Journal of Environmental Health and Sustainable Development

The Effect of UV Light on Increasing the Arsenate Resistance of Acidithiobacillus Ferrooxidans

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ARTICLE INFO

ORIGINAL ARTICLE

Article History:
Received:25 November 2018
Accepted: 20 January 2019

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Keywords:
Acidithiobacillus Ferrooxidans,
Ultraviolet Rays,
Mutation,
Copper.

ABSTRACT

Introduction: Mutations are the most popular way to increase the efficiency of mineral waste bioleaching. *Acidithiobacillus ferrooxidans* are used as an important microorganism in biohydrometallurgy. Arsenate is one of the toxic elements in mines, which reduces the efficiency of *A. ferrooxidans* leaching. The purpose of this research was to increase the resistance of *A. ferrooxidans* to high concentrations of arsenate.

Materials and Methods: This research was an analytical – descriptive study. The studied population was isolated *A. ferrooxidans* bacterium from the Sarcheshmeh copper mine in Kerman. The highest tolerable concentration of arsenate was determined by successive cultures of this bacterium at increasing concentrations of arsenate. The bacteria were exposed to UV radiation at different times and then cultured in higher concentrations of arsenate.

Results: The results showed that the wild strain was able to grow in the medium containing 20 mM of arsenate. With adaptation, this bacterial strain could grow in medium containing increasing concentrations (40, 60, 80, 100, 120, and 140 mM) of arsenate. When the bacterium was exposed to UV ray for 60 minutes, it was able to grow at a concentration of 120 mM of arsenate.

Conclusion: The results indicated a very good effect of UV ray on increasing the arsenate resistance of *A. ferrooxidans*. It is suggested that this modified strain can be used in real environments for bioleaching.

Citation: Dehghani A, Dousti B, Gilavand F, et al. **The Effect of UV Light on Increasing the Arsenate Resistance of Acidithiobacillus Ferrooxidans**. J Environ Health Sustain Dev. 2019; 4(1): 557-66.

Introduction

Arsenate is a very toxic element that causes threats to human health and environment throughout the world. One of the biggest problems associated with arsenate is that even very low amount of it can lead to cancer [1]. Removal of arsenate from contaminated water due to the oxidation state (-3, 0, +3 and +5) with various physicochemical characteristics is very difficult [2]. Microorganisms play a significant role in the geochemical cycle of arsenate [3]. Metabolic mechanisms lead to resistance of micro-organisms against the harmful effects of arsenate [4]. However, some microbes actively use various combinations of arsenate in their metabolism as an electron

donor or recipient of electrons in their respiration. Some microorganisms can also methylate inorganic arsenate, or demethylate organic arsenate, which is probably a mechanism of resistance. So, understanding microbial responses to arsenate is very important for improving the arsenate bioremediation from contaminated environment and water. The release of arsenate into the environment can occur either by human activities or by natural geochemical processes [2]. Arsenate toxicity is dependent on its actual state of the chemical. Arsenate (As^{+5}) is a phosphate analog structure. Arsenate and phosphate ions compete together in many enzymatic reactions [5, 6]. In addition, trivalent arsenate has a high affinity for thiol groups and may inhibit many enzymes that are associated with thiols groups [7]. Arsenate can also lead to the destruction of Fe-S clusters in the proteins [2]. *Acidithiobacillus ferrooxidans* as a chemoautotrophic bacterium has been considered as one of the most important and frequently used microorganism in commercial biohydrometallurgy due to its acidophilic specifications. As far as growth energy is concerned, this bacterium can obtain energy from the oxidation of inorganic reduced sulfur compounds as well as the ferrous ions [8].

Bacterial leaching technology is a very effective method to treat low purity wastes. Low cost, simple equipment, fast processing, wide application, easy management, and environment-friendly are its advantages. Unfortunately, a few studies were conducted on bioleaching of mineral wastes. So, we should screen the new and suitable strain for bioleaching the mineral wastes [9]. The main bacterial screening methods include indigeneity, mutagenesis, and genetic modification [10]. The popular UV radiation-induced mutagenesis is the most effective physical method for screening [11]. The efficient ultraviolet wavelengths are mainly about 250 nm that are similar to the DNA absorption spectrum in most bacteria [11, 12]. Excessive UV rays can cause loss of the large segment of DNA or prevent the transection of DNA from opening, thereby preventing DNA replication and transcription. On the other hand, excessive UV rays can cause cell death. Therefore, high-efficiency strains may be chosen from several strains of positive mutation [12– 14].

The main objective of this study was to develop the arsenate resistance of the mutated *A. ferrooxidans*, so that it can tolerate high concentrations of arsenate in mines and perform the bio-leaching process well.

Materials and Methods

This research was an analytical - descriptive study. The studied population was isolated *A. ferrooxidans* bacterium from the Sarcheshmeh copper mine in Kerman.

Microorganism and media

A. ferrooxidans bacterium was prepared from Sarcheshmeh copper mine (Kerman, Iran). The composition of the culture medium (9K medium) for bacterial growth was as follows: 3 g/L $(NH_4)_2SO_4$; 0.1 g/L KCl; 0.5 g/L K_2HPO_4; 0.5 g/L $MgSO_4.7H_2O$; 0.01 g/L Ca $(NO_3)_2$; 44.22 g/L $FeSO_4.7H_2O$; 1ml/L H_2SO_4. This chemical material was bought from Sigma – Aldrich. A 10 percent bacterium was inoculated in the 9K liquid medium and incubated for 4 days at 30 ° C, pH 2, and 150 rpm. The bacterial growth curve was also plotted [15, 16].

Bacterial growth in presence of arsenate

The tolerance of *A. ferrooxidans* was analyzed using the different concentrations of arsenate. In order to measure the growth rate of the bacteria, the optical density of the culture medium was measured every 12 hours at 430 nm (OD_{430nm}), until the optical density of the culture medium reached 0.8 (OD_{430nm} = 0.8). A wavelength of 430 nm is the best wavelength for measuring the cell density in a salt medium. At first, the primary bacterium was inoculated by 10 percent into a medium containing 20 mM arsenate ($Na_2HAsO_4.7H_2O$). After the bacterial growth in this medium, the bacterium was inoculated in culture media containing 40, 60, 80, 100, and 120 mM arsenate respectively and the highest tolerable concentration was determined [5, 17].

UV light effect on increasing resistance to arsenate

A. ferrooxidans bacterium from different concentrations of arsenate medium (40, 60, 80, 100, and 120 mM arsenate) were distributed in five separate plates and placed at a distance of 30 cm from the UV lamp (270 nm) for 10, 30, 60, 90, and 120 minutes. After the irradiation, the 9k medium containing 120, 140, and 160 mM arsenate were inoculated by treated *A. ferrooxidans* bacterium. The optical density of the culture medium was measured at 430 nm (OD_{430nm}) every 12 hours. To ensure the stability of the mutation, the mutated bacterium was cultured in 9 K medium for three generations. Culture conditions were similar for each generation [11, 12].

Ethical issues

This study does not contain any studies with human participants or animals.

Results

Acidithiobacillus ferrooxidans growth

The growth curve of *A. ferrooxidans* is shown in Figure 1. As it is shown, the highest growth of the optical density was observed at 72 hours. In the following experiments, this time was used as the optimal growth time of *A. ferrooxidans*. The growth of the bacteria resulted in a change in the color of the culture medium from clear to dark brown.

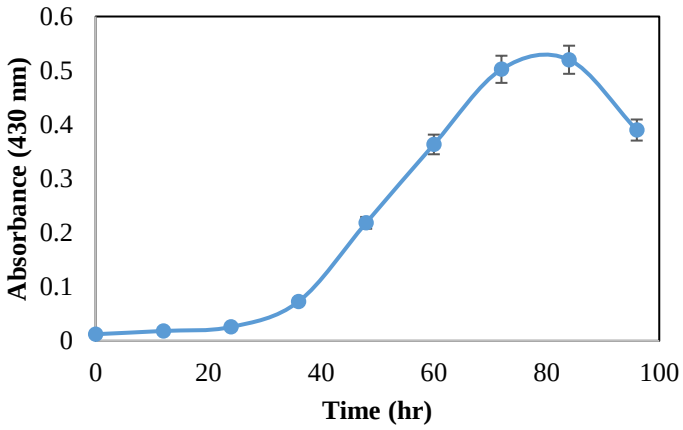

Figure 1: The growth curve of *A. ferrooxidans*

Growth of bacteria in different concentrations of arsenate

A.ferrooxidans was able to grow in concentrations of 20 – 100 mM arsenate. In these culture medium, optical density were increased over the time. The results are represented in Figure 2. The bacterium was not able to grow in a culture medium containing 120 mM arsenate. Over the time, there was no change in the opacity and the optical density of the medium.

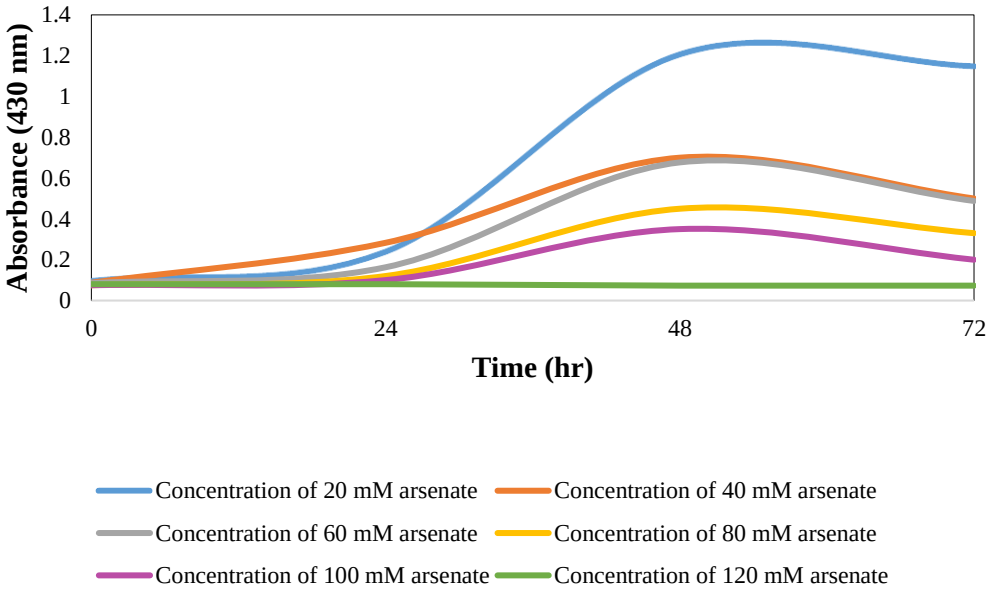

Figure 2: Growth of *A. ferrooxidans* in different concentrations of arsenate.

(*A.ferrooxidans* was able to grow in concentrations of 20 – 100 mM arsenate but could not grow at a concentration of 120 mM of arsenate)

The effect of UV radiation on increasing resistance to arsenate

As indicated in Figure 3, the best effective time of UV light on mutation and increased resistance of bacteria to the high concentration of arsenate was 60 minutes. The mutated bacterium was capable of growing at a concentration of 120 mM of arsenate. The results are shown in Figure 4. The curve (a) is related to the growth of unreacted strains at a concentration of 100 mM arsenate, the curve (b) is related to the growth of mutated strains in the concentration of 120 mM arsenate, and the curve (c) is related to the non-growth of the mutated bacterium at a concentration of 140 mM arsenate. The mutated bacterium growth after three concentrations in 9K medium containing 120 mM of arsenate was similar to the initial growth, which indicates a stable mutation in the mutant bacteria.

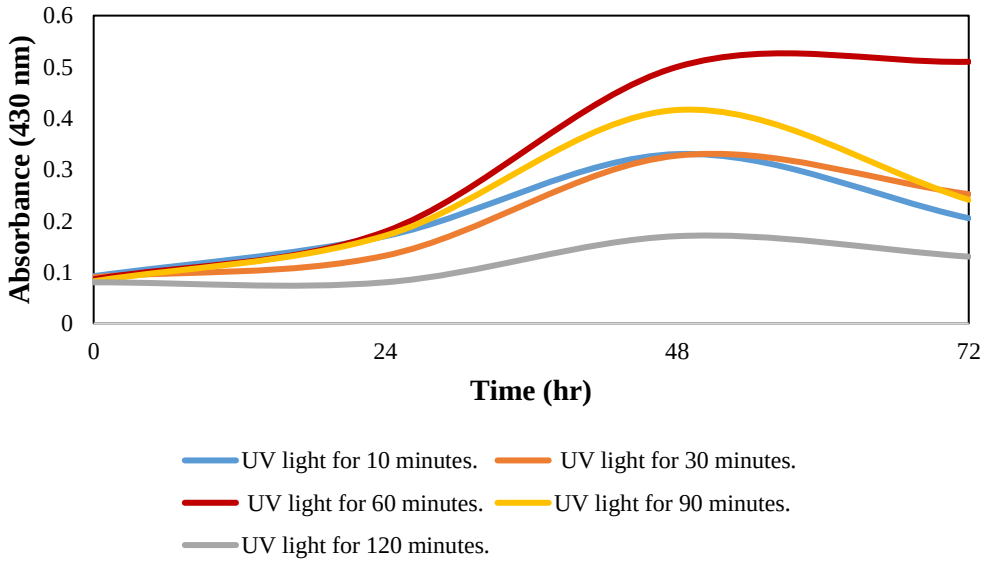

Figure 3: Effect of UV radiation on *A. ferrooxidans*

(The best effective time of UV light was 60 minutes)

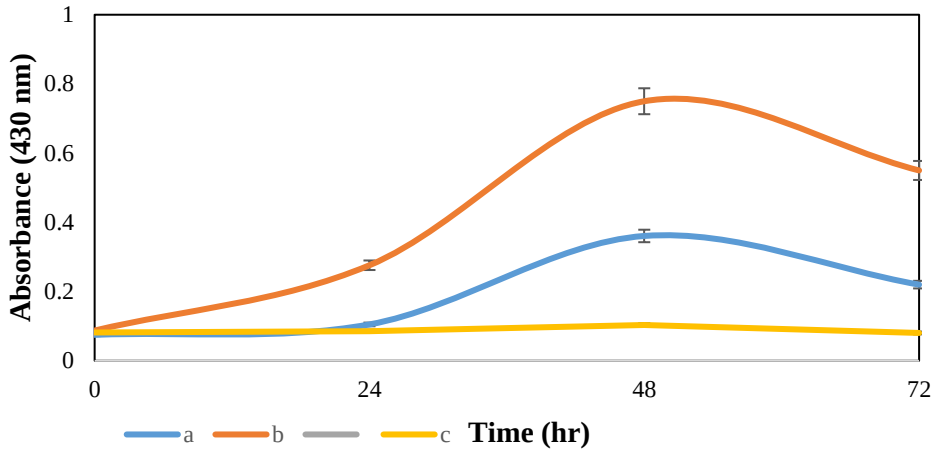

Figure 4: Growth of mutated *A. ferrooxidans* in high concentrations of arsenate

(a): Growth of unreacted strains at a concentration of 100 mM arsenate.
(b): Growth of mutated strains in the concentration of 120 mM arsenate.
(c): Non-growth of a mutated bacterium at a concentration of 140 mM arsenate.

Discussion

In our study, a wild species of *A. ferrooxidans* was used which was previously isolated from the Sarcheshmeh copper mine. The results of the preliminary experiments showed that this species can tolerate only the concentration of 20 mM of arsenate. This wild species was not able to grow at a concentration of 40 mM of arsenate.

In order to increase the adaptability of bacteria with high concentrations of arsenate, this species was cultured in the 9K medium containing increasing concentrations (40, 60, 80, 100, 120, and 140 mM) of arsenate. At the end of the adaptation, this bacterium was able to grow at a concentration of 100 mM of arsenate. Yan and et al isolated a native *A. ferrooxidans* from Wudalianchi volcanic lake, northeast China. The bacterium could grow well at concentrations of 8 to 64 mM of arsenate but at concentrations of 96 and 128 mM arsenate, the growth of the bacterium was sharply reduced [5].

Since mutation is used to genetically modify in bacteria, it was decided to use one of the mutation methods to investigate the possibility of increasing bacterial tolerance to arsenate. UV ray is the most usual physical mutagen. The most important effect of UV is the formation of thymine dimers, which can change the biological activity of DNA, lead to bacterial mutation, and ultimately cause it to die [13,14,18].

For this purpose, the bacterium was affected by UV radiation in different periods of time. The bacteria that were exposed to UV radiation for 60 minutes were able to grow at a concentration of 120 mM of arsenate. Tillich and et al induced a point mutation in Synechocystis sp. by ultra violet radiation and prompted temperature tolerance in this organism [19]. In order to improve production of endoglucanase and β-glucosidase from newly isolated thermo-tolerant *Streptomyces griseoaurantiacus*, Kumar applied UV mutagenesis treatment [20].

The results of this study showed that UV radiation could be used to increase the resistance of *A. ferrooxidans* to arsenate. The mutated bacteria can be applied to leach elements from contaminated environments with arsenate. We suggest other researchers to identify mutated genes in mutagen strains and mechanisms of increasing resistance to arsenate at the molecular level.

Conclusion

The results of this study showed that the treated bacteria with UV light exhibited great efficacy growth in arsenic-containing media under natural condition, the same conditions that exist in the

natural environment. We recommend other researchers to investigate application of these mutated microbial strains in the real environment. Eventually, the role of microbial interactions as well as physical and chemical parameters should be considered in the bioleaching process in real ecosystems. Bioleaching in natural environments can be a cooperative process; therefore, the role of each factor should be determined.

Acknowledgements

This article is an excerpt from the master's thesis of Abdolrahman Dehghani. Samaneh Sedighi-Khavidak conceived and supervised the study, designed experiments and analysed the data; Behrooz Dousti, Farhad Gilavand, and Amirarsalan Kavyanifard provided help in the experimental design and data analysis. All authors read and approved the final manuscript.

Funding

The work was not funded.

Conflict of interest

Authors have no competing interests.

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