Bioremediation- a new biological technique for removal of effluents by microorganisms and growth profile and characterization of the bioaccumulator Pseudomonas sp.

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Abstract— **This study deals with the use of microorganisms to curb heavy metal contamination in the environment. Expansion of existing industries or setting up new industries results in the disposal of industrial effluents, which discharge untreated effluents causing air, water, soil and soil solid waste pollution. These disposed materials have high persistence capacities and can also change into toxic recalcitrant upon combining with other eco-materials or manmade products. Although several methods have been implemented for degrading these recalcitrant, bioremediation has a significant impact on them. Heavy metal containing industrial effluents which is a major source of contamination may cause serious environmental issues. Removal of heavy metals from waste water and soil has been a big challenge for quite a long time. Even though several methods are practised for decontamination process, bioremediation acts as an effective tool for removing heavy metals. Microorganisms are applied as bioaccumulators for heavy metal removal due to high surface to volume ratio; large availability, rapid kinetics of adsorption and desorption and low cost. The action of Pseudomonas sp. as bioaccumulator has been carried out and discussed in this article.**

Index Terms— **Bioremediation, Heavy Metals, Microorganisms, Industrial effluents, Bioaccumulator, Pseudomonas sp., Growth kinetics**

I. INTRODUCTION

Environmental pollution caused by the release of toxic waste effluents (i.e. persistent organic pollutants,POPs) from industries are creating disturbance to the ecosystem, causing climatic changes, reduction of water levels in the ground as well as oceans, melting of icecaps, global warming, ozone layer depletion due to photochemical oxidation etc. and this made ecologists to focus more on impacts of pollution and its reduction. Anthropogenic compounds (synthetic) plays a major role in polluting the environment.

Most of the heavy metal ions are toxic to living organisms. These metal ions are non-degradable and are persistent in the environment. These effluents are coming from many industries such as corrosion of water pipes, dumping waste, electroplating, electrolysis, electro-osmosis, mining, surface finishing, energy and fuel producing, fertilizer, pesticide, iron and steel, leather, metal surface treating, photography, aerospace and atomic energy installations etc. Thus the removal and recovery of heavy metals from effluent streams are essential to protect the environment.

Bioremediation can be defined as any process that uses microorganisms or their enzymes to return the environment altered by contaminants to its original condition. Bioremediation uses biological agents, mainly microorganisms, yeast, fungi or bacteria to clean up contaminated soil and water. This technology relies on promoting the growth of specific microflora or microbial consortia that are indigenous to the contaminated sites that are able to perform desired activities. Establishment of such microbial consortia can be done in several ways, e.g. by promoting growth through addition of nutrients, by adding terminal electron acceptor or by controlling moisture and temperature conditions, among others. In bioremediation processes, microorganisms use the contaminants as nutrient or energy sources.

Natural organisms are the primary agents used for bioremediation. The organisms that are utilized vary, depending on the chemical nature of the polluting agents and are to be selected carefully as they only survive within a limited range of chemical contaminants. Since numerous types of pollutants are to be encountered in a contaminated site, diverse types of microorganisms are likely to be required for effective mediation.

II. HEAVY METALS

Heavy metals have been excessively released into the environment due to rapid industrialization and have created a major global concern. Heavy metal pollution is one of the most important environmental problem because of their toxicity, bio-accumulation tendency, threat to human life and the environment. The term "heavy metal" is applied to a group of metals with density greater than 5 g/cm^3, atomic number above 20 and is toxic or poisonous at low concentrations.

Heavy metals are natural components from the earth's crust. They cannot be destroyed or degraded. However, most of these heavy metals become toxic at high concentrations due to their ability to accumulate in living tissues. Lead, mercury, cadmium and chromium (VI) are at the top on the toxicity list among various metal ions; the first three, called 'the big three', are in the limelight due to their major impact on the environment.

Metals play an integral role in the life processes of living organisms. Some heavy metals are essential and serve as micronutrients, while others have no biological role and are non-essential. Toxicity of nonessential metals occurs through the displacement of essential metals from their native binding sites or through ligand interactions.

Factors Effecting Bioremediation

- Nutrient availability

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- Moisture content
- pH
- Temperature of soil matrix

Appropriate and optimum level of nutrients and pH are factors which will directly control whether or not the microorganisms are able to survive within the environment. Nutrients are crucial for the growth of microorganisms. Temperature effects bioremediation by changing the properties of the effluents. Temperature influences rate of biodegradation by controlling rate of enzymatic reactions within microorganisms. Moisture content effects the cell growth and function. Availability of water affects circulation of water and soluble nutrients into and out of microorganism cells.

Pseudomonas sp. as a bioaccumulator

Pseudomonas sp. is an effective agent for bioremediation. It breaks down hazardous substances into less toxic or nontoxic compounds. These organisms consume and break down environmental pollutants and removes heavy metals from the environment.

Isolation

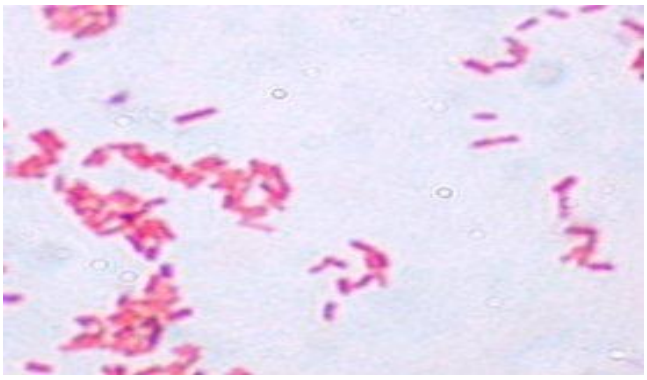
Pseudomonas sp. from soil and water sample was isolated by pour plate method and streak plate technique.

Staining

Gram staining used to determine the morphology of the isolated bacteria. Pseudomonas sp. is a Gram negative, rod shaped, anaerobic bacteria. Crystal violet (primary stain), Gram's iodine, ethanol (decolourizer) and Safranin (secondary stain) used in Gram staining.

Gram positive cells, due to their thick peptidoglycan layer retains the Crystal violet-Iodine complex even after it is subjected to decolourizaation with ethanol. Hence the counter stain has no action on Gram positive cells.

As a result of thin peptidoglycan layer and more lipid contents in the cell wall of Gram negative bacteria, they are easily susceptible to the action of decolourizer and hence the Crystal violet-Iodine complex is easily washed out. Thus these bacteria takes up the colour of the counter stain Safranin.



III. Growth Kinetics

The increase in cell size and cell mass during the development of an organism is termed as growth. The organism require certain basic parameters for their energy generation and cellular biosynthesis. The growth of the organism is affected by both physical and nutritional factors. After reaching a certain size, they divide by binary fission. The growth of bacteria is modeled with four different phases: lag phase, log phase, stationary phase and death phase.

Lag phase- During this phase bacteria adapt themselves to growth conditions. Lag phase increases in the presence of any inhibitor. The length of the lag phase depends on the previous growth condition of the organism.

Log phase- The log phase is a period characterized by cell doubling.

The number of new bacteria appearing per unit time is proportional to the present population .The metabolic activity increases and the organism begin the DNA replication by binary fission at a constant rate. The growth medium is exploited at the maximal rate, the culture reaches the maximum growth rate and the number of bacteria increases logarithmically (exponentially) and finally the single cell divide into two, which replicate into four, eight, sixteen, thirty two and so on.

Stationary phase- The stationary phase is often due to a growth-limiting factor such as the depletion of an essential nutrient, and/or the formation of an inhibitory product such as an organic acid. Stationary phase results from a situation in which growth rate and death rate are equal.

Death phase- At death phase (decline phase), bacteria die. This could be caused by lack of nutrients, environmental temperature above or below the tolerance band for the species, or other injurious conditions. The depletion of nutrients and the subsequent accumulation of metabolic waste products and other toxic materials in the media facilitates the bacterium to move on to the Death phase.

The viable cells of the bacterium are inoculated on to the sterile broth and incubated under optimal growth conditions. The bacterium utilises the components of the media and it increases in its size and cellular mass. The dynamics of the bacterial growth are studied by plotting the cell growth (absorbance) versus the incubation time or log of cell number versus time. The curve thus obtained is a sigmoid curve and is known as a standard growth curve. The increase in the cell mass of the organism is measured by using the Spectrophotometer.

The Spectrophotometer measures the turbidity or Optical density which is the measure of the amount of light absorbed by a bacterial suspension.

The degree of turbidity in the broth culture is directly related to the number of microorganism present, either viable or dead cells, and is a convenient and rapid method of measuring cell growth rate of an organism.

Preparation of lead solution (heavy metal solution) :-

$V1$ = 200 ml = volume of solution in which the isolated bacteria was grown.

$V2$ = volume of lead solution to be added to the broth.

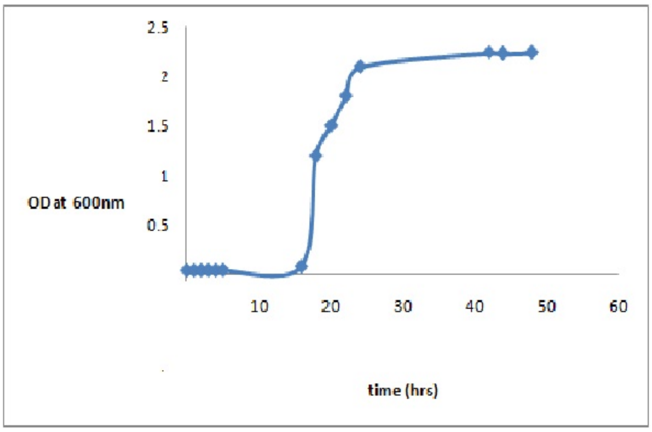
$S1$ = 400 ppm = concentration of lead solution to be added to the broth.

$S2$ = 1000 ppm = concentration of stock(lead) solution prepared.

To prepare 1000 ppm lead solution, 1.6 g of lead nitrate was dissolved in 1000 ml of Millipore water.

(V1 * S1) = (V2 * S2)
(200 * 400) = (V2 * 1000)
V2 = (80000 ÷ 1000)
V2 = 80 ml
Hence, volume of lead solution to be added to the broth = 80 ml
Thus, volume of water = (200 - 80) ml = 120 ml

The growth profile of Pseudomonas sp. observed is given:



IV. CONCLUSION

It is observed that bioremediation is the most economical and eco-friendly method for removal of heavy metal from domestic as well as industrial wastewater. It is being an alternative to conventional methods for the removal of toxic heavy metals from industrial effluents. It offers several advantages including cost effectiveness, high efficiency, minimization of chemical/biological sludge. The microbes play a vital role in the remediation of heavy metals and other pollutants. The isolated *Pseudomonas* sp. is a potent bioremediation agent. It may be concluded that microbes can tolerate against the heavy metals and they are armed with various resistance and catabolic potentials. This catabolic potential of microbes is enormous and is advantageous to mankind for a cleaner and healthier environment through bioremediation.

The new biological – based technologies need not necessarily replace conventional treatment approaches but may complement them. So, the bioremediation technology becomes more beneficial and attractive than currently used technologies.

REFERENCES

[1] Bouwer EJ. and Zehnder AJB, (1993). Bioremediation of organic compounds putting microbial metabolism to work. Trends in Biotechnology 11: 287-318.
[2] Blackburn JW. and Hafker WR, (1993). The impact of biochemistry, bioavailability and bioactivity on the selection of bioremediation techniques. Trends in Biotechnology 11: 328-333.
[3] LE. Macaskie and ACR. Dean, (1989). Microbial metabolism, desolubilisation and deposition of heavy metals: metal uptake by immobilized cells and application to the detoxification of liquid wastes. *Adv. Biotechnol. Process* 12:159-72.
[4] CL. Brierley, (1993). Bioremediation of metal-contaminated surface and ground- water. *Geomicrobiol. J.* 8: 201-213.
[5] Bruins MR, Kapil S, Oehme FW (2000). Microbial resistance to metals in the environment. Ecotoxicol Environ. Saf., 45: 198–207
[6] Chatterjee, Sandipan, Chattopadhyay Pritam, Roy, Subhasita and Sen, Sukanta K. (2008). Journal of Applied Biosciences, 11: 594 - 601.
[7] Whyte LG, Bourbonnière L, Greer CW (1997) Biodegradation of petroleum hydrocarbons by psychrotrophic Pseudomonas strains possessing both a Alkane (alk) and naphthalene (nah) catabolic pathways. Appl Environ Microbiol 63: 3719-3723.
[8] Mirdamadian SH, Emtiazi G, Golabi MH, Ghanavati H (2010) Biodegradation of Petroleum and Aromatic Hydrocarbons by Bacteria Isolated from Petroleum- Contaminated Soil. J Pet Environ Biotechnol 1: 102.
[9] Murray, William (1992) PULP AND PAPER: THE REDUCTION OF TOXIC EFFLUENTS. Environment Canada, Science and Technology Division.
[10] Elbanna K, Hassan G, Khider M, Mandour R (2010) Safe Biodegradation of Textile Azo Dyes by Newly Isolated Lactic Acid Bacteria and Detection of Plasmids Associated With Degradation. J Bioremed Biodegrad 1: 110.
[11] Hadad D, Geresh S, Sivan A (2005) Biodegradation of polyethylene by the thermophilic bacterium *Brevibacillus borstelensis*. Journal of Applied Microbiology 98: 1093-1100.
[12] Gupta R. and Mahapatra H, (2003). Microbial biomass: An economical alternative for removal of heavy metals from waste water. Ind Jou Exp Biol 41: 945-966.
[13] Malik A (2004); Metal bioremediation through growing cells. Environ Int., 30: 261–278.
[14] Mondal, P. Maumder C.B. and Mohanty B. (2008). Treatment of arsenic contaminated water in a laboratory scale upflow bio-column reactor. J. Hazard. Material. 153 (1-2):126-145.
[15] Nies DH (1999). Microbial heavy-metal resistance. Appl Microbiol Biotechnol 51: 730–750.
[16] Roane TM, Pepper IL (2000). Microorganisms and metal pollutants. In: Maier RM, Pepper IL, Gerba CP (eds) Environmental Microbiology. Academic, San Diego , Pp 403–423.
[17] Shukla KP, Singh NK, Sharma S (2010) Bioremediation: Developments, Current Practices and Perspectives. Genetic Engineering and Biotechnology Journal 3: 1-20.
[18] Wong .P.K. and So. C.M. (1993). Copper accumulation by a strain of Pseudomonas putida. Microbiol., 73:113-121.
[19] G.M. Gadd (2010), Metals, minerals and microbes: geomicrobiology and bioremediation. Microbiology, 156:609-643.
[20] Watanabe K, Kodoma Y, Stutsubo K, Harayama S, (2001). Molecular characterization of bacterial populations in petroleum-contaminated ground water discharge from undergoing crude oil storage cavities. Applied and Environmental Microbiology 66: 4803-4809.
[21] N. Goyal, SC. Jain, and UC. Banerjee (2003). Comparative studies on the microbial adsorption of heavy metals. *Adv. Environ. Res.* 7:311-319.
[22] A. Demirbas (2008). Heavy metal adsorption onto agro-based waste materials: a review. *J. Hazard. Mater.* 157: 220-229.
[23] C. White, JA. Sayer, and GM. Gadd(1997). Microbial solubilization and immobilization of toxic metals: key biogeochemical processes for treatment of contamination. *FEMS Microbiol. Rev.* 20:503-516.

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