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Microbial bioremediation of heavy metals from effluent discharge of metal processing industries

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Abstract
In this study the microbial bioremediation was carried out from effluent discharge of metal processing industries using the isolate bacterial strains. The microbiological analysis total of 6 industrial effluents samples were collected from various industries, and total of 42 isolates were obtained among these isolates *Achromobacter* sp. (8), predominantly obtained followed by *Bacillus* sp. (5), *Shigella* sp. (7), Salmonella sp. (7), *Pseudomonas* sp. (5), *Corynebacterium* sp. (3), *Staphylococcus* sp. (3), *Proteus* sp. (2), *Exigoubacterium* sp. (1) and *Microbacterium* (1), among them one isolate SR6a (*Achromobactor*) was resistant to Cu at higher concentration (2000ppm) and considered as potential heavy metal degrading isolate. The some isolates such SR3f (*Bacillus*), SR4g (*Achromobactor*), SR5c (*Bacillus*), and SR3a (*Pseudomonas*) were multi heavy metal degrading ones. All these isolates from metal industrial effluents showed the heavy metal resistance against Copper, Nickel, Cadmium, Zinc and Mercury. the isolate SR6a identified as *Achromobactor* 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.

Keywords: microbial bioremediation, heavy metals, effluent and metal processing industries

Introduction
In recent years, concern has increased over heavy metal pollution, as all heavy metals are potentially harmful to most organisms at some level of exposure. The release of increasing quantities of heavy metals and their salts in terrestrial and aquatic environment and their accumulation in living and non-living systems endanger life. Since the second part of 20th century, there has been growing concern over the diverse effects of heavy metals on humans and aquatic ecosystems. Environmental impact of heavy metals was earlier mostly attributed to industrial sources. In recent years, metal production emissions have decreased in many countries due to strict legislation, improved cleaning/ purification technology and altered industrial activities. Today and in the future, dissipate losses from consumption of various metal containing goods are of most concern. Therefore, regulations for heavy metal containing waste disposal have been tightened (McGrath *et al.*, 1995) [8].

Effluents from textile, leather, tannery, electroplating, galvanizing, pigment and dyes, metallurgical and paint industries and other metal processing and refining operations at small and large-scale sector contain considerable amounts of toxic metal ions (Ahluwalia *et al.*, 2007) [1]. The toxic metals and their ions are not only potential human health hazards but also to another life forms. Toxic metal ions cause physical discomfort and sometimes life-threatening illness including irreversible damage to vital body system (Malik, 2004) [6]. Bioremediation is a state-of-the-art technique used for heavy metal removal and/or recovery from polluted environments. The technique utilizes inherent biological mechanisms to eradicate hazardous contaminants using microorganisms and plants, or their products, to restore polluted environments to their original condition. It is an environmentally friendly and cost-effective technique for heavy metal removal/recovery, when compared to the Conventional chemical and physical techniques, which are often more expensive and ineffective, especially for low metal concentrations. (Dixit *et al.*, 2015; Mani and Kumar, 2014) [7].

A wide variety of microorganisms including bacteria, fungi, yeast and algae interact with metals. The structural and functional complexity of microbes help them to interact with heavy metals in several ways. The interactions of microorganisms with heavy metals can be broadly classified as metabolism dependent (active) and metabolism independent (passive). They can also be classified on the basis of the site of metal interaction *viz*. extracellular, exocellular and intracellular (Veglio and Beolchini, 1997) [13].

Microbe related technologies may provide an alternative or addition to conventional method of metal removal or metal recovery (Chellaiah *et al.*, 2009) [3]. Therefore, there is an urgent need for the treatment of metal processing industry waste. Waste management strategies adopted in India have failed to keep pace with the industrial growth and urbanization. So, we have proposed to undertake the studies with the help of microorganisms. The majority of heavy metals disrupt microbial cell membranes, but microorganisms can develop defense mechanisms that assist them in overcoming the toxic effect. Thus, the response of microorganisms to heavy metal toxicity is of importance for re-establishing polluted sites.

Materials and Methods
Collection of Effluent Samples: The effluents sample were collected from different metal processing industry of Marathwada region. Samples were collected in a plastic bucket and then thoroughly mixed on a piece of clean cloth

and the lumps were broken using wooden pestle and mortar and were air dried (Tandon, 1993) [12]. After collection, a portion of each sample was immediately transferred to laboratory and stored at 4^0C for microbial analysis.

Isolation and identification of Bacteria

One mL of water sample was added to 9 mL of sterile distilled water and a tenfold serial dilution was done, and the lower, middle, and high dilutions were plated in duplicate into nutrient agar (Himedia, Mumbai), MacConkey agar, and potato dextrose agar plates already prepared. These were incubated at 37°C for 18–24 hrs for total bacteria and coliforms. Colonies on plates were observed and counted and the population density was estimated; bacterial colonies were picked according to their cultural morphology on the plates and these were streaked on new nutrient agar plates for pure colonies (Nwachukwu and Apata, 2013) [9].

Morphological characterisation of bacterial isolates

The morphological characteristics of isolates were observed and recorded and this was the basis for the isolation of colonies. The cell shape and arrangements of isolates were determined following the standard procedures of basic stain, gram stain (Nwachukwu and Apata, 2013) [9].

Inoculum preparation

The suspension of 2 days old cultures of bacteria was used for the degradation of heavy metals from the effluent discharge of metal processing industries. They were prepared in saline solution (0.85% sodium chloride). A loopful of above bacterial cultures were inoculated into 50 ml of saline and incubated at 37^0C for 3 hours.

Bioremediation of Heavy metals from effluent discharge of metal processing industries

Degradation of heavy metals experiments were carried out in 250 ml of separate flasks containing 100 ml of effluent discharge of metal processing industries. Then, the flasks were autoclaved at 121^0C for 15 minutes. The autoclaved flasks were inoculated with 5ml of bacterial inoculum of each isolates. The flasks were kept in mechanical shaker and incubated at 37^0C for 24, 48 and 72 hours. The heavy metal content was assessed by measuring supernatant with the help of inductively coupled plasma mass spectrometry (ICP-AES).

Results and Discussion

In this preliminary study, the bacterial isolates from the effluent from metal processing industries of Marathwada region investigated revealed metals resistance strains probably as a result of selective pressure from metal pollution in waste wastewater and this is of public health concern. Thus, there is a need to exploit this opportunity to evaluate them for safety and toxicity to humans, animals, and the environment. The waste discharged from metal processing industries is degradable waste, though it is hazardous to microorganism in the costal water. The extent of pollution was high as expressed by physicochemical properties. Analysis of industrial effluent discharge showed that the various parameters are beyond the permissible limit. The effluent from two industries shows acidic pH while remaining samples have alkaline pH. These values are generally due to the decomposition of the proteinaceous matter and emission of ammonia.

Table 1: Microbial analysis of Effluent Samples Collected from different Metal processing Industries

Sr. No	Name of isolates	No.of Isolates
1.	*Achromobacter* sp.	8
2.	*Bacillus* sp.	5
3.	*Shigella* sp.	7
4.	*Salmonella* sp.	7
5.	*Pseudomonas* sp.	5
6.	*Corynebacterium* sp.	3
7.	*Staphylococcus* sp.	3
8.	*Proteus* sp	2
9.	*Exigoubacterium* sp.	1
10.	*Microbacterium*	1
Total isolates		42

Table 2: Analysis of Heavy Metal degradation at 2000 ppm by isolates after 24hr, 48hr and 72hr respectively

SN	Code of Isolate	Time interval 24hr	Removal Efficiency (%)	Time interval 48hr	Removal Efficiency (%)	Time interval 72hr	Removal Efficiency (%)	Name of Isolates
		Metal removed		Metal removed		Metal removed		
1	SR2c	756	38	823	41	900	45	*Exigoubacterium*
2	SR3a	931	47	980	49	1034	52	*Pseudomonas*
3	SR5c	978	49	1010	51	1180	59	*Bacillus*
4	SR4g	1080	54	1165	58	1180	59	*Achromobactor*
5	SR3f	1078	54	1170	59	1200	60	*Bacillus*
6	SR6a	1090	55	1180	59	1200	60	*Achromobactor*

The ICP-AES analysis of the isolates incubated for the 24hr shows that the isolate SR3a and SR2c have the removal efficiency of 44 and 45 percent respectively. Isolate SR5c can remove the heavy metal with removal efficiency of 52 percent. All the three remaining isolates such as SR6a, SR3f and SR4g have the same removal efficiency of the heavy metal mercury, copper, and nickel respectively, which was calculated as 55 percent (Table 2 and Fig. 1). The ICP-AES analysis of the isolates incubated for the 48hr shows that the isolate SR3a and SR2c have the same removal efficiency of 50 percent. Isolate SR5c can remove the heavy metal with removal efficiency of 56 percent while SR4g removed 57 percent. SR6a and SR3f have the same heavy metal removal efficiency of 59 percent. The heavy metal removal efficiency of the isolates after 72hr have shown that the isolate SR2c shows 56 percent and SR3a shows 58 percent removal efficiency.

The isolate SR4g and SR5cwere 59 percent efficient in the

removal of the heavy metals.

The remaining two isolates SR6a and SR3fwere showing the highest heavy metal removal efficiency of 60 percent as compared to other isolates.

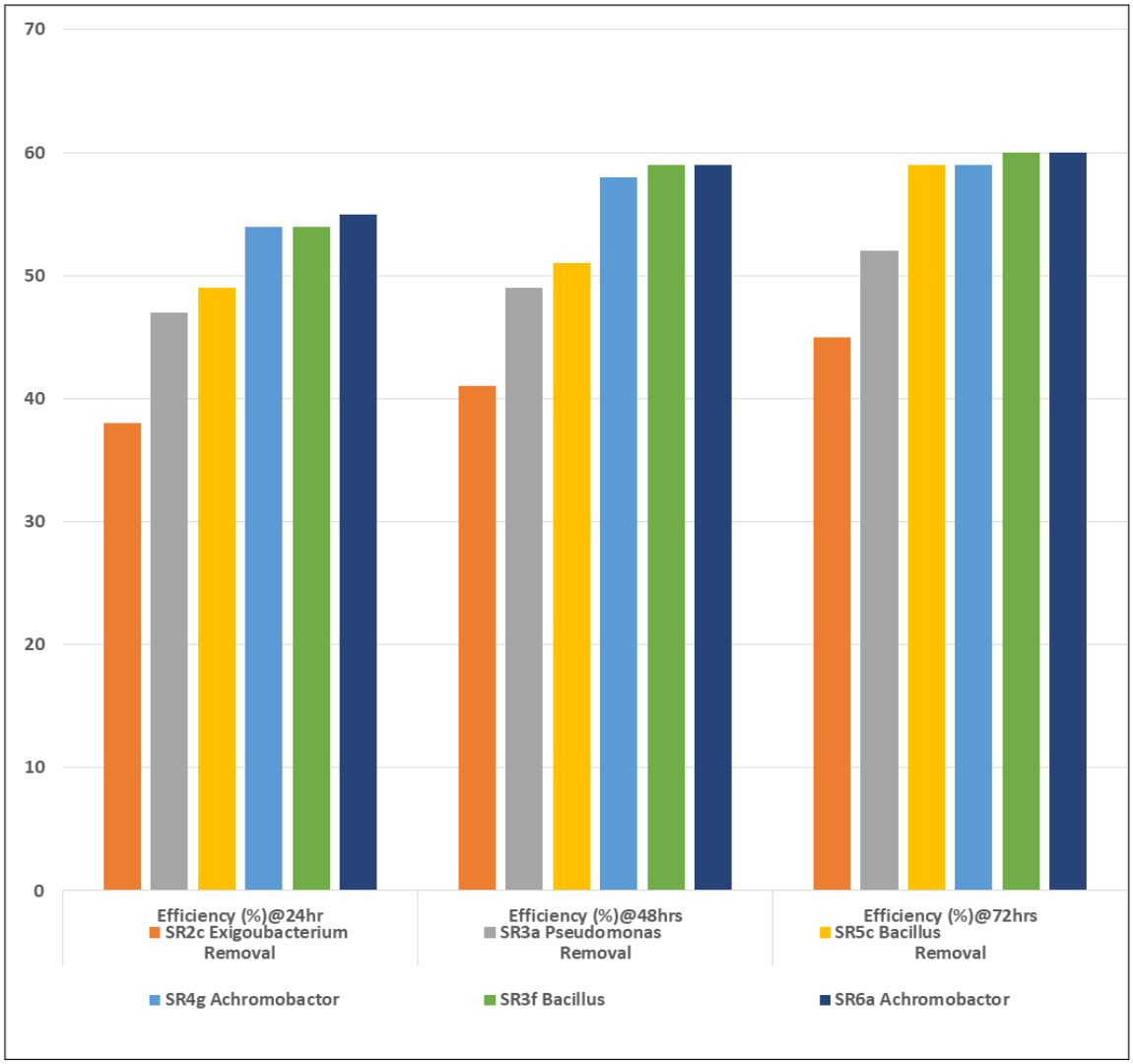

Fig 1: Heavy metal degradation at 2000 ppm by isolates after 24hr, 48hr and 72hr

It has shown that the degradation efficiency is increasing as the incubation time increases. Isolates SR3f and SR6a were found to be the most efficient heavy metal degrading isolates. Similar study was done on *Pseudomonas putida* and *Pseudomonas fluorescens* species in Egypt. The percentage of Cu (II) removal ranged between 50 and 93% (Hussein *et al.*, 2004) [5]. The study carried out by (Smrithi, A. and Usha, K. 2012) [11]. with an objective to remediate the tannery effluent contaminated soil by microorganisms, have shown that the leather tanning effluent contaminated soil having higher pH and large amount of total suspended and total dissolved solids, minerals and metals like sodium, potassium, chromium, zinc and copper contains *Bacillus* spp., that was found to reduce 85.9% of chromium from the medium after 96 h. The results also showed that the isolated *Bacillus* sp has the capacity to remove other heavy metals (Ni, Cr, Cu, Zn and Cd) in the tannery effluent. The metal removing capacity increased with increase in concentration of the metals. 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 (Bhutada and. Dahikar, 2017; Singanan, 2011) [2].

Conclusion

To conclude that the present study revealed that all the bacterial isolates were able to tolerate different concentration of heavy metals recovered from the different contaminated sites. The most prominent species that were revered from all the six sites were *Achromobactor* sp. and *Bacillus* spp., furthermore all the isolates were resistant various metals. The present study suggests that, these bacterial isolates can be used for the bioremediation of heavy metals.

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