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Effect of Environmental Factors in Biological Removal by Halophilic Bacteria

Zahra Kahrarian[1*], Mojtaba Taran[2] and Ahmad Tajehmiri[2,3]

[1]Department of Biology, Faculty of Science, Razi University, Kermanshah, Iran

[2]Department of Biology, Faculty of Science, Razi University, Kermanshah, Iran

[3]Department of Biotechnology and Chemical Engineering, Islamic Azad University, Kermanshah Branch, Kermanshah, Iran

Research Article

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*For Correspondence:

Zahra Kahrarian, Department of Biology, Faculty of Science, Razi University, Kermanshah, Iran

Email: zahra.kahrarian@gmail.com

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ABSTRACT

Background and Objective: With the advent of the industry, the risk of heavy metals entering the environment has increased. Industrial sewage is considered as an important source of water pollution. Heavy metals, including cadmium, have toxic effects on humans and the environment. Today, the use of microorganisms, including bacteria, in biological refining has been considered as an environmentally friendly and cost effective method. In this study, the ability of *Halomonas elongata* IBRC-M10433 strain isolated from sediments of Lake Urmia to remove cadmium from metals has been considered. The aim of this study was to optimize the environmental conditions of removal of Cadmium metal by *Halononas elongata* IBRC-M10433.

Method: Design of Experiments Taguchi method to determine the optimum conditions relative to environmental factors such as Ammonium chloride (NH_4Cl) as a source of nitrogen, and phosphate potassium (K_2HPO_4) as a source of phosphorus and cadmium concentrations in biological removal of cadmium was studied by the bacterium *Halomonas longata* IBRC- M10433.

Materials and Methods: Taguchi experimental design was designed to determine the relative optimal conditions of environmental factors such as ammonium chloride (NH_4Cl) as a source of nitrogen, potassium phosphate (K_2HPO_4) as a source of phosphorus, cadmium concentration in cadmium biodegradation by *Halomonas Elongata* IBRC-M10433 was studied. Data analysis was performed using Qualitek-4 software and the relative importance of each of the factors was determined.

Results: Optimum absorption conditions at Potassium phosphate 0.3 g/L, 100 mg cadmium and Ammonium chloride 2 g/L was determined at 37°C and pH 7.

Discussion and Conclusion: Due to high costs and environmental problems, conventional methods of removing

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heavy metals are an effective and cost effective biological removal. The use of relative salt bacteria is beneficial in bio-refining with a high uptake of heavy metals.

INTRODUCTION

With the development of the industry, living conditions have improved, but the release of large quantities of pollutants into the environment that has been carried out through air, water and soil has affected life [1]. The heavy use of heavy metals has resulted in the release of toxic substances into the environment [2]. In low concentrations, metals often play important functions in the production of enzymes as important components of biological processes. But at concentrations above a certain level, they can be toxic [3].

The main dangerous of metal is deal of stability and resist to decomposition in environment [4]. Heavy metals are harmful for cells, because inhibited of proteisynthesis, protected of enzymatic process and prevented of cell dividing [5].Cadmium causes acute and chronic diseases such as cancer, kidney disease, pulmonary disorder, weight loss, kidney damage, digestive and bone discomfort [6]. Cadmium absorption has the same mechanism of calcium and iron absorption, and thus replaces an essential element, thereby increasing the iron content [7].Biological cleanup is a process in which cellular components or metabolic activities of microorganisms can be used to remove toxic substances from the environment and convert them into non-toxic and harmless substances [8]. The process of biological absorption is economical, environmentally friendly and has high absorption efficiency [9]. Different mechanisms of microbial resistance facilitate the biological cleansing function of germs [10].Increasing salt concentration can be fatal to microorganisms. However, resistance to salt soluble bacteria depends on sodium and potassium ions and is essential for the growth and activity of enzymes and pumps [11]. Optimum environmental conditions make it possible for bacteria to grow and multiply, and the adverse environment reduces their growth [12].

The purpose of this study was to investigate the effect of environmental factors on the removal of Cadmium metal from aqueous solution by *Halomonas elongata* IBRC-M10433.In this study, with the Taguchi method, the relationship between the factors affecting the absorption process was obtained. Using the optimum conditions, factors were determined. Optimizing environmental conditions will increase efficiency. The temperature range is a natural temperature that does not require cooling and heating costs at the industrial scale. With the lowest amount of absorbent consumption, the most absorbing capacity was economically economical.

MATERIAL AND METHODS

The *Halomonas elongate* IBRC-M10433 bacterium was prepared from Iranian Biological Resource Center. Culture media contains 1.5 g/L Magnesium Sulfate, 2.3 g/L Ammonium Chloride, (1.5,2,2.5)g/L Potassium Phosphate (0.03,0.6,0.9) g/L Ferric Sulfate, Glucose 10 g/L, NaCl 150g/L, Cadmium chloride (50, 100 and 200 ppm) were prepared from Different dilutions of Cadmium (50, 100 and 200 ppm) added to culture media (pH, 7). They were then sterilized by autoclave. Cultures media were inoculated by 10^7 CFU/ml of *Halomonas elongate* IBRC-M10433 bacterium, 5% per culture, and were grown at 37°C in shaking incubator (100 rpm). After 48 h, when growth phase finished, bacteria were precipitated at 10000 rpm for 12 min. The quantity of Cadmium in 1 mL of supernatant was carried by Atomic Absorption (Inc. Varian Spectr AA220) as recommended by last researchers [13].

Design of Experiments

For design of experiments we used Taguchi method and Quailtek-4 software (V. 14.5, Nute Inc., MI, USA). The effected factors in biologic removal were selected. The factors such as concentration of metal, NH_4Cl and KH_2PO_4 in tests for Cadmium removal were segregated (**Table 1**).

Table 1: Factors and their levels used in Taguchi DOEs to investigate bioremediation of cadmium by *H. elongate* IBRC-M1043.

Level 3	Level 2	Level 1	Factors
2.5	2	1.5	NH_4Cl g/L
0.9	0.6	0.3	KH_2PO_4 g/L

200	100	50	Concentration Cd ppm

Statistical Analysis

In order to investigate the effects of different conditions on removed of Cadmium ion we used ANOVA and Data were analyzed by QULITEK-4 software.

RESULTS

Results from this research indicated that halophilic bacteria has high efficiency for biologic refinement and control of pollution from heavy metals in industrial wastewaters. Taguchi is a suitable method for experiments design and achievement to optimization conditions. In this method at first the effects of factors and their concentrations were determined. In recent study the effects of three factors Cadmium metal (50, 100 and 200 ppm), NH_4Cl (1.5, 2 and 2.5) g/L and KH_2PO_4 (0.03, 0.6, 0.9) g/L for removed of heavy metal by *H. elongate* IBRC-M10433 were examined. The higher efficiency for removed of Cadmium metal (80%) in concentration of 50 ppm happened in culture medium that contains KH_2PO_4 0.3 g/L, NH_4CL g/L in pH 7 and 37°C (Table 2). In different bacteria that biological removal was optimized the ranges of pH were 5-7 [14].

Table 2: Taguchi DOEs and corresponding bioremediation of cadmium by *H. elongate* IBRCM1043.

Experiment number	NH_4Cl g/L	KH_2PO_4 g/L	Concentration Cd ppm	Bioremediation rate [%]
1	1.5	0.3	50	80
2	2	0.6	50	74
3	2.5	0.9	50	35
4	1.5	0.3	100	69.50
5	2	0.6	100	77.50
6	2.5	0.9	100	59
7	1.5	0.3	200	63.50
8	2	0.6	200	60.75
9	2.5	0.9	200	60.50

The highest removal of Cadmium metal was attained in different conditions of KH_2PO_4 (0.3 g/L), NH_4CL (1.5 g/L) and Cadmium (100 ppm) (Table 3).

Table 3: The effects of different levels of factors on bioremediation of cadmium by *H. elongate* IBRC-M1043.

Factors	Level	Bioremediation rate [%]	Bioremediation rate [%]
NH_4Cl g/L	1.5	63	481.6 ± 416.64
	2	68.7	
	2.5	61.6	
KH_2PO_4 g/L	0.3	71	376.19 ± 416.64
	0.6	70.75	
	0.9	51.5	
Concentration Cd ppm	50	66.6	78.8 ± 416.64
	100	68	

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	200	58.7	

With increasing primary concentration of Cadmium, the percentage of removal was decreased. The percentage removal of Cadmium in 50, 100 and 200 concentrations decreased the percentage of Cadmium removal with increased primary concentration of this metal (**Figure 1**). With increasing concentration of NH_4Cl, the percentage removal of Cadmium was decreased (**Figure 2**). With increasing concentration of KH_2PO_4, the percentage removal of Cadmium was decreased (**Figure 3**).

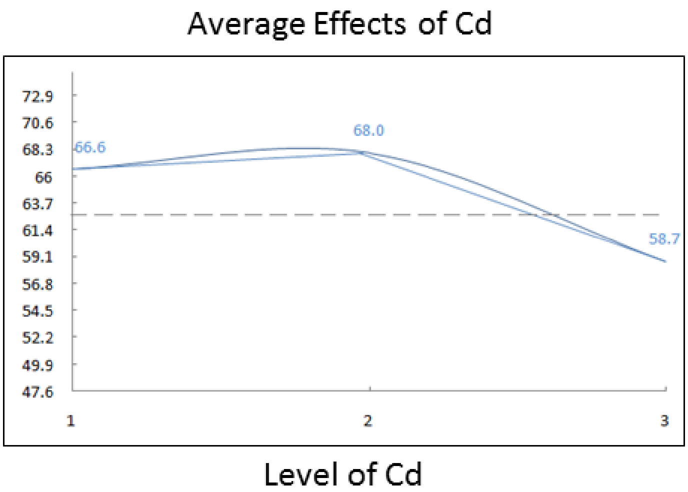

Figure 1: Graph representing decrease in percentage removal of Cadmium with increase in primary concentration of Cadmium.

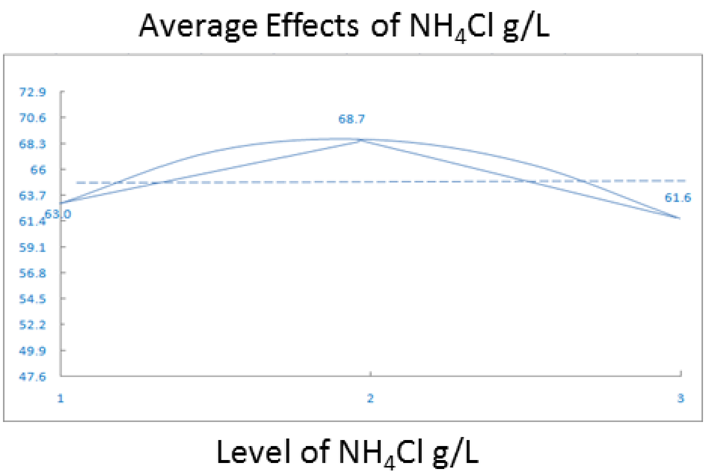

Figure 2: Graph representing decrease in percentage removal of Cadmium with increase in concentration of NH_4Cl.

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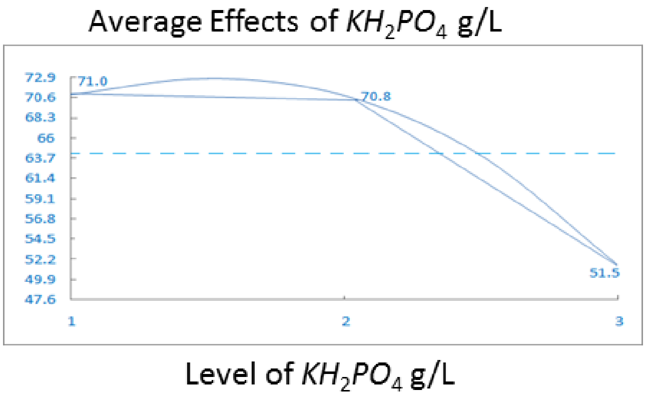

Statistical Analysis

In order to investigate the effects of different conditions on removed of Cadmium ion we used ANOVA and Data were analyzed by QULITEK-4 software. Differences of L_1 and L_2 were calculated (5.665). The relative effect of level 2 on level 1 was additive. In contrary KH_2PO_4 has the lowest difference of average (-2.5). The effect of level 2 on level 1 was decreasing (Table 4).

Table 4: The effects of different levels of factors on bioremediation *by H. elongate* IBRC-M1043.

Columns #/Factors	Level 1	Level 2	Level 3	L2-L1
NH_4Cl g/l	63	68.666	61.583	5.665
KH_2PO_4 g/l	71	70.75	51.5	-.25
Cd ppm	66.583	68	58.666	1.417

Interaction of dual factors in biological removal of Cadmium showed that KH_2PO_4 g/L x Cd (26.38%) and NH_4Cl g/L x Cd (18.33%) on removing of Cadmium as a heavy metal have important effects (Table 5). Results from this study indicated that the effect of one factor in biological removing depends on interaction this factor with the other factors.

Table 5: Effects of interacting factor pairs on bioremediation of cadmium by H. elongate IBRC-M1043elongate IBRC-M1043.

Interacting Factor	Columns	SI (%)	Col	Opt.
KH_2PO_4 g/L x Cd	2 x 3	26.38	1	[1,1]
NH_4Cl g/L x Cd	1 x 3	18.33	2	[1,1]
NH_4Cl g/L x KH_2PO_4 g/L	1 x 2	15.55	3	[1,1]

Statistical analyses showed that NH_4Cl and KH_2PO_4 have highest and lowest effects on heavy metal removing (Table 6).

Table 6: ANOVA test for Taguchi DOEs re.

Factors	DOF (f)	Sum of Squares. (S)	Variance (V)	F-Ratio (F)	Pure Sum (S)	Percent (%)
NH_4CL g/L	2	84.29	42.145	0.179	0	0
KH_2PO_4 g/L	2	750.875	375.437	1.6	281.833	19.356
Cd	2	151.793	75.896	0.323	0	0
Other/Error	2	469.041	234.52			80.644

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Total	8	1456				100.00

The effective parameter for biological removing of Cadmium was level 3 of glucose. Optimized quantity for biologic removal of Cadmium was detected in level 1 of KH_2PO_4 (0.3 g/L), NH_4CL (2 g/L), level 1 of Cadmium (100 ppm), pH 7 and 37°C. Expected Result at Optimum Conditions was detected in Contribution 78.831% (**Table 7**).

Table 7: Prediction of the optimum conditions for the maximum bioremediation of cadmium by *H. elongate* IBRC-M1043.

Columns #/Factors	Level Description	Level	Contribution[%]
NH_4Cl g/L	Fact A-Level 2	2	4.249
KH_2PO_4 g/L	Fact B-Level 1	1	6.583
Cd	Fact C-Level 2	2	3.583
Total Contribution From All Factor			14.414
Current Grand Average Of Performance			64.416
Expected Result At Optimum Conditions			78.831

DISCUSSION

By increasing the concentration of metal ions, the number of adsorbent increases which increases the process of adsorption [15,16]. By comparing of results from this research with last publications on *Halomona seurihalina* persist to Cadmium [17]. Data from recently paper indicated that the *Halomonas elongate* IBRC-M10433 is very useful for removing of heavy metals such as Cadmium from industrial wastewater. The effect different concentrations of factors for Cadmium removal was favorable that confirmed last studies [18]. Sendstatein (1976) stated that the addition of nitrogen and phosphate inorganic increases the process of biodegradation, but does not affect the amount of biodegradation [19]. Manasy et al (2014) reported that *Halomonas* BVR 1 had a maximum cadmium uptake of 12.23 mg and a concentration of 200 mg/L. This bacterium has a high capacity of cadmium adsorption, and absorption depends on the pH, temperature and contact time and depends on the high salt concentration [20]. In this research the ability of biological removal of Cadmium from arouse environment by *H. elongate* IBRC-M10433 was examined. Results were analyzed by Taguchi method. The effective factor for largest biological removal of Cadmium by *H. elongate* IBRC-M10433 was determined. Results from this research indicated that *H. elongate* IBRC-M10433 has ability to remove of Cadmium and the other heavy metals from industrial wastewaters

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