Al-Muttairi and Al-Mayaly Iraqi Journal of Science, 2023, Vol. 64, No. 8, pp: 3859- 3866
DOI: 10.24996/ijs.2023.64.8.13

ISSN: 0067-2904

The Potential Efficiency of *Bacillus subtilis* AIK to Remove Nickel from Aqueous Solutions

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Received: 20/8/2022 Accepted: 28/10/2022 Published: 30/8/2023

Abstract

In this study a new strain of mesophilic *Bacillus subtilis* AIK, recorded for the first time in Iraq, was used to remove nickel (Ni) from aqueous solutions. The factors that affect bioremediation include temperature, pH value and metal concentrations. The results showed that the highest removal efficiency (R%) was 54, 52 and 48% at 25ºC and pH of 5, 7 and 9, and with 10 ppm Ni concentration respectively. Whereas the highest R% recorded was 47, 45 and 52% at 30ºC and of pH 5, 7, and 9 with 1 ppm Ni concentration respectively. On the other hand, the highest R% at 40ºC was 49, 46, 42 % at pH 5, 7 and 9, with 5, 10 and 10 ppm Ni concentrations respectively. The results also showed that the optimum pH value for Ni removal at both 25 and 40ºCwas 5, while at 30ºC it was 9. The optimum temperature was kept at 25ºC. In general, *B. subtilis* AIK showed an admissible capacity to remove Ni from aqueous solutions.

Keywords: Heavy metals, Nickel, Bacillus subtilis, Mesophilic bacteria, Bioremediation.

الفعالية المحتملة لبكتريا (*Bacillus subtilis AIK*) لإزالة النيكل من المحاليل المائية

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الخلاصة

في هذه الدراسة تم تسجيل سلالة جديدة من بكتريا (*Bacillus subtilis*) و هي *B. Subtilis* AIK و لأول مرة في العراق، كما تم استخدام هذه السلالة لدراسة مدى قدرتها على إزالة النيكل من المحاليل المائية. تم دراسة العوامل التي تؤثر على كفاءة المعالجة الحيوية، حيث وجد انه اعلى إزالة كانت عند درجة حرارة 25 مئوي 54, 52 و 48 % عندما كانت قيمة الاس الهيدروجيني للمحلول 5, 7 و9 وبتركيز اولي للنيكل 10 جزء بالمليون على التوالي. في حين بينت النتائج عند درجة حرارة 30 مئوي فان اعلى إزالة كانت 47, 45 و52 عندما كانت قيمة الاس الهيدروجيني 5, 7 و9 وعند تركيز اولي للنيكل 1 جزء بالمليون على التوالي. في حين كانت اعلى إزالة عند درجة حرارة 40 مئوي هي 49, 46 و42 عندما كانت قيمة الاس الهيدروجيني 5, 7 و9 عند تركيز اولي للنيكل 5, 10و 10 جزء بالمليون على التوالي. وقد بينت النتائج ان قيمة الاس الهيدروجيني

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المثلى لإزالة النيكل كانت 5 عند درجة حرارة 25 و40 مئوي على التوالي بينما كانت 9 عند درجة حرارة 30 مئوي. وكانت درجة الحرارة المثلى هي 25 مئوي. لقد أظهرت البكتريا المقترحة إمكانية مقبولة في إزالة النيكل.

1. Introduction

Water is a critical element and an important part of the environment and all life forms on the planet. Through pollution, human activities have put a tremendous pressure on this valuable source that has caused degradation in the water quality [1]. One of the biggest problems that began to appear on the surface in Iraq is water contamintaion due to the pollutants like fertilizers, pesticides, chemicals and heavy metals that have entered the water systems. Removal all such foreign chemicals requires effective and sustainable treatment methods [2].

Heavy metals are inorganic pollutants that are harmful and can easily accumulate in the human body, thus causing a number of health issues. As a result of developmental industrialization, mining, farming as well as the release of untreated wastewater, higher concentrations of heavy metals are present in water bodies [3]. Some trace metals like copper, iron and nickel are required for the growth and development of plants at low levels. Whereas others including lead, arsenic, cadmium and mercury are considered toxic at any present level [4]. Nickel is found in nature both in the water and soil, and is required by plants at low concentrations. A high concentration of Ni can be toxic for humans. It can enter water bodies from wastewater released from factories, fertilizers and pesticides, and can cause a variety of health issues in human beings [5]. It is known that using chemicals to treat heavy metals ends up adding more chemicals to the water. Whereas, the physiochemical methods can't remove the metals to the required extent. On the other hand, biological treatment is more appealing from both the cost and environmental point of view [6]. Many methods are proposed for Ni removal [7, 8, 9]. With the emphasis being on the methods used need to be sustainable and do not damage the environment, biosorption has been extensively used [10].

The stability of halophiles enzymes in a hypersaline environment gives the possibility of using these bacteria in the bioremediation of hydrocarbons and fatty acids within days from a saline environment where even other conventual bioremediating bacteria cannot work [11, 12]. Many studies have been conducted using halophilic bacteria in bioremediation. *Halomonas venusta* H9 was tested by Zmorrod *et al*.[13] for its ability to remove lead and cadmium. Their results showed that this bacterium was able to accumulate these chemicals in its cells. While Farag *et al*. [14] used the marine halophilic *B. subtilis* AAK to remove 2,4-dichlorophenol and other phenolic compounds. They found that the used bacillus strain had the potential to degrade six different types of phenolic compounds. On the other hand, Sahoo and Goli [15] found that *B. pumilus* was able to adsorb Pb ions on its cell surface quite effectively. They also found that this strain of *Bacillus* was tolerant to a group of trace metals such as lead, cadmium, barium, chromium, iron and copper.

This study was carried out to test the ability of the newly identified moderately halophilic *Bacillus subtilis* AIK, isolated from local agricultural drainage in Babylon province in Iraq, for the biosorption of nickel.

2. Materials and Methods

2.1. Bacteria isolation

This new halophilic bacterial species was isolated from the Al-Saddah agricultural drainage water in Al-Saddah District in Babylon province in Iraq at site 1 (N 44º19′58, E 26′32º32) and site 2 (N 44º18′25, E 02′35º32) respectively. It was then cultured and purified on selective halophilic agar (M590) [16]. After that, the purified isolate was sent for sequencing (16 RNA) and the new strain was recorded in NCBI as *Bacillus subtilis* AIK as shown in NCBI link: Bacillus subtilis strain AIK 16S ribosomal RNA gene, partial sequence - Nucleotide - NCBI (nih.gov)

2.2. *Heavy Metal Preparation*

A stock solution of nickel (1000 ppm) was prepared by dissolving $Ni(NO_3)_2.H_2O$ in one litre of de-ionized water, and then the required concentration was prepared by applying the following equation :

$$N1V1=N2V2 \quad \ldots (1)$$

2.3. *Experimental Work*

In general, the experiment work was done to estimate the ability of *B. subtilis* AIK to remove nickel from aqueous solutions. Five initial metal concentrations (0.5, 1, 2, 5, and 10 ppm) were used at 5, 7, and 9 pH values respectively. The samples were incubated at 25, 30 and 40ºC respectively. With three replicates for each sample, a sample size of 250 ml and inoculum size of 10 ml of *B. subtilis* AIK was created. The samples were collected over 10 days period. Each sample was filtrated before measuring the nickel concentration using the flame atomic spectrometer. Optical density was used to determine the bacterial density [17].

3. Results and Discussion

The results showed that *B. subtilis* AIK was able to remove nickel at 25ºC (Figure 1A, C, and E). The R% ranged from 33% to 54 %, 40% to 52%, and 39% to 48% at 5, 7, and 9 pH values respectively, with an initial Ni concentration of 0.5 to 10 ppm. In general, there was an increase in R% with the raise in the initial metal concentration which could be due to the increase in initial concentration inducing a higher interaction between the bacteria and the metal ions, thus significantly causing an increase in the removal efficiency [18]. This study results agree with the findings of Al-Gheethi *et al*.[19] who found that the removal ability of both treated and untreated *B. subtilis* cells increases with an increase in initial metal concentration.

The optical density is representative of bacterial density at 5, 7 and 9 pH values respectively. As illustrated in Figure 1B, D, and F, the bacterial biomass increased with time from day 1 to 10.

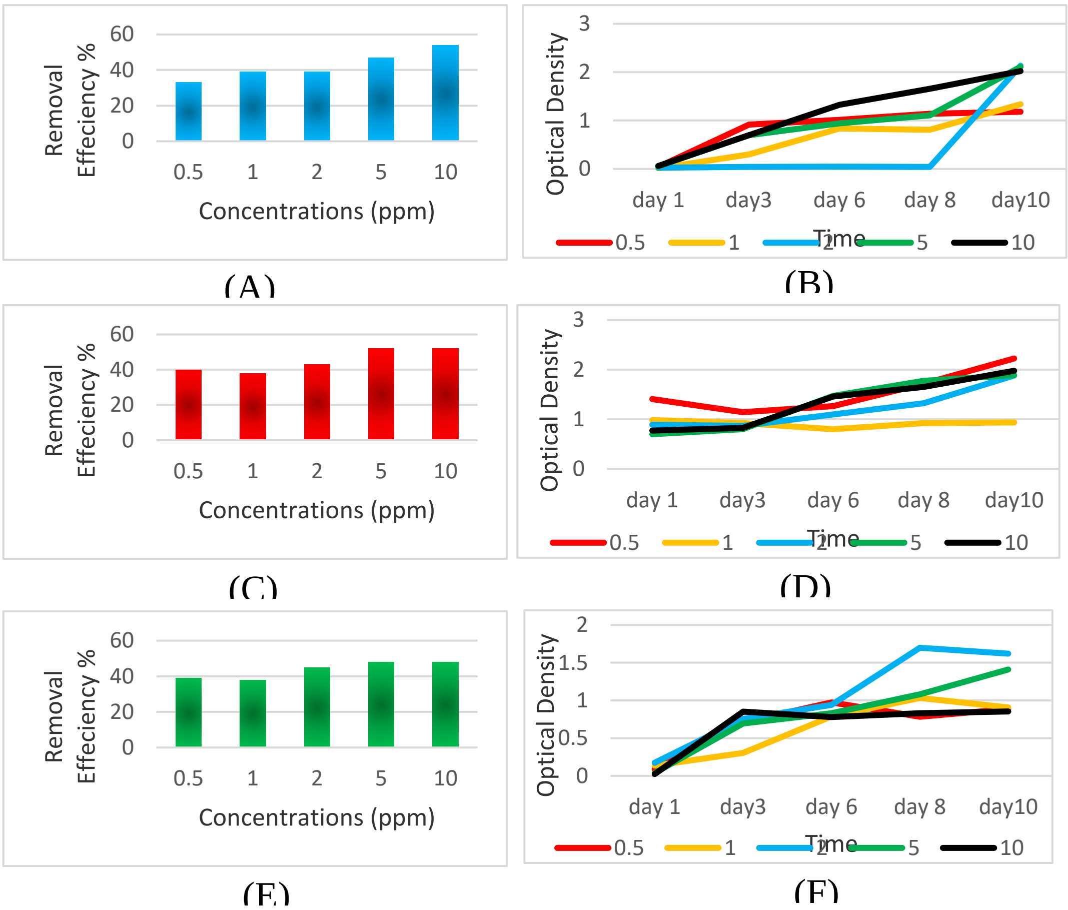

Figure 1: Removal efficiency of nickel by *B. subtilties* and optical density of bacterial growth at 25^{0}C (A) and (B) under pH=5, (C), and (D) under pH=7, (E) and (F) under pH=9 respectively.

Figures 2A, B, and C illustrate the removal efficiency of nickel by *B. subtilis*, with the R% ranging 47, 45 and 52% at 5, 7, and 9 pH values respectively, and at Ni initial concentration of 1 ppm. The removal efficiency however decreased as the concentration of the Ni increased which could be explained by the fact that with high initial nickel concentration a higher number of nickel ions remained in the solution because of the saturation of the active site available for the metal to bind [20]. The results of the current study are similar to the findings of another study that found there was a decrease in Ni removal by five bacterial species as the initial Ni concentration was increased from 10 to 40 ppm [21].

The optical density for the bacterial growth was recorded throughout the study and the results are depicted in Figure 2B, D and F. In general, bacterial growth increased with time.

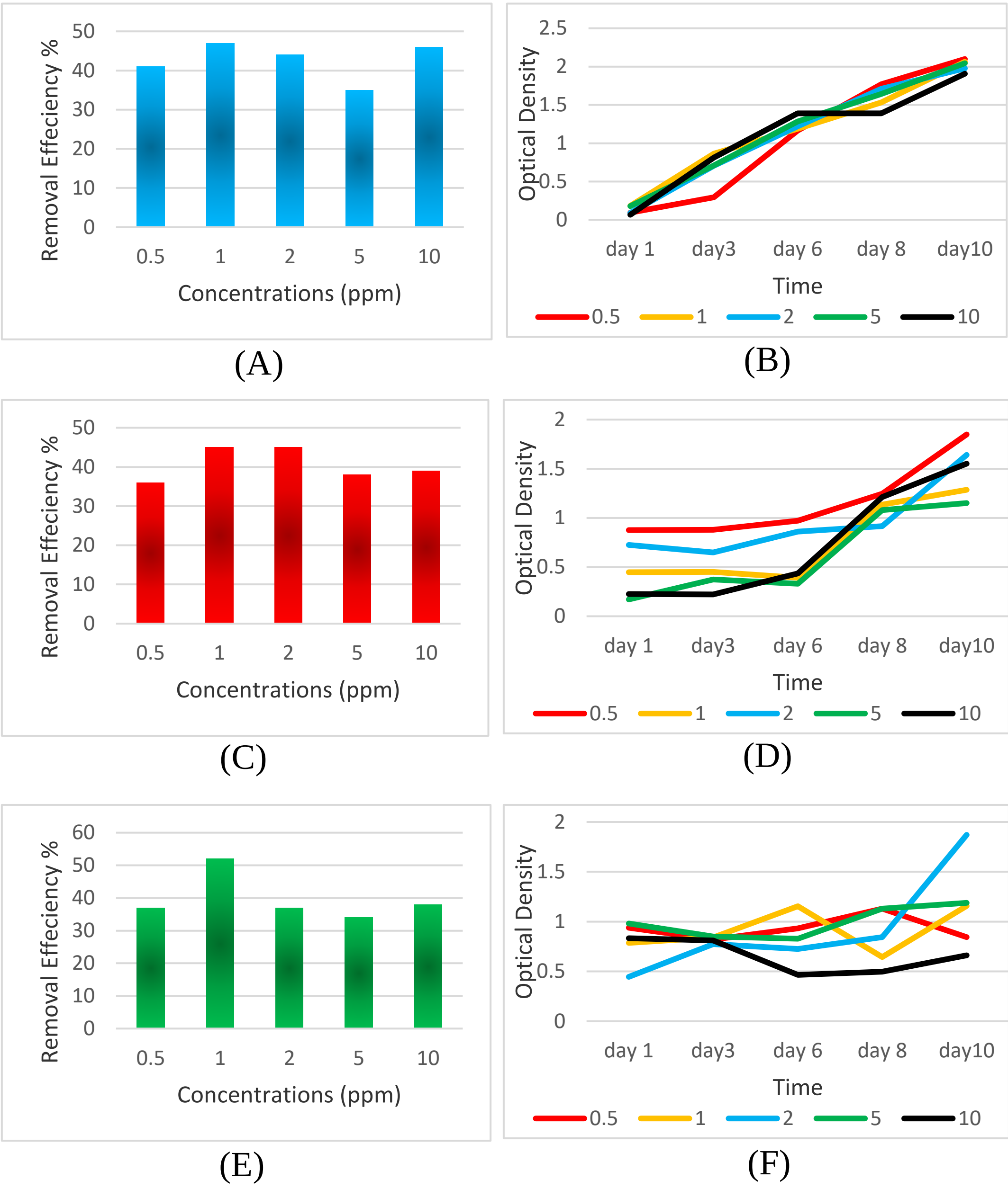

Figure -2: Removal efficiency of nickel by *B. subtilties* and optical density of bacterial growth at 30^{0}C (A) and (B) under pH=5, (C), and (D) under pH=7, (E) and (F) under pH=9 respectively.

The removal of nickel by *B. subtilis* AIK at 40ºC is shown in Figure 3A, B, and C. The R% increased as the initial metal concentration increased from 0.5 ppm to 10 ppm. R% ranged between 37-49, 28-46, and 34-42% at 5, 7 and 9 pH values respectively. This is due to the increase in the initial metal concentration giving the driving force required to overcome the resistance to transfer between the liquid and solid phase produced by the metal ions mass [22]. The results of this study aligned with the results of Karakagh *et al*. [23] who found that the Ni and Cd removal by *Bacillus* Sp., *Streptomyces* sp. and *actinomycetes* sp. increased with the increase in metal concentration from 1 to 4 mg/l. The highest removal efficiency recorded was 49, 46 and 42% at 5, 7 and 9 pH values respectively. The optical density is illustrated in Figure 3B, D and F.

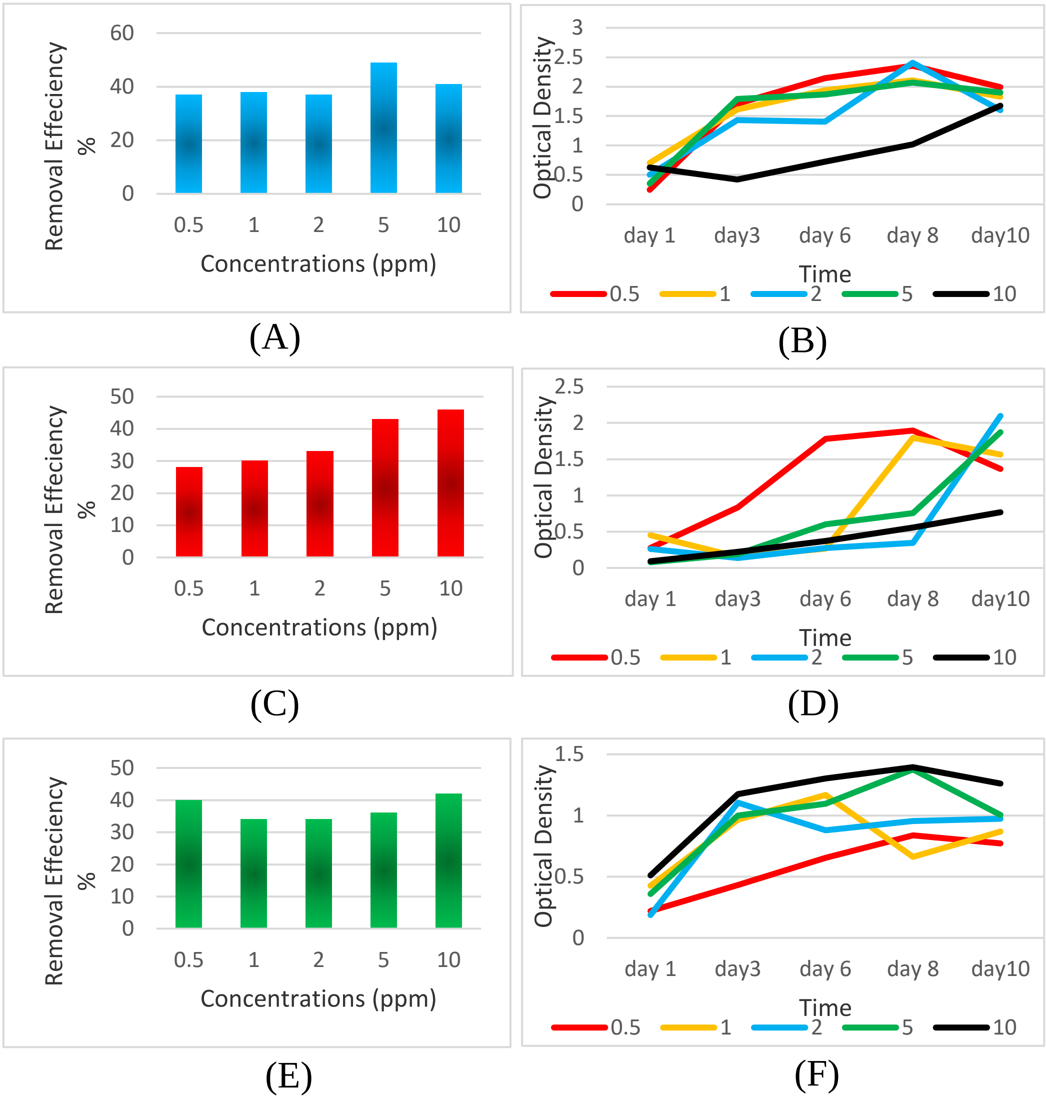

Figure 3: Removal efficiency of nickel by *B. subtilties* and optical density of bacterial growth at 40ºC (A) and (B) under pH=5, (C), and (D) under pH=7, (E) and (F) under pH=9 respectively.

Throughout the three experiments, the results showed that the highest removal efficiency was achieved at 25ºC incubation temperature followed by 30ºC, and the lowest reading being at 40ºC. The results are similar to the findings of Goel and Kaur [24] who found that to achieve the highest removal efficiency of nickel (60.7%), the optimum temperature is 25ºC. They also found that the enzyme began to denaturized with an elevation of temperature. The decrease in the removal efficiency of the metal with an increase in temperature could possibly be due to the fact that an increase in temperature causes the metal attached to the active site on the bacteria to be released back into the solution [25].

The study results showed that the highest removal efficiency was achieved in acidic conditions (pH=5), followed by R% in neutral conditions (pH=7), and the lowest in alkaline conditions (pH=9) at both 25ºC and 40ºC respectively. While at 30ºC, the increase in R% can be attributed to the fact that, at pH 9, the metal may precipitate to insoluble form which is related to the increase of bacterial accumulation in the metal [26]. It is known that the pH of the solution can impact the uptake of the metal by affecting the cell binding sites as well as the metal chemistry in the solution [27]. The decrease at a higher pH value is perhaps due to the presence of hydroxide complex and with an increase in pH value, the ligands such as carboxylate may uptake the metal ions. The results agree with the findings of Uthra and Kadirvelu [28] concerning that the highest removal of Ni can be achieved by a mixture of *Pseudomonas aeruginosa* and *Bacillus subtilis* at pH5 and it decreases as the pH value is increased to 7.

4. Conclusions

Throughout the study, the *B. subtilis* AIK showed an acceptable ability to remove nickel from the aqueous solutions. The bacteria showed the highest removal at 25ºC incubation temperature, with the best results recorded under acidic conditions (pH=5).

5. Acknowledgements

We would like to express our thanks to the Biology Department, College of Science at the University of Baghdad for allowing us to conduct our studies in the lab of Environmental Science for higher studies.

6. Conflict of Interest

The authors declared that they had no conflicts of interest.

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