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Full Length Paper.

Nickel (II) bio recovery from industrial wastewater by *Azotobacterial vineland* during Two stage processes

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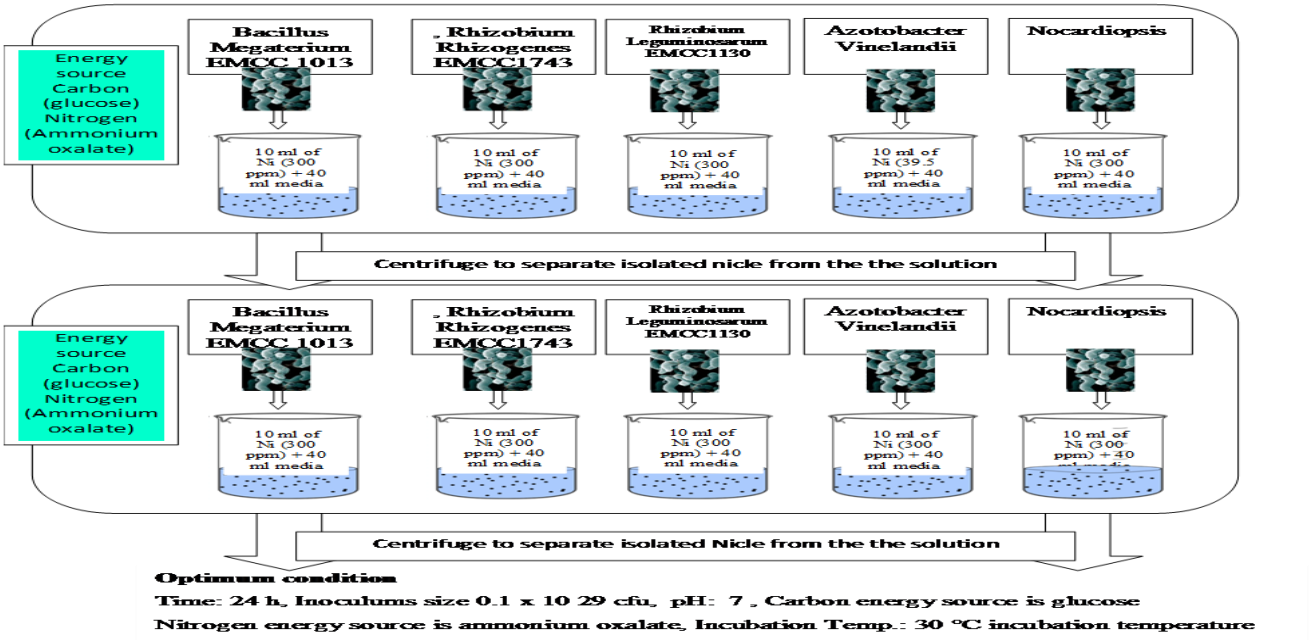
The ability of heavy metal removal, especially nickel (II) by five different bacterial species was evaluated during this work. Industrial plating waste water contains high concentrations of heavy metals that may contaminate food chain during cultivation. Washing water using industrial water without pre-treatment causes sever health weakness and mutagenesis in many ecosystem living. Nickel (II) is discarded during wastewater each year. Nickel (II) bio recovery as less expensive and environmentally friendly process were screened during this work by five different bacterial species Bacillus megaterium EMCC 1013, Rhizobium rhizogenes EMCC1743, Rhizobium leguminosarum EMCC1130, Azotobacter vinelandii and Nocardiopsis Dassenvillei Azotobacter vinelandii exhibited maximum recovery of Nickel (II) at concentration 10 ppm with 88 % recovery during two stages recovery processes and 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 30o C as incubation temprature. The principle target of this work was confirming the bio recovery of Nickel (II) from industrial waste water through two stages to raise the percentage of Nickel (II) removal. In addition, evaluation the bio recovery ability of Nickel (II) by different five microorganisms to utilize them in further studies in the removal of Nickel (II) from plating waste water. Azotobacter vinelandii as the most potent Nickel (II) removal in this study will be used in biotechnology for the bio recovery of Nickel (II) and also used as biomarkers for the detection of Nickel (II)

Keywords: Nickel, Azotobacter vinelandii, Waste water, Heavy metal, bioremoval

Graphical Abstract of Nickel



I. INTRODUCTION

Bioremediation process is the most charming where naturally occurring biomass or used microbial biomass from many fermentation industries can be effectively utilized [1]. Bioremediation process also has prospect incentives for bio absorption as viable cleanup methods for heavy metal pollution. Immobilization process advantages of bioremediation are its stability also can be used alternately with ease for the process of adsorption/ desorption [2]. Successful results have been achieved with reference to the bio sorption of chromium, nickel and zinc [3].

Plating industrial activities such as waste dumping, industrial effluents, burning fuels, corrosion of water pipes and natural phenomena like acid rains and acid mine drainage result in 'higher than tolerable' levels of metals in environment. These metals can cause severe problems when they are present in too high levels as they would if they are present in too low levels. Unlike organic contaminants, which can be degraded to harmless chemical species, heavy metals cannot be mineralized [4].

Industrialization and several technology development increase burden on the environment by accumulation huge tons of toxic waste, heavy metals and organic contaminants that have severe destruction on the environment and ecosystem. The accumulation of heavy metals in soils and waters continues to create serious global health concerns, these heavy metals cannot be degraded into non-toxic forms, but persist in the ecosystem. Contamination of the environment with heavy metals has increased beyond the recommended limit and is detrimental to all life forms [5].

Most of these heavy metals are toxic with low doses and are able to enter the food chain, where they accumulate and inflict damage to living organisms. heavy metals also, have the potential to exhibit harmful effects at higher concentrations and the toxicity of each metal depends on the amount available to organisms, the absorbed dose, the route and the duration of exposure [5].

Bioremediation is used for heavy metal removal from polluted water and soil. This method uses inherent microbial mechanisms to remove hazardous contaminants using microorganisms and plants, or their products, to restore polluted environments to their original condition [6].

Microorganisms exhibit fabulous different metabolic pathways that degrade many toxic compounds as energy source for their growth. Due to their characteristic degradative enzymes for a particular contaminant, they have evolved diverse mechanisms for maintaining homeostasis and resistance to heavy metals, in order to adapt to toxic metals in the ecosystem [7].

This study aimed to screening the bio removal of Nickel (II) as heavy metals during two stages by different bacterial species to reduce Nickel (II) concentration in painting waste water factories after the manufacturing process

II. MATERIAL AND METHODS

A. Microorganisms

Three bacterial species were purchased from Egyptian 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 [9] and *Nocardiopsis Dassenvillei* was obtained by Elbarbary et al. [10].

B. Nickel (II) stock solution:

Synthetic Nickel (II) sample was prepared by dissolving Nickel Sulphate of 4.476 g in one liter of distilled water to make 1000 ppm stock solution. The solution was diluted to get the concentrations

C. Industrial waste water polluted by copper

The concentration of Nickel (II) in industrial electroplating wastewater was evaluated as 300 ppm.

D. Nickel (II) bioremediation Experiments

Nickel (II) Removal from industrial plating industry waste water was evaluated through two stages processes of bio recovery by different bacterial species to maximize the efficacy of Nickel (II) removal. First stage Nickel (II) removal percentage evaluated for different bacteria first then second stage we used the waste water treated by bacteria to second Nickel (II) removal to maximize bio recovery in plating waste water

LB (Luria-Bertani) liquid medium (Oxoid) was used as basal media consists of different ppm concentration of nickel (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 nickel removal by Optical Emission Spectrometer Model: Optima 2000 DV Perkin Elmer (Inductive Couple Plasma). Bio removal of nickel ion in basal media inoculated with five different bacterial species separately were evaluated by following equation $\text{Bioremediation of Ni (II) \%} = \frac{\text{S cont} - S\ sampl}{\text{S cont}}\ x\ 100$. All the glassware was cleaned with 5% HNO_3.

E. Relative effects of different copper 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 Nickel (II) for each bacterial species. After 24 h of incubation the bio recovery percentage of Nickel (II) concentration on each bacterial growth was evaluated

F. Relative effects of different inoculum size on Nickel (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 bio recovery percentage of Nickel (II) concentration on each bacterial growth was evaluated

G. Relative effects of different Temperature on Nickel (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 bio recovery percentage of Nickel (II) concentration was evaluated

H. Relative effects of different pH on Nickel (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 Nickel (II) concentration was screened

I. Relative effects of different Carbon sources on Nickel (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 copper concentration was evaluated

J. Relative effects of different Nitrogen sources on Nickel (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 Nickel (II) concentration on each bacterial growth was assessed

III. RESULTS AND DISCUSSION:

Microbial bioremediation considered as environment friendly and less expensive alternative compare with chemical methods. Bio recovery of heavy metals by microbial cells has been established as a unique alternative to existing technologies for recovery of heavy metals from industrial waste surroundings. Our work suggests that

Azotobacter vinelandii had high applications efficacy in degrading and removing Nickel (II) from industrial plating water wastes.

A. Relative effects of different Nickel (II) concentration during two bio removal stages 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 Nickel (II) bio recovery by various concentration of Nickel (II) after two stages treatment with 63, 62, 60, 75 and 70 % respectively for 10 ppm of Nickel (II) on two stages. Decrease in Nickel (II) recovery by all bacterial species by increase in Nickel (II) concentration as shown in (figure No. 1 and 2). The bioremoval uptake of Nickel (II) depend on its concentration in the medium and was increased with increase in nickel concentration in the liquid medium up to 20 ppm whereas maximum recovery of Nickel (II) was observed [11]. Microorganisms had different capacity to remove and uptake heavy metals in base of the different factors like concentration of inoculum so different optimization was evaluated during this work to improve bio-removal of Nickel (II).

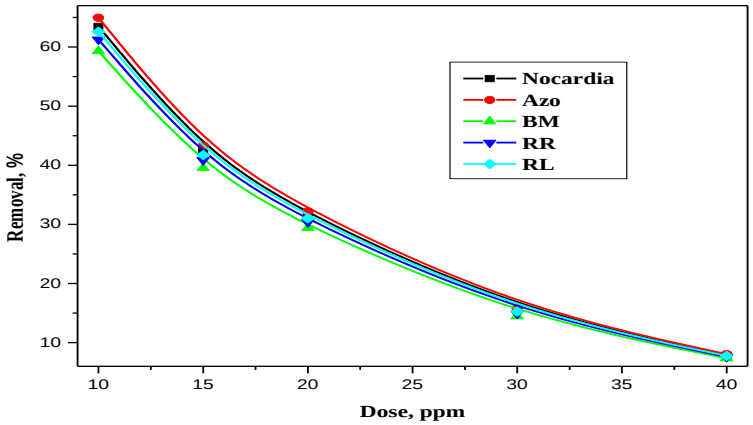


Figure 1: Relative effects of different Nickel (II) (ppm) concentration bioremediation by different bacterial species first stage

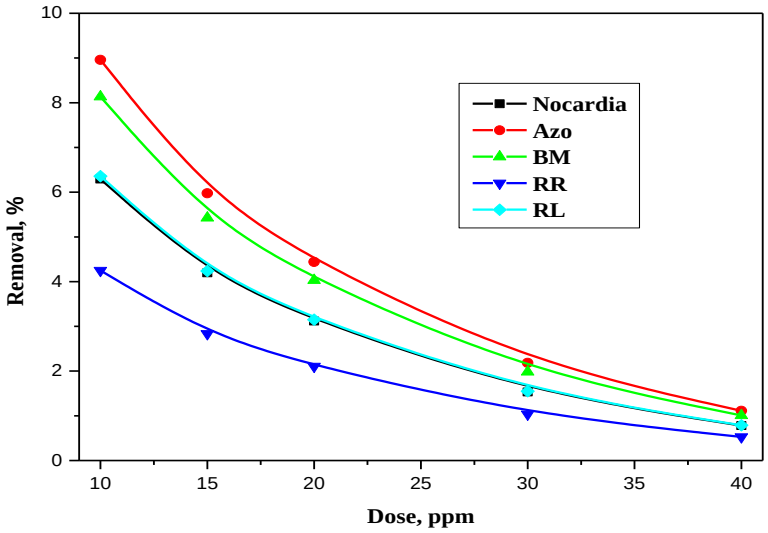


Figure 2: Relative effects of different Nickel (II) (ppm) concentration bioremediation by different bacterial species second stage

B. Relative effects of different inoculum size on Nickel (II) bio removal during two bio removal stages on microbial growth

Different inoculum size of five bacterial Nickel (II) bioremediation evaluated was screened for their ability to bio removal of Nickel (II) with 10 ppm concentration of Nickel (II). The results indicated as increase in bacterial cell count decrease percentage of copper bioremediation. The highest bioremedation was by using inoculum size 0.1 x 10^{29} cfu of five different bacterial species as Azotobacter vinelandii was 80.2 % Nickel (II) removal after second stage of Nickel (II) as shown in figure No 3 and 4. Bioremediation of Zn (II) by Bacillus megaterium EMCC 1013 which the optimum inoculum size was 0.1 x 10^{29}

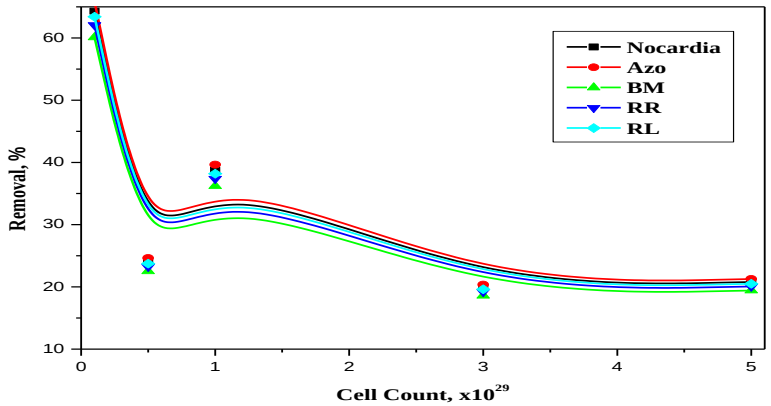


Figure 3: Relative effects of different inoculum size on Nickel (II) bio removal by different bacterial species first stage

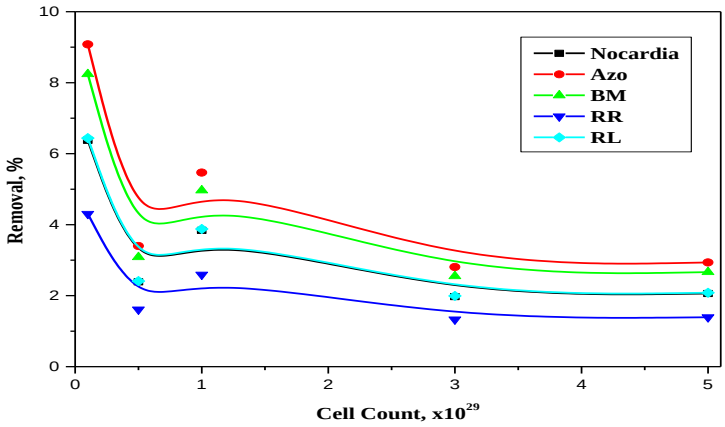


Figure 4: Relative effects of different inoculum size on Nickel (II) bio removal by different bacterial species second stage

C. Relative effects of different Temperature on Nickel (II) bio removal during two bio removal stages on microbial growth

Different incubation temperature of five bacterial species was evaluated for their ability to bio removal of Nickel (II) with 10 ppm concentration of Nickel (II) and inoculum size 0.1 x 10^{29} cfu. *Azotobacter vinelandii* was the most potent Nickel (II) removal percentage with 83.7 % at 30°C as shown in fig. 5 and 6

Temperature had great affect at the stability of the cell wall, its configuration which also makes ionization of chemical moieties. The binding sites on the bacteria 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 [12]. whereas, some examples of endothermic adsorption have also been reported [13].

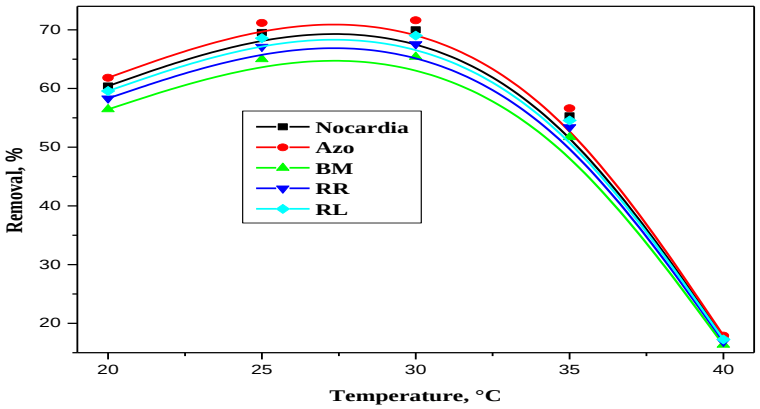


Figure 5: Relative effects of different temperature on Nickel (II) bio removal by different bacterial species first stage

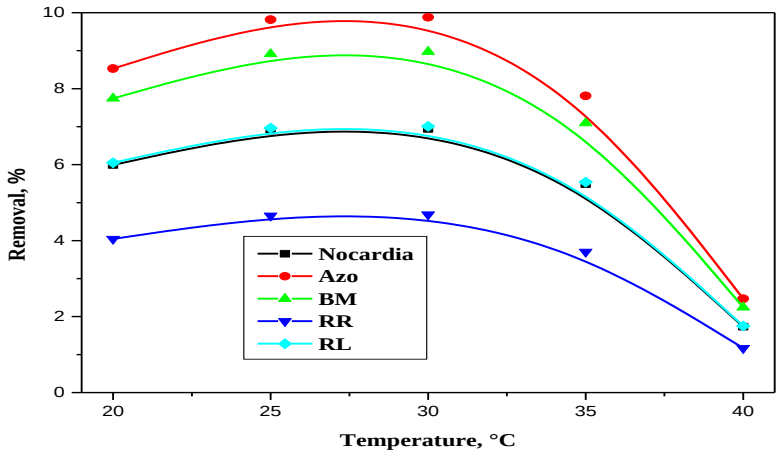


Figure 6: Relative effects of different temperature on Nickel (II) bioremoval by different bacterial species second stage

D. Relative effects of different pH on Nickel (II) bio removal

Effect of different pH for Nickel (II) bioremediation evaluated by using five different species of bacteria *Azotobacter vinelandii* was the most potent Nickel (II) bioremediation percentage with 56 % at PH 7.

Samarghandi et.al. [14] revealed that the bio removal of Nickel (II) from waste water. And the impact of his work exhibit that the bio removal potential increased by increasing pH and decreased with initial Nickel (II) concentration. Experimental. The optimum adsorption activity was 22.47 mg/g at a pH value of 7.

From the results of this work, the maximum bioremediation percentage rates were 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 [15] Figs. 7 and 8.

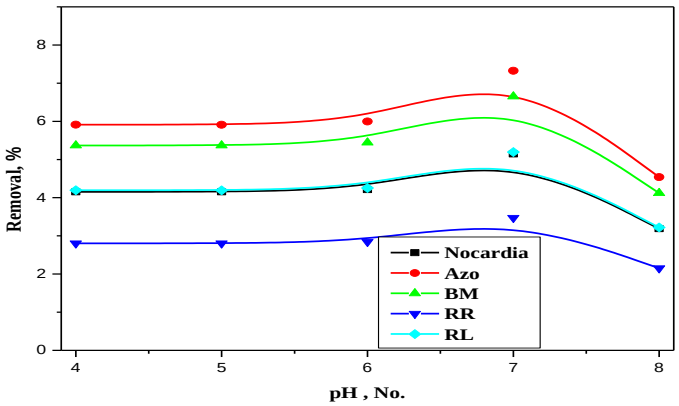


Figure 7: Relative effects of different temperature on Nickel (II) bio removal by different bacterial species first stage

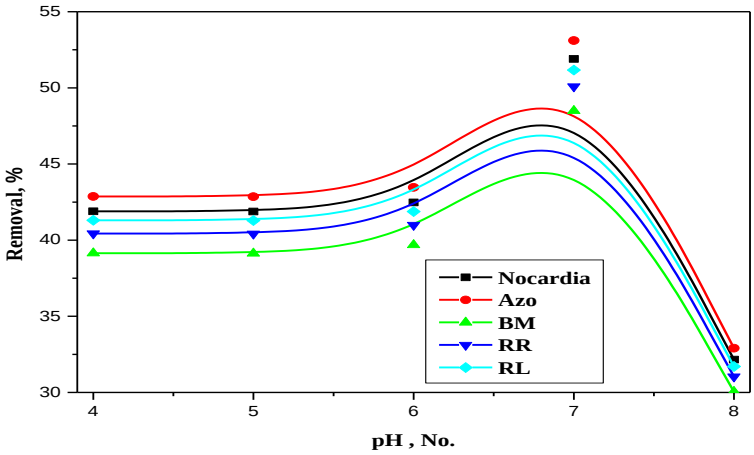


Figure 8: Relative effects of different temperature on Nickel (II) bio removal by different bacterial species second stage

E. Relative effects of different Carbon sources on Nickel (II) bioremoval

Different carbon source for Nickel (II) bioremoval was studied using five different bacterial species. Azotobacter *vinelandii* was the most potent Nickel (II) bioremediation percentage with 88 % Nickel (II) bioremediation with glucose utilization as carbon source figures No 9 and 10. This results the similar to work as reported by El-badry et al. [9]. Azotobacter vinelandii for phosphate dissolution with high efficacy with glucose which reaches to 52.8% then dextrose with low 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

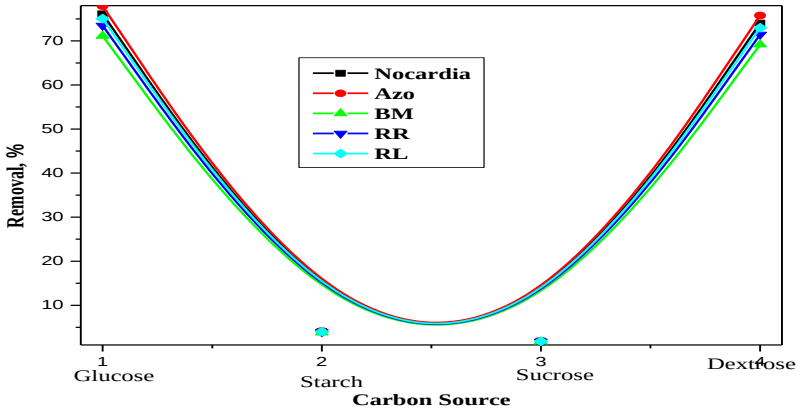


Figure 9: Relative effects of different carbon source on Nickel (II) bio removal by different bacterial species first stage

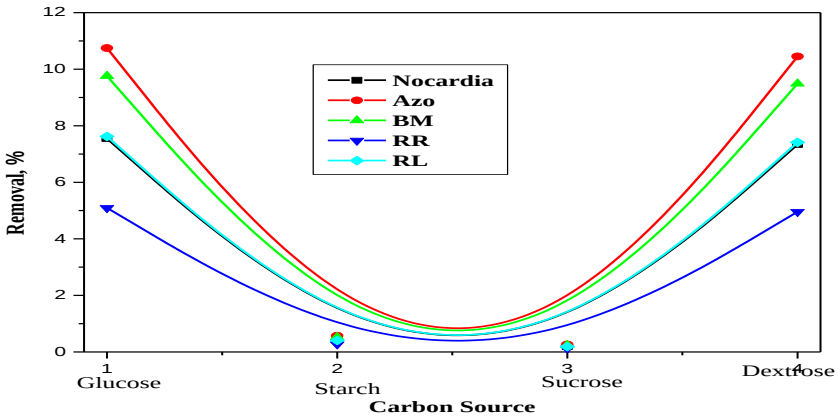


Figure 10: Relative effects of different carbon source on Nickel (II) bio removal by different bacterial species second stage

F. Relative effects of different Nitrogen sources on Nickel (II) bioremoval

Different nitrogen sources for Nickel (II) bio removal was studied by using five different bacterial species. Azotobacter *vinelandii* was the most potent Nickel (II) bio removal percentage with 56 % Nickel (II) bio removal with ammonium sulphate utilization as nitrogen source figures 11 and 12. As mentioned by El-Badry et al., [9]. Nocardiopsis dassenvillei dissolution high quantitiy of phosphorus from rock phosphate ore using ammonium oxalate as nitrogen source followed by ammonium sulphate

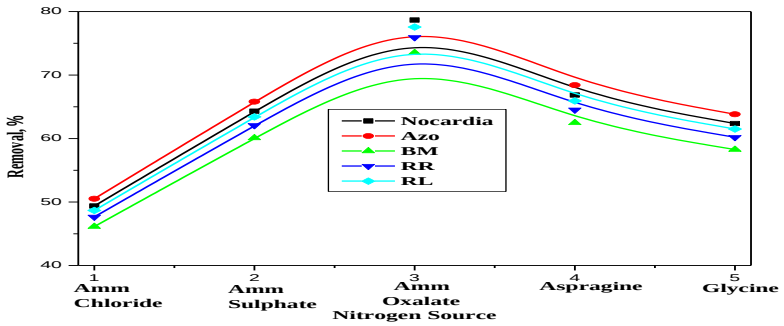


Figure 11: Relative effects of different nitrogen source on Nickel (II) bio removal by different bacterial species first stage

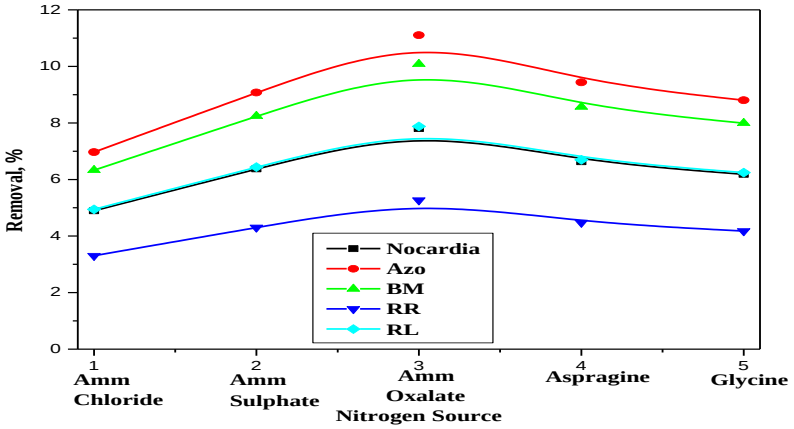


Figure 12: Relative effects of different nitrogen source on Nickel (II) bio removal by different bacterial species second stage

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