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Full Length Paper

Two Stages Chromium from Industrial Waste Water by *Azotobacter Vinelandii*

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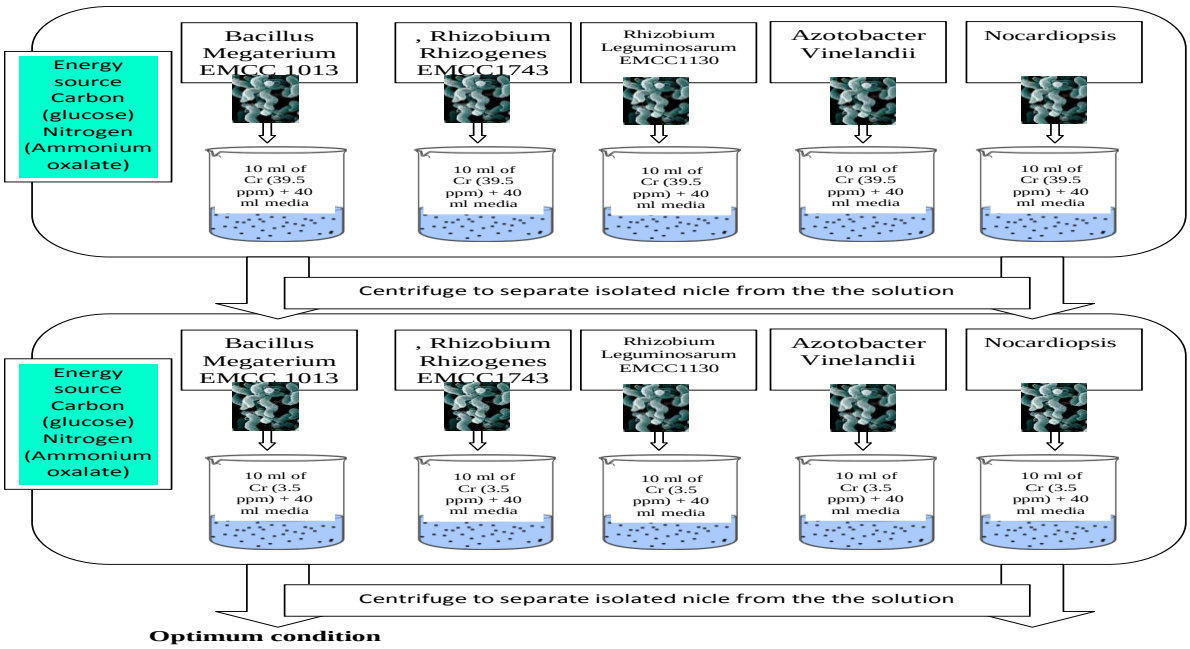
Wastewaters containing chromium are produced from different industries. In food chains it causes dangerous health weakness, carcinogenicity and mutagenesis in different living systems. Thousands Tons of industrial waste water containing chromium are discarded every year. Chromium biomass can be used as a cheap source for chromium. So, In this work five different bacterial species, namely Bacillus Megaterium EMCC 1013, Rhizobium Rhizogenes EMCC1743, Rhizobium Leguminosarum EMCC1130, Azotobacter Vinelandii and Nocardiopsis Dassenvillei were evaluated potential for its activity in bioremediation of chromium. The results show that the five bacterial species have different capacities for chromium removal. Two stage of chromium adsorption. Azotobacter vinelandii has the highest capacity for bioremediation of chromium amounting 10ml (39.5 ppm) with 93.52 % removal during two steps under the optimization condition of 24 h with inoculum size 0.1 x 10^{29} cfu at PH 7 and energy source is glucose and ammonium oxalate as carbon and nitrogen source respectively at 30 °C incubation temprature. The main purpose of this study is to improve the bioremediation of chromium from wastewater during two steps to maximize the percentage of chromium removal. Screening the bioremediation capacity of chromium as heavy metal by different bacterial species encourage using them in future study for removal of chromium from electroplating waste water. In addition, Azotobacter vinelandii as the most effective chromium resistant microorganism will be very useful in biotechnology for the remediation of Cr contaminated environments and can also be used in the construction of biomarkers for the detection of chromium.

Keywords: Environmental pollutants, Azotobacter Vinelandii , Wastewater, Biotechnology

Graphical Abstract of Chromium



Optimum condition

Time: 24 h, Inoculums size 0.1 x 10 29 cfu, pH: 7 , Carbon energy source is glucose

Nitrogen energy source is ammonium oxalate, Incubation Temp.: 30 °C incubation temperature

I. INTRODUCTION

Heavy metals such as chromium, lead, zinc, arsenic, copper, manganese, cadmium, nickel and mercury are considered to be toxic pollutants of freshwater reserves [1]. The toxic effect, non-biodegradablty and long standing accumulation in nature cause cancer and several health problems like organ damage, reduced growth, nervous system impairments and oxidative stress [2]. The increase industrial growth is the main source of heavy metals supplied to the environment including air, water, soil and biosphere. The effected industrial sectors include mining, smelting, surface finishing, electroplating, electrolysis, electric appliances and electric boards/circuits manufacturing also in agriculturel such as fertilizers and pesticides[3].

Many methods have been attempted for the removal of heavy metals from waste water environment. Traditional methods as filtration, flocculation, ion exchange are effective in some cases but are expensive, and less applied in case of normal environmental conditions. Whereas bioremediation is cheap. It includes the utilize of renewable resources like microorganisms and plants to solve heavy metal problems and subsequently to restore the lost fertility of soils [4]. Betwwen several bioremediation scientists utilizes live or dead cells of bacteria [5], fungi [6], yeast [7] and algae [8] to eliminate heavy metals from water and soil.

Volesky and Holan [9] indicated that several types of biomass absorption had efficiente ability to accumulate heavy metal outer their cells. The varity of microbial communities and the bacterial cell mass were one of the most important biosorbents used for metal removal and detoxification,so the adsorption of heavy metals onto bacterial cell walls has met considerable awerness in recent experimental and modelling studies [10&11].

Chromium ordinary found from industrial effluents, seepage, water from refuse dumps, pesticides or corrosive water that have come into contact with fittings and pipes containing chromium. Chromium ions inhibit macromolecule synthesis and other enzymatic reactions [12], which alter microbial community structure which lead to change diversity of microbial community [13], and result to above minimize cell of microbial numbers [14].

Azotobacter vinelandii is a widespread gram-negative, strict-aerobic, and free-living bacterium that fixes nitrogen through the action of nitrogen [15]. This organism has evolved a number of physiological mechanisms to allow it to fix nitrogen aerobically, despite the inherent oxygen sensitivity of nitrogen enzyme. The biochemical study of nitrogen fixation by Azobacter vinelandii is presented in many literatures [16].

The aim of this work is to estimate the removal of chromium as a heavy metal during two stages by evaluate the bioremediation efficiency of five different bacterial species in reducing chromium which pollutes painting waste water.

II. MATERIAL AND METHODS:

A. Microorganisms:

Three bacterial species were purchased from Egyptian Microbial Culture Collection unit, Ain shams university (*Bacillus megaterium*EMCC 1013*, Rhizobium rhizogenes* EMCC1743*, Rhizobium leguminosarum* EMCC1130). *Azotobacter vinelandii* was obtained by El -Badry et al [17] and *Nocardiopsis Dassenvillei* was obtained by Elbarbary et al. [18].

B. Chromium Stock Solution:

Chromium stock solution was prepared using potassium dichromate (KCr_2O_7) dissolved in distilled water. Different Chromium concentrations media were prepared using this solution.

C. Industrial Waste Water Polluted by Chromium:

The percentage of chromium in waste water from painting industry amounts 39.5ppm.

D. Chromium Bioremediation Experiments:

Chromium removal from industrial painting factory waste water was evaluated during two stages of bioremediation using different species of bacteria to increase chromium removal. The first stage of chromium removal was evaluated for different bacteria then for the second stage water treated by bacteria was used in the second stage of chromium removal to increase the amount of chromium removal from waste water.

LB (Luria-Bertani) liquid medium (Oxoid) was used as base media consisting of different concentrations of chromium solution. different pH solutions with values were prepared by adjustment of 0.1(N) HCl and 0.1(N) NaOH solutions. The media was autoclaved in 250 ml conical flasks containing 100 ml media. The media were inoculated with five different bacterial species. After the incubation time the samples were collected and centrifuged at 6000 rpm for 10 minutes. Supernatant solution were assayed for chromium removal ions by Optical Emission Spectrometer Model: Optima 2000 DV Perkin Elmer (Inductive Couple Plasma) at wave length 460 nm by using Chromium complexing agent as sodium diethyl di-thiocarbamate. Bioremediation of chromium ion in base media inoculated with five different bacterial species separately were evaluated applying the following equation:

$$\text{Bioremediation of chromium } \% = \frac{S\ \text{cont} - S\ sampl}{S\ \text{cont}}\ x\ 100$$

E. Relative Effects of Different Chromium Concentration Bioremediation on Microbial Growth

Different species of bacteria 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 chromium for each bacterial species. After 24 h incubation the remediation percentage of chromium concentration on each bacterial growth is assessed**.**

F. Relative Effects of Different Inoculum Size on Chromium Bioremediation

Different species of bacteria were grown in a rotary shaker at 150 rpm and pH 7.0, while the temperature was 37 C in LB 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 incubation the remediation percentage of chromium concentration for each bacterial growth is assessed

G. Relative Effects of Different Temperatureon Chromium Bioremediation

Different species of bacteria were grown in a rotary shaker at 150 rpm and pH 7.0, at a temperature of 37 °C in LB broth medium at different incubation temperatures (20, 25, 30, 35 and 40) °C. After 24 h of incubation the remediation percentage of Chromium concentration on each bacterial growth was assessed

H. Relative Effects of Different PH on Chromium Bioremediation

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

J. Relative Effects of Different Carbon Sources on Chromium Bioremediation

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

K. Relative Effects of Different Nitrogen Sources on Chromium Bioremediation

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

III. RESULTS AND DISCUSSION:

Numerous biological systems have been improved for bioremediation of industrial wastewater containing heavy metal ions [19]. Microorganisms, mainly bacteria, have been found to be efficient for environmental clean-up [20]. It has been proved that genetically determined resistance systems against heavy metal stress after a continuous

exposure to heavy metals have been developed. These bacteria not only resist pollutants but also show great potential for practical use in bioremediation due to the genetic basis of the resistance mechanism [21].

A. Effects of Chromium Concentration on Microbial Growth During Two Stages Bioremediation.

Different species of bacteria namely *Bacillus megaterium* EMCC 1013, *Rhizobium rhizogenes* EMCC1743, *Rhizobium leguminosarum* EMCC1130, *Azotobacter vinelandii* and *Nocardiopsis Dassenvillei* were evaluated for their effect on chromium bioremediation under different concentrations of chromium with 88.87, 88.36, 88.48, 91.20 and 89.95 % respectively for the first stage while it was 20.47, 20.49, 45.96, 26.89 and 35.89 respectively for the second stage 10 ml 39.5 ppm to become 91.14, 90.75, 93.79, 93.52 and 93.59 of chromium on two stages. Decrease in chromium bioremediation for all tested microorganism by increasing chromium concentration is shown in (Figs. 1 and 2). The resistance of rhizobia species to the heavy metals Pb, Cu and Zn was evaluated and found to rise high potency to heavy metal resistance as proved by Khalid and Abdel-lateif [22] which agrees with the results obtained. *Azotobacter vinelandii* has the most chromium removal capacity with 93.52 % in first stage and 91.20 % in second stage of bioremediation with removal 26.89 %. Also, most potent organism isolated from contaminated soil that showed multi resistance to all heavy metals tested were identified as *A. chroococcum* as reported by Aly, et al.[23]. On the other hand chromium removal with 91.14 % was achieved using *Bacillus megaterium*. Similar results were obtained in removal of chromium from polluted environments were reported by Kumar and Achyuthan [24].

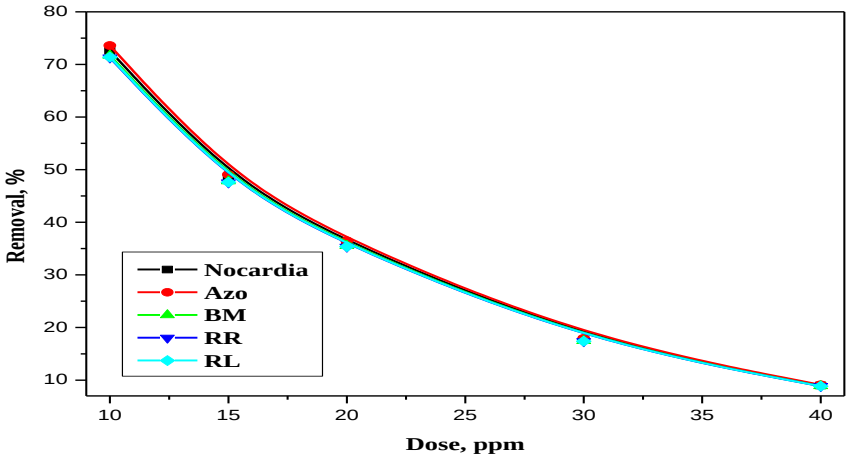


Figure 1: Effects of different chromium concentrations on first stage using bioremediation by different bacterial species.

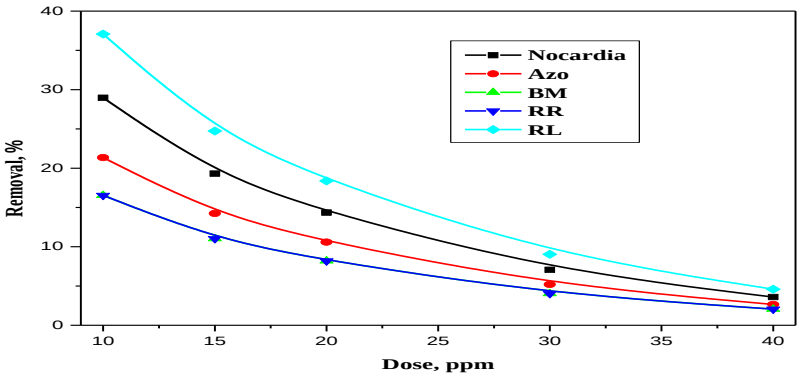


Figure 2: Effects of different chromium concentrations on bioremediation by different bacterial species during second stage

B. Effects of Different Inoculum Size on Chromium Bioremediation

Different inoculum size of five bacterial chromium bioremediation were studied and the results are shown in figure 3 with 10 ml of 39.5ppm concentration of chromium. The results indicate that increase in bacterial cell count decreases the percentage of chromium bioremediation. The highest bioremedation was achieved by using inoculum size 0.1 x 10^{29} cfu of five different bacterial species as Azotobactesr vinelandii was 74.55 % after first stage of chromium removal, as shown in Figs. 3 and 4.

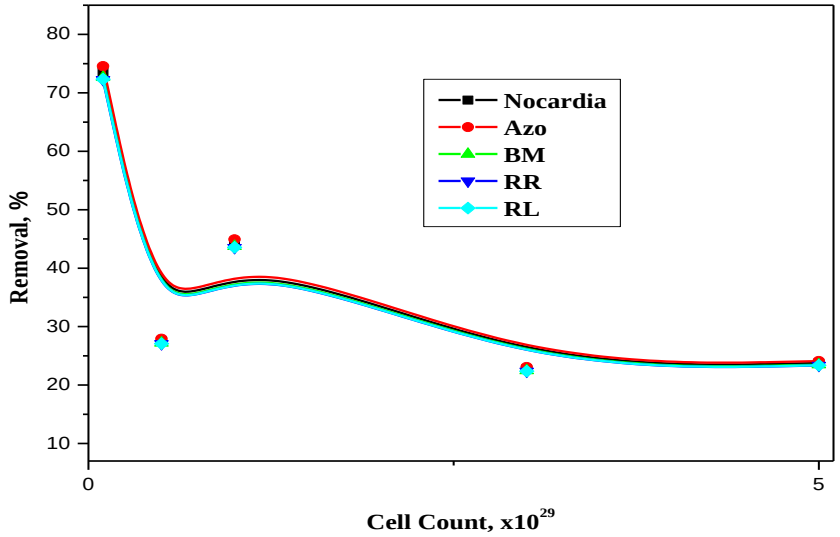


Figure 3: Effect of different inoculum size on chromium bioremediation using different bacterial species in the first stage

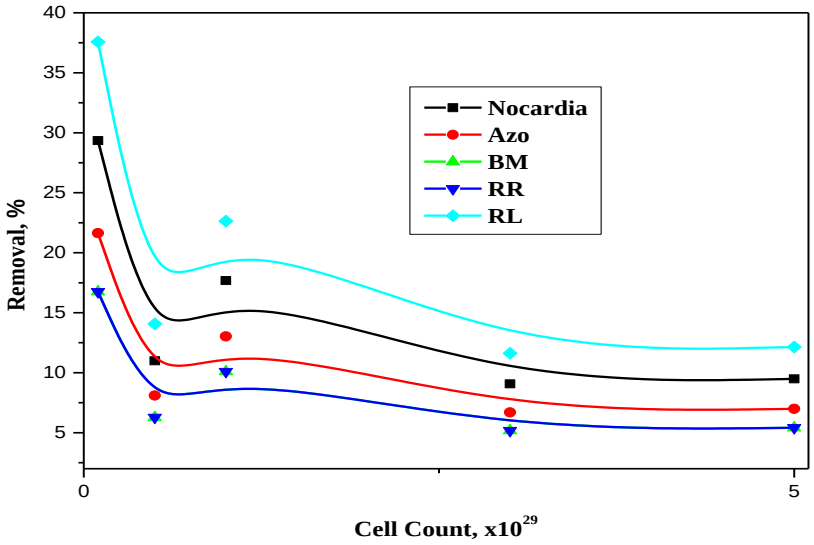


Figure 4: Effects of different inoculum size on chromium bioremediation using different bacterial species in the second stage

C. Effect of Temperature on Chromium Bioremediation

The effect of different incubation temperatures on chromium bioremediation using *Bacillus megaterium* EMCC 1013, *Rhizobium rhizogenes* EMCC1743, *Rhizobium leguminosarum* EMCC1130 *Azotobacter vinelandii* and

*Nocardiopsis Dassenvillei***.** *Azotobacter vinelandii* was studied. It is found that the most potent chromium bioremdation percentage with 84.15 % at 30 °C. El-barbary et al [25] reported that copper bioremediation of *Azotobacter vinelandii* was amounts 70.05 % at 20 °C. as shown in Figs. 5 and 6.

Temperature effects the stability of bacterial wall, and alter with ionization of its chemical structure. The binding sites of the isolated bacterial species affected by these factors and cause reduction in metal removal. Energy-independent mechanisms are less likely affected by temperature since the processes responsible for removal are largely physiochemical in nature [26]. Mostly adsorption is an exothermic process, some examples of endothermic adsorption have also been reported.

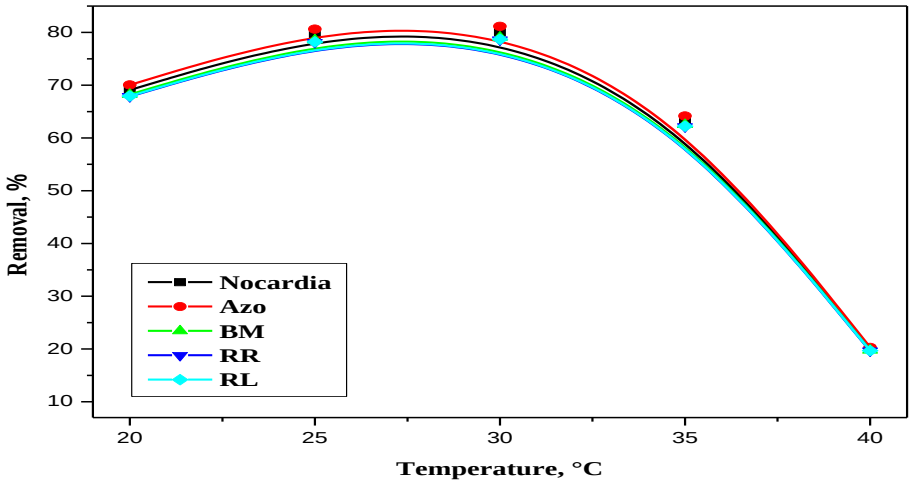


Figure 5: Effect of different temperatures on chromium bioremediation using different bacterial species in the first stage

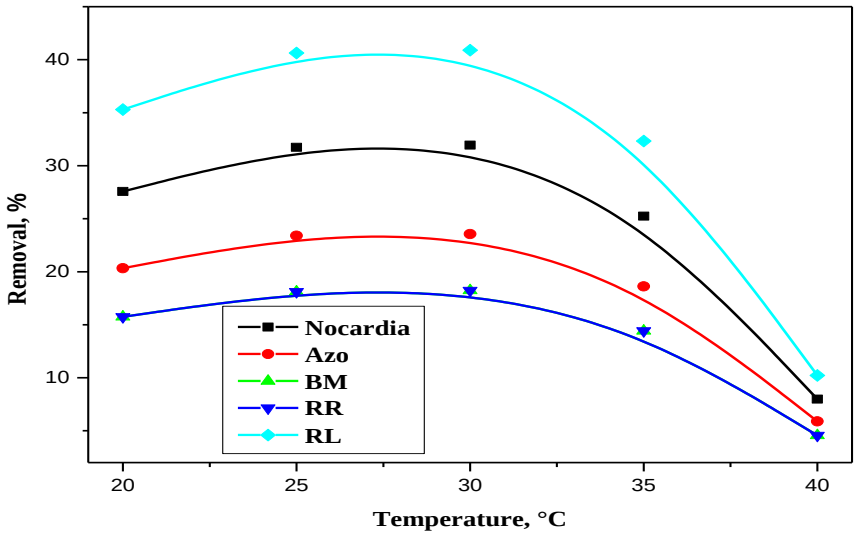


Figure 6: Effect of different temperatures on chromium bioremediation by different bacterial species second stage

D. Effect of Different pH on Chromium Bioremediation

The influence of pH values in chromium removal using five different species of bacteria were evaluated. the most potent chromium removal with 60.16 % at PH 7. And at PH 8 chromium bioremediation sharply decreased to

37.27 % which indicated that alkaline conditions decrease the percentage of chromium bioremediation rather than the slightly acidic conditions. Bacterial cell wall has negative charge while chromium positive charge so attached with the bacterial cell wall as shown in Fig. No 4. The results show that with the optimum pH with a maximum removal of 60.16% removal of chromium whereas the results obtained which show that 7 was the optimum pH with 60.16 % chromium bioremediation. Literature reports from various authors have shown that the pH level is the main prevailing factor in bioremediation efficiency by different microrganisms[27].

The optimum removal percentage was noted by all bacterial species in this study at neutral pH 7 which follow this fact the ideal pH range for bio removal for bacteria ranged from 6.0-8.5 [28] Figs. 7 and 8.

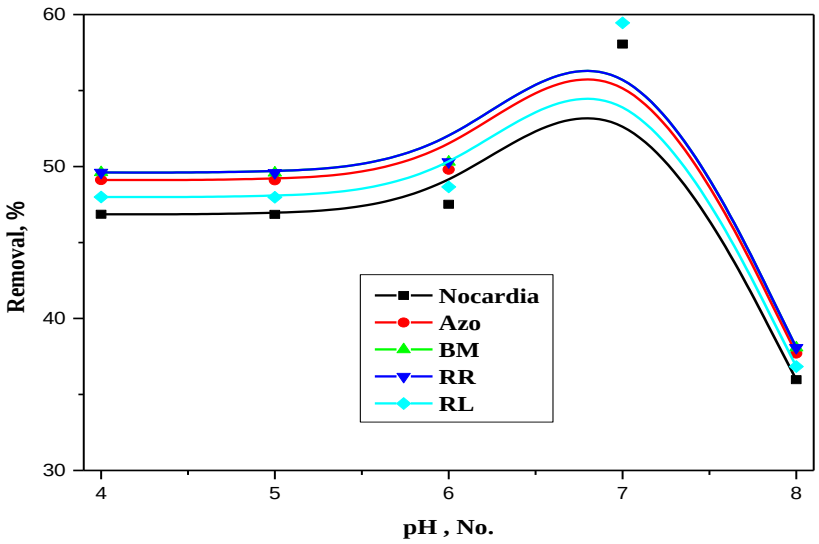


Figure 7: Effect of different pH on chromium bioremediation by different bacterial species during first stage

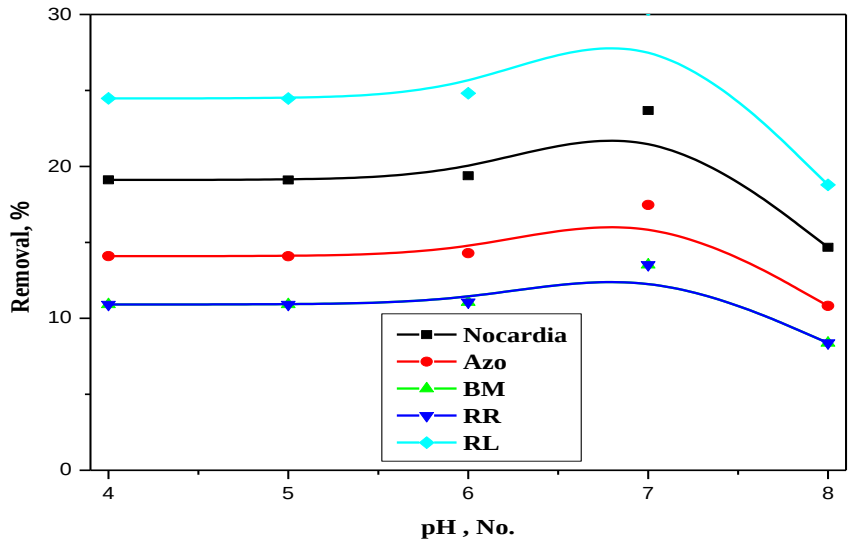


Figure 8: Effect of different pH on chromium bioremediation by different bacterial species during second stage

E. Effect of Different Carbon Sources on Chromium Bioremediation

The effect of different carbon sources on chromium bioremediation using *Bacillus megaterium* EMCC 1013, *Rhizobium rhizogenes* EMCC1743, *Rhizobium leguminosarum* EMCC1130 Azotobacter *vinelandii* and *Nocardiopsis Dassenvillei*. *Azotobacter vinelandii* was found to be most potent as chromium bioremediation percentage of 88.25 % Chromium bioremediation with glucose utilization as carbon source Figs. 9 and 10. These results showed similarities with El-barbary et al.[25] as glucose is the best carbon sources for chromium bioremediation.

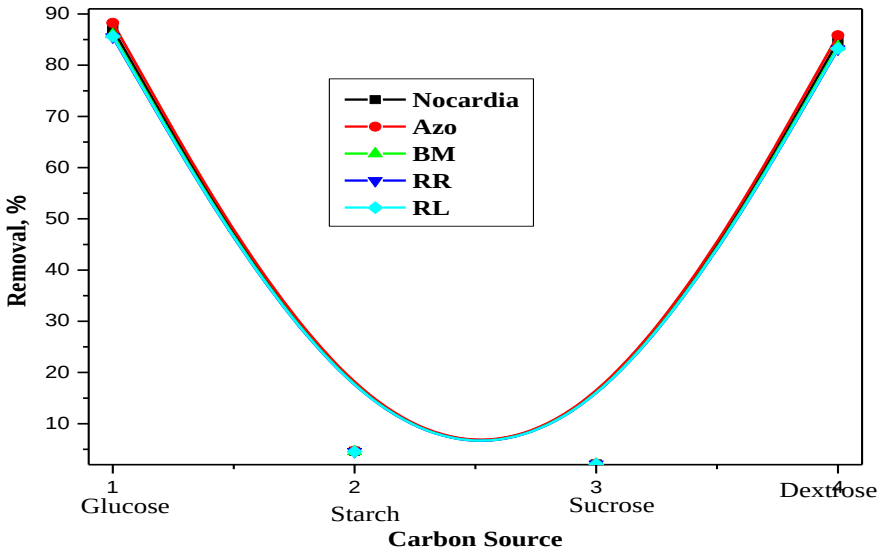


Figure 9: Effects of different carbon source on chromium bioremediation by different bacterial species during first stage

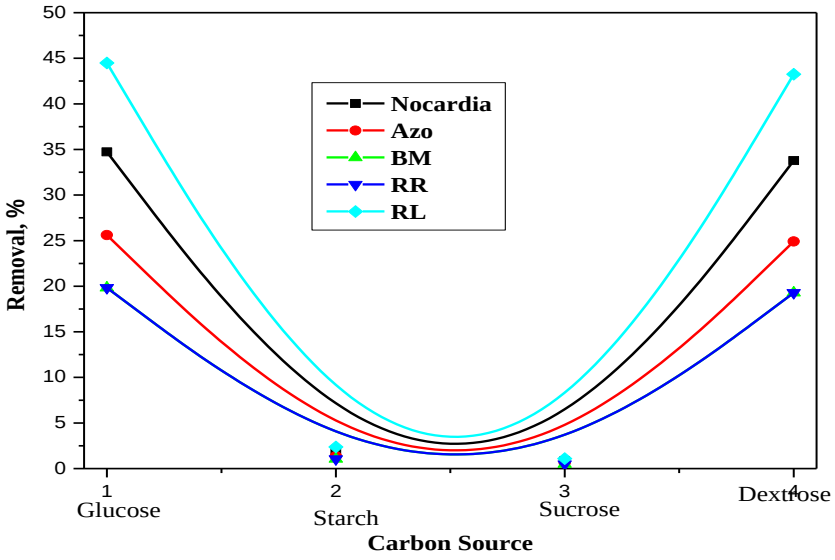


Figure 10: Effect of different carbon source on chromium bioremediation by different bacterial species during second stage

F. Effects of Different Nitrogen Sources on Chromium Bioremediation

The effect of different nitrogen sources for chromium bioremediation using *Bacillus megaterium* EMCC 1013, *Rhizobium rhizogenes* EMCC1743, *Rhizobium leguminosarum* EMCC1130 Azotobacter *vinelandii* and *Nocardiopsis Dassenvillei* was studied. Azotobacter *vinelandii* was found to be the most potent chromium bioremediation organism with percentage with 93.52 %. Chromium bioremediation with ammonium sulphateutilization as nitrogen source Figs. 11 and 12. As reported by El-barbary et al.[25] as ammonium oxalate as nitrogen source is the best nitrogen sources for Chromium bioremediation.

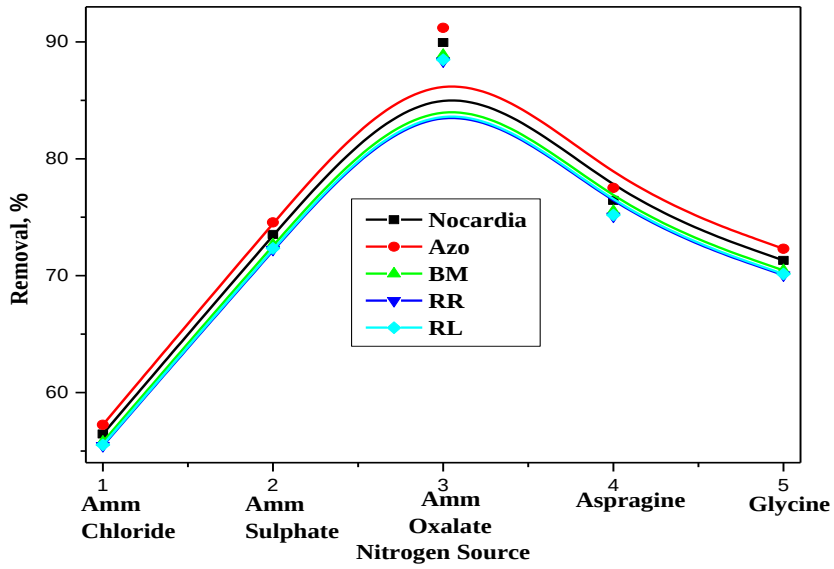


Figure 11: Effects of different nitrogen source on chromium bioremediation by different bacterial species during first stage

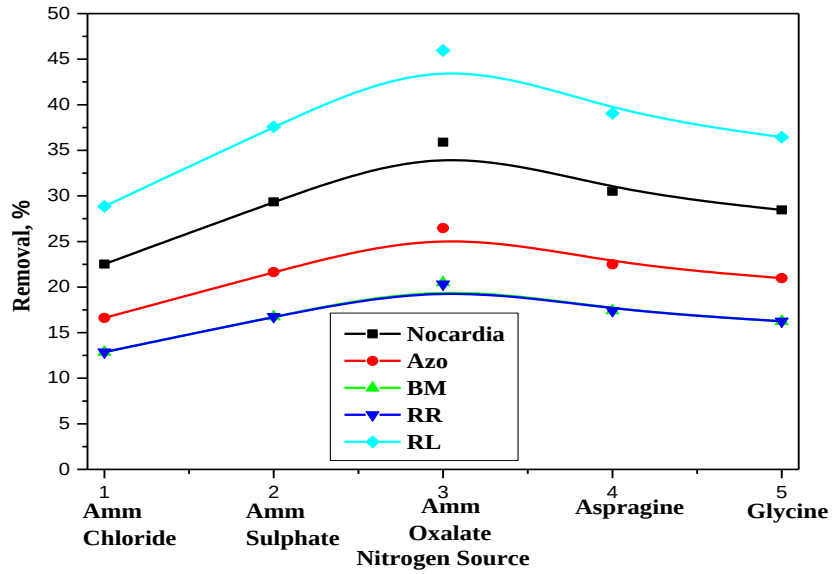


Figure 12: Effects of different nitrogen source on chromium bioremediation by different bacterial species during second stage

VI. CONCLUSIONS

It was found that Azotobacter vinelandii had the highest capacity for bioremediation of Chromium 10ml of 39.5ppm with 97.67 % removal during two steps the first one remove 91.20 while the second remove 26.47 after optimization condition 24 h with inoculum size 0.1 x 10^{29} cfu at PH 7 and energy source glucose and ammonium oxalate as carbon and nitrogen source and 30 °C as incubation temprature. The main value of this study was to improve the bioremediation of chromium from water during two steps to maximize percentage of chromium removal. Also, screening the bioremediation abiliy for chromium as heavy metals usind varity of bacterial specieswill open gates for other work in removal of chromium from plating waste water. Also, Azotobacter vinelandii as the most potent chromium resistant microorganisms will be intrested in biotechnology work for the removal heavy metal from environments with chromium and also aplied in the construction of biomarkers for the detection of chromium**.**

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