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Optimal conditions for the biological removal of arsenic by a novel halophilic archaea in different conditions and its process optimization

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Recently, concerns about arsenic have been increased due to its high acute toxicity to human and serious environmental problems. In this study, the ability of *Halorcula* sp. IRU1, a novel halophilic archaea isolated from Urmia lake, Iran for arsenic bioaccumulation was investigated and optimized by Taguchi experimental design. The optimum conditions for high arsenic bioaccumulation by *Haloarcula* sp. IRU1 could be achieved in the presence temperature 40°C, pH 8 and $NaAsO_2$ at 90 mg/L. Under optimum conditions, the microorganism was able to perform their desired function with a 60.89 percent removal of arsenic. In conclusion, *Haloarcula* sp. IRU1 is resistant to arsenic and removes it in different conditions.

Keywords: Arsenic, bioaccumulation, optimization, *Haloarcula*.

INTRODUCTION

Arsenic is one of the most prevalent and toxic metalloids present in the environment. It is usually originated geogenically but can be intensified by human activities such as applications of pesticides and wood preservatives, mining and smelting operations, coal combustion, and industrial activities[1–3]. Arsenic can occur in the environment in several forms but in natural waters and drinking water, it is mainly found as trivalent arsenite (As III) or pentavalent arsenate (As V). Arsenate generally is the dominant form in oxic waters, while arsenite dominates in sulfidic and methanic waters including most geothermal water. Both forms are toxic; comparatively arsenite is the most toxic form[4, 5]. Chronic arsenic poisoning in the general population has been widely reported in many areas of the world today[9]. Consequentially, elevated levels of arsenic have been reported in soils and groundwater worldwide. The maximum concentration limit (MCL) of arsenic recommended for drinking water is 0.01 mg/L by the World Health Organization (WHO). Elevated levels of arsenic in drinking water can affect human health and have been implicated in human diseases and mortality[3, 7]. Chronic arsenic can have immediate toxic effects in humans. Organs involved with arsenic absorption, accumulation, and/or excretion such as gastrointestinal tract, circulatory system, liver, kidney and skin are most affected by arsenic[8, 9]. Signs of chronic arsenic toxicity include skin lesions (e.g., hyperpigmentation, hyperkeratosis, desquamation, and loss of hair), cancers of skin, bladder, kidney and lung, diseases of the blood vessels of the legs and feet, high blood pressure and reproductive disorders[10].

Given high toxicity of arsenic, its efficient removal from natural waters intended for drinking water, using low-cost methods, is considered of great importance[11]. Conventional physicochemical methods such as electrochemical treatment, ion exchange, precipitation, reverse osmosis, evaporation, and sorption for heavy metal removal from waste streams are not cost effective[12–14]. Recent recognition of the need to develop low cost environmentally friendly technologies for water treatment has stimulated interest in studies on the bioremediation of metals[15–17]. While arsenic is a well-known poison, some taxonomically diverse microorganisms have evolved biochemical mechanisms that either prevent arsenic from entering cells or rapidly extrude it back to the environment if it does enter[4, 5]. Some microorganism capable of removing arsenic from their surroundings could thus be ideal candidates for bioremediation, and could therefore be used as an alternative or to supplement the existing physico-chemical methods of arsenic removal[17]. This paper presents for the first time, the use of *Haloarcula* sp. IRU1 as a novel halophilic archaea for bioremediation of arsenic.

EXPERIMENTAL DETAILS

Microorganism and growth conditions

Haloarcula sp. IRU1 (identificatied on comparison of the 16S rRNA gene sequence) isolated from hypersaline Urmia lake, Iran was provided from Alzahar University. This microorganism was cultivated in 100 ml Erlenmeyer flasks containing 20 ml of defined basal salt medium and incubated in an orbital shaker for 5 days. The basal salt medium contained (g/L) NaCl, 250; $MgCl_2 \times 6\ H_2O$, 34.6; $MgSO_4 \times 7H_2O$, 49.4; Glucose, 50; yeast extract, 5; $CaCl_2 \times 2\ H_2O$, 0.92; NaBr, 0.058; KCl, 0.5; KH_2PO_4, 0.1; NaH_2CO_3, 0.17 in distilled water.

Bioaccumulation assay

Bioaccumulation assay of arsenic by *Haloarcula* sp. IRU1 was carried out in 20 ml of culture supplemented with various factors by varying pH [7, 8, and 9], temperature [37, 40, and 43°C] and arsenic as sodium arsenate concentrations [0.03, 0.06, and 0.09 (g/l)], as shown in Table 1). After 5 days incubation, 5ml of the culture broth was centrifuged at 10.000 rpm for 10 min, and then the pellet was washed three times with 10% (wv.) NaCl solution to remove arsenic that is bound to the bacterial surface. Finally, the pellet was air dried for a day in 25°C, treated with 500 µl of conc. HNO_3 and

Table 1. Factors and their levels employed in the Taguchi experimental design for bioaccumulation of arsenic by *Haloarcula* sp. IRU1

Factor	Levels		
	1	2	3
Temperature (°C)	37	40	43
pH	7	8	9
$NaAsO_2$ (mg/L)	30	60	90

bioaccumulation of arsenic in cell mass was estimated using atomic absorption spectrophotometry (AAS, Philips model PU 9100)[5].

Taguchi methodology experimental design

All the combination experiments using the assigned parameter values were conducted with the aim of obtaining the final optimum conditions. The Qualitek-4 software was used to design and analyze Taguchi experiments. Each experiment was replicated two times in a completely randomized design.

RESULTS AND DISCUSSION

The biological removal of arsenic by microorganisms is potentially important in present days because of the toxic effects of arsenic and its compounds. The activity of microorganisms is greatly influenced by different factors such as pH and temperature. Thus, the optimization of the conditions that facilitate the microorganisms could result in high bioaccumulation of heavy metals. In recent years, studies have been extended into newer areas, exploring its potential use for the removal of arsenic. Biovolatilization and biosorption using some microorganisms have great potential for the bioremediation of arsenic contaminated sites[18].

Before we could analyze the effect of different factors on the bioaccumulation of arsenic by *Haloracula* sp. IRU1, the significant factors and their levels must be determined. Table 1 shows the factors (variable) and their levels in the Taguchi experimental design for the bioaccumulation of arsenic by *Haloarcula* sp. IRU1. Each of these factors is assigned with three levels. We evaluated the effects of different factors (temperature, pH, and $NaAsO_2$ concentration) on the bioaccumulation of arsenic by this microorganism. Table 2 shows the layout of the L9 orthogonal array and the amount of arsenic bioaccumulation in each experiment. As shown in Table 2, *Haloarcula* sp. IRU1 grew in all conditions. Moreover, the bioaccumulation of arsenic by this microorganism occurred in all trials. The minimum and maximum bioaccumulation was observed in trial 1 and 5, respectively. These data suggested *Haloarcula* sp. IRU1 is a good candidate for the removal of arsenic because of its resistance to the arsenic toxicity.

Table 2. The orthogonal array of Taguchi experimental design and corresponding arsenic bioaccumulation by *Haloarcula* sp. IRU1

Trial	Temperature	pH	$NaAsO_2$	As (mg/L)	Arsenic removal (%)
1	1	1	1	6.73	22.43
2	1	2	2	14.64	24.40
3	1	3	3	26.54	29.49
4	2	1	2	18.32	30.53
5	2	2	3	54.80	60.89
6	2	3	1	23.40	78.01
7	3	1	3	9.42	10.47
8	3	2	1	45.14	66.46
9	3	3	2	37.44	62.40

The main effects of three factors (temperature, pH, and $NaAsO_2$ concentration) and their interaction at the assigned levels on *Haloarcula* sp. IRU1 were presented in Table 3. Among different factors, pH was very influential at level 2 (pH 8), whereas the effects of temperature and $NaAsO_2$ concentration were higher in level 2 (40°C) and level 3 (90 mg/L), respectively. Based on the studied factors showed in Table 3, pH showed stronger influence on the bioaccumulation of arsenic by *Haloarcula* sp. IRU1 followed by temperature and $NaAsO_2$ concentration.

Table 3. Main effects of different factors on arsenic bioaccumulation by *Haloarcula* sp. IRU1

Factors	Level 1	Level 2	Level 3
Temperature	15.97	32.173	30.666
pH	11.49	38.193	29.126
$NaAsO_2$	25.089	23.466	30.253

The main objective of ANOVA is to extract from the results how much variations of each factor causes relative to the total variation. The last column of Table 4 indicates the influence of each factor and column 3 gives a sum of squares (S)[19, 20]. The ANOVA results indicate that pH plays a significant role in arsenic bioaccumulation by *Haloarcula* sp. IRU1. It is clear that among the three studied factors, pH with maximum variance (V), sum of squares (S) and percentage influence (553, 1106.324, and 27.753, respectively) is the most influential factor for arsenic removal by *Haloarcula* sp. IRU1. It is also obvious from this Table that temperature and $NaAsO_2$ concentration (both with percentage influence 0) have no significant effect on arsenic removal.

The point prediction for achieving the highest arsenic bioaccumulation by *Haloarcula* sp. IRU1 in terms of contribution for the levels of factors is shown in Table 5. According to the obtained results, pH plays a significant role in arsenic removal than other selected parameters and their levels. The speciation of As is significantly affected by pH. As (III) is mostly found as an uncharged species at neutral pH, whilst As (V) is mostly found as negative species under the conditions of pH higher than 2.3[21]. Increasing pH can result in the formation and precipitation of metal hydroxides or oxides. Likewise, the pH of the medium can affect metal-microorganism responses by its effects on metal speciation and cell physiology and metabolism, indirectly[18]. The results indicate that the expected result under optimal conditions is 48.1% and the total contribution from all factors is

Table 4. ANOVA for bioaccumulation of arsenic by *Haloarcula* sp. IRU1

Factor	DOF (f)	Sum of Squares (S)	Variance (V)	F-Ratio (F)	Pure Sum (S)	Percent P (%)
Temperature	2	480.809	240.404	0.952	0	0
pH	2	1106.324	553	2.191	601.503	27.753
$NaAsO_2$	2	37.676	37.676	0.149	0	0

Table 5. Point prediction for optimum conditions arsenic bioaccumulation by *Haloarcula* sp. IRU1

Factor	Level	Contribution
Temperature	2	5.903
pH	2	11.923
$NaAsO_2$	3	3.983
Total Contribution From all factors…		21.809
Current Grand Average Of Performance…		26.269
Expected Result At Optimum Condition		48.078

21.81. With these selected factors and levels, the grand average performance is 26.27%.

CONCLUSION

Altogether, we introduced *Haloarcula* sp. IRU1 as an efficient microorganism for the bioaccumulation of arsenic for the first time. A combination of factors and their levels involved in the bioaccumulation of arsenic by *Haloarcula* sp. IRU1 was identified for its maximum yield. In the present paper, we showed that the yield of arsenic bioaccumulation can be significantly improved by optimization of the factors involved in bioaccumulation of arsenic by *Haloarcula* sp. IRU1. The optimal factor levels are pH 8, temperature 40°C, and $NaAsO_2$ concentration 90 mg/L. It is clear that pH is the most significant process factor affecting the arsenic bioaccumulation.

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