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Biological removal of nickel (II) by *Bacillus* sp. KL1 in different conditions: optimization by Taguchi statistical approach

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Bioremediation is the removal of heavy-metals such as nickel (Ni) using microorganisms and has been considered as an important field in the biotechnology. Isolation and characterization of microorganisms exhibiting bioremediation activities and their optimization to treat polluted wastewaters is a vital and difficult task in remediation technologies. In this study, investigation was carried out to isolate Ni (II) remediating microbial strains from soils contaminated with municipal solid waste leachate. Furthermore, Taguchi design of experiments were used to evaluate the influence of concentration, pH, temperature, and time on bioremediation of Ni (II) using isolated bacteria. This study concluded that *Bacillus* sp. KL1 is a Ni (II)-resistant strain and had Ni (II) bioremediation activity. The highest bioremediation of Ni (II) was observed as 55.06% after 24 h at 30°C, pH 7, and 100 ppm concentration. Moreover, it was also observed that concentration is the most effective factor in the bioremediation process. In conclusion, we have demonstrated that bacteria isolated from soils contaminated with garbage leachate have the *Bacillus* sp. KL1 bacteria which can efficiently uptake and eliminate Ni (II) from contaminated sites and thus makes it possible to treat heavy-metal containing wastewaters in industry by using this microorganism at optimized conditions.

Keywords: Nickel, Bioremediation, Garbage leachate, *Bacillus* sp. KL1, Taguchi method.

INTRODUCTION

Heavy-metals are a group of elements, including Cd, As, Cr, Cu, Pb, Hg, Ni, Se, and Zn, which are commonly used in industry and are generally toxic to animals. Some heavy-metals in low concentrations are very useful for living cells. For example, enzymes like racemases in bacteria and ureases in plants contain nickel which is necessary for their normal functioning[1, 2]. On the other hand, they are highly toxic in higher concentrations and bind to DNA. Their highly binding affinity affect the biology of organisms, inhibiting or inducing cell division, disruption of cell membranes, and blocking of the activity of translocators and enzyme proteins[3–5]. Thus, heavy-metal pollution is a major environmental concern which is rapidly increasing because of industrial operations. Therefore, developing methodologies to remove environmental heavy-metals is the necessity of time. A number of inefficient, expensive, and conventional methodologies such as exchange filtration, reverse osmosis, chemical precipitation, electrochemical treatment, chemical oxidation or reduction, membrane technologies, and evaporation recovery are being used to decontaminate heavy-metal pollutions[6–8]. Development of effective tools and techniques to manage the environmental pollutions is an interesting research field in the biotechnology and currently bioremediation has been considered as an art-of-state technique in which living organisms such as plants and microorganism can be used to remove or minimize various contaminants like heavy-metals due to their natural biological activity[9–11].

Microorganisms such as bacteria are main biological agents used in the remediation of heavy-metals from contaminated sites. They can reduce the toxic effects of these pollutants using different mechanisms such as bioadsorbtion, biotransformation, and bioaccumulation. Isolation and characterization of these microorganisms and their optimization for bioremediation activities may improve remediation technology and these microorganisms can be used as heavy-metal remediating agents. Waste leachate production is one of the biggest problems in our world, because liquid wastes contaminate surrounding soil surfaces and ground water. Garbage leachate contaminated soils are good sources to isolate such kind of bioremediation agents having potential to degrade the waste compounds. To access these microorganisms, soft soil samples can be collect from the surface and depth of leachate contaminated sites and can be identified in laboratory.

The present study was designed to identify the bacteria having bioremediation activity for Ni (II) in the municipal solid waste leachate contaminated soils and optimization conditions were evaluated using Taguchi method to have highest bioremediation activity.

MATERIAL AND METHODS

Sample collection

Soil samples were collected from both surface and depth (15 cm) of municipal solid waste leachate contaminated sites in Kermanshah province of Iran. All samples were kept in sterile bags, labeled and transferred to the laboratory. Samples were homogenized by passing them through a 2 mm sieve.

Isolation of Ni (II)-resistant bacteria

Isolation of bacteria from the soil samples was carried out using a serial dilution procedure and up to 10^{-5} dilutions were prepared. For isolation of the Ni (II)-resistant bacteria, 100 μl of each suspension was spread on Ni (II)-containing (0.5 mM) nutrient agar medium. The plates were incubated at 37°C for 48–72 h, and then the presence of bacterial colonies on plates were investigated. For purification of the Ni (II)-resistant bacteria, single emerged colony on plates were picked up and streaked on Ni (II)-containing (0.5 mM) nutrient agar media. Finally, automated 16S rDNA gene sequencing and

morphological analysis were used for characterization of the bacteria which were grown on the Ni (II)-containing media. Gene sequencing and morphological analysis were carried out at Iranian Biological Resource Center.

Detection of the minimum inhibitory concentration of Ni (II)

The minimum inhibitory concentration (MIC) was assessed using a preparation of serial dilutions of Ni (II) (40, 50, 60, 70, 80, 90, 100, 110, 120, 130 ppm). One hundred microliter of each dilution of Ni (II) was added to a nutrient agar plate. The bacterial suspension was prepared and adjusted spectrophotometrically at 800 nm (OD800) to match a turbidity of 1.5×10^8 CFU mL (equivalent to 0.5 McFarland standard). Then, 20 μl of 0.5 McFarland of Ni (II)-resistant bacteria suspension was dropped and spread on the surface of the plates. These plates were then incubated at 37ºC for a week. The plates were assessed daily for bacterial growth against control to ensure reliable results. The minimum inhibitory concentration was defined as the lowest concentration of an antimicrobial agent that inhibits the visible growth of a microorganism after overnight incubation. A single colony of bacteria strain with highest MIC was selected and identified by the Iranian Biological Resource Center, according to 16s rDNA gene analysis. Finally, the bioremediation activity of this strain for Ni (II) was evaluated under different culturing conditions.

Design of experiments (DOEs)

Qualitek-4 software (V. 14.5, Nutek Inc., MI, USA) was used to design experiments by Taguchi statistical method for evaluating the effects of concentration, pH, temperature, and time on bacterial bioremediation of Ni (II). Table 1 shows considered variable factors and their levels.

Table 1. Factors and their levels used in Taguchi DOEs to investigate bioremediation of Ni (II) by *Bacillus* sp. KL1

Factor	Level 1	Level 2	Level 3
Time [h]	12	24	36
pH	5	7	9
Concentration [ppm]	50	75	100
Temperature [°C]	30	35	40

Bioremediation of Ni (II) according to DOEs

A single colony of bacteria with the highest MIC was cultured in 100 ml nutrient broth and was incubated in a shaking incubator at 37ºC and 120 rpm. The Ni (II) stock solutions were prepared according to DOEs. Five ml of each Ni (II) stock solution was mixed with 5 ml of bacterial suspension in different sterile Erlenmeyer to prepare working concentrations as designed by Taguchi approach. Then, the pH of suspensions was adjusted with sodium hydroxide or Hydrochloric acid (0.1 M) according to DOEs. All samples were incubated in a shaking incubator at 120 rpm, variable temperature and time set for each experiment. After incubation times, contents of each Erlenmeyer flask were transferred into sterile tubes and centrifuged at 12000 rpm for 5 min and supernatants were transferred to new centrifuge tubes. Ni (II) concentrations in these solutions were measured by atomic absorption spectrophotometry (GBC-q02)[12]. All the experiments were done in triplicate to ensure the reproducibility of these experiments.

Statistical analysis

Statistical analysis of Taguchi DOEs results were done using fixed-effects model of analysis of variance (ANOVA) in Qualitek-4 software.

RESULTS AND DISCUSSION

Microorganisms have received considerable attention in recent years for being efficient heavy-metals bioremediation agents. The results of our study showed that soils contaminated with municipal solid waste leachate were contained Ni (II)-resistant microorganisms. Out of different colonies which were emerged on the nutrient agar plates, several clones were selected and purified for further studies on bioremediation of Ni (II). The 16S rDNA gene sequencing and morphological analysis confirmed that the bacteria in selected colonies were belongs to *Bacillus* species. The minimum inhibitory concentrations of Ni (II) were determined for these colonies. One colony from the plate with the maximum tolerance for Ni (II) (100 ppm) was selected and characterized as *Bacillus* sp. KL1 by 16S rDNA gene sequencing. We inferred that soils contaminated with municipal solid waste leachate are good sources of Ni (II)-resistant bacteria which may be useful for nickel bioremediation.

Studies indicate that initial concentration and duration of exposure to pollutant agent, pH, and temperature are important parameters affecting bioremediation activity of microorganisms[13, 14]. In this study, Taguchi approach was applied to detect optimum temperature, pH, concentration, and time for bioremediation of Ni (II) by *Bacillus* sp. KL1. The results of the Taguchi optimization method demonstrated 9 orthogonal arrays of experiments which are introduce in Table 2. The last column of Table 2 shows the percentages of Ni (II) removal for each DOE. These results indicated that the highest bioremediation of Ni (II) by *Bacillus* sp. KL1 (55.06%) was achieved at 5th run (30ºC, 100 ppm, pH 7, and 24 h).

Table 2. Taguchi DOEs and corresponding bioremediation of Ni (II) by *Bacillus* sp. KL1

Experiment number	Time [h]	pH	Concentration [ppm]	Temperature [°C]	Bioremediation rate [%]
1	12	5	50	30	18.96
2	12	7	75	35	33.88
3	36	9	50	40	42.66
4	24	5	75	40	31.44
5	24	7	100	30	55.06
6	24	9	50	35	10.99
7	36	5	100	35	40.33
8	36	7	50	40	8.30
9	36	9	75	30	36.12

The effects of parameters such as time, pH, concentration, and temperature were assessed on Ni (II) bioremediation activity of *Bacillus* sp. KL1. The rate of Ni (II) bioremediation by the bacteria was measured at three different levels of factors (Time: 12, 24, 36 h; pH: 5, 7, 9; Concentration: 50, 75, 100 ppm; Temperature: 30, 35, 40°C). Maximum bioremediation of Ni (II) (32.5, 32.41, 46.02 and 36.71 %) was observed with different parameters at levels 2 (24 h; pH 7), at level 3 (100 ppm), and at level 1 (30°C) (Table 3). In accordance with previous studies on the other bacteria[13, 15, 16], this study demonstrated that the highest uptake of Ni (II) by *Bacillus* sp. KL1 was achieved in a lower time. Moreover, the optimal pH range 5–7 reported as the efficient bioremediation of heavy-metals by different bacterial strains[14, 17, 18]. We reported that pH 7 is optimum for the maximum bioremediation of Ni (II) by *Bacillus* sp. KL1. In addition, the results of current study showed the stronger bioremediation of Ni (II) in cultures with increasing concentration of nickel, although this effect was limited in higher concentrations only. It may be due to constraints in number of metal uptake sites on the bacterial surfaces. These results are in accordance with previous studies in which influence of increased concentrations on uptake of Ni (II) by *Micrococcus* isolates was studied[14]. Furthermore, it is demonstrated that bioremediation of Ni (II) by *Bacillus* sp. KL1 reduced at higher temperatures. Sari and colleagues reported that heavy-metal uptake is an exothermic reaction and raising the temperature destroys metal uptake sites on the surface of bacteria or induces ion exudation into solution by bacteria[13].

The effects of interacting factor pairs on the Ni (II) bioremediation activity of *Bacillus* sp. KL demonstrated that the interactions of time and temperature is the most effective pair (63.07%) (Table 4). On the other hand, interacting factor pair time and concentration showed the minimum effect on the bioremediation activity of this microorganism (5.91%). These results suggest that the influence of one factor on bioremediation activity is dependent on its interaction with the other factors. In this regard, it shows that concentration in the optimum level (100 ppm) is the most influential factor on bioremediation activity of *Bacillus* sp. KL (Table 3), but this effect got declined when concentration interacts with other factors (Table 4). Moreover, in comparison with other factors, time at the optimum level (24 h) shows the minimum effect on bioremediation activity of the bacteria, but when it interacts with the optimal level of temperature (30°C) the maximum bioremediation of Ni (II) was achieved. In addition, interacting factor pair pH and temperature in their optimal levels induce effective rate of Ni (II) bioremediation. Together these results indicate that time and temperature at their optimal levels have the most synergistic effect as compared to other interacting factor pairs.

In the present study, ANOVA test was used to analysis the effects of each factor and their variations on bioremediation of Ni (II). The ANOVA results indicated that concentration with the maximum sum of squares (S), variance (V), and percentage influence (1699.26, 849.63, and 89.57, respectively) is the most influential factor (Table 5) and pH with the minimum sum of squares (S), variance (V), and percentage influence (11.01, 5.51, and 0.58, respectively) has no significant effect on bioremediation of Ni (II).

Importance of each factor on bioremediation of Ni (II) has been given in Table 6. The maximum bioremediation was obtained by setting temperature 30°C, pH 7, concentration 100 ppm, and time 24 h. Our statistical analysis predicted that the removal rate of nickel under optimal conditions is 55.06% and concentration is the most influential factor (15.16%) on bioremediation activity of *Bacillus* sp. KL1.

Table 3. The effects of different levels of factors on bioremediation of Ni (II)

Factor	Level	Bioremediation rate [%]
Time [h]	12	31.83
	24	32.5
	36	28.25
pH	5	30.24
	7	32.41
	9	29.92
Concentration [ppm]	50	12.75
	75	33.81
	100	46.02
Temperature [°C]	30	36.71
	35	28.4
	40	27.47

CONCLUSION

This study reported the presence of *Bacillus* sp. KL1 as a Ni (II)-resistant bacterium in soils contaminated with municipal solid waste leachate. The results of our study proved that Taguchi approach is effective in optimizing the influential factors for the maximum bioremediation of Ni (II) by *Bacillus* sp. KL1. Moreover, the optimal

Table 4. Estimating effects of interacting factor pairs on bioremediation of Ni (II)

Interacting factor pairs	Levels	DOE	Contribution [%]
Time × Temperature	24 h × 30°C	5	63.07
pH × Temperature	7 × 30°C	6	45.49
pH × Concentration	7 × 100 ppm	1	14
Time × pH	24 h × 7	3	9.3
Concentration × Temperature	100 ppm × 30°C	7	6.12
Time × Concentration	36 h × 100 ppm	2	5.91

Table 5. ANOVA test for Taguchi DOEs results

Factor	DOF [f]	Sum of Sqrs [S]	Variance [V]	Pure sum [S´]	Percent [%]
Time [h]	2	31.31	15.66	31.31	1.65
pH	2	11.01	5.51	11.01	0.58
Concentration [ppm]	2	1699.26	849.63	1699.26	89.57
Temperature [°C]	2	155.48	77.74	155.48	8.2

Table 6. Prediction of the optimum conditions for the maximum bioremediation of Ni (II) by *Bacillus* sp. KL1

Parameters	Level	Bioremediation rate [%]
Time [h]	24	1.64
pH	7	1.55
Concentration [ppm]	100	15.16
Temperature [°C]	30	5.85
Sum of rates Mean of rates Total bioremediation at the optimal conditions		24.2 30.86 55.06

conditions for efficient Ni (II) bioremediation were determined as 30°C, pH 7, 100 ppm concentration, and 24 h time. **C**oncentration was observed as the most influential factor on bioremediation activity of this microorganism. Furthermore, our results showed that the influence of one factor on bioremediation activity of the bacteria is dependent on its interactions with the other factors. It was also demonstrated that interactions of time and concentration is the most effective pair for efficient bioremediation of Ni (II) by *Bacillus* sp. KL1. In general, this study indicates the effectiveness of *Bacillus* sp. KL1 for bioremediation of Ni (II) and thus application of this microorganism as a promising tool to treat industrial wastewaters.

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LITERATURE CITED

1. Desguin, B., Goffin, P., Viaene, E., Kleerebezem, M., Martin-Diaconescu, V., Maroney, M.J., Declercq, J. P., Soumillion, P. & Hols, P. (2014). Lactate racemase is a nickel-dependent enzyme activated by a widespread maturation system. *Nat. Commun.* 5, 3615. DOI: 10.1038/ncomms4615.

2. Polacco, J.C., Mazzafera, P. & Tezotto, T. (2013). Opinion: nickel and urease in plants: still many knowledge gaps. *Plant Sci.* 199–200, 79–90. DOI: 10.1016/j.plantsci.2012.10.010.

3. Ding, J., He, G., Gong, W., Wen, W., Sun, W., Ning, B., Huang, S., Wu, K., Huang, C., Wu, M., Xie, W. & Wang, H. (2009). Effects of nickel on cyclin expression, cell cycle progression and cell proliferation in human pulmonary cells. *Canc. Epidem. Biomark. Prev.* 18, 1720–9. DOI: 10.1158/1055-9965.EPI-09-0115.

4. Ahemad, M. (2012). Implications of bacterial of resistance against heavy metals in bioremediation: a review. *IIOABJ.* 3, 39–46.

5. Magaye, R., Zhou, Q., Bowman, L., Zou, B., Mao, G., Xu, J., Castranova, V., Zhao, J. & Ding, M. (2014). Metallic Nickel Nanoparticles May Exhibit Higher Carcinogenic Potential than Fine Particles in JB6 Cells. *PloS One.* 9, e92418. DOI: 10.1371/journal.pone.0092418.

6. Eccles, H. (1999). Treatment of metal-contaminated wastes: why select a biological process? *Trends Biotechnol.* 17, 462–5. DOI: http://dx.doi.org/10.1016/S0167-7799(99)01381-5.

7. Hunsom, M., Pruksathorn, K., Damronglerd, S., Vergnes, H. & Duverneuil, P. (2005). Electrochemical treatment of heavy metals (Cu2+, Cr6+, Ni2+) from industrial effluent and modeling of copper reduction. *Water Res.* 39, 610–6. DOI: http://dx.doi.org/10.1016/j.watres.2004.10.011.

8. Barakat, M.A. (2011). New trends in removing heavy metals from industrial wastewater. *Arab. J. Chem.* 4, 361–377. DOI: http://dx.doi.org/10.1016/j.arabjc.2010.07.019.

9. Bhatnagar, S. & Kumari, R. (2013). Bioremediation: a sustainable tool for environmental management – a review. *Ann. Rev. Res. Biol.* 3, 974–993.

10. Bestawy, E.E., Helmy, S., Hussien, H., Fahmy, M. & Amer, R. (2013). Bioremediation of heavy metal-contaminated effluent using optimized activated sludge bacteria. *Appl. Water Sci.* 3, 181–192. DOI: 10.1007/s13201-012-0071-0.

11. Guo, H., Luo, S., Chen, L., Xiao, X., Xi, Q., Wei, W., Zeng, G., Liu, C., Wana, Y., Chen, J. & He, Y. (2010). Bioremediation of heavy metals by growing hyperaccumulaor endophytic bacterium Bacillus sp. L14. *Bioresour. Technol.* 10, 8599–8605. DOI: 10.1016/j.biortech.2010.06.085.

12. Taran, M., Safari, M., Monaza, A., Reza, J.Z. & Bakhtiyari, S. (2013). Optimal conditions for the biological removal of arsenic by a novel halophilic archaea in different conditions and its process optimization. *Pol. J. Chem. Technol*. 15, 7–9. DOI: 10.2478/pjct-2013-0017.

13. Sari, A., Mendil, D., Tuzen, M. & Soylak, M. (2008). Biosorption of Cd(II) and Cr(III) from aqueous solution by moss (Hylocomium splendens) biomass: Equilibrium, kinetic and thermodynamic studies. *Chem. Engin. J.* 144, 1–9. DOI: 10.1016/j.jhazmat.2008.05.112.

14. Congeevaram, S., Dhanarani, S., Park, J., Dexilin, M. & Thamaraiselvi, K. (2007). Biosorption of chromium and nickel by heavy metal resistant fungal and bacterial isolates. *J. Haz. Mat.* 146, 270–7. DOI: http://dx.doi.org/10.1016/j.jhazmat.2006.12.017.

15. Yilmaz, E.I. & Ensari, N.Y. (2005). Cadmium biosorption by Bacillus circulans strain EB1. *World J. Microb. Biotech.* 21, 777–779. DOI: 10.1007/s11274-004-7258-y.

16. Ozturk, A. (2007). Removal of nickel from aqueous solution by the bacterium Bacillus thuringiensis. *J. Haz. Mater.* 147, 518–23. DOI: http://dx.doi.org/10.1016/j.jhazmat.2007.01.047.

17. Tunali, S., Cabukb, A. & Akar, T. (2006). Removal of lead and copper ions from aqueous solutions by bacterial strain isolated from soil. *Chem. Engin. J.* 115, 203–211. DOI: http://dx.doi.org/10.1016/j.cej.2005.09.023.

18. Kumar, R., Verma, D., Singh, B.L., Kumar, U. & Shweta (2010). Composting of sugar-cane waste by-products through treatment with microorganisms and subsequent vermicomposting, *Bioresour. Technol.* 101, 6707–11. DOI: 10.1016/j.biortech.2010.03.111.