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Influence of metal ions concentration on phenol degradation by *Rhodococcus pyridinivorans* GM3

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Abstract

Phenol is discharged in large quantities to in the environment, and because of its persistence and high toxicity, can be a potential threat to human health; therefore, biodegradation be recognized as a best way for removing phenol from the environment. Industrial water is often polluted with metal ions, which may effect on phenol degradation. Some metal ions are useful for microorganisms but other metal ions may have toxic effects, perhaps increased metal ions in wastewater have negative impact on microorganisms. Phenol degrading bacterium was isolated from soil samples by enrichment method and has been identified as *Rhodococcus pyridinivorans* GM3. *Rhodococcus pyridinivorans* was studied to degrade phenol (1.5 and 2.0 g/L concentrations of phenol) with different concentrations of 19 different metal ions. The results showed that *R. pyridinivorans* GM3 was degraded phenol on concentrations 1.5 and 2.0 g/L at 150 ppm concentrations of Ba^{2+}, $As^{5-}$and Pb^{2+}, while metal ions Ag^{+}>Cd^{2+}>Hg^{2+}>Zn^{2+}>Cu^{2+}>Co^{2+}have inhibited degradation of phenol (2.0 g/L concentration) was observed. The results clearly indicated that *R. pyridinivorans* GM3 can degrade phenol with many of metal ions and has may be employed for degradation of phenol in industrial wastewaters that are contaminated with metal ions.

Keywords; Phenol, *Rhodococcus pyridinivorans*, Metal ions, Degradation.

Introduction

Among the different toxic compounds, phenol is recognized as a pollutant and phenol contaminated water is a potential threat to human health because it is hematotoxic and hepatotoxic, provoke mutagenesis toward humans and other living organisms [1].

Biodegradation of phenol by bacteria has been a central subject in environmental microbiology, as well as a major mechanism of removal of organic pollutants from a contaminated site [2]. Thus, biological

removal or treatment technology has turned out to be a favourable alternative [3].Today biodegradation is considered as a new tool to eliminate environmental pollution using naturally occurring microorganisms to degrade hazardous phenol into less toxic or nontoxic compounds with relatively low cost, simple technology, which generally have a high public acceptance and can often be carried out.

One of the most important factors that affect biodegradation is the role and interactions of the metal ions in the metabolism of phenol degradation. Many reasons were thought that may be powerful inhibition or stimulation effect by metal ion on phenol degradation, as the different concentration of metal ions often found in industrial wastes. Sandrin and Maier [4] reported that metals appear to affect organic biodegradation through impacting both the physiology and ecology of organic degrading microorganisms. The metals Cr^{+6} and Hg^{+2} have inhibitory effect in the assimilation of phenol [5]. Dimethylsulphide degradation by intact cells of *Thiobacillus thioparus* TK-m was stimulated by the addition of divalent metal ions ($Ca^{2+} > Mg^{2+} > Mn^{2+}$) [6].

Thus, biodegradation of phenol may be affected much by metal ions; bacterial resistances to metals are heterogeneous in both their genetic and biochemical basis. Metal resistance may be encoded chromosomally, plasmid or transposon encoded, and one or more genes may be involved. Other important selective factors include the nature of the uptake systems for the metal, the role and interactions of the metal in the normal metabolism of the cell and the availability of plasmid (or transposon) encoded resistance mechanisms [7]. Native plasmids that mediate heavy metal characteristics, resistance such as arsenate, arsenite, cadmium, and thallium resistance characteristics, have been described in several norcardioform actinomycetes, including *Rhodococcclus fascians* [8]. The toxic metals interact with essential cellular components through covalent and ionic bonding. At high levels, both essential and nonessential metals can damage cell membranes, alter enzyme specificity, disrupt cellular functions, and damage the structure of DNA [9].

Phenol is toxic to several biological reactions. However, biological transformation of phenols to non-toxic entities exists in specialized microbes, owing to enzymatic potential involving enzymes of phenol catabolic pathways. To predict the effect of metal ions on degradation of phenol and intermediate products, it is better to check the phenol degradation in presence of metal ions.

Materials and Methods

Isolation

Enrichment of phenol degrading bacteria was carried out to screen soils sample, one of the bacterium strain that isolated showed high phenol degradation under aerobic condition and has been identified as *Rhodococcus pyridinivorans* GM3 by microscopic, morphological and biochemical characteristics.

Growth medium

The mineral salts medium (MSM) consists of (g/L), 1.25 of yeast extract, 0.35 of K_2HPO_4, 0.35 of $MgCl_2.6H_2O$, 0.2 of $Ca(NO_3)_2$, 0.12 of $FeCl_2$ and trace elements (0.1 mg/L $ZnSO_4.7H_2O$, 0.2 mg/L $CuSO_4.5H_2O$, 0.2 mg/L $MnSO_4.2H_2O$ and 0.1 mg/L Na_2MoO_4) with phenol as the sole carbon source.

Phenol estimation

Phenol was estimated by direct photometric method [10] in portion of the medium withdrawn and centrifuged at 5000 rpm for 10 mins to remove cell pellet and was analyzed by U.V/visible recording spectrophotometer SHIMADZU 160A (Tokyo, Japan) at 500 nm. To the supernatant was added 4-amino-antipyrene at pH 7.9 ± 0.1 by using ammonium hydroxide (0.5N) and phosphate buffer (pH 6.8), followed by oxidation with alkaline $K_3Fe(CN)_6$ giving a red color when phenol is present.

Inoculum preparation

R. pyridinivorans GM3, isolated from soil in lab by enrichment culturing with phenol. Actively growing culture of *R. pyridinivorans* GM3 was inoculated (loop full) into MSM broth with 1% glucose and 0.05% phenol and incubated at 32°C and with agitation 200 rpm (optimization conditions) for 20 hours (approximately 10^9 CFU/mL).

Effect of metal ions on phenol degradation

Living organisms require some metal ions at very low concentrations such as Zn^{2+}, Cu^{2+}, Mn^{2+}, Mo^{6+}, Ni^{2+}, B^{3+} and Co^{2+} for normal growth, but other metal ions may have toxic effects, perhaps increased metal ions in media have negative impact on microorganisms. Industrial water is often polluted with metal ions which may effect on phenol degradation Therefore, effect of various concentrations of metal ions (from 150 ppm to less than that selected based on the inhibitory effect metal ion was used) was investigated. Following metal ions were studied for phenol degradation: Al^{3+}, Mo^{6+}, Cu^{2+}, Ba^{2+}, Zn^{2+}, B^{3+}, Mn^{2+}, Se^{4+}, Li^{+}, Hg^{2+}, Co^{2+}, Ni^{2+}, Sn^{2+}, Cs^{+}, As^{5-}, Cd^{2+}, Ag^{+}, Cr^{3+} and Pb^{2+}. Stock solutions of these ions were prepared by dissolving in water (Table 1), working test metal solutions were prepared by diluting stock solutions of all metal ions and were sterilized as required and added after autoclaving.

All glassware was washed before use to avoid binding of element. Triplicates of MSM (50 mL) was taken containing 1.5 and 2.0 g/L concentrations of phenol in 250 mL flask at pH 8.5, inoculated with 1% inoculum and incubated at 32ºC with 200 rpm agitation (optimization conditions).

Table 1. Different compounds used as metal ions source in experiments

Compound	Metal ions	Compound	Metal ions	Compound	Metal ions
$Al_2(SO_4)_3.16H_2O$	Al^{3+}	$MnCl_2.4H_2O$	Mn^{2+}	$SnCl_2$	Sn^{2+}
H_3BO_3	B^{3+}	$NiCl_2$	Ni^{2+}	CsCl	Cs^{+}
$Na_2MoO_4.2H_2O$	Mo^{6+}	SeO_2	Se^{4+}	Na_2HAsO_4	As^{5-}
$CuCl_2.2H_2O$	Cu^{2+}	$Li_2SO_4.H_2O$	Li^{+}	$CdCl_2.H_2O$	Cd^{2+}
$BaCl_2.2H_2O$	Ba^{2+}	$HgCl_2$	Hg^{2+}	Ag_2SO_4	Ag^{+}
$ZnCl_2$	Zn^{2+}	$CoCl_2.6H_2O$	Co^{2+}	$Cr_2(SO_4)_3.2H_2O$	Cr^{3+}
$PbCl_2$	Pb^{2+}				

Results

One bacterial isolate labeled GM3 showed potential phenol degradation was identified as *Rhodococcus pyridinivorans* by microscopic, morphological and biochemical characteristics and this isolate was studied further. This research focuses on *R. pyridinivorans* GM3 interaction with 19 different metal ions on phenol degradation, of them few are essential metals that are required by microbes at low concentrations while other metal ions are not involved in any known biological processes (nonessential metal) and may be quite toxic and may get accumulated in organisms ($Cd^{2+}$and Hg^{2+}).

All the metal ions were tested at two different concentrations of phenol (1.5 and 2.0 g/L). The results of the investigation showed effect of metal ions on the degradation of phenol varied with each metal ion tested and they are inferred into three types, as shown in Tables (2, 3, and 4). Table 2 showed metal ions Pb^{2+}, $Ba^{2+}$and As^{5-} that these three metal ions only delayed the phenol degradation and had no inhibitory effect on phenol degradation at the concentrations tested here (upto 150 ppm for each metal ion). Effect of metal ions Al^{3+}, Mo^{6+}, B^{3+}, Mn^{2+}, Li^{+}, Sn^{2+}, Se^{4+}, Cr^{3+} and Cs^{+} showed that there was moderate effect on phenol degradation (Table 3). However, at 12.5 ppm concentrations of the above metal ions, there was no effect at any given time interval. Each metal ion at a specific concentration was effective in phenol degradation. For example Al^{3+}, Cr^{3+}, Se^{4+} and Mo^{6+} at concentrations 12.5, 12.5, 25 and 50 ppm respectively did not show any effect on phenol degradation at 1.5 g/L phenol concentration. However, concentrations of 100 ppm (Al^{3+} and Cr^{3+}) and 150 ppm (Se^{4+} and Mo^{6+}) inhibited phenol (1.5 g/L) biodegradation.

Table 4 shows the effect of metal ions Cd^{2+}, Ag^{+}, Hg^{2+}, Zn^{2+}, Co^{2+}, Cu^{2+} and Ni^{2+} that have stringent effect on phenol degradation by *R. pyridinivorans* GM3. These metal ions were powerful inhibitors of phenol degradation at low concentrations used in this study.

Inhibitory concentration of metal ions on phenol degradation by *R. pyridinivorans* GM3 is shown in Table 5. *R. pyridinivorans* GM3 showed capacity to degrade phenol in presence of high concentration and high degree of metal tolerance. In presence of As^{5-}, $Ba^{2+}$and Pb^{2+} did not exhibit any significant inhibitory effect on the phenol degradation or microbial growth throughout the incubation. However, Ag^{+} (0.5 ppm) and Cd^{2+} (2.0 ppm) at low concentrations used in this study did exhibit significant inhibitory effect on degradation of phenol at 2.0 g/L concentration. Also, it was observed that metal ions inhibited phenol (2.0 g/L) degradation in the following order $Ag^{+}>Cd^{2+}>Hg^{2+}>Zn^{2+}> Cu^{2+}>Co^{2+}$ (Table 2).

The increasing phenol concentration might result in increased inhibition role of metal ions, synergism of metal and phenol toxicity towards growth of *R. pyridinivorans* GM3. The concentration 1, 25 and 5 ppm of metal ions Ag^{+}, Cd^{2+} and Hg^{2+} respectively inhibited *R. pyridinivorans* GM3 for degradation of phenol (1.5 g/L) whereas the degradation of phenol (2.0 g/L) was inhibited at concentrations 0.5, 2 and 3 ppm of same metal ions respectively.

Table 2. Metal ions that have less effect on phenol degradation by R. pyridinivorans GM3

Metal ion	Degradation of phenol at 1.5 g/L concentration		Degradation of phenol at 1.5 g/L concentration	
	Concentration rang of metal ion (ppm)	Time rang for phenol degradation (hours)	Concentration rang of metal ion (ppm)	Time rang for phenol degradation (hours)
Pb^{2+}	150-100	48-24	150-40	96-48
Ba^{2+}	150-100	48-24	150-40	72-48
As^{5-}	150-50	48-24	150-50	72-48

Table 3. Metal ions that have moderate effect on phenol degradation by R. pyridinivorans GM3

Metal ion	Degradation of phenol at 1.5 g/L concentration		Degradation of phenol at 2.0 g/L concentration	
	Concentration rang of metal ion (ppm)	Time rang for phenol degradation (hours)	Concentration rang of metal ion (ppm)	Time rang for phenol degradation (hours)
Al^{3+}	50-12.5	48-24	50-12.5	120-48
Cr^{3+}	50-12.5	48-24	75-25	72-48
Se^{4+}	100-25	168-24	50-12.5	72-48
Mn^{2+}	100-50	48-24	125-100	72-48
Li^{+}	150-50	96-24	100-50	72-48
Sn^{2+}	150-100	72-24	125-100	72-48
Cs^{+}	150-50	72-24	100-25	72-48
Mo^{6+}	150 -50	72-24	100-50	72-48
B^{3+}	150-100	48-24	125-100	72-48

Table 4. Metal ions that have more effect on phenol degradation by R. pyridinivorans GM3

Metal ion	Degradation of phenol at 1.5 g/L concentration		Degradation of phenol at 2.0 g/L concentration	
	Concentration rang of metal ion (ppm)	Time rang for phenol degradation (hours)	Concentration rang of metal ion (ppm)	Time rang for phenol degradation (hours)
Ag^{+}	0.5-0.15	72-24	0.4-0.15	72-48
Hg^{2+}	3-0.5	120-24	1.5-0.5	72-48
Co^{2+}	10-2	120-24	5-1.25	72-48
Cd^{2+}	20-3	96-24	1.5-1	120-48
Cu^{2+}	20-5	96-24	4-2	72-48
Zn^{2+}	20-5	48-24	3-2	72-48
Ni^{2+}	25-5	120-24	20-2	168-48

Table 5. Inhibitory concentration of metal ions on phenol degradation by R. pyridinivorans GM3

Metal ion	Conc. of metal ion at 1.5 g/L phenol	Conc. of metal ion at 2.0 g/L phenol	Metal ion	Conc. of metal ion at 1.5 g/L phenol	Conc. of metal ion at 2.0 g/L phenol
Al^{3+}	100 ppm	100 ppm	Li^{+}	> 150 ppm	150 ppm
Cr^{3+}	100 ppm	100 ppm	Hg^{2+}	5 ppm	3 ppm
Mo^{6+}	>150 ppm*	150 ppm	Co^{2+}	15 ppm	10 ppm
Zn^{2+}	25 ppm	5 ppm	Ni^{2+}	30 ppm	25 ppm
B^{3+}	>150 ppm	150 ppm	Sn^{2+}	> 150 ppm	150 ppm
Cu^{2+}	30 ppm	8 ppm	Cs^{+}	> 150 ppm	150 ppm
Se^{4+}	150 ppm	100 ppm	Cd^{2+}	25 ppm	2.0 ppm
Ag^{+}	1.0 ppm	0.5 ppm	Mn^{2+}	150 ppm	150 ppm

*****The experiments were conducted at 150 ppm as there was no effect at 150 ppm concentration, great than that need to be tested for further information on concentration of maximum tolerance.

Discussion

Commonly, the wastewater polluted with phenol contains metal contaminants such as arsenic, cadmium, chromium, copper, lead, mercury, nickel, zinc and others. Industrial wastewater polluted with trace elements and heavy metals, which may effect phenol degradation, in order to resolve phenol contamination by bioremediation. Therefore, it is necessary to investigate interaction between various concentrations of metal ions with phenol degradation through optimization of growth conditions.

Concentrations of around 150 ppm of Ba^{2+}, As^{5-} and Pb^{2+} did not show any inhibition on phenol degradation at both concentrations (1.5 and 2.0 g/L) of phenol. Almost all known bacterial resistance mechanisms are encoded on plasmids and transposons [11]. Rosen [12] noticed that bacteria have evolved

various types of resistance mechanisms to toxic metals and metalloids including mercury, cadmium/zinc, copper/silver and arsenic/antimony, active efflux of the metal is a frequently utilized stratagem, lowering the intracellular concentration to subtoxic levels. Metal resistance in bacteria may be chromosomally linked plasmid or transposon encoded, and one or more genes may be involved [7]. El-Deeb [13] reported that the subsequent plasmid curing experiments demonstrated that the ability of *Enterobacter* sp. to grow in presence of $Cd^{2+}$and Zn^{2+} was encoded by the 98 kb plasmid, whereas the ability to grow in presence of Pb^{2+} appeared to be encoded by the chromosome.

At high levels, both essential and nonessential metals can damage cell membranes, alter enzyme specificity, disrupt cellular functions and damage the structure of DNA. Microorganisms have adapted to the presence of both metals by developing a variety of resistance mechanisms [9] as well as some heavy metal ions are essential at trace concentrations for growth of microorganisms. Nies [14] observed that most essential or non-essential heavy metals are toxic at higher concentrations.

Metal ions Cd^{2+}, Ag^{+}, Hg^{2+}, Zn^{2+}, Co^{2+}, Cu^{2+} and Ni^{2+} have stringent effect on phenol degradation by *R. pyridinivorans* GM3. According to the literature, Cd^{2+} or Cu^{2+} was found to exert a strong inhibitory effect on the catechol 2,3-dioxygenase enzyme activity of *Pseudomonas putida* strain PhCN in the presence of cyanide as a nitrogen source [15]. Clearly, when considering the impact of metals on biodegradation, metals appear to affect organic biodegradation that has impact on both the physiology and ecology of microorganisms [4]. Talley and Sleeper [16] mentioned that metals such as copper, silver, and mercury are typically very toxic particularly as ions.

The current work demonstrated that the tolerance of *R. pyridinivorans* GM3 to metal ions varied even though each metal have specific effect. Rathnayake et al. [17] reported that the Gram positive bacteria *Paenibacillus* sp. and *Bacillus thuringiensis* were highly sensitive to Cu^{2+} than the Cd^{2+} and Zn^{2+}.

The effects of metal ions as inhibitors of enzyme activity of catechol 1,2-dioxygenase from *Geobacillus* sp. G27 strain were determined. Among metal ions tested, the enzyme was completely inhibited by $AgNO_3$ and $CuSO_4$ [18]. Metals exert their toxic effects on microorganisms through one or more mechanisms. An excellent review is available that describes modes of metal toxicity and the mechanisms by which microorganisms resist such toxicity. Nweke et al. [19] showed that the silver has more effect on phenol degradation and the contamination and accumulation of Zn^{2+} in the sediment likely impact negatively on carbon metabolism and respiratory activities of the bacterial strains. Inhibitory effect of metal ions lead to arrested degradation process or retarded rate of degradation, and took more time for phenol degradation than without addition of these metal ions, also, it was observed that the resistance of *R. pyridinivorans* GM3 to metal ions varied depending on nature of metal.

Yeom and Yoo [20] observed that among 12 tested metal ions, Cu^{2+}, Ni^{+2}, Co^{2+} and Ag^{+} inhibited the degradation of benzene and toluene severely by *Alcaligenes xylosoxidans* Y234 and Cu^{2+} was found to inhibit catechol 1,2-dioxygenase in the degradation process.

The data also suggest that most of the metal ions at low concentration are not effecting phenol degradation. Similarly, Kuo and Genthner [21] reported that the addition of some metals at low levels

stimulated biodegradation. Similarly, Adoki [6] indicated that the dimethylsulphide degradation by intact cells of *Thiobacillus thioparus* TK-m was stimulated by the addition of divalent metal ions (Ca^{2+} > Mg^{2+} > Mn^{2+}). In the present study, the mineral salts medium consists Mn^{2+}, Cu^{2+} and Zn^{2+} (trace elements) at low levels which were not affect phenol degradation by *R. pyridinivorans* GM3. *R. pyridinivorans* GM3 tolerance for metals such as $Ba^{2+}$and Pb^{2+} clearly indicated that these metal ions at 100 ppm had negligible effect on the biodegradation of phenol (1.5 g/L concentration).

It is common that the wastewater polluted with phenol also contains metal contaminants. To understand better the diverse responses of bacterium to metal ions resistance, and may interact with phenol degradation the resistance pattern towards metal ions was studied. Effect of 19 different metal ions on phenol degradation by *R. pyridinivorans* GM3 was studied. The results of the investigation showed that the effect of metal ions on the degradation of phenol varies, and they can be categorized into three types of effect. Firstly, metal ions Pb^{2+}, $Ba^{2+}$and As^{5-} have less effect on phenol degradation and degradation process was not inhibited at concentration of 150 ppm of these metal ions. Secondly, metal ions Al^{3+}, Mo^{6+}, B^{3+}, Mn^{2+}, Li^{+}, Sn^{2+}, Se^{4+}, Cr^{3+} and Cs^{+} have moderate effect on phenol degradation and at 12.5 ppm concentration have not shown inhibition on phenol degradation. Other metal ions Cd^{2+}, Ag^{+}, Hg^{2+}, $Zn^{2+,}$ Co^{2+}, Cu^{2+} and Ni^{2+} have stringent effect on phenol degradation by *R. pyridinivorans* GM3, these metal ions were powerful inhibitors of phenol degradation, and that have inhibitory effect when fed to microorganism can neither degrade substrate nor uptake the metal ions.

Conclusion

R. pyridinivorans GM3 proved that it degraded phenol at various concentrations of metal ions. The concentrations of Ba^{2+}, $As^{5-}$and Pb^{2+} on 150 ppm had no inhibition of phenol degradation process at 1.5 and 2.0 g/L phenol concentrations was observed. While, metal ions Ag^{+}>Cd^{2+}>Hg^{2+} >Zn^{2+}>Cu^{2+}>Co^{2+} have inhibited *R. pyridinivorans* GM3 for phenol degradation at 2.0 g/L concentration.

References

[1] **J. Michałowicz, and W. Duda**, "Phenols–Sources and toxicity," Polish Journal of Environmental Studies, 2007, vol. 16, pp.347–362.
[2] **J. S. Seo, Y. S. Keum, and Q. X. Li**, "Bacterial degradation of aromatic compounds," International journal of Environmental Research and Public Health, 2009, vol. 6, pp.278–309.
[3] **R. Thavasi, and S. Jayalakshmi**, "Bioremediation potential of hydrocarbonoclastic bacteria in Cuddalore harbour waters (India)," Research Journal of Chemistry and Environment, 2003, vol. 7, pp.17–22.
[4] **T. R. Sandrin, and R. M. Maier**, "Impact of metals on the biodegradation of organic pollutants," Environmental Health Perspectives, 2003, vol. 111, pp.1093–1101.
[5] **A. A. L. Silva, M. P. Pereira, R. G. S. Filho, and E. Hofer**, "Utilization of phenol in the presence of heavy metals by metaltolerant nonfermentative gram-negative bacteria isolated from wastewater," Revista Latinoamericana de Microbiología, 2007, vol. 49, pp.68–73.
[6] **Adoki, A**, "Influence of divalent metal ions on degradation of dimethylsulphide by intact cells of *Thiobacillus thioparus* TKm," African Journal of Biotechnology, 2007, vol. 6, pp.1343–1347.

[7] **D. A. Rouch, B. T. O. Lee, and A. P. Morby**, "Understanding cellular responses to toxic agents: a model for mechanismchoice in bacterial metal resistance, Journal of industrial Microbiology, 1995, vol. 14, pp.132–141.
[8] **B. Dabrock, M. Kebeler, B. Averhoff, and G. Gottschalk**, "Identification and characterization of a transmissible linear plasmid from *Rhodococcus erythropolis* BD2 that encodes isopropylbenzene and trichloroethene catabolism," Applied and Environmental Microbiology, 1994, vol. 60, pp.853–860.
[9] **M. R. Bruins, S. Kapil, and** F. W. **Oehme**, "Microbial resistance to metals in the environment," Ecotoxicology and Environmental Safety, 2000, vol. 45, pp.198–207.
[10] **L. S. Clesceri, A. E. Greenberg, and A. D. Eaton**, "Standard Methods for Examination of Water and Waste Water," 20th ed. American public health association. Washington, 1998, 5530 D, pp5–(43–44).
[11] **A. Silver, and M. Walderhaug**, "Gene regulation of plasmid- and chromosome-determined inorganic ion transport in bacteria," Microbiological Reviews, 1992, vol. 56, pp195–228.
[12] **B. P. Rosen**, "Bacterial resistance to heavy metals and metalloids," Journal of Biological Inorganic Chemistry, 1996, vol. 1, pp273-277.
[13] **B. El-Deeb**, "Plasmid mediated tolerance and removal of heavy metals by *Enterobacter* sp.," American Journal of Biochemistry and Biotechnology, 2009, vol. 5, pp47–53.
[14] **D. H. Nies**, "Microbial heavy-metal resistance," Applied Microbial Biotechnology, 1999, vol. 51, pp730–750.
[15] **B. El-Deeb, and A. D. Altalhi**, 2009. Degradative plasmid and heavy metal resistance plasmid naturally coexist in phenol and cyanide assimilating bacteria. American Journal of Biochemistry and Biotechnology, 1997, vol. 5, pp84–93.
[16] **J. W. Talley, and P. M. Sleeper**, "Roadblocks to the implementation of biotreatment strategies," Annuals of New York Academic of Sciences, vol. 829, pp16–29.
[17] **I. V. N. Rathnayake, M. Megharaj, N. Bolan, and R. Naidu**, "Tolerance of heavy metals by gram positive soil bacteria," World Academy of Science. Engineering and Technology, 2009, vol. 53, pp1185–1189.
[18] **G. Giedraityte, and L. Kalėdiene**, "Catechol 1,2-dioxygenase from α-naphthol degrading thermophilic *Geobacillus* sp. strain: purification and properties," Central European Journal of Biology International Journal of Environmental Research, 2009, vol. 4, pp68–73.
[19] **C. O. Nweke, C. S. Alisi, J. C. Okolo, and C. E. Nwanyanwu**, "Toxicity of zinc to heterotrophic bacteria from a tropical river sediment," Applied Ecology and Environmental Research, 2007, vol. 5, pp123–132.
[20] **S. H. Yeom, and Y. J. Yoo**, "Overcoming the inhibition effects of metal ions in the degradation of benzene and toluene by *Alcaligenes Xylosoxidans* Y234," Korean Journal of Chemical Engineering, 1997, vol. 14, pp204–208.
[21] **C. W. Kuo, and B. R. S. Genthner**, "Effect of added heavy metal ions on biotransformation and biodegradation of 2- chlorophenol and 3-chlorobenzoate in anaerobic bacterial consortia," 1996, Applied and Environmental Microbiology, vol. 62, pp2317–323.