Vol II Issue V Feb 2013 ISSN No : 2249-894X

Monthly Multidisciplinary Research Journal

Review Of Research Journal

Chief Editors

Welcome to Review Of Research

RNI MAHMUL/2011/38595 **ISSN No.2249-894X**

Review Of Research Journal is a multidisciplinary research journal, published monthly in English, Hindi & Marathi Language. All research papers submitted to the journal will be double - blind peer reviewed referred by members of the editorial Board readers will include investigator in universities, research institutes government and industry with research interest in the general subjects.

Advisory Board

Address:-Ashok Yakkaldevi 258/34, Raviwar Peth, Solapur - 413 005 Maharashtra, India
Cell : 9595 359 435, Ph No: 02172372010 Email: ayisrj@yahoo.in Website: www.isrj.net

Review Of Research
Vol.2, Issue.5, Feb. 2013
ISSN:-2249-894X
Available online at www.reviewofresearch.net

ORIGINAL ARTICLE

BIOREMEDIATION OF HEAVY METAL FROM PAPER MILL AND USING POTENTIAL OF MERCURY RESISTANT BACTERIAL STRAIN

S.R.PARANTHAMAN AND B.KARTHIKEYAN.

Department of Microbiology, Annamalai University, Chidambaram.

Abstract:

Use of microorganisms for removing mercury is an effective technology for the treatment of industrial wastewaters and can become an effective tool for the remediation of man-impacted ecosystems with this metal.Nonviable biomass of an estuarine Bacillus sp. was employed for adsorbing Hg ions from aqueous solutions at six different concentrations. Most of the mercury adsorption occurred during the first 24 -72 hrs. It was found that changes in pH have a significant effect on the metal adsorption capacity of the bacteria, with the optimal pH value between 6.0 and 9.0 at 300C The impact of concentration on biomass production was also evaluated using various concentration (0, 0.5, 1.0 1.5, 2.0 and 2.5%) in NB with 10ppm mercury as substrate were used.

KEYWORDS:

Water Resource and Protection, Microorganisms, Degradation, Bacteria, Heavy metal.

INTRODUCTION

Environmental pollution is one of the major problems of the world and it is increasing day by day due to urbanization and industrialization. Over the last few decades large scale usage of chemicals in various human activities has grown very fast, particularly in a country like India which has to go for rapid industrialization in order to sustain over growing large problem of population (Mustafa et al., 2010). The current pattern of industrial activity alters the natural flow of materials and introduces novel chemicals into the environment. The released organic compounds and heavy metals are one of the key factors that exert negative influences on man and environment causing toxicity to plants and other forms of biotics and abiotics that are continually exposed to potentially toxic heavy metals (Chandra et al., 2010).

Pulp and paper manufacturing is one of the oldest and largest industry in India with an installed capacity of about three million metric tone per annual finished product.Since on an average about 300m3 water is consumed per tone of paper produced, the industry generates ahuge volume of highly coloured and toxic effluents (Piyush malaviya and Rathore, 2007). The effluent colour may increase water temperature and decrease photosynthesis, both of which probably lead to a decrease concentration of dissolve oxygen (Kingstad and Lindstorm 1984). The waste water colour is primarily due to lignin and its derivatives, which are dischared in the effluent mainly from the pulping ,bleaching and chemical recovery stages of the plant (selvam et al.,2002)

Microorganisms play an important role in the biodegradation of such hazardous chemicals in the environment. It has been established that contaminated environments harbour a wide range of unidentified pollutant-degrading microorganisms that have crucial role in bioremediation (Margesinet et al. 2003). Pulp

Title: BIOREMEDIATION OF HEAVY METAL FROM PAPER MILL AND USING POTENTIAL OF MERCURY RESISTANT BACTERIAL STRAIN Source:Review of Research [2249-894X] S.R.PARANTHAMAN AND B.KARTHIKEYAN. yr:2013 vol:2 iss:5

and paper mill is the major industry in India. The heavy demand for the paper helps in steady expansion of paper industries. Since early fifties, the number of paper pulp mills in India has increased from 17 to more than 406 in 2008, with simultaneous increase in paper production from 0.13 to 1.9 million tons per annum (Singh and Thakur 2006).

Molecular approaches may enable the design of biomass with specific metal-binding properties through the expression of metal-chelating proteins and peptides, the improvement of metal precipitation processes and the introduction of metal transformation activities in robust environmental strains. In spite of this, large-scale biological applications are still rare. This is due to the reluctance of the market to embrace new technologies, especially those involving recombinant microorganisms, but also to the inherent difficulty of reproducing these processes at large scale. The fact that the bacterially mediated precipitation of metal in the form of sulfides as well as the use of biosurfactants are the objects of large-scale commercial development holds the promise that other biotechnologies could be used in the field of metal decontamination.The present study was aims to carry out the degradation of mercury using mercury resistant bacteria isolated from paper mill effluent.

MATERIALS AND METHODS

Collection of the Samples

The effluent was obtained from RAJAGANAPATHY PAPER MILL (Board mill private ltd) Vadamangalam, Pondicherry, A south Indian base integrated pulp and paper mill industry. The sample was collected from the outlet of primary settling tank were used for investigation. The sample was collected in a plastic container and transported to Annamalai University Microbiological Research laboratory situated in Chidambaram within 4 hours. The effluent was stored at 4oC until further investigation.

Methods

All the testing was performed according to standard microbiological method for the examination of water and wastewater as described (Washington, D.C., 1995).

Bacterial isolation

Bacteria were isolated by serially diluting 1 ml of the water sample in sterile dilutions were plated by spread plate technique on Nutrient agar plates (Hi-media India) supplemented with 10ppm of mercuric chloride. Later, the plates were incubated at 37°C for 24 h and observed for bacterial growth.

Biochemical identification of different bacteria:

The different bacterium was identified morphologically and biochemically using standard procedures (Barrowand Feltham 1993).

MICROBIAL IDENTIFICATION

Identification was done based on morphological, cultural, biochemical and physiological characteristics based on Cappuccino et al., (1999) and Schaad et al., (2001) and the results were cross checked with Bergey's Manual of Determinative Bacteriology (Buchanan et al., 1974).

Growth study

Optimal growth of potential strain was estimated at various parameters such as temperatures (25, 30, 35 and 40°C), different incubation periods (0-60hr) and pH (6.0, 7.0, 8.0, 9. 0 and 10.0). The impact of concentration on biomass production was also evaluated using various concentration (0, 0.5, 1.0 1.5, 2.0 and 2.5%) in NB with 10ppm mercury as substrate.

Growth Media

Nutrient broth was used for the growth and maintenance of the organisms. Cells were grown at pH 7.0 on a NB medium.

Mercury removal/reduction

Mercury removal was done in 3 sets of experiments with increasing concentration of mercury used in mineral medium.Mercury removeal by different concentration (10-100ppm,25-200ppm and 200-600ppm).

The above experiments were carried out in 250ml conical flasks containing 100ml of medium with the respective mercury concentration. The flasks were incubated at room temperature. Based on the turbidity, the frequency of analysis was decided as represented in Figs (4-6). Thereafter, mercury contents in medium and cells were measured by cold vapour atomic absorption spectrometry (Perkin Elmer AA700) using the procedure of Sadhukhan et al., 1997.

SDS-PAGE (protein profile)

Protein profile of control and mercury treated cells of B. subtilis was observed in the present study. Control strain originally isolated using 10ppm mercury was transferred to nutrient broth without mercury for 3 consecutive times. Each time log phase cultures were centrifuged, and transferred to nutrient broth which was free from mercury.

To study the protein profile of treated culture, the culture obtained through 100ppm mercury amended broth was used as at this concentration growth was comparatively better.

Protein Separation- SDS-PAGE-(Laemmli, 1973)

The proteins were separated by SDS-PAGE electrophoresis and size of polypeptide chains of given protein can be determined by comparing its electrophoretic mobility in SDS-PAGE gel with mobility marker proteins of known molecular weight.

Gel Casting

Chemistry Involved in Gelling

Polyacrylamide gel results from the polymerization of acryl amide monomers into long chains and cross linkages are brought by N-N-methylene bisacrylamide. Polymerization of acrylamide is initiated by the addition of either ammonium per sulphate or riboflavin. TEMED (N-N-N-N) Tetra methylene diamine act as accelerator of polymerization. Effective pore size of polyacrylamide gel is greatly influenced by the total acryl amide concentration in polymerization mixture. Buffer system in PAGE is designed in such a way that the protein is separated into individual polypeptide.

To study the homogeneity of the proteins, polyacrylamide gel electrophoresis was carried out for the separation of protein according to their electric charges. Most commonly the strong anionic detergent SDS is used in combination with a reducing agent and heated to disassociate the proteins before they are loaded on the gel. The amount of SDS bound is always proportional to molecular weight of the poly peptides and is independent of their sequence. Sodium dodecyl sulphate polyacrylamide gel electrophoresis (i.e.) SDS-PAGE is an excellent tool to identify and monitor proteins during purification and to access the homogeneity of the purified proteins.

Sample Preparation and Electrophoresis

The sample was mixed with equal amount of sample buffer and heated at 95°C for 5 min. Then the samples were loaded into the wells and allowed for electrophoresis at 50V initially. After the dye front had reached the end of the stacking gel, the voltage was increased to 100V and proteins were allowed to migrate through resolving gel.

Procedure

The casting apparatus was assembled and the gel volume was determined (1-2cm stacking gel is used). The monomer solution was prepared for the supporting gel. The solution was poured into the casting tray and allowed to solidify then prepare the solution for the resolving gel (12%) concentration. The solution was poured into the tray and overlay it with a layer of butanol:water (1:1) to prevent exposure of the gel to air and allowed to solidify and then prepare the solution for the stacking gel. The butanol layer was removed and washed it with twice or thrice to remove traces of butanol. The components of the stacking gel (12%) were added and place the comb, allowed to solidify.

Loading of the Sample

Protein marker and samples were loaded into the wells of the stacking gel. This is followed by the addition of electrophoresis buffer in to top and bottom reservoir. Power supply was given and it was turned off when the dye front reaches three-fourth of the gel.

Detection of Proteins in Gel

Protein detection was done in many ways. Widely used staining method is coomassie brilliant blue staining.

Coomassie Brilliant Blue Staining

This is the standard method of protein detection. Easy visibility requires the order of 0.1-1mg of protein per band. After electrophoresis, the gel assembly was removed and the glass plates were separated. The gel was soaked in the staining solution and left it overnight for staining and then the gel was destained. The protein bands were observed in the Gel-documentation system (syngene).

Plasmid isolation

Cells were pelleted by centrifugation at 5000rpm for 15 min. at 4°C and suspended in 100µl of solution I and centrifuged for 5 min. Then 200µl of solution II and 150µl of solution III were added. The suspension was mixed vigorously and precipitated with ethanol and stored in TE buffer at -20°C. 0.8% of agarose gel was casted by dissolving 0.8g of agarose in 100ml of IX TAE buffer and the plasmid DNA was loaded along with the loading buffer in the wells formed on the gel and allowed to run in an electrophoresis tank by providing 50mA of current, and the DNA bands were viewed under UV trans-illuminator in a Gel-documentation system. The plasmids were isolated from mercury-resistance strain adopting the method of Sambrook and Russel (2001).

Agarose Gel Electrophresis of Plasmid DNA

The isolated plasmid was analyzed by agarose gel electrophoresis using 1.5% agarose gel in lX TE buffer and with 2µl of ethidium bromide was incorporated for plasmid DNA staining. 25µl of samples were added to each well and was run in lX TE buffer at 100V for 30 min. Hind III digested DNA was used as the molecular weight marker.

Curing of plasmid DNA

Curing of the plasmids was performed by incubating the isolates overnight at 30°C in LB broth containing 25 mg/ ml of acridine orange. Sample of 0.1 ml from culture broth after appropriate dilution were separately plated on LB agar plates containing various concentration of mercury by spread plate technique ad the plates were incubated at 30°C for 24 h. The cultures were inoculated to LB broth also, which contained the same concentration as in plates.

RESULTS

Isolation of Mercury-resistance bacteria

A number of isolates obtained from paper and pulp effluent grown at 37°C on Nutrient Agar containing mercury in the medium .The resistant culture were further analyzed for certain characteristics were gram positive, sporulating, non-motile, rods shaped, and further test for hydrolysis of starch is negative, gelatin test and vp test is positive, It also test for different carbon sources, nitrate reduction is negative and phenyl alanine were positive give in Table 1 The microorganisms is Bacillus spp

Identification of potential strain

Gram staining was performed on logrithmic phase cultures and confirmed using positive control (Bacillus sp.). A modified version of the schaeffer-Fulton stain was used for the detection of spores. Other tests were as per the Bergey's manual of determinative bacteriology.

Growth optimization

Maximum growth occurred was observed at 36h under the conditions of pH 8, Temperature 30ºC, Salinity 2.0% and mercury concentration 10ppm/100ml in shake flask. Growth was measured spectrophotometrically at 600nm (Figs. 1-3).

Mercury degradation

The heavy metal mercury degradation study was done in 3 sets of concentration of mercury using mineral medium. At lower concentration mercury induced growth, compared to the growth in higher concentration. The lower concentration of mercury in culture broth was found to be supportive to growth and at 10ppm of mercury concentration maximum growth was observed at 24hr itself. Time lag in degradation was very much obvious especially at the concentration level of 200-600ppm.

Mercury was readily degraded within 24 to 72hr when the concentration was lower. When the concentration was increased to 100-200ppm, total degradation observed from 72-160h. In higher concentration (i.e.) 300ppm to 400ppm/l upto 200-400h no visible growth was observed (Figs. 4-6).

The study addressed many key issues involved in bioremediation of an important pollutant (i.e.) mercury. Bacillus spp seemed to be an ideal organism for bioremediation of mercury

Mercury analysis using Atomic absorption spectrometry (AAS)

The concentration of total soluble Hg in the water samples collected at paper mill effluent was 118.22 µg/L, as determined by AAS. The amount of mercury removed was calculated in cell free extract as well as in cells based on AAS analysis. Cells didn't contain any mercury.

SDS-PAGE

The protein profile of treated and control strains of B. subtilis were analyzed on 12% SDS-PAGE. The protein pattern between higher concentration (100 ppm) grown and the control strains showed significant variations. A 61 KDa protein which was expressed in the control strain was not expressed in the strain treated with higher concentration of mercury. Whereas a new protein of 82 KDa, which was expressed in the treated strain was not found in the control strain. Compared to the control 3 prominent proteins in the treated sample ranged 28, 19 and 16 KDa were expressed higher in the control strain. Further study is needed in this regard to reveal the exact function of these proteins.

Plasmid isolation and curing

The Bacillus subtilis strain screened to possess a single plasmid of 19.5Kb (nicked form) and 12.8Kb (linear form). After curing the strain lost mercury resistance ability.

DISCUSSION

In the present study, mercury degradation by a high tolerant bacterial strain B. subtilis isolated from a polluted environment was attempted. Mineral medium was used to check the degradation pattern in oligotrophic waters including waters.

In the present study the potential strain Bacillus subtilis growth was estimated at various parameters such as temperatures (25, 30, 35 and 40°C), different incubation periods (0-60hr) and pH (6.0, 7.0, 8.0, 9. 0 and 10.0). The impact of NaCl concentration on biomass production was also evaluated using various concentration (0, 0.5, 1.0 1.5, 2.0 and 2.5%) in NB with 10ppm mercury was used as substrate. Maximum growth was observed at 36h at pH 8, temperature 30ºC, 2.0% salinity. Growth was measured spectrophotometrically at 600nm.

The Bacillus spp strain used in the present study tolerated mercury upto 600ppm concentration. The reason may be the origin of the strain is from a highly polluted environment. The strain might have acclimatized to a higher concentration of mercury. At lower concentration (10-100ppm) the degradation process started by 24h. When the concentration was in the range of 100-200ppm it took 78h to start the degradation process. In 300-500ppm range it took 200h to observe degradation. Surprisingly when the strain was acclimatized to a particular concentration, the next higher concentration it degraded fully (i.e.) total degradation was observed. (i.e.) when the strain was acclimatized to 100ppm for a week time, it degraded 125ppm concentration within a stipulated time (i.e.) 160h. Likewise when the strain was

acclimatized to 200ppm 100% degradation was observed when it was transferred to 300ppm (within 400h). However further acclimatization at 600ppm was not done in the present study. The results clearly indicated that there is a strong correlation exists between the prevailing concentration a particular environment and the exposure of the organism to it.

According to Ramaiah and De, (2003) in all, 37 of the 120 HgS isolates tested were found to be extremely sensitive to Hg as discerned by their inability to grow in SWNA with 10 ppm Hg. Of the 37 that did not grow on SWNA with 10 ppm Hg, only 25 were able to grow in the proximity of Mercury Resistant Bacteria MRB). All these 25 isolates were confirmed to have acquired mercury resistance as they all inside the Copenhagen Harbor, which had higher abundance of MRB. These authors reported 62% of the isolates from this location to contain plasmids while about 30% of the isolates had plasmids from a noncontaminated site, though there were no differences in Shannon–Weaver diversity indices of the isolates from polluted and non-polluted locations. As seen in this study, about 40% (15 of 37 isolates) of the HgS sensitive isolates tolerated 50 ppm Hg when grown in very close proximity to known MRB. Although this is not direct evidence, as Rasmussen and Sorensen, 2001 also suggested, it is likely that many species of marine and estuarine bacteria continue acquiring mercury resistance through interspecies horizontal gene transfer. In the present study also the Bacillus spp strain possessed a plasmid of 19.5Kb (nicked form) and 12.8Kb (linear form) molecular weight the same thing might be true in this strain. When plasmid curing was done the strain totally lost its degradation ability which confirmed the mercury resistance in this strain is plasmid mediated.

The facts that a minimum of 13% of CFU and 69% of 120 isolates from routine in chloralkali electrolysis waste water samples but well-defined SWNA grew on 10 ppm Hg itself is a strong indication that significant numbers of native bacteria are already resistant to Hg (Canstein et al., 1999). Such environmental isolates are potentially useful in heavy-metal pollution bioremediation. Unlike all the Hgs strains including the reference one, many strains tolerating 50 ppm Hg were able to grow in seawater nutrient broth with a single or combinations of toxic xenobiotics. Compared to these strain, the one isolated in the present seemed to be more potential as it degraded up to 500ppm level.

In the present study the potential strain Bacillus subtilis contains the concentration of total soluble Hg in the water samples collected at this site 118.22 µg/L, as determined by AAS. Though this strain was not tested against other pollutants, it might be capable of degrading many pollutants including xenobiotics as it was found more potential compared to any other strain reported.

P. aeruginosa PU21 (Rip64) resting cells were reported to take up as much as 110 mg Pb g- 1 dry cell mass whereas inactivated cells absorbed 70 mg Pb g- 1 dry cell mass (Chang et al., 1997). This bacterium could adsorb Hg2+ up to 400 mg Hg g- 1 dry cell mass. Cysteine-rich transport proteins associated with the cell membrane were postulated to be important in metal adsorption in this bacterium.

Elevated level of mercury whether from natural or anthropogenic (Compeau and Bartha 1984) sources, acts as selection pressure for the presence of adapted and resistant bacteria (Barkay and Olson 1986; Muller et al., 2001 and Ramaiah and De 2003). Many bacteria possessing resistance to Hg and some other toxic chemicals have been reported (Barbieri et al., 1996) but marine MRB possessing resistance to such a variety of toxic heavy metals and xenobiotics .

According to a study conducted by Baldi, 1997 mercuric ion tolerance in the newly-isolated thermophilic Bacillus sp. and Ureibacillus sp. was apparent and was not attributable to classical mer operon-mediated mercury reduction, but to some other mechanism, since NADP-dependent mercuric reductase activity was negligible and mer genes were not found. When considering features of mercuric ion resistance it is important to distinguish between Hg resistance and Hg tolerance. The former can be regarded as a genetically-encoded detoxification mechanism which is specifically induced in response to mercurial, whereas mercury tolerance is a detoxification mechanism which is a by-product of normal metabolism, and is not specifically induced (Baldi 1997). From these results it is likely that Ureibacillus sp. and Bacillus sp. are exhibiting mercury tolerance mechanisms. However in the present study mercury concentration was very much reduced in broth compared to control some detoxification and mercury removal might have happened. Hence this might be 'mercury removal' rather than tolerance resistance.

McKibben's work was followed by that of Brookman, 1995 who conducted similar studies on 22 strains of Gluconobacter species. Brookman found that 18 of these 22 strains contained plasmids ranging in size from 2 to >54 kb. Plasmids of similar size were found among different strains in both McKibben and Brookman's research. Brookman speculated that these size similarities may indicate the transfer of plasmids among Gluconobacter species over time.

Several authors have reported the presence of metallothionein- like proteins in bacterial system that exhibited resistance to several heavy metals like zinc, cadmium, nickel cobalt, mercury and arsenic (Silver and Ji 1994 and Robinson et al., 2001).

Bacterial mobile genetic elements, such as plasmids or transposons, can carry multiple genes

encoding metal and antibiotic resistance. Thus, exposure to one agent may select for microorganisms resistant to several toxicants. Such organisms may be important in performing biological processes in contaminated habitats. Metal-resistant strains also may have application in remediation of metal-contaminated environments.

A genetically engineered Escherichia coli strain with Hg2+ transport system and metallothionein has been used to bioaccumulate mercury from wastewater (Deng and Wilson, 2001). Deinococcus radiodurans has been engineered to remediate radioactive mixed waste as well as to vaporize mercury (Brim et al., 2000).

Previous studies in terrestrial and freshwater environments have shown that as many as 100% culturable mercury resistant environmental isolates contain genes that have homology to merTn21 (Barkay et al., 1989) or merTn501 (Bruce et al., 1995). However, there are conflicting findings on the frequency of prototypic mer genes in culturable mercury-resistant isolates obtained from marine samples (Rasmussen and Sørensen 1998 and Reyes et al. 1999).

Further research is going on in B. subtilis strain isolated in the present study, especially regarding the gene responsible. However the present study revealed the enormous bioremediation potential of this strain and the details related to use it in field application

REFERENCES

Abou-Shanab, R.A., Angle, J.S., Delorme, T.A., Chaney, R.L., van Berkum, P., Moawad, H., Ghanem, K. and Ghozlan, H.A., 2003. Rhizobacterial effects on nickel extraction from soil and uptake by Alyssum murale. New Phytol 158:219–224

Adriano, D. C., 2001. Trace Elements in the Terrestrial Environment: Biogeochemistry, Bioavailability and Risks of Metals, 2nd edn. New York: Springer.

Adriano, D. C., Bolan, N. S., Vangronsveld, J. and Wenzel, W. W., 2004a. Heavy metals. In Encyclopedia of Soils in the Environment, pp 175–182. Edited by D. Hillel. Amsterdam: Elsevier.

Ahmady-Asbchin, S., Andres, Y., Gerente, C. and Cloirec, P.L., 2008. Biosorption of Cu(II) grom aqueous solution by Fucus serratus: surface characterization and sorption mechanism. Bioresource Technol 99:6150–6155.

Aiking, H., Govers, H. and vant Riet, J., 1985. Detoxification of mercury, cadmium and lead in Klebsiella aerogenes NCTC 418 growing in continuous culture. Appl. Environ. Microbiol. 50: 1262–1267.

Bae, W., Wu, C. H., Kostal, J., Mulchandani, A. and Chen, W., 2003. Enhanced mercury biosorption by bacterial cells with surface displayed MerR. Appl Environ Microbiol 69, 3176–3180.

Baeuerlein, E., 2000. Biomineralization. Weinheim, Germany: Wiley-VCH.

Baldi, F., 1997. Microbial transformation of mercury species and their importance in the biogeochemical cycle of mercury. Metal Ions Biol. Syst. 34: 213–257.

Baldi, F., Pepi, M. and Filippelli, M., 1993. Methylmercury resistance in Desulfovibrio desulfuricans strains in relation to mercury degradation. Appl. Environ. Microbiol. 59: 2479–2485.

Banfield, J. F. and Nealson, K. H. (editors) 1997. Geomicrobiology: Interactions between Microbes and Minerals, Reviews in Mineralogy and Geochemistry, vol. 35. Washington, DC: Mineralogical Society of America.

Banfield, J. F., Cervini-Silva, J. and Nealson, K. H. (editors) 2005. Molecular Geomicrobiology, Reviews in Mineralogy and Geochemistry, vol. 59. Washington, DC: Mineralogical Society of America.

Bang, S.W., Clark, D.S. and Keasling, J.D., 2000. Engineering hydrogen sulphide production and cadmium removal by expression of the thiosulfate reductase gene (phsABC) from Salmonella enterica serovar typhimurium in Escherichia coli. Appl. Environ. Microbiol., 66: 3939–3944.

Barbeau, C., Deschenes, L., Karamanev, D., Comeau, Y. and Samson, R., 1997. Bioremediation of pentachlorophenol-contaminated soil by bioaugmentation using activated soil. Appl Microbiol Biotechnol., 48: 745–752.

Barbieri, P., Bestetti, G., Reniero, D. and Galli, E., 1996. Mercury resistance in aromatic compound degrading Pseudomonas strains. FEMS Microbiol Ecol., 20:185–194

Barbieri, P., Galassi, G. and Galli, E., 1989. Plasmid-encoded mercury resistance in a Pseudomonas stutzeri strain that degrades o-xylene. FEMS Microbiol Ecol., 62:375–384.

Da Silva, M.L., Daprato, R.C., Gomez, D.E., Hughes, J.B., Ward, C.H. and Alvarez, P.J., 2006. Comparison of bioaugmentation and biostimulation for the enhancement of dense non aqueous phase liquid source zone bioremediation. Water Environ Res., 78: 2456–2465.

Dash, R.R., Balomajumder, C. and Kumar, A., 2009. Removal of cyanide from water and wastewater using granular activated carbon. Chem Eng J 146:408–413.

De Flora, S., 2000. Treshold mechanisms and site specificity in chromium (VI) carcinogenesis.

Carcinogenesis 21:533–541.
De Flora, S., Bennicelli, C. and Bagnasco, M., 1994. Genotoxicity of mercury compounds: a review. Mut Res., 317:57–79.
Vieira, R. H. and Volesky, B., 2000. Biosorption: a solution to pollution? Int Microbiol., 3, 17–24.
Vincent, J.B., 2004. Recent developments in the biochemistry of chromium (III). Biol Trace Elem Res., 99:1–16.
Vogel, T.M., 1996. Bioaugmentation as a soil bioremediation approach. Curr Opin Biotechnol., 7: 311–316.
Volesky, B., 2001. Detoxification of metal-bearing effluents: biosorption for the next century. Hydrometallurgy 59:203–216.
Wang, J. and Chan, C., 2009. Biosorbents for heavy metals removal and their future. Biotechnol Adv 27:195–226.
Wang, J.L. and Chen, C., 2009. Biosorbents for heavy metals removal and their future. Biotechnol Adv 27:195–226.
Warren, L. A. and Haack, E. A., 2001. Biogeochemical controls on metal behaviour in freshwater environments. Earth Sci Rev., 54, 261–320.
Wenzel, W.W., Bunkowski, M., Puschenreiter, M. and Horak, O., 2003. Rhizosphere characteristics of indigenously growing nickel hyper accumulator and excluder plants on serpentine soil. Environ Poll., 123:131–138.
Yu, K.S., Wong, A.H., Yau, K.W., Wong, Y.S. and Tam, N.F., 2005. Natural attenuation, biostimulation and bioaugmentation on biodegradation of polycyclic aromatic hydrocarbons (PAHs) in mangrove sediments. Mar Pollut Bull., 51: 1071–1077.
Zayed, A. and Terry, N., 2003. Chromium in the environment: factors affecting biological remediation. Plant Soil., 249: 139–156.

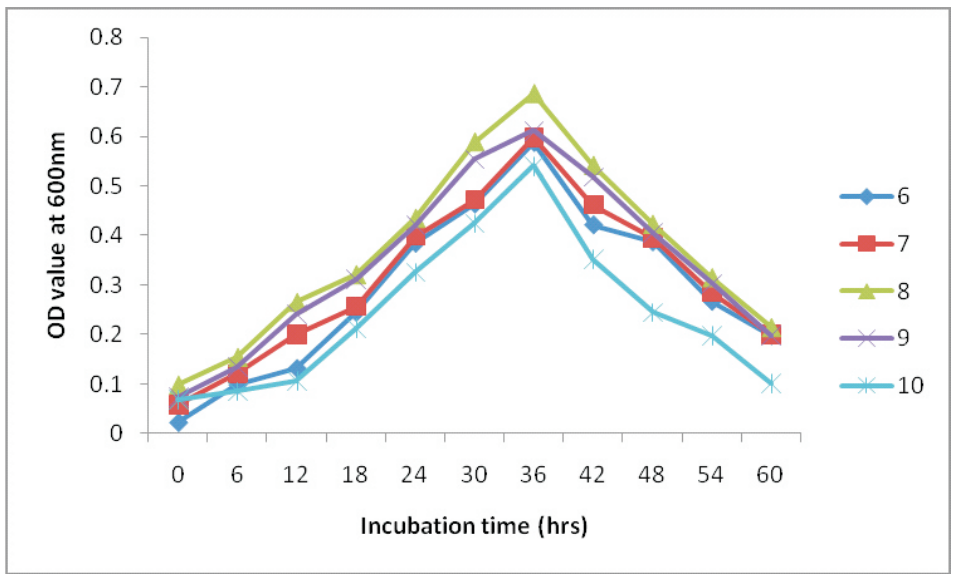


Fig.1 Effect of pH on growth

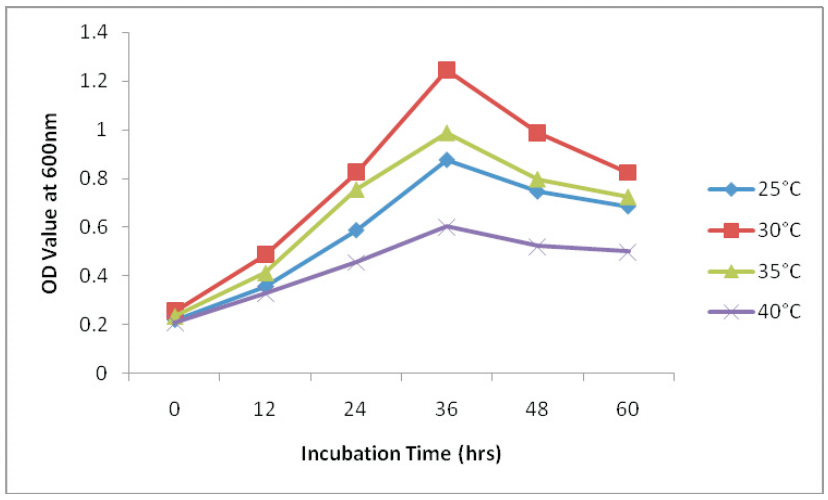


Fig.2 Effect of Temperature on growth

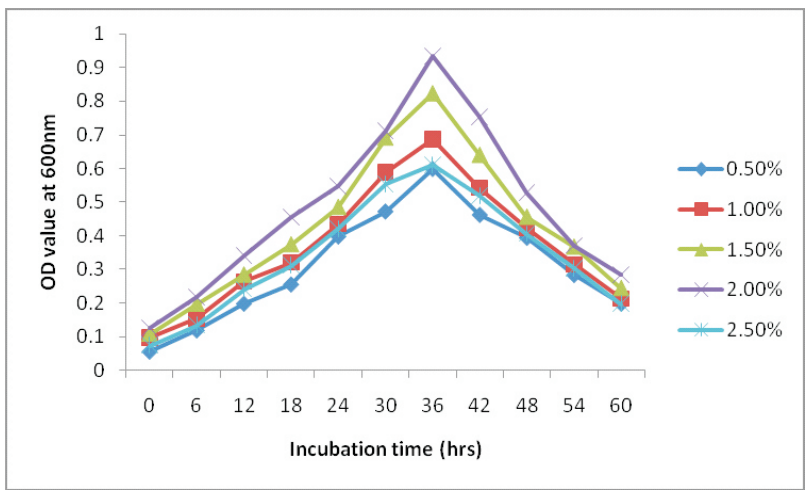


Fig.3 Effect of salinity on growth

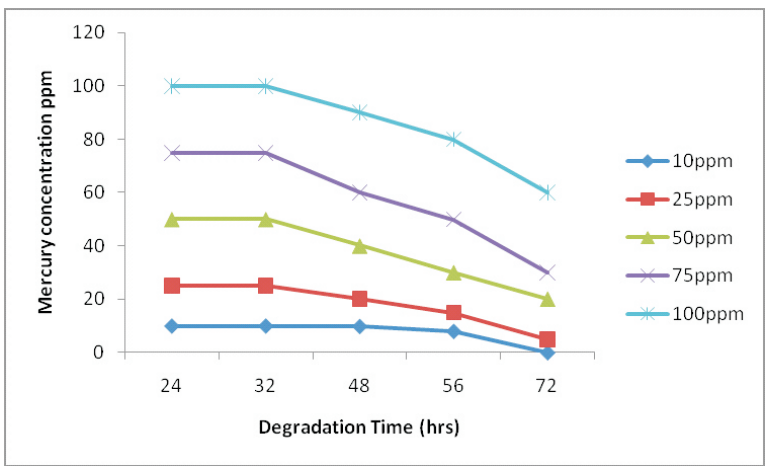


Fig.4 Mercury degradation pattern at different concentration

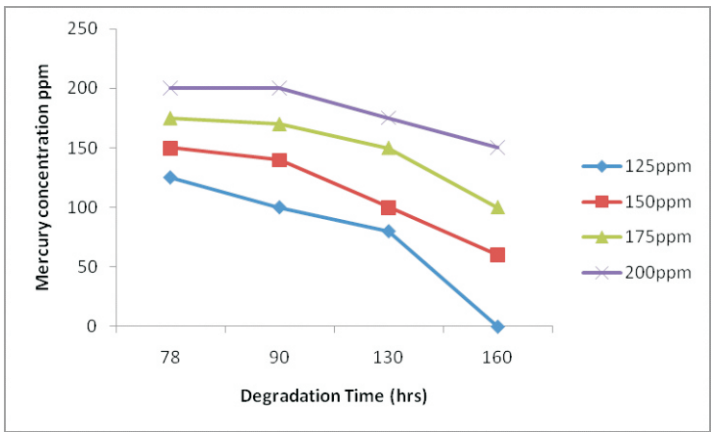


Fig.5 Mercury degradation pattern at medium concentration

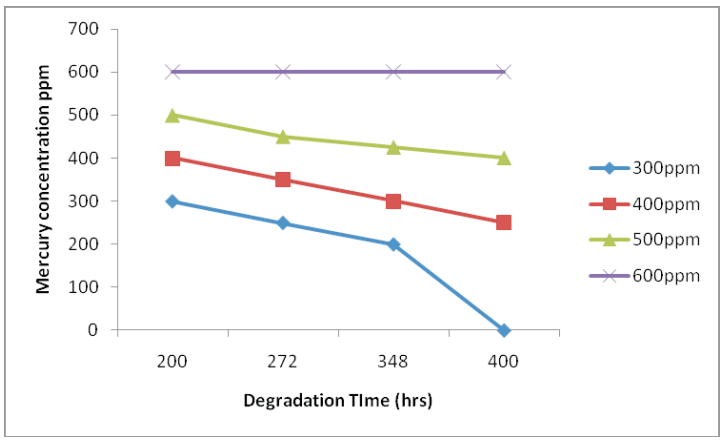
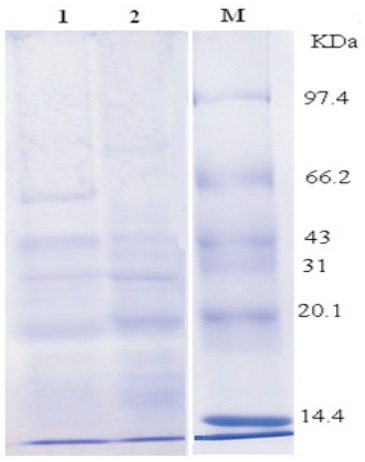


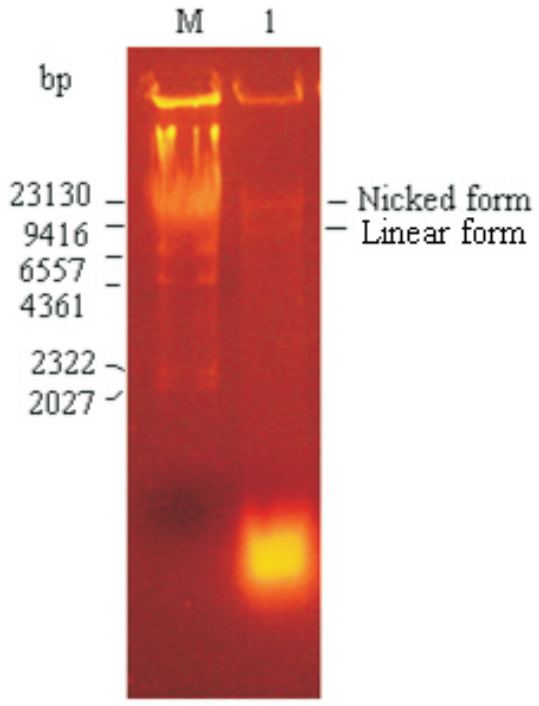
Fig.6 Mercury degradation pattern at higher concentration

SDS-PAGE Profile of Mercury Resistant Bacteria *B.subtilis*



Lane 1 : Control Sample
Lane 2 : Mercury Treated Sample
Lane M : Standard Protein Molecular Weight Marker

Plasmid DNA Profile of Mercury Resistant *Bacillus subtilis*



Lane 1 : Plasmid DNA isolated from mercury resistant bacteria
Lane M : Lambda DNA+Hind III Digest Marker