Journal of Applied and Advanced Research 2017, 2(3): 156–160
doi.: 10.21839/jaar.2017.v2i3.85
http://www.phoenixpub.org/journals/index.php/jaar
ISSN 2519-9412 / © 2017 Phoenix Research Publishers

Research Article – Microbiology

Evaluation of removal of heavy metals by microorganisms isolated from industrial effluents

S.A. Bhutada*, S.B. Dahikar

Department of Microbiology, Sanjivani Arts, Commerce and Science College, Kopargaon, Ahmednagar – 423603, Maharashtra, India

Abstract

At present various microorganisms are used for bioremediation of heavy metals from soil and water bodies. The aim of present work was to isolate the potential heavy metal degrading organisms and to apply for bioremediation of heavy metals from the domestic as well as industrial waste. The study involves the isolation of the bacterial species residing the natural habitat of such environments and screening of these isolates to degrade different heavy metals such as Cu, Cd, Hg, Ni, and Zn up to the concentration 2000 ppm. There were six bacterial potential isolates found namely *Pseudomonas* spp., (3), *Achromobacter* spp., *Uncultured Microbacterium* spp., and *Exigoubacterium* spp., which showing the growth up to the concentration of 2000 ppm. The potency of the six potential isolates was determined by using the conventional plate count technique. The percentage removal of analyzed by the use of ICP-AES technique. The study shows isolation of the species which can remove heavy metal up to 60%. It was also found that the increase in the incubation time causes more reduction in the heavy metal concentration. The mutational analysis of the isolates for the strain improvement process shows that the *Exigoubacterium species* can grow at 3000 ppm heavy metal concentration and showed 60% reduction in heavy metal. This highly potential species can be used for the removal of different heavy metals which is also a viable, eco friendly and cost effective technology for cleanup of the environment.

Key words: Heavy metal, Microorganisms, Industrial effluent, Sewage

Introduction

Due to Industrialization, environmental pollution became a conundrum for human race. This industrialization introduced pollutants like Lead (Pb), cadmium (Cd), mercury (Hg), Uranium (U), Arsenic (As), Chromium (Cr), Copper (Cu), Selenium (Se), Nickel (Ni), Gold (Au), Silver (Ag), and Zinc (Zn) in to the environment (Ahalya *et al.,* 2003). The most abundant pollutants in the industrial wastewater and in sewage are heavy metals. Human activities, such as mining operations and the discharge of industrial waste, have resulted in the accumulation of metals in the environment and eventually are accumulated through the food chain, leading to serious ecological and health problems. Damage caused by heavy metals to the ecosystem has been confirmed (Hrynkiewicz and Baum, 2014).

Among the heavy metal such as zinc, nickel, cadmium mercury and copper ions are toxic to human being as well as living creature. When they are taken up by organism may cause adverse reaction in different organs and biological function, including reproduction and birth defects (Hobman *et al*., 1997; Hussein *et al*., 2004). The physico-chemical techniques such as incineration, ion exchange, filtration, chemical precipitation, adsorption membrane separation and reverse osmosis are available for removal of heavy metal (Pandit, *et al.,* 2013). These techniques are having some lacuna such as high energy requirements,

Received: 01-05-2017; Accepted 08-06-2017; Published Online 10-06-2017

*Corresponding Author

Dr. S.A. Bhutada, *Department of Microbiology, Sanjivani Arts, Commerce and Science College, Kopargaon, Ahmednagar – 423603, Maharashtra, India, Email: sabhutada13@gmail.com*

incomplete removal, waste products generation and expensiveness. Waste minimization is an important aspect to any industry, as it is not only reduces the consumption of potable water but also decreases the volume of wastewater generated (Nanda *et al*., 2011). Hence bioremediation is advantageous technique for removal of heavy metals and comparatively safer and cheaper. Wide varieties of microorganisms are capable of growing in the presence of heavy metal ions and tolerant high concentration (Niles, 1992). There is no general mechanism for resistances to all heavy metal ions.

Shendra MIDC (19°53'20"N 75°29'38"E) and Chikhalthana MIDC (19°53'15"N 75°21'59"E) are industrially populated area in Marathwada region. This region has different type of industries including metal processing industries, pharmaceutical industries, paint industry, distilleries and also the domestic waste disposal sites. The region is never studied before for presence of potential microbial bioremediation agent. The attempt of the present work was to evaluate potential heavy metal degrading microbial flora from the heavy metal contaminated industrial effluent and domestic sewage, for bioremediation.

Materials and Methods

Collection of Samples

The study area for research paper was selected based on the need, diversity and extent of pollutants produced by different industries located in Shendra and Chikhalthana MIDC, Aurangabad, Maharashtra. A total of 50 samples were collected, among them 25 effluent samples were collected from various industries like pharmaceutical, distilleries, metal processing plants, paint industry etc and 25 samples from domestic sewage water samples were collected respectively. Effluent samples were collected in dry, sterile polypropelene containers and transported immediately to the Sakolkar Life Sciences and Research Centre, Shendra MIDC, Aurangabad. These containers were maintained at 4^0C or less to ensure the minimal biological activity. Processing of the samples for the isolation of bacteria was carried out within 3hr of sample collection.

Physico-Chemical Analysis

The parameter like the pH, color, odor, turbidity, temperatures, BOD and COD of all the collected samples were analyzed and also heavy metal degradation analysis was carried out in laboratory.

Isolation of Bacteria

The bacterial species were isolated from the collected water samples with the help of conventional serial dilution technique as described by Harley and Prescott (1993). For the pure culture of bacteria single colonies were picked and streaked on the nutrient agar plates containing different concentrations (200 ppm to 2000 ppm) of different heavy metals namely Cu, Cd, Zn, Hg, and Ni under sterile conditions.

Screening of Heavy Metal Degrading Microorganisms

Pure cultures of strains which showed growth on plates containing 2000 ppm heavy metal concentration were grown on slants by stab and streak method for storage purpose and subsequently for identification and biochemical characterization of the bacterial isolates were done. The isolates which have shown growth on heavy metal concentration 2000 ppm were considered as the potential degraders and for confirmative identification, these isolates were sent to NCCS Pune for the 16s rRNA. Further this confirmative identification these isolated species then inoculated in nutrient broth containing the heavy metal concentration of 2000ppm, incubated for three different time periods (24h, 48h and 72h) at 20-30^0C temperature. After the completion of the incubation the samples were centrifuged to separate the supernatant and the separated supernatant samples were then sent to IIT, Powai, Mumbai for analysis of the heavy metal degradation by using ICP-AES.

Mutational analysis of the isolates from domestic and industrial effluents for degradation of heavy metals

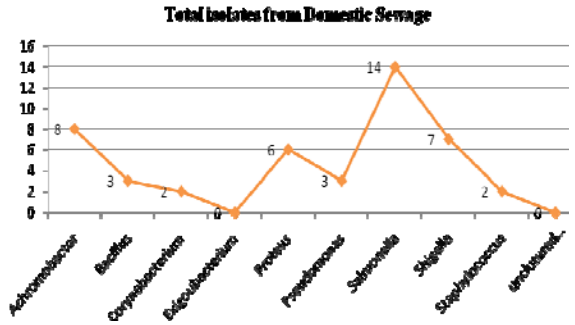
The plates were inoculated with potential six isolates on the heavy metal concentration 2000ppm-3000ppm and exposed to UV radiation for 10 minutes and then incubated at 37^0C overnight and heavy metal degradation results were recorded by using ICP-AES.

Results and Discussion

A total of 50 samples were collected, among them 25 effluent samples were collected from variety of industries like pharmaceutical, distilleries, metal processing plants, paint industry and 25 samples from domestic sewage. Effluent samples were collected in dry, sterile polypropelene containers and transported immediately to the Sakolkar Life Sciences and Research Centre, Shendra MIDC, Aurangabad. These containers were maintained at 4^0C or less to ensure the minimal biological activity. Processing of the samples for the isolation of bacteria was carried out within 3 hr of sample collection.

The physicochemical characteristics of the samples collected from industrial effluents and domestic sewage water shows that the samples were black, grayish, brown in color having pH in the range of 5-9 and having pungent and irritating odor. The temperatures of samples were in the range of 20-30^{0}C. All the samples were found turbid. BOD of industrial effluent was 12-25 mg/g whereas domestic sewage was 20-25 mg/g. COD of industrial effluents was 180-230mg/g and of domestic sewage was 190-210mg/g.

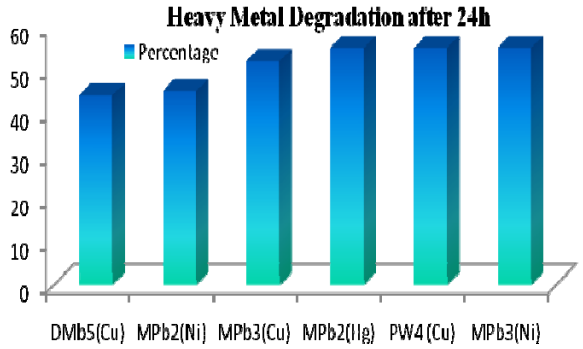
Fig. 1: Total Isolates from Domestic Sewage samples



From these 25 industrial effluent samples, a total of 58 isolates were obtained whereas from domestic sewage samples, a total of 45 isolates were obtained and identified on basis of standard morphological, biochemical and sugar fermentation characteristics by using determinative bacteriology of Bergey's manual. from 25 industrial effluent samples, a total of 58 isolates were obtained, among these isolates *Shigella* sp. (11) predominantly obtained followed by *Bacillus* sp. (9), *Salmonella* sp. (9), *Achromobacter* sp. (8), *Pseudomonas* sp. (7), *Corynebacterium* sp. (5), *Staphylococcus* sp. (4), *Proteus* sp. (3), *Exigoubacterium* sp. (1) and *Uncultured Microbacterium* (1).

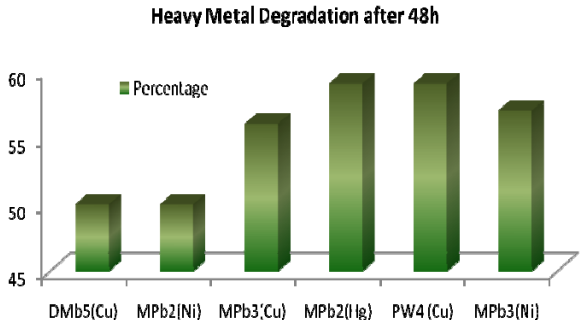
From domestic sewage samples isolates were identified as *Salmonella* sp. (14) predominantly obtained followed by *Achromobacter* sp. (8), *Shigella* sp. (7), *Proteus* sp. (6), *Bacillus* sp. (3), *Pseudomonas* sp. (3), *Corynebacterium* sp. (2), and *Staphylococcus* sp. (2), shown in Fig. 2.

Fig. 2: Heavy Metal degradation after 24h



A total six isolates, five from industrial effluents and one from domestic sewage samples were shown growth on 2000 ppm concentration of heavy metals are considered as potential heavy metal degrading isolates. These isolates included as *Pseudomonas* spp. (DMb5(Cu), *Achromobacter* spp. (MPb2(Ni), *Pseudomonas* spp. (MPb3(Cu), *Exigoubacterium* spp. (MPb2(Hg), *Pseudomonas* spp. (PW4 (Cu), *Uncultured Microbacterium* (MPb3(Ni). For the confirmative identification purpose these isolates were proceed for 16s rRNA.

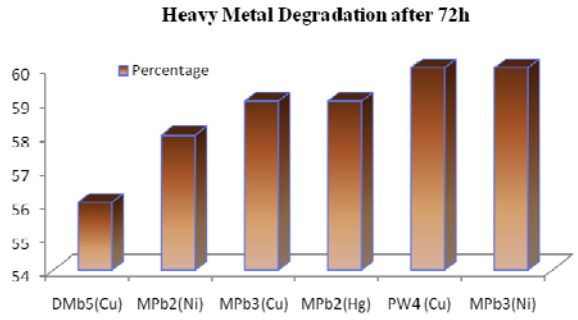
Fig. 3: Heavy Metal degradation after 48h



The ICP-AES analysis of the isolates incubated for the 24hr shows that the isolate

DMb5(Cu) and MPb2(Ni) have the removal efficiency of 44 and 45 percent respectively. Isolate MPb3(Cu) can remove the heavy metal with removal efficiency of 52 percent. All the three remaining isolates such as MPb2(Hg), PW4 (Cu), MPb3(Ni) have the same removal efficiency of the heavy metal mercury, copper, and nickel respectively, which was calculated as 55 percent (Fig. 3)

The ICP-AES analysis of the isolates incubated for the 48hr shows that the isolate DMb5(Cu) and MPb2(Ni) have the same removal efficiency of 50 percent. Isolate MPb3(Cu) can remove the heavy metal with removal efficiency of 56 percent while MPb3(Ni) removed 57 percent. MPb2(Hg) and PW4(Cu) have the same heavy metal removal efficiency of 59 percent (Fig. 4).



The heavy metal removal efficiency of the isolates after 72hr have shown that the isolate DMb5(Cu) shows 56 percent and MPb2(Ni) shows 58 percent removal efficiency. The isolate MPb3(Cu) and MPb2(Hg) were 59 percent efficient in the removal of the heavy metals. The remaining two isolates PW4 (Cu) and MPb3(Ni) were showing the highest heavy metal removal efficiency of 60 percent as compared to other isolates (Fig. 5).

It has shown that the degradation efficiency is increasing as the incubation time increases. Isolates PW4 (Cu) and MPb3(Ni) were found to be the most efficient heavy metal degrading isolates. Also some of the biosorption studies have shown that as the concentration of the biomass increases the removal efficiency of the heavy metal degradation increases consequently (Singanan, 2011).

After inducing the exposure of the UV radiation, the heavy metal degrading activity of the isolates at higher concentrations (2000 ppm-3000 ppm) was analyzed and it was shown that the isolate MPb2(Hg) identified as *Exigoubacterium* sp., was able to degrade the heavy metal up to 3000 ppm concentration and can be very useful for the application in the environmental bioremediation. It can degrade heavy metal about 60% whereas all the other isolates were unable to grow on the heavy metal concentration above 2000 ppm.

Results of different studies carried out have demonstrated that the bacteria saliently affected the metal degradation rate. Also the mutation was found to be effective way to increase the ability of the degradation of the heavy metal. At low concentrations, metals can serve as important components in life processes, often serving important functions in enzyme productivity. However, above certain threshold concentrations, metals can become toxic to many species (Vilensky, *et al*., 2002). Fortunately, microorganisms can affect the reactivity and mobility of metals. Microorganisms that affect the reactivity and mobility of metals can be used to detoxify heavy metals and prevent further metal contamination (Vilensky *et al*., 2002).

All these information clearly indicated that isolated potential bacteria from industrial effluents and domestic sewage samples are efficient for the removal or degradation of heavy metals.

Conclusion

The present study on removal of heavy metals by microorganisms isolated from industrial effluents concluded that, the isolated species can remove heavy metal up to 60%. It was also found that the increase in the incubation time causes more reduction in the heavy metal concentration. The mutational analysis of the isolates for the strain improvement process shows that the *Exigoubacterium species* can grow at 3000 ppm heavy metal concentration and showed 60% reduction in heavy metal. This highly potential species can be used for the removal of different heavy metals which is also a viable, eco friendly and cost effective technology for cleanup of the environment. Therefore, the study is very useful to suggest that the possible impact of metal contaminated locations in human life may be greater than the direct consequence of the pollution.

References

Ahalya N, Ramachandra TV, Kanamadi RD, (2003). Biosorption of heavy metals. Res J Chem Environ 7: 71-78.

Hobman, JL, Wilson, JR, and Brown, NL, (1997). Mercury ions in biological systems In:Mercury and its effects on environmental biology. pp- 503-568, Mareel Dekker, New York.

Hrynkiewicz, K., & Baum, C (2014). Application of microorganisms in bioremediation of environment from heavy metals Environmental Deterioration and Human Health:215-227

Hussein H, Ibrahim SF, Kandeel K, Moawad H (2004). Biosorption of heavy metals from waste water using *Pseudomonas sp*. Electron. J. Biotechnol. 7: 38-46.

Nanda, M, Sharma, D, Kumar, A, (2011). Removal of Heavy Metals from Industrial Effluent Using Bacteria. International Journal of Environmental Science. 2 (2): 0976-4402.

Niles DH (1992). Resistance to cadmium, cobalt, zinc and nickel in microbes. Plasmid ; 27: 17-28.

Pandit, R., Patel, B., Kunjadia, P., & Nagee, A (2013). Isolation, characterization and molecular identification of heavy metal resistant bacteria from industrial effluents, Amala-khadiAnkleshwar, Gujarat. Inter J Environ Sci, 3(5):1689-1699.

Singanan M., (2011). Removal of lead(II) and cadmium(II) ions from wastewater using activated biocarbon. ScienceAsia 37 (2011): 115–119.

Vilensky MY, Berkowitz B, Warshawsky A (2002). In situ remediation of groundwater contaminated by heavy-and transition-metal ions by selective ion-exchange methods. Environ. Sci. Technol., 36(4): 1851- 1855.