-170

Muralisastry, Ahmad A, Khan MI, Rajivkumar (2004) Microbial nanoparticles production. In: Niemeyer C, Mirkin C (eds) Nanobiotechnology, WILEY-VCH Verlag GmbH& Co.KgaA, Weinherim, pp 126-135

Nair AS, Pradeep T (2003) Halocarbon mineralization and catalytic destruction by metal nanoparticles. Curr Sci 84(12):1560-1564

Nair B, Pradeep T (2002) Coalescence of nanoclusters and the formation of sub-micron crystallites assisted by Lactobacillus strains. Cryst Growth Des 2:293-298

Poole CP Jr, Owens FJ (2003) Introduction to nanotechnology. Wiley interscience, A John Wiley & Sons, Inc, Publication, Hoboken, New Jersey

Thomas JP, Kulkarni GU (2003) From colloids to nanotechnology: Investigations on magic nuclearity palladium nanocrystals. Curr Sci 85:(12)1760-1766

Tolles WM, Rath BB (2003) Nanotechnology, a stimulus for innovation. Curr Sci 85(12):1746-1759

Vincent SGP (2003) Nanotechnology. In: Selvin J (ed) Biotechnology Emerging Trends, Biotech books, New Delhi, pp 4-7

Whan RE (1986) Materials characterization Vol 10 of Metals hand book, American Society for metals, Metals Park OH