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Full Length Paper.

Bioremediation potential of cupper by different bacterial species

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Microbial biomass is one of the low-cost and efficient bio sorbents of heavy metals removal from solutions. Five different bacterial species Bacillus megaterium EMCC 1013, Rhizobium rhizogenes EMCC1743, Rhizobium leguminosarum EMCC1130,AzotobactervinelandiiandNocardiopsis Dassenvillei were evaluated their potential activity in bioremediation of copper. Our results showed that five bacterial species have good potential for copper bioremediation. Bacillus megaterium EMCC has the highest capacity for bioremediation of cupper 10 ppm with 99 % removal after 24 h with inoculum size 0.1 x 10 29cfu 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 cupper as heavy metals by five different bacterial species to use them in further study in removal of cupper from plating waste water. In addition Bacillus megaterium EMCC as the most potent cupper resistant microorganisms will very useful in biotechnology for the remediation of metal contaminated environments with cupper and can also be used in the construction of biomarkers for the detection of cupper.

Keywords: Bioremediation, Bacillus megaterium, heavy metal, cupper

INTRODUCTION

Heavy metal pollution cause excessive environmental problem in the last several decades. A large amount of heavy metal ions exist in final industrial effluents which are extremely undesirable due to their toxicity even at low concentrations**(**Vijayaraghavan, and Yun, 2008). Copper is one of vital conductive metal of electricity and heat, a building material, and a constituent of various metal alloys in several industries that include electric motors, electronics, and architecture. However, copper, at high levels, is toxic posing some dangerous threats to human, animals and plants. The extreme use of copper precipitates in water resources which damage and alters the osmo-regulatory mechanism of water organisms (Lee et al., 2010)**.** Process of bio adsorption considers one of the most efficient methods for removal of heavy metal at low concentrations. It is a simple physicochemical process resembling conventional adsorption using fungal, bacterial as biological sorbent which can bind the soluble chemicals to its cellular surfaces through surface complexation and precipitation, physical adsorption or ion exchange (Chojnacka, 2010). Inexpensive material, speed and regeneration of bioadsorbents are advantages of usesthis method (Demirbas, 2008). Bioremediation of copper onto the surface of a microorganism is affected by several factors such as initial pH, initial copper ion concentration, time and temperature etc. The level of the three variables: pH 6.18; initial copper concentration, 32.50 mg L-1 , time 30 hours, were found to be optimum for maximum copper removal. The corresponding removal in optimum condition was found to be 60.264% as experimented by (Ghosh and Saha. 2013). An increase in copper concentration had a negative impact on biosorption efficiency (Das et al. 2008). *Bacillus megaterium* is mentioned in the literature as producing nitric acid as secondary metabolic pathway in which glycine is converted to hydrogen cyanide after the oxidative decarboxylation reaction catalyzed by the glycine decarboxylase (Faramarzi et al 2004). *Bacillus megaterium* contribute to the immobilization (bioaccumulation) of metal by sorption in organelles or precipitation as organic or inorganic compounds, such as oxalates, sulfites or phosphates (Selenska et al., 2006) The literature reports a growth sporulation of Bacillus megaterium in the presence of heavy metals in their growth medium, an increase in the amount of extracellular proteins that this bacterium produces and a most significant cell biomass accumulation (Ravikumar et al., 2009). World attention increased towards discharge of industrial effluent into the environment due to rising population and rapid industrialization. One major public and environmental problem is pollution of soil and aquatic system with heavy Metals from industrial effluent. The removal of heavy metals from industrial effluent using conventional methods are often costly and less efficient, therefore, biological removal considered as a cheap treatment method, which proved high capability for pollutants removal (Lopez et al., 2000).The aim of this work was to estimation the bioremediation capacity of cupper as heavy metals by five different bacterial species to use them in further study in removal of cupper from plating waste water

MATERIAL AND METHODS

Microorganisms:

Three bacterial species were purchased from Egyptian Microbial culture collection, Ain shams university (*Bacillus megaterium EMCC* 1013*, Rhizobium rhizogenes*EMCC1743*, Rhizobium leguminosarum*EMCC1130). *Azotobactervinelandii*was obtained by El -Badry et al 2016 and *Nocardiopsis Dassenvillei* was obtained by Elbarbary et al., 2015

Cupper stock solution

Copper stock solution was prepared using Pentahydrate copper sulfate ($CuSO_4$,5H2O) dissolving in distilled water. Using this stock solution different copper concentration media was prepared.

Cupper bioremediation Experiments

LB (Luria-Bertani) liquid medium (Oxoid) was used as basal media consists of different concentration of Cu solution. Different pH was prepared by adjustment 0.1(N) HCl and 0.1(N) NaOH 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 copper removal ions by Optical Emission Spectrometer Model: Optima 2000 DV Perkin Elmer (Inductive Couple Plasma) at wave length 460 nm by using cupper complexing agent as sodium diethyl di-thiocarbamate (Snell and Snell, 1959). Bioremediation of copper ion in basal media inoculated with five different bacterial species separately were evaluated by following equation$\text{Bioremediation of cupper } \% = \frac{\text{S cont} - S\ sampl}{\text{S cont}}\ x\ 100$

Relative effects of different cupper 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 cupper for each bacterial species. After 24 h of incubation the remediation percentage of cupper concentration on each bacterial growth was assessed

Relative effects of different inoculum size on Cupper 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 x 10^{29}, 0.5 x 10^{29} , 1 x 10^{29}, 3 x 10^{29} and 5 x 10^{29}) cfu of each bacterial species. After 24 h of incubation the remediation percentage of cupper concentration on each bacterial growth was assessed

Relative effects of different Temperature on Cupper 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 cupper concentration on each bacterial growth was assessed

Relative effects of different PH on Cupper 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 cupper concentration on each bacterial growth was assessed

Relative effects of different Carbon sources on Cupper 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, sucroseand dextrose). After 24 h of incubation the remediation percentage of cupper concentration on each bacterial growth was assessed

Relative effects of different Nitrogen sources on Cupper 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 cupper concentration on each bacterial growth was assessed

RESULTS AND DISCUSSION

Copper is one of the toxic heavy metals of concern in the environment. Toxicity of heavy metals is largely due to their presence in aqueous systems in ionic forms, which are easily absorbed by living organisms (Arica and Bayramoglu, 2005). There is increasing interest in the use of microbial biomass for biosorption of heavy metals from the environment. Biosorption of heavy metals involve accumulation of the metals in microbial biomass with subsequent recovery and remediation through bioremediation or chemical technologies. Copper resistant microorganisms with the capacity to adsorb copper on biomass can be used as bioremediation tools to remove copper from contaminated terrestrial and aquatic environments (Andreazza et al., 2010).

Relative effects of different cupper concentration bioremediation on microbial growth

Five different bacterial species *Bacillus megaterium EMCC* 1013*, Rhizobium rhizogenes*EMCC1743*, Rhizobium leguminosarum*EMCC1130,*Azotobacter vinelandii and Nocardiopsis Dassonvillei* were evaluated for their potential percentage of cupper bioremediation under different concentration of Cupper with 30, 30, 31, 31 and 28 % respectively for 10 ppm of copper. Decrease in copper bioremediation for all tested microorganism by increase in copper concentration as shown in (figure 1) the resistance of rhizobia species to heavy metals Pb, Cu and Zn heavy metal elements was evaluated with high potency to heavy metal resistance as proved by Khalid and Abdel-lateif , 2017. In addition The most potent isolates from contaminated soil that showed multiresistance to all heavy metals tested were identified as *A. chroococcum* as reported by Ali, et al., 2013. 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 2007

Relative effects of different inoculum size on Cupper bioremediation

Different inoculum size of five bacterial cupper bioremediation evaluated test organisms was studied as shown in figure with 10 ppm concentration of cupper. The results indicated as increase in bacterial cell count decrease percentage of cupper bioremediation. The highest bioremedation was by using inoculum size 0.1 x 10 ^{29}cfu of five different bacterial speciesas *Bacillus megaterium EMCC* 1013 was 81 %*, Rhizobium rhizogenes*EMCC1743 was77 %*, Rhizobium leguminosarum*EMCC1130 was 78 % and by *Azotobacter vinelandii and Nocardiopsis Dassonvillei*78 % respectively. From the above results *Bacillus megaterium EMCC* 1013 showed the most potent cupper bioremediation organism as shown in figure 2

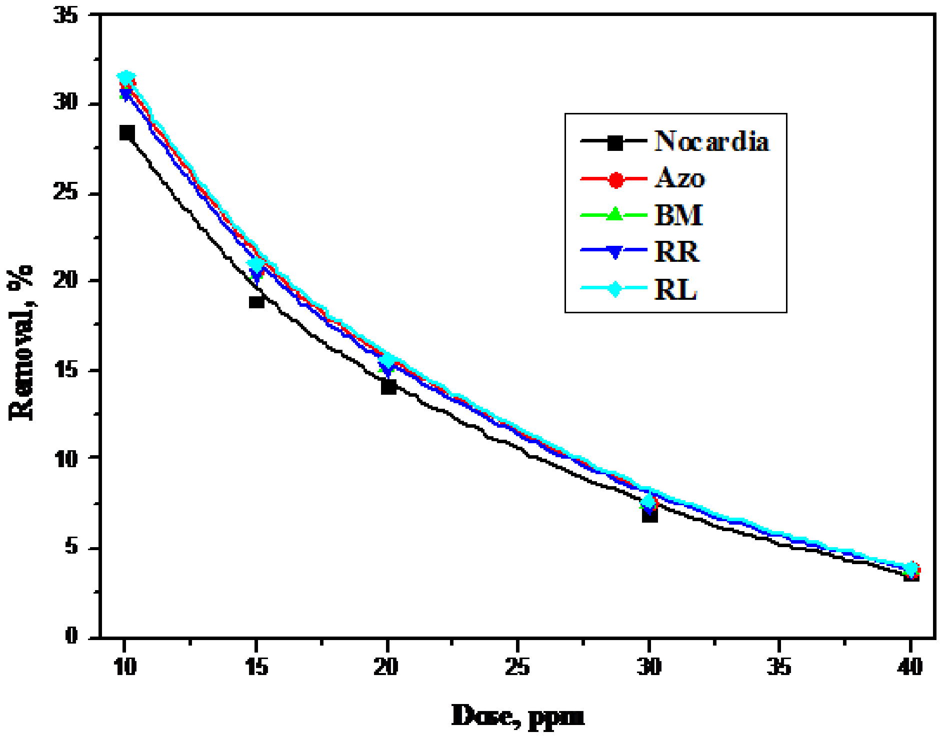

Figure 1: Relative effects of different cupper (ppm) concentration bioremediation by

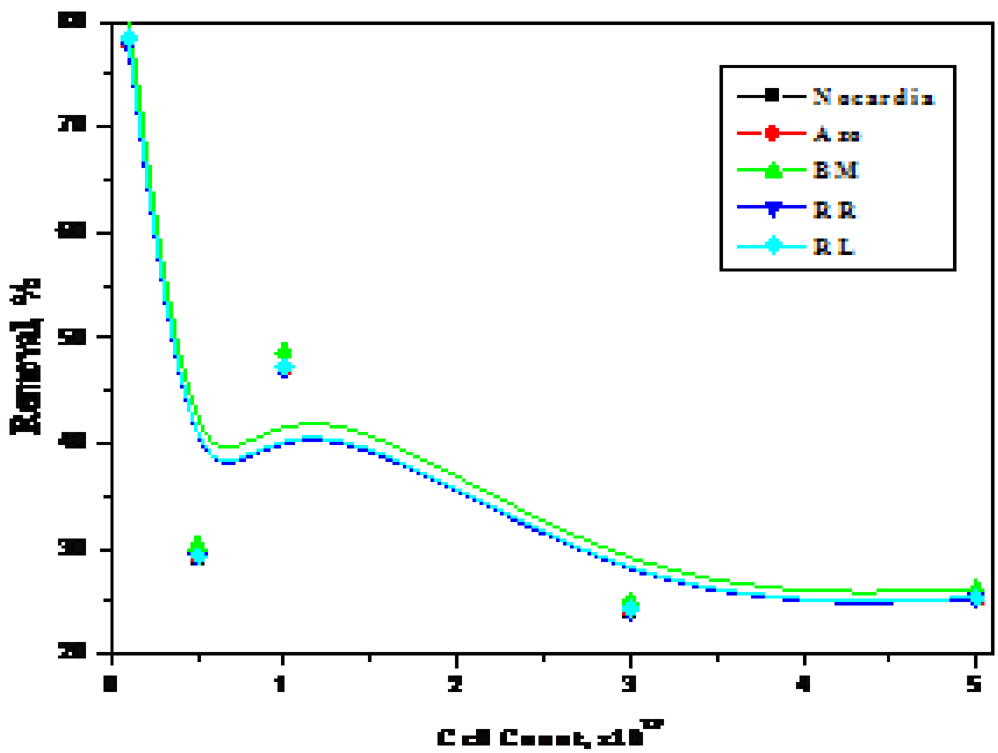

Figure 2: Relative effects of different inoculum size on Cupper bioremediation by different bacterial species

Relative effects of different Temperature on Cupper bioremediation

Effect of different incubation temperature for cupper bioremediation using *Bacillus megaterium EMCC* 1013, *Rhizobium rhizogenes*EMCC1743*, Rhizobium leguminosarum*EMCC1130 *Azotobacter vinelandii and Nocardiopsis Dassonvillei.Bacillus megaterium EMCC* 1013 was the most potent cupper bioremdation percentage with 76 % at 20 ^{o} C followed by *Rhizobium rhizogenes*EMCC1743*, Rhizobium leguminosarum*EMCC1130 *Azotobacter vinelandii and Nocardiopsis Dassonvillei* by 73 % cupper bioremediation at 20 ^{o} C as shown in figure 3. As mentioned by Rajeshkumar et al., 2011

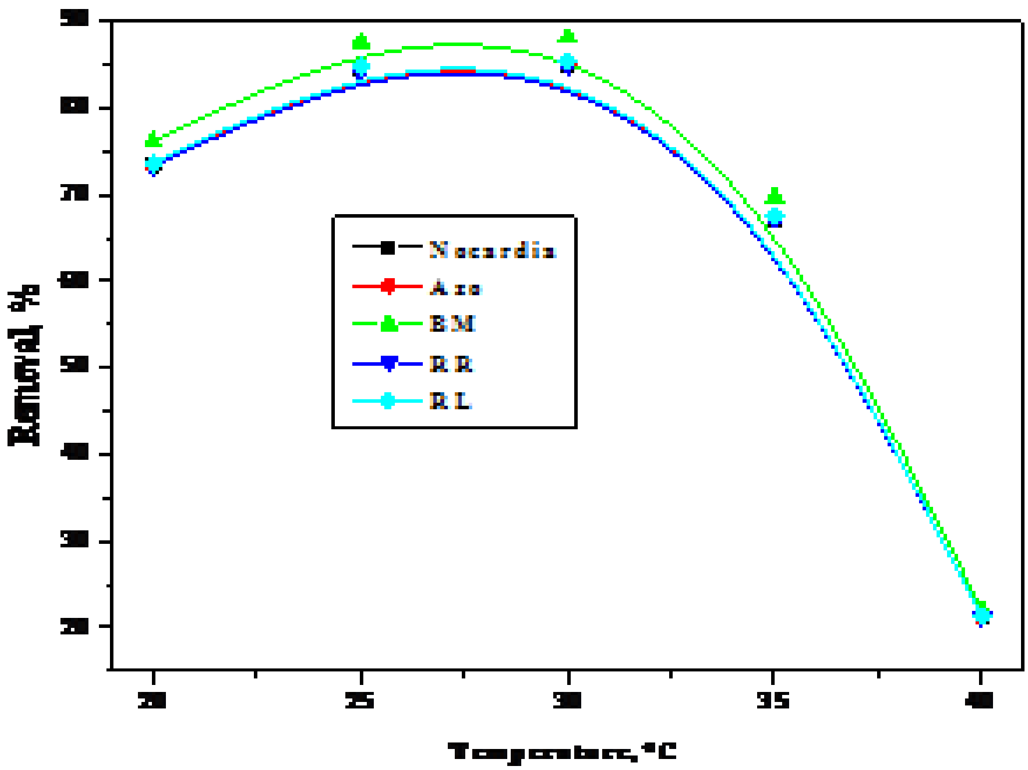

Figure 3: Relative effects of different Temperature on Cupper bioremediation by different bacterial species

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 (Gulay and Yakup, 2003). Mostly adsorption is an exothermic process (Martins et al., 2006), whereas, some examples of endothermic adsorption have also been reported (Davis et al., 2003). Which agree with our bacterial species which included under endothermic adsorption. The results from Rajeshkuma and Kartic, 2011 showed that Bio sorption studies were carried out in the Bacillus sp. With optimum temperature was determined to be 35°Cwith The maximum removal of 88% of cupper which disagree with our results which show that 20 °C was optimum temperature

Relative effects of different PH on Cupper bioremediation

Effect of different PH for cupper bioremediation using *Bacillus megaterium EMCC* 1013*, Rhizobium rhizogenes*EMCC1743*, Rhizobium leguminosarum*EMCC1130 *Azotobacter vinelandii and Nocardiopsis Dassonvillei.Bacillus megaterium EMCC* 1013 was the most potent cupper bioremediation percentage with 65 % at PH 7 followed by other tested bacterial species by 62 % cupper bioremediation at PH 7. At PH 8 cupper bioremediation sharply decreased to 40 % which indicated that alkaline condition is decrease percentage of cupper bioremediation rather than slightly acidic conditionAs mention by Chowdhury and Das, 2012 Bacterial cell surface was negatively charged whereas Cu(ll) was positively charged, and as a result copper may attached with the bacterial cell surface so copper salt’s solution started precipitating above pH 7 as shown in figure 4. The results from Rajeshkuma and

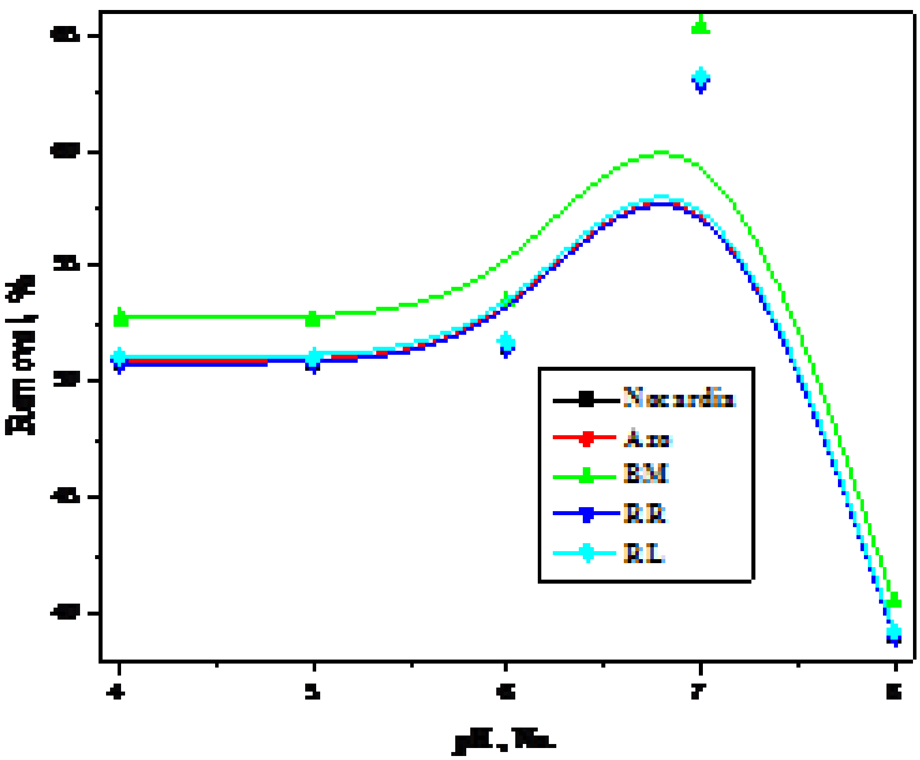

Figure 4: Relative effects of different PH on Cupper bioremediation by different bacterial species

Kartic, 2011 showed that Bio sorption studies were carried out in the Bacillus sp. With optimum PH was determined to 8 with The maximum removal of 88% removal of cupper which disagree with our results which show that 8 was optimum PH with 65 % cupper bioremediation. Literature reports emanating from various authors have shown that pH level is main prevailing factor in bioremediation efficiency by different microrganisms (Pandey et al., 2007). 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 (Sag et al., 2000). At low pH values, lead ions compete with hydrogen ions at the binding sites of the microbial cells.

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 (Van Nostrand et al., 2007)

Relative effects of different Carbon sources on Cupper bioremediation

Effect of different PH for cupper bioremediation using *Bacillus megaterium EMCC* 1013*, Rhizobium rhizogenes*EMCC1743*, Rhizobium leguminosarum* EMCC1130 Azotobacter vinelandii and Nocardiopsis Dassonvillei. Bacillus *megaterium EMCC* 1013 was the most potent cupper bioremediation percentage with 95 % followed by other tested bacterial species by 92 % cupper bioremediation with glucose utilization as carbon source. Utilization of starch and sucrose as carbon source showed sharply decrease in cupper bioremediation with 4 % and 2 % respectively with all tested bacterial species as presented in figure 5. As reported by Rohiniand Jayalakshmi, 2015 evaluation of cupper removal of *Bacillus cereus* by different carbon sources in copper accumulation was recorded highest bioremediation when glucose was used as the sole carbon source (copper content of 42%) as recorded in our work with 95 % cupper removal by *Bacillus megaterium EMCC*(Figure 5)

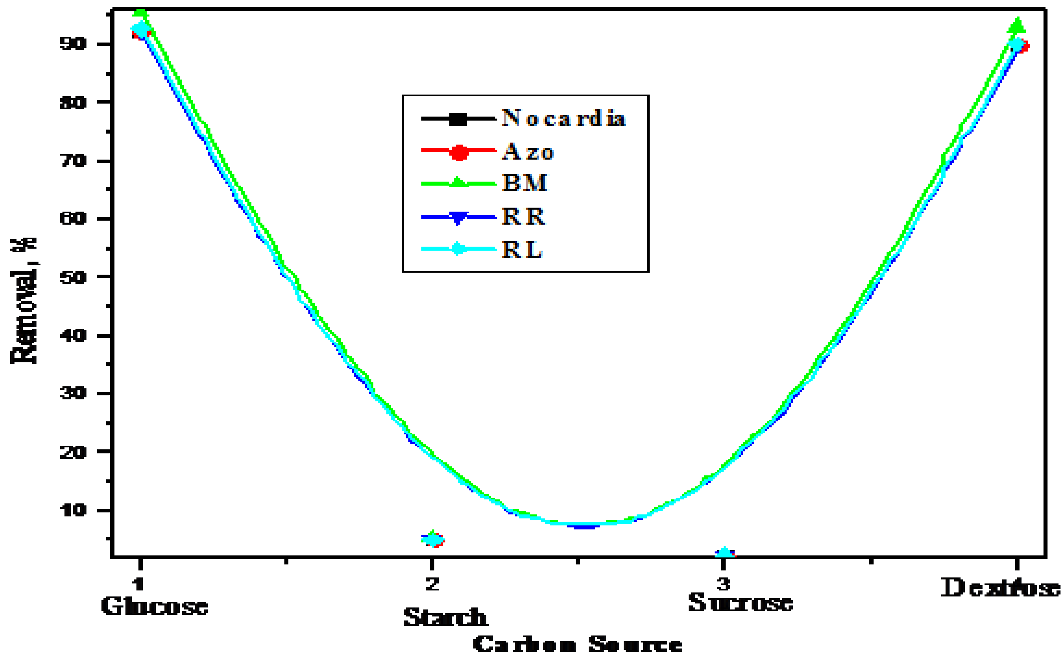

Figure 5: Relative effects of different Carbon sources on Cupper bioremediation by different bacterial species

Relative effects of different Nitrogen sources on Cupper bioremediation

Effect of different PH for cupper bioremediation using *Bacillus megaterium EMCC* 1013*, Rhizobium rhizogenes*EMCC1743*, Rhizobium leguminosarum*EMCC1130 Azotobacter vinelandii and Nocardiopsis Dassonvillei. Bacillus megaterium *EMCC* 1013 was the most potent cupper bioremediation percentage with 99 % followed by other tested bacterial species by 95 % cupper bioremediation with ammonium oxalate utilization as nitrogen source. Utilization of ammonium chloride as nitrogen source showed decrease in cupper bioremediation with 59 % with all tested bacterial species as presented in figure 6.

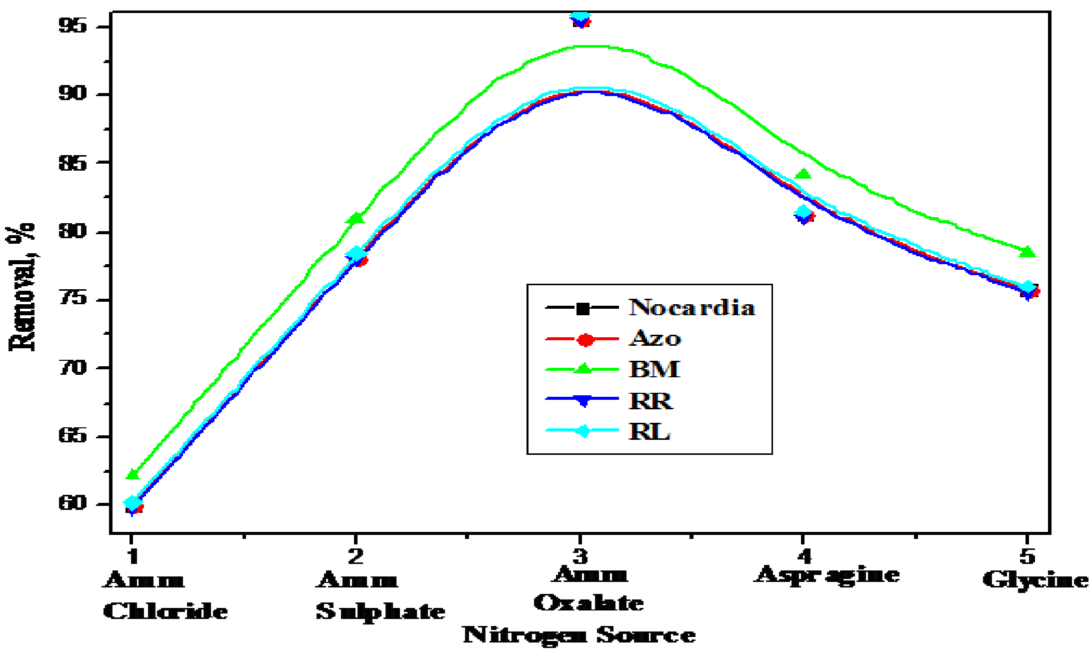

Figure 6: Relative effects of different nitrogen sources on Cupper bioremediation by different bacterial species

As reported by Rohini and Jayalakshmi, 2015 evaluation of cupper removal of *Bacillus cereus by* different nitrogen sources in copper accumulation was recorded highest bioremediation when Beef extract was used as the sole nitrogen source (copper content of 48%)which different recorded in our work with 99 % cupper removal by *Bacillus megaterium EMC*

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