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Bioremediation Potential of Zn (II) By Different Bacterial Species

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Original Research Article

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Abstract: Heavy metals are generally toxic to microorganisms, especially if they exist at high concentrations. Environmental pollution particularly in soil with heavy metals can stem from industrial activities or sewage discharges. In this study, Five different bacterial species *Bacillus megaterium* EMCC 1013, *Rhizobium rhizogenes* EMCC1743, *Rhizobium leguminosarum* EMCC1130, *Azotobacter vinelandii* and *Nocardiopsis Dassenvillei* were evaluated their potential activity in bioremediation of Zn (II). Our results showed that five bacterial species have great variation potential for zinc bioremediation.–*Bacillus megaterium* EMCC has the highest capacity for bioremediation of Zn (II) 650 ppm with 99 % removal after 24 h with inoculum size 0.1 x 10^{29} cfu and incubation temperature 30^0C at PH 7 and energy source glucose and ammonium oxalate as carbon and nitrogen source. The aim of our study was to evaluation the bioremediation capacity of zinc as heavy metals by five different bacterial species to use them in further study in removal of Zn (II) from plating waste water. In addition *Bacillus megaterium* EMCC as the most potent Zn (II) resistant microorganisms will very useful in biotechnology for the remediation of metal contaminated environments with Zn (II) and can also be used in the construction of biomarkers for the detection of zinc ions.

Keywords: Bioremediation, *Bacillus megaterium*, heavy metal, Zinc.

INTRODUCTION

Heavy metals are present in the environment in different concentrations in water, soil, and in all biological objects. So, Exposure to heavy metals has been linked with development retardation [1]. Zinc consider one of the metals found in effluents discharged from industries involved in galvanization, electroplating, manufacturing of batteries, and metallurgical industries. Zinc in its metallic form has limited bioavailability and poses no ecological risk. However, zinc can react with other chemicals like acids and oxygen to form compounds, which can be potentially toxic and can cause serious damage to biological systems [2].

Wide paying attention on management of environmental pollution and its control due to hazardous materials like heavy metals was been interested. Heavy metals bioremediation in water behind industrial factories has been a challenge for a long time. A lot of physicochemical strategies, such as, membrane technology, electrochemical treatment, ion exchange, oxidation/reduction, filtration and reverse osmosis, have been developed for bioremediation of heavy metals from the polluted water [3].

A lot of records had indicated that microbe–plant symbioses and native microbes resist heavy metal concentrations in different ways and may play a significant role in the restoration of polluted water soil [4].

Bioremediation involves the utilize of microorganisms to remove and detoxify environmental contaminants, has received increasing attention to remove up a contaminated environment [5].

The bioremediation of heavy metals from contaminated environments and reducing their toxicity by applying different microorganisms was developed, so, *Bacillus megaterium* has great role in the biogeochemical cycle of heavy metals and processes involved in bioremediation [6]. Microorganisms are using their secondary metabolites that participate in the bioremediation of heavy metals by production of organic and inorganic acids, oxidation or reduction

reactions or excretion of chelating agents [7]. The aim of this paper was to evaluation the bioremediation efficacy of heavy metal zinc as by different species of microorganisms to use them in further study in removal of zinc from waste water plating industries

MATERIAL AND METHODS

Microorganisms

Three bacterial species were purchased from Egyption Microbial culture collection, Ain shams university (*Bacillus megaterium* EMCC 1013, *Rhizobium rhizogenes* EMCC1743, *Rhizobium leguminosarum* EMCC1130). *Azotobacter vinelandii* was obtained by El -Badry *et al*., [8] and *Nocardiopsis Dassenvillei* was obtained by Elbarbary *et al*., [9].

Chemicals and instrumentation

Zinc stock solution

Zn (II) (1 mg/mL) stock solution was prepared using by dissolving 4.398 g of $ZnSO_4 \cdot 7H_2O$ in distilled water containing a few drops of conc. H_2SO_4 and standardized by 8-hydroxyquinoline [10]. Using this stock solution with different zinc ppm concentration was prepared for culture media supplements.

Zn (II) bioremediation Experiments:

LB (Luria-Bertani) liquid medium (Oxoid) was used as basal media consists of different ppm concentration of Zn (II) solution. Different pH was prepared by adjustment 0.1(N) HCl and 0.1(N) Na OH solutions. After that media was autoclaved in 250 ml conical flasks containing 100 ml media. The media was inoculated with five different bacterial species. After incubation time samples were collected and centrifuged at 6000 rpm for 10 minutes. Supernatant was assayed for the zinc removal by Optical Emission Spectrometer Model: Optima 2000 DV Perkin Elmer (Inductive Couple Plasma). Bioremediation of zinc ion in basal media inoculated with five different bacterial species separately were evaluated by following equation Bioremediation of Zn (II) $\% = \frac{S\ \text{cont} - S\ sampl}{S\ \text{cont}}\ x\ 100$. All the glassware was cleaned with 5% HNO_3.

Relative effects of different Zn (II) concentration bioremediation on microbial growth

Five different bacterial species were grown in a rotary shaker at 150 rpm and pH 7.0, while the temperature was 37 C in LB broth medium supplemented by Different concentration (10, 15, 20, 25, 30, 35, and 40) ppm of Zn (II) for each bacterial species. After 24 h of incubation the remediation percentage of Zn (II) concentration on each bacterial growth was assessed.

Relative effects of different inoculum size on Zn (II) bioremediation

Five different bacterial species were grown in a rotary shaker at 150 rpm and pH 7.0, while the temperature was 37 C in LB broth medium supplemented by Different inoculum size (0.1×10^{29}, 0.5×10^{29}, 1×10^{29}, 3×10^{29} and 5×10^{29}) cfu of each bacterial species. After 24 h of incubation the remediation percentage of Zn (II) concentration on each bacterial growth was assessed.

Relative effects of different Temperature on Zn (II) bioremediation

Five different bacterial species were grown in a rotary shaker at 150 rpm and pH 7.0, while the temperature was 37 C in LB broth medium supplemented by Different incubation temperature (20 ^{o}, 25 ^{o}, 30 ^{o}, 35 ^{o} and 40 ^{o}) C. After 24 h of incubation the remediation percentage of Zn (II) concentration on each bacterial growth was assessed.

Relative effects of different PH on Zn (II) bioremediation

Five different bacterial species were grown in a rotary shaker at 150 rpm and pH 7.0, while the temperature was 37 C in LB broth medium supplemented by Different PH (4, 5, 6, 7 and 8). After 24 h of incubation the remediation percentage of Zn (II) concentration on each bacterial growth was assessed.

Relative effects of different Carbon sources on Zn (II) bioremediation

Five different bacterial species were grown in a rotary shaker at 150 rpm and pH 7.0, while the temperature was 37 C in LB broth medium supplemented by Different carbon sources (glucose, starch, sucrose and dextrose). After 24 h of incubation the remediation percentage of Zn (II) concentration on each bacterial growth was assessed.

Relative effects of different Nitrogen sources on Zn (II) bioremediation

Five different bacterial species were grown in a rotary shaker at 150 rpm and pH 7.0, while the temperature was 37 C in LB broth medium supplemented by Different nitrogen sources (ammonium chloride, ammonium sulphate, ammonium oxalate, glycine and asparagine). After 24 h of incubation the remediation percentage of Zn (II) concentration on each bacterial growth was assessed.

RESULTS AND DISCUSSION

Bioremediation is elimination processes that apply microbial way to minimize cytotoxicity of heavy metal pollutant to limit of an acceptable value. In this method, biotransformation by microorganisms for different heavy metal pollutants in the environment was carried out. Bioremediation follows of various

biochemical reactions which help in activity, growth and reproduction of microorganisms. This microbial metabolism system allows microorganisms to obtain carbon, electrons and other necessary components for their existence. During bioremediation process, heavy metals are penetrated into the cells, get attached to intracellular proteins and then are associated with vacuoles or other intracellular sites [5, 11].

Different industries as metal cutting, milling, mining and surface finishing are the largest source of many toxic heavy metal ions such as Zn (II). It is very harmful for humans as they adversely affect the liver, brain and skin, kidney and respiratory tracts. Recently, the levels of zinc metals in drinking water have increased at an alarming rate. Thus the removal, recovery and recycling of these metals have greater significance.

Relative effects of different Zn (II) concentration bioremediation on microbial growth

Five different bacterial species *Bacillus megaterium* EMCC 1013, *Rhizobium rhizogenes* EMCC1743, *Rhizobium leguminosarum* EMCC1130, *Azotobacter vinelandii* and *Nocardiopsis Dassenvillei* were evaluated for their potential percentage of *Zn (II)* bioremediation under different concentration of *Zn (II)* with 80, 76, 77, 77 and 77 % respectively for 10 ppm of Zn (II). Its removal decreased in bioremediation for all tested microorganism by increase in Zn (II) concentration as shown in (Figure-1). Rhizobia species have great efficacy to removal heavy metals Zn elements was studied with high potential to removal of heavy metal resistance as proved by Khalid and Abdel-lateif [12]. On the other hand, the most potent isolates from contaminated soil that showed multi resistance to all heavy metals tested were identified as A. chroococcum as reported by Ali et al., [13]. On the other hand the removal of heavy metals from polluted environments of their toxic potential can be realized by *Bacillus megaterium*, so it plays an important role in the biogeochemical cycle of heavy metals and processes involved in bioremediation was reported by Kumar and Achyuthan [7]. Ahemad and Malik [14] characterized and identified five Zn (II) resistant Bacillus spp. from Indian agricultural soils irrigated with metal polluted wastewater.

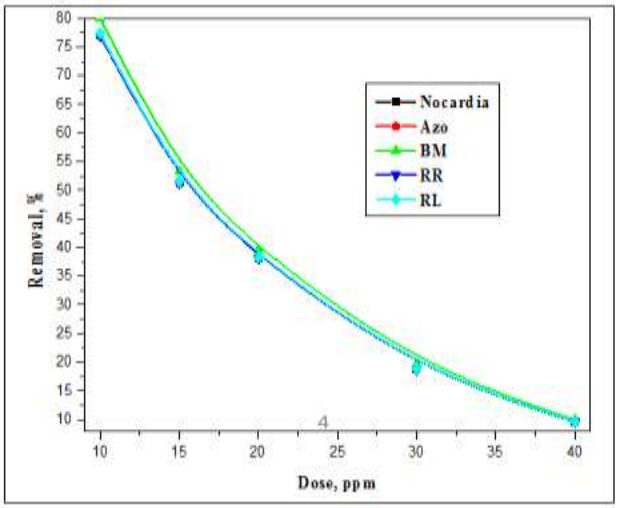

Fig-1: Relative effects of different Zn (II) (ppm) concentration bioremediation by different bacterial species

Relative effects of different inoculum size on Zn (II) bioremediation

Different inoculum size of five bacterial Zn (II) bioremediation evaluated test organisms was studied as shown in figure no with 10 ppm concentration of Zn (II). The results indicated as increase in bacterial cell count decrease percentage of Zn (II) bioremediation. The highest bioremedation was by using inoculum size 0.1 x 10^{29} cfu of five different bacterial species as *Bacillus megaterium* EMCC 1013 was 81. From the above results *Bacillus megaterium* EMCC 1013 showed the most potent Zn (II) bioremediation organism as shown in Figure-2.

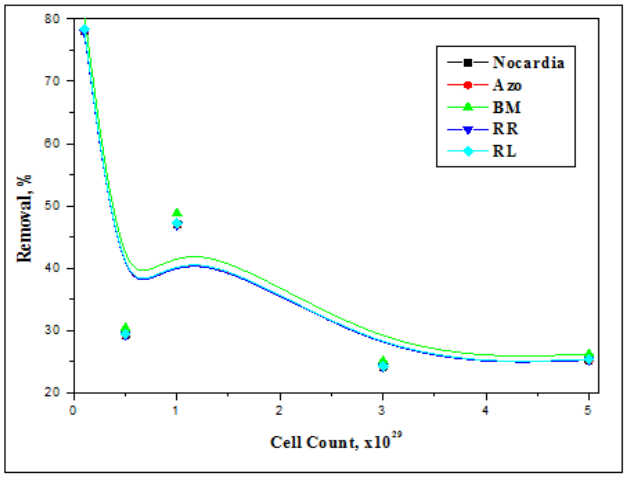

Fig-2: Relative effects of different inoculum size on Zn (II) bioremediation by different bacterial species

Relative effects of different Temperature on Zn (II) bioremediation

Effect of different incubation temperature for Zn (II) bioremediation using *Bacillus megaterium* EMCC 1013, *Rhizobium rhizogenes* EMCC1743, *Rhizobium leguminosarum* EMCC1130 *Azotobacter vinelandii* and *Nocardiopsis Dassenvillei***.** *Bacillus megaterium* EMCC 1013 was the most potent Zn (II) bioremdation percentage with 88 % at 30°C followed by *Rhizobium leguminosarum* EMCC1130 by 85 % Zn (II) bioremediation at 30 ^{o}C as shown in Figure-3. As mentioned by Rajeshkumar *et al*., [15] Temperature can affect the stability of the cell wall, its configuration and can also cause ionization of chemical moieties. The binding sites on the isolated bacterial species might be simultaneously affected by these factors and may cause reduction in metal removal. Energy-independent mechanisms are less likely to be affected by temperature since the processes responsible for removal are largely physiochemical in nature [16]. Li *et al*., [17] reported that the optimum temperature for Zinc removal by *Rhodobacter sphaeroides* was 40°C which larger than form our work by using *Bacillus megaterium* EMCC 1013. These results was explained by the influence of incubation temperature on the microbial cells and in this case the bioremoval process appeared as endothermic process, where the bio-removal process increase with the increasing of temperature to limited values, or based on the influence of incubation temperature on the metal ions where the removal process decreased with the increasing of temperature due to the releasing of metal ions form the active site to the solution [18, 19].

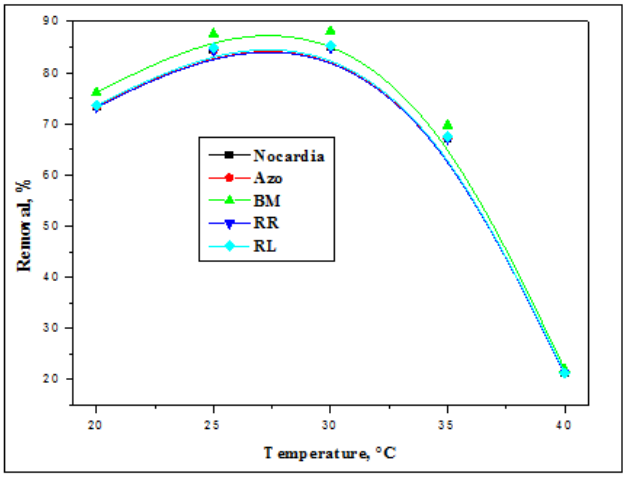

Fig-3: Relative effects of different Temperature on Zn (II) bioremediation by different bacterial species

Relative effects of different PH on Zn (II) bioremediation

Effect of different PH for Zn (II) bioremediation using *Bacillus megaterium* EMCC 1013,*Rhizobium rhizogenes* EMCC1743, *Rhizobium leguminosarum* EMCC1130 *Azotobacter vinelandii* and *Nocardiopsis Dassenvillei***.** *Bacillus megaterium* EMCC 1013 was the most potent Zinc II bioremediation percentage with 65 % at PH 7. It has been shown that low pH affect the network or chemistry of the cell wall as well as its physio-chemistry and the hydrolysis of the heavy metals [20].

From the results of this work, the maximum bioremediation percentage rates was observed in all five different bacterial species at Neutral pH 7 which agrees with the evidence that the optimal pH range for bioremediation by bacteria is 6.0-8.5 With increase in pH, there will be a resulting increase in negative charge on the surface of the cell which favoured electrochemical attraction and adsorption of metal [21]. Li *et al*., [17] reported that the optimum PH for Zinc removal by *Rhodobacter sphaeroides* was 7 which agree with our work by using *Bacillus megaterium* EMCC 1013. Many of authors have indicated that optimum removal efficacy for microbial biomass is indicated between 6 and 8, while little removal is recoded at pH less than 3 due to the cation competition effects with oxonium (hydronium) ion $H3O^{+}$ [22].

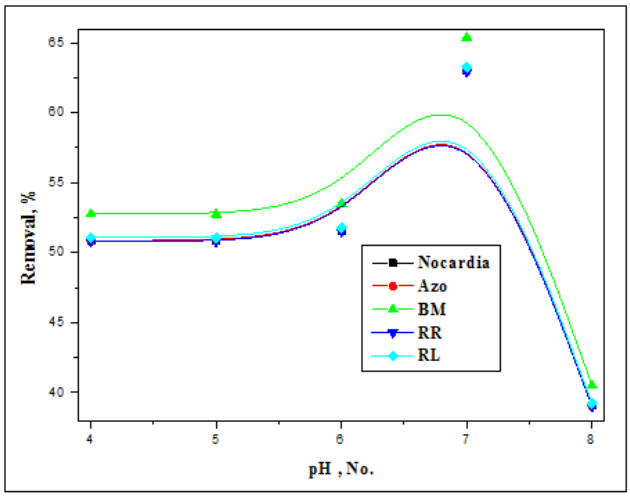

Fig-4: Relative effects of different PH on Zn (II) bioremediation by different bacterial species

Relative effects of different Carbon sources on Zn (II) bioremediation

Effect of different PH for Zn (II) bioremediation using *Bacillus megaterium* EMCC 1013, *Rhizobium rhizogenes* EMCC1743, *Rhizobium leguminosarum* EMCC1130 Azotobacter *vinelandii* and *Nocardiopsis Dassenvillei* was evaluated. *Bacillus megaterium* EMCC 1013 was the most potent Zn (II) bioremediation percentage with 95 % followed by other tested bacterial species by 92 % Zn (II) bioremediation with glucose utilization as carbon source. Utilization of starch and sucrose as carbon source showed sharply decrease in Zn (II) bioremediation with 2 % with all tested bacterial species as presented in Figure-5. Our results was agree with results As reported by El-badry *et al*., [8] *Azotobacter vinelandii* isolate grows well on modified PVK liquid medium containing different carbon sources. Whereas, high amounts of soluble phosphate is detected only in the culture filtrate of bacterium with glucose which reaches to 52.8% then dextrose with low pH value, while starch and sucrose exhibited low amount of soluble phosphate with high pH value. The bacterial growth exhibited remarkable variation according to the utilized carbon source, the best bacterial growth to produce enzyme and organic acids reached when glucose is utilized as a carbon source

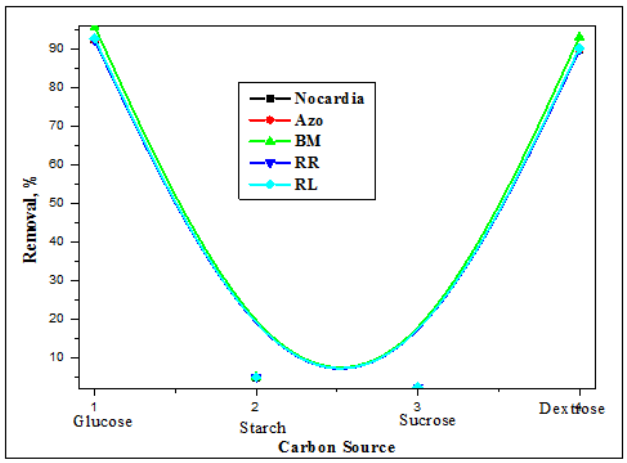

Fig-5: Relative effects of different Carbon sources on Zn (II) bioremediation by different bacterial species

Relative effects of different Nitrogen sources on Zn (II) bioremediation

Effect of different PH for Zn (II) bioremediation using *Bacillus megaterium* EMCC 1013, *Rhizobium rhizogenes* EMCC1743, *Rhizobium leguminosarum* EMCC1130 Azotobacter *vinelandii* and *Nocardiopsis Dassenvillei*. *Bacillus megaterium* EMCC 1013 was the most potent Zn (II) bioremediation percentage with 99 % followed by other tested bacterial species by 95 % Zn (II) bioremediation with ammonium oxalate utilization as nitrogen source. Utilization of ammonium chloride as nitrogen source showed decrease in Zn (II) bioremediation with 59 % with all tested bacterial species as presented in figure No 6. As a nitrogen source, ammonium oxalate was found to give maximum soluble Phosphate. Oxalate ions have the ability to form stable complexes with calcium, iron and aluminum to liberate phosphates [23]. As reported by Elbarbary *et al*., [8] Nocardiopsis dassenvillei solublized high amount of phosphorus from rock phosphate ore ammonium oxalate was found to be the best nitrogen source utilized by Nocardiopsis dassenvillei isolate for maximum phosphate solublization that reached to 53.5% followed by ammonium sulphate and lowest dissolution of phosphate content of the ore at using glycine as nitrogen source.

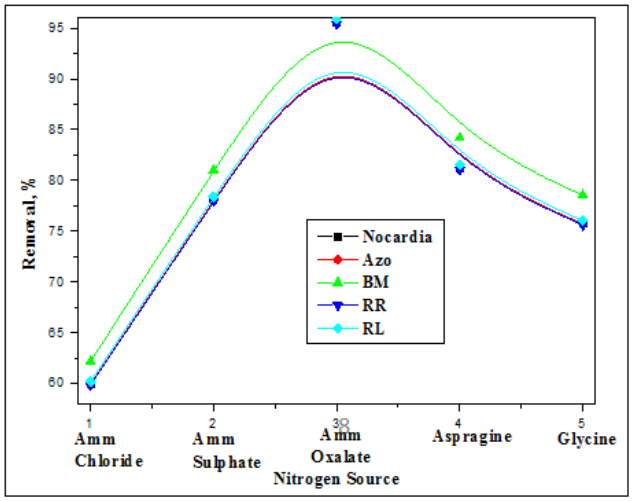

Fig-6: Relative effects of different nitrogen sources on Zn (II) bioremediation by different bacterial species.

CONCLUSION

Zinc as heavy metal elements was removal evaluation by different bacterial species. *Bacillus megaterium* EMCC 1013 was the most potent of zinc removal with 99 %. The results from this work is important to be well understand the bioremediation mechanism of *Bacillus megaterium* EMCC, and is significant for its pilot test and future practical application.

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