

Effect of heavy metals on the induction of arsenate reductase of *Bacillus megaterium*

Mst. Nusrat Jahan Arbi, Sabrina Sharmin¹, Shafia Mustafa Marzan,
Nasrin Akhter², Md. Zahidul Islam*, Umme Salma Zohora and
Mohammad Shahedur Rahman

Department of Biotechnology and Genetic Engineering, Jahangirnagar University, Savar,
Dhaka-1342, Bangladesh

Abstract

Throughout the world, millions of people are affected by arsenic as they drink contaminated water. Almost all the districts in Bangladesh are under the alarming incursion of arsenic due to its ubiquitous underground presence. Arsenic is responsible for triggering many diseases, such as kidney failure, vertigo, diarrhoea, even cancer in the lung and skin and so on. Widespread arsenic has made it impossible to remove it from the environment. However, management measures can be taken to withstand its effect. Therefore, bioremediation can be one of the ways to remove arsenic from contaminated water. *Bacillus megaterium* was isolated from the arsenic-contaminated soil. This bacterium produces an arsenate reductase enzyme that can convert arsenate [As(V)] to arsenite [As(III)]. Arsenate reductase enzyme was induced by arsenate. Pretreatment with other heavy metals, like lead and bismuth, also induced this enzyme. Induction also took place in both moving and static culture conditions. In static culture, biofilm was formed, and the most significant amount of enzyme induction occurred in this condition compared to any other culture condition. Enzymatic treatment to separate the cells in biofilm released more enzymes in the medium than mechanical treatment. This strain was able to grow aerobically and anaerobically as well. This strain can be used to further study the effect of various circumstances on the induction of the arsenate reductase enzyme. The results of this research will help the detoxification of arsenic contamination to protect the water, soil, aquaculture, agriculture and global ecosystem. Subsequently, a more meaningful way of bioremediation can be attained.

Keywords: *Bacillus megaterium*, Arsenate reductase, Arsenate, Arsenite, Lead, Bismuth.

INTRODUCTION

Due to the omnipresence of arsenic in the environment, exposure to it is a daily event seen in numerous countries. Geomorphological contamination of drinking water is the main reason behind this exposure (IARC, 2004). Indeed, around 330 million people in the Indian Peninsula are vulnerable to arsenic diseases because of the incidence of exposure to arsenic. Nowadays, it is considered one of the significant causes of illness in the world, especially in several territories of countries like Bangladesh, India, Argentina and Mexico due to the poisoning of arsenic. Consequently, the widespread occurrence of arsenicosis

¹ Faculty of Pharmacy, Level 11, FF1 Building, UiTM Selangor, Puncak Alam Campus, 42300 Bandar Puncak Alam, Selangor, Malaysia.

² Khulna Medical University, Khulna-9100, Bangladesh.

*Corresponding author. Email: zahidul@juniv.edu

occurred in Bangladesh due to the poisonous drinking water wells containing micro molar concentrations of arsenic, leaving it as a crucial demand to scrutinize the process of its conversion and mobilization (Dhar *et al.*, 1997). Either a single time exposure or a long time, slow repeated accumulation can cause toxicity in the body. Numerous shallow and deep wells submerged in different parts of the country are setting up another level of toxicants. Studies have shown that Bangladesh's groundwater contains arsenic that is above the maximum safe threshold for drinking water. In 1996, around 400 measurements were taken in Bangladesh (Smith *et al.*, 2000). Among the 64 districts of Bangladesh, almost 59 have been hard hit by arsenic contamination (Safiuddin & Karim, 2001).

The level of arsenic in water inhabitants such as fishes and seafood are likely to be high, as they ingest it directly (IARC, 2004). Arsenic accumulation in soil and water occurs through chemical spills or incautious disposal strategies. Plants' absorption of arsenic is a fairly easy process, so higher amounts of arsenic in food would not be a matter of wonder. Arsenic causes neurotoxicity and reproductive and developmental problems in exposed organisms (Mukhopadhyay *et al.*, 2002). Coming across excessive and consistent inorganic arsenic can lead to sterility and frequent abortion in women, and epidermal disorders, vulnerability to infection, heart diseases, and neurological disorders in men and women (Lee-Feldstein, 1989). Adverse reproductive and developmental cases, increased stillbirth, frequent miscarriage, underweight deliveries, neonatal and post-neonatal mortality cases are vastly seen in pregnant women owing to high intake of arsenic and its ability to cross the placental barrier (Hopenhayn *et al.*, 2000; Ahmad *et al.*, 2001).

About 50 years of mortality statistics were analyzed for lung and bladder cancers, considerably expanding a prior analysis (Smith *et al.*, 1998). More study could augment the comprehension of disease latency and the consequences of less exposure. Eventually, in northern Chile, increased arsenic water drinking was found to be oddly correlated with lung cancer (Ferreccio *et al.*, 2000). The same outcome was found in the case of skin and bladder cancer, where monomethylarsonic acid (MMA) to dimethylarsinic acid (DMA) ratios are involved (McCarty *et al.*, 2007; Pu *et al.*, 2007), however, no genetic confirmation has been found yet. Another way of arsenic pollution could be producing reactive oxygen species (Pi *et al.*, 2002).

Long-term actions should be taken immediately to reduce the spread of arsenic from different sources. No matter what process is used, continuous monitoring is the key to stopping future contamination (Jain & Singh, 2012). The ars operon in the system contains the *ArsC* gene that encodes the arsenate reductase enzyme. The binding of this enzyme with arsenate ion led to the detrimental conversion of arsenate to arsenite (Arbi *et al.*, 2022). Microbial remediation of arsenic could be one of the methods of choice, even though sometimes microbial metabolism apparently exacerbates environmental arsenic problems. The success of these bioremediation processes depends upon a better understanding of how microbial populations respond to different concentrations of metals, optimization of biological and environmental conditions of these bacteria, scrutinizing the best conditions of their enzyme induction and their enzymes for their arsenic metabolism.

MATERIALS AND METHODS

Reagents and Medium: In this study, yeast extract mannitol (YEM) medium was used for bacterial growth. However, No. 3 medium was used for the production of biofilm (Zohora *et al.*, 2009). No. 3 medium contains peptone 3.0g (Titan Biotech LTD, India), starch 1.0g (Scharlau, Spain), KH_2PO_4 1.0g (Scharlau, Spain), and $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ 0.05g (MERCK, India). Stocks of 2 mM and 10 mM As (III) and As (V) were prepared by dissolving sodium arsenite $[(\text{NaAsO}_2)]$ (LOBA Chemie, India)] and sodium arsenate heptahydrate $[(\text{Na}_2\text{HAsO}_4 \cdot 7\text{H}_2\text{O})]$ (LOBA Chemie, India)] in sterile PBS solution. Stock of 2 mM Lead nitrate $[\text{Pb}(\text{NO}_3)_2]$ (Uni-Chem, China), bismuth nitrate $[\text{Bi}(\text{NO}_3)_3 \cdot 5\text{H}_2\text{O}]$ (MERCK, Spain) were also prepared by dissolving them in sterile PBS. Lugol's iodine was prepared by using 10g of KI and 5g of I in a 100 mM PBS solution. A solution of 50 mL (Sigma Aldrich, Switzerland, potency 30 U/mL) of alpha amylase was made by dissolving 50 mg of the enzyme into 50 mL of buffer solution. Proteinase *k* (Bioneer Corporation, Korea) enzyme was dissolved to a concentration of 25 mg/mL. For lipase enzyme preparation, 1mg lipase enzyme (Sigma Aldrich, Switzerland) was dissolved in 30 μL of PBS solution.

Sample collection and isolation of bacteria: Subsurface soil was collected from an arsenic-contaminated area, and samples were diluted up to 10^5 times using a sterile 0.85% NaCl solution. Diluted samples (100 μL) were spread on YEM and incubated at 37°C for 24-48 h. The pure bacterial strain was obtained by successive cross-streaking processes (Islam *et al.*, 2019).

Arsenate-resistant bacteria were screened using the methods described by Mandal *et al.* (Mandal *et al.*, 2007). In brief, arsenate-resistant bacteria were cultured in arsenate (2 mM) containing YEM medium and incubated at 37°C and 120 rpm in a shaking water bath for 24 h. Supernatants were collected after 10 min centrifugation at 10,000 rpm and kept on ice. Positive and negative controls were 2 mM sodium arsenite and 2 mM sodium arsenate solution. 30 μL of 10% Lugol's iodine solution in 1% starch complex was mixed to each 1.5 mL supernatant. The samples were kept in the dark for 10 minutes at 37°C and the optical density (OD) of the supernatants was measured immediately at 570 nm wavelength. In this study, all experiments were performed using an isolated bacterial strain *B. megaterium* (Islam *et al.*, 2019 and Khanam *et al.*, 2019). *B. megaterium* was cultured in No. 3 medium for biofilm production (Zohora *et al.*, 2009). Three independent experiments were carried out for each sample. Average values and standard deviations were calculated.

Screening and comparison of other metal resistance and induction activity: Whether the arsenate reducing enzyme is only induced by the same molecule that it converts, or also by some other molecules. Resistance and induction activity were tested for some other heavy metals, too. This bacterium was cultured in the same manner as cultured for the isolation of arsenic-resistant bacteria (Islam *et al.*, 2019). Bacteria were cultured in different plates containing lead, bismuth, and arsenite (2mM), along with arsenate-containing plates. The plates were kept for 48 h in the incubator and then inoculated into 2 mM arsenate-containing YEM broth medium. These inoculated broths were incubated for 24 h at 37°C and 120 rpm. Then all the cultures were centrifuged for 10 minutes, the supernatant was collected, and 1.5 mL of each was taken into different test tubes. 30 μL of 10% Lugol's iodine solution in 1% starch complex was mixed with each of them. After

keeping those in the dark for 10 minutes, the color of these solutions was checked by the naked eye. The deep blue color of the Lugol's iodine solution disappears if the arsenate [As(V)] is converted to arsenite [As(III)].

Determination of arsenate reductase enzyme-induced metabolism by other metals:

The exact procedure mentioned in the previous section was followed, and induction by arsenite, lead and bismuth was investigated. Bacteria were cultured in plates containing 2mM of lead, bismuth, arsenite and arsenate. The plates were kept for 48 h in the incubator and then inoculated into 2 mM arsenate-containing YEM broth medium. These inoculated broths were incubated for 24 h at 37°C and 120 rpm. Then all the cultures were centrifuged for 10 minutes, the supernatant was collected, and 1.5 mL of each was taken into test tubes. 30 µL of 10% Lugol's iodine solution in 1% starch complex was mixed with each of them. After keeping those in the dark for 10 minutes, absorbance was taken at 570 nm. Positive and negative controls were 1.5 mL of 2 mM sodium arsenite and sodium arsenate (Islam *et al.*, 2019).

Induction of arsenate reductase enzyme in various culture conditions: Induction of the enzyme was tested in both static and shaking culture conditions.

Induction of arsenate reductase enzyme in shaking culture conditions: A few test tubes of YEM medium were taken to investigate the induction mechanism in shaking conditions. Among them, some were added with 2 mM arsenate (AsV) while some were not. All the test tubes were inoculated and kept in the water shaking bath at 37°C and 120 rpm for 24 h. Then, each of the cultures was centrifuged, and the pellet was collected and washed with an autoclaved buffer twice. A 5 mL autoclaved medium was then poured into those tubes. Test tubes were kept in the water shaker bath at 37°C and 120 rpm. After 24 h, the culture was centrifuged for 10 minutes, and the supernatant was collected. 2 mM arsenate was mixed and incubated for 2 h at 37°C. Then 30 µL of 10% Lugol's iodine solution in 1% starch complex was mixed and kept in the dark for 10 minutes to observe the color disappearance in the presence of arsenite. Optical density (OD) was measured at 570 nm immediately. Positive and negative controls were 2 mM sodium arsenite and sodium arsenate, respectively.

Induction of arsenate reductase enzyme in static culture conditions: At the same time, to investigate the induction in sessile conditions, No. 3 medium and 2 mM arsenate (V) containing beaker, along with a control were inoculated and kept in the incubator at 37°C for 24 h to allow the formation of biofilm. After 24 h, the culture from the static condition was observed and centrifuged, and the pellet was collected. In the case of static plate culture, the colony was transferred from the plate into No. 3 medium-containing tubes and incubated at 37°C for 24 h. The rest of the procedure was done in the same manner as mentioned before in the case of shaking culture condition.

Mechanical treatment of biofilm: In the procedure mentioned above, in the case of static culture in a beaker, after 24 h, the culture was centrifuged, and the pellet was inoculated again into autoclaved No. 3 medium. After 24 h, the culture was homogenized by homogenizer for 2 minutes with a few seconds break after every 30 seconds and

centrifuged to collect the supernatant. The rest of the procedure was as mentioned before (2.5.1). Absorbance was then taken at 570 nm.

Enzymatic treatment of biofilm: Here, instead of homogenizer, enzymes such as alpha amylase, lipase and proteinase *k* were used to break down the extracellular polymeric substance into which bacterial adherent cells were embedded. As this substance is mainly made of polysaccharides and proteins, hence various enzymes were used to dissolve that material. After 10 minutes, the culture was centrifuged, and the supernatant was taken. Supernatant was treated with 2 mM arsenate, then incubated, and absorbance was taken at 570 nm.

Culture in anaerobic conditions: An anaerobic culture was performed to examine the presence of another mechanism of arsenate conversion to arsenite by an arsenate reductase enzyme. As the *Arr* or arsenate respiratory reductase is responsible for using arsenate as the electron acceptor and respiratory acetate as the electron donor, this enzyme can conserve energy by arsenate respiration (Afkar *et al.*, 2003; Glasser *et al.*, 2018). This process is only possible when an anaerobic condition is present (Salticov *et al.*, 2005). An anaerobic jar was used to culture the bacteria in the medium containing 2 mM arsenate for 24 h at 37°C without shaking. The rest of the procedure and optimization kept alike the process used in static aerobic culture.

RESULTS AND DISCUSSION

Isolation of arsenate-resistant bacteria: Various isolated bacterial strains were grown in 2 mM arsenate-containing YEM medium. Among them, only four converted arsenate to arsenite, and the arsenite acted as a reducing agent. The bacteria that produce more arsenite from arsenate can be identified by observing the faster disappearance of the blue color of the starch-Lugol's iodine. *B. megaterium* was the most potent as it quickly changed the iodine color (Islam *et al.*, 2019). Optical density (OD) was taken at 570 nm, and it was confirmed that the culture supernatant had lost the blue color of iodine.

Screening and comparison of resistance and reduction activity by other heavy metals: Resistance against other metals like lead and bismuth, arsenate, and arsenite was found in the case of the *B. megaterium* (strain I-34). Bacterial growth in plates containing lead, bismuth, and arsenite was found (Figure 1).

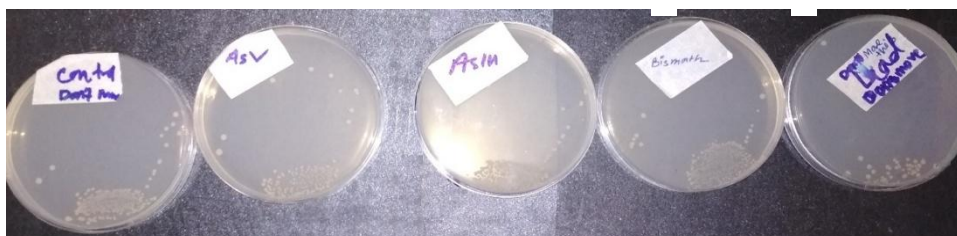


Fig. 1. Resistance of *B. megaterium* (I-34) strain against arsenate, arsenite, lead and bismuth-containing medium. Bacterial growth contained these metals at a final concentration of 2 mM.

These pretreated bacteria also grew in the YEM broth medium containing 2 mM of arsenate. The supernatant of the broth lost the blue color of Lugol's iodine. Induction of the arsenate converting enzyme was also found because it changed the color of Lugol's iodine. The blue color of iodine disappeared in the samples induced by arsenate, arsenite, lead and bismuth (Figure 2 and 3). Three independent experiments were carried out for each sample. Average values and standard deviations were calculated.

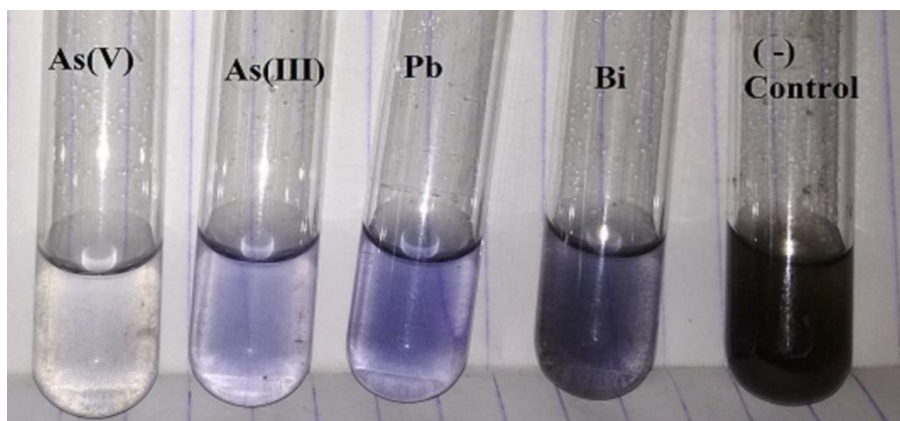


Fig. 2. Induction activity of *B. megaterium* (I-34) by arsenate, arsenite, lead and bismuth. The negative control is sodium arsenate. The less intense the color, the more arsenate was converted to arsenite.

The starch-iodine complex is very useful for indicating redox titrations that involve iodine because of the distinct color change. When there is an excess oxidizing agent, the complex is blue, and in the presence of a reducing agent, the I_5^- breaks up into iodine and iodide, resulting in color disappearance. The arsenite [As (III)] solution, a reducing agent, reacts with the starch-iodine complex, resulting in color ranging from blue-black to colorless (Mandal *et al.*, 2007).

In the tube, arsenate is converted to arsenite, which is responsible for the disappearance of the deep blue color of the Lugol's iodine solution, whereas in the absence of arsenite in the control, which contains only arsenate, the color remains intact. The presence of arsenite in all the tubes indicates the conversion of arsenate to arsenite. As all of them were pretreated with various metals, it can be said that all the metals induce arsenate-reducing enzymes to some extent.

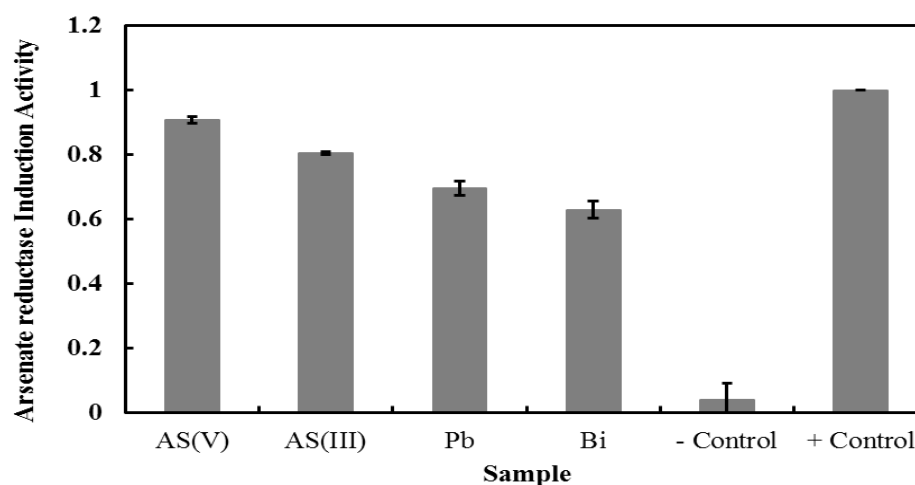


Fig. 3. Resistance and induction activity of various metals. The mean values and standard deviation of arsenate concentration were shown (N=3). Unlike the negative control (sodium arsenate), the concentration of arsenate in all the samples was lower. It indicates the conversion of arsenate to arsenite. The concentration of arsenate was zero in the positive control (sodium arsenite).

Heavy metals such as lead and bismuth often occur in areas contaminated with arsenic, mainly due to mining or industrial emissions. Multiple toxic elements are released into soils, water and the aqueous environment, leading to combined exposure. Mixed heavy metals may contribute to cross-metal induction (Xu *et al.*, 2024). Therefore, the co-occurrence of heavy metals with arsenic may influence the toxicity of soil and water. Here, the studied strain showed resistance to various heavy metals.

Induction of the arsenate reducing enzyme also occurred not only by arsenate itself but also by its reduced form, arsenite, and also by lead and bismuth. Lead and bismuth were selected based on both chemical and environmental considerations. Bismuth, being in the same group as arsenic in the periodic table, may exhibit similar chemical behavior and thus potentially influence arsenic reductase induction. In addition, lead was included as a related heavy metal to assess its comparative effect. Moreover, these metals frequently co-occur in contaminated environments, where mixed exposure and potential cross-contamination can affect biological responses. Therefore, their inclusion allows for a more realistic evaluation of enzyme induction under environmentally relevant conditions. The concentration of arsenate seems low compared to the negative control (Figure 3). In all the samples, the concentration of arsenate varied depending on the amount of arsenite that was reduced from arsenate.

Induction in various culture conditions: In both the culture conditions, static and shaking, induction took place. However, the amount of enzymatic induction of the enzyme in various conditions was not the same. Different results in different conditions may indicate a correlation between the culture conditions of enzyme induction.

Induction in shaking culture conditions: Induction took place in shaking culture conditions (Figures 4 and 6). After subsequent steps, when incubated with 2 mM arsenate and then mixed with starch iodine, the blue color of Lugol's iodine disappeared due to the presence of arsenite in the supernatant.

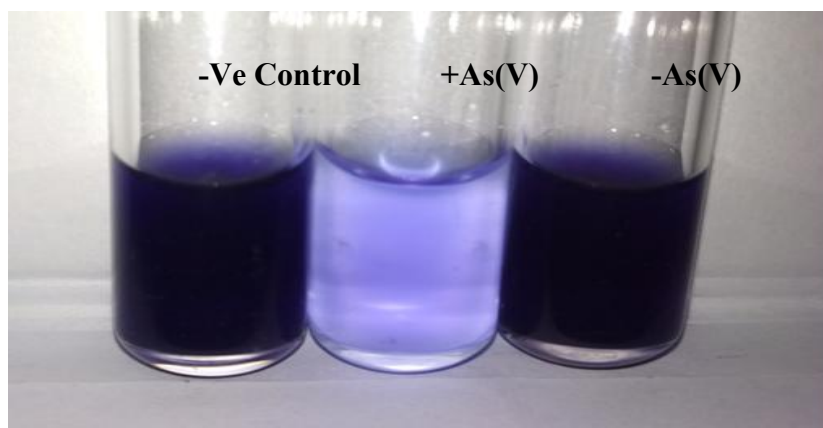


Fig. 4. Induction in shaking culture conditions in the presence of arsenate and the absence of arsenate. Negative control was the same amount of Lugol's iodine

Induction in static culture conditions: Induction also took place in the case of static culture (Figure 3.6). *B. megaterium* formed biofilm when it was inoculated in the beaker containing No. 3 medium (Figure 5).

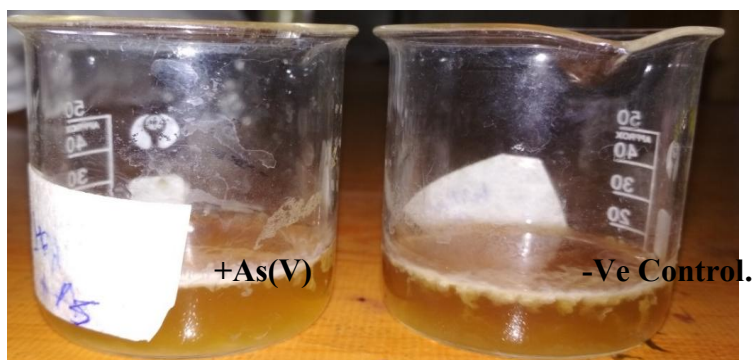


Fig. 5. Biofilm formation of the I-34 strain in No. 3 Medium

Mechanical treatment of biofilm and induction: In this experiment, the induction of enzymes was more than shaking culture (Figure 6). Mechanical treatment was used to separate the bacteria which were attached to the biofilm formation; there was a high chance that the cell itself would be broken down. Therefore, more enzymes could have been released into the medium if the cells were separated successfully without cell wall disruption.

Enzymatic treatment of the biofilm and induction: Here, it can be seen that induction occurs mostly in the biofilm (Figure 6). When it is treated with enzymes to separate the extracellular polymeric substances (EPS) between cells, it gives the best result compared to the mechanical treatment of the biofilm, as the chance of a breakdown of the cell itself is minimized. As the enzyme is extracellular, the experiment conducted in the separation of cells in the biofilm is an important part, so that enzymes can be released successfully into the medium.

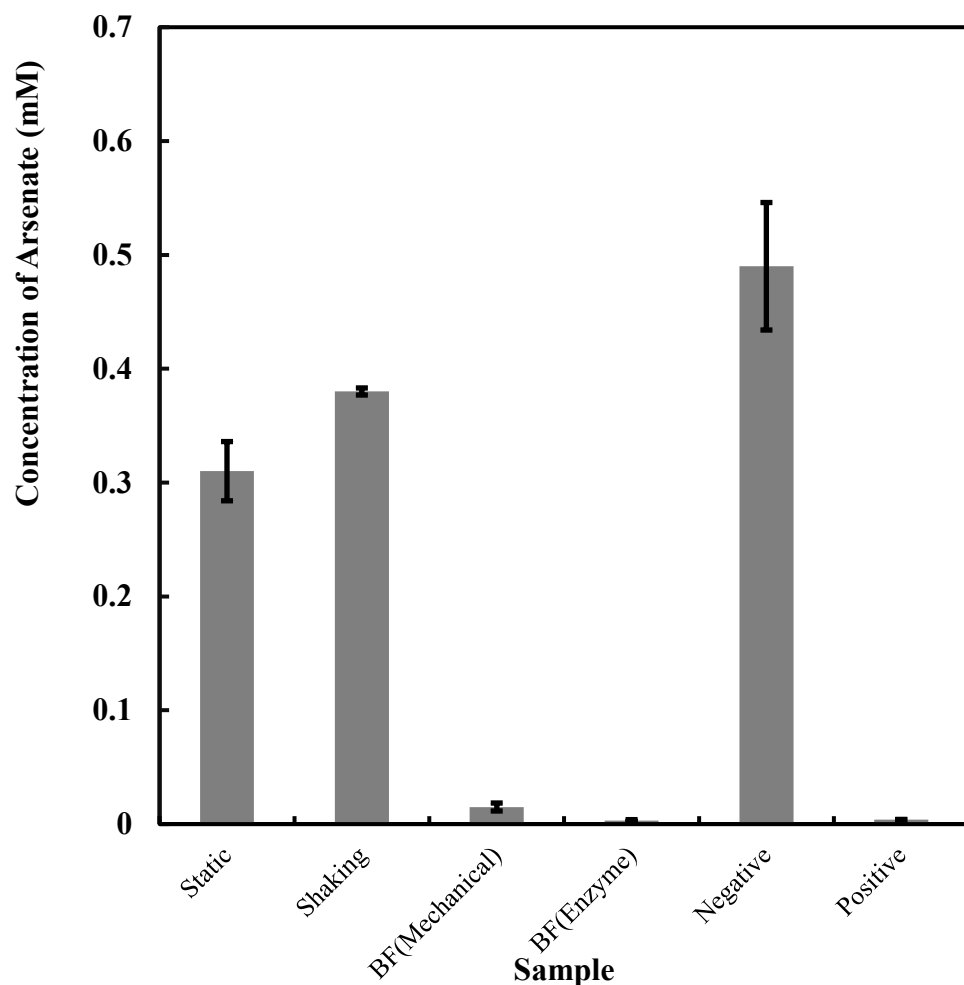


Fig. 6. Induction of arsenate reductase enzyme in various conditions (N=3). In all culture conditions, induction took place. In biofilm, it is notable, particularly when enzymatically treated to separate the cells. Negative and positive controls were sodium arsenate and arsenite respectively. BF= biofilm.

Anaerobic culture: Induction of the arsenate-reducing enzyme was also observed when bacteria were cultured anaerobically (Figure 7). The presence of the arsenate respiratory

reductase can be hypothesized here. Because this enzyme, expressing the *Arr* gene, is only expressed in anaerobic conditions.

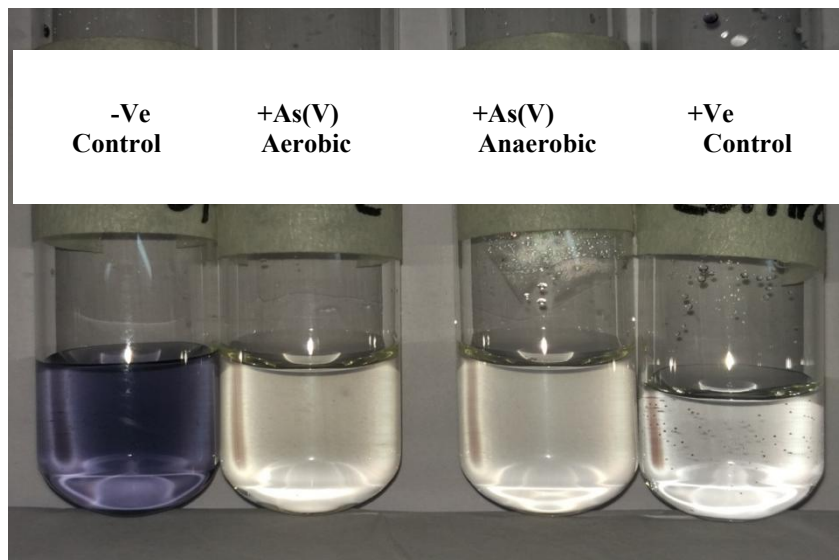


Fig. 7. Aerobic and anaerobic induction of the arsenate reductase enzyme

This result shows the presence of the *ArsC* and *Arr* genes of the *B. megateerium* strain I-34. *Bacillus selenitireducens* strain MLS10 also showed the same mechanism in the presence of air. Subsequent research confirmed the presence of the *Arr* gene, which is responsible for respiration through arsenate, using it as an electron acceptor (Afkar *et al.*, 2003)

Conclusion: Microbial metabolism undoubtedly intensifies environmental arsenic problems by releasing arsenite into drinking water, including shallow wells. Arsenic is removed by oxidation of As(III) to As(V) through microbial batch reactors. The microbial anaerobic respiratory arsenate reductase releases previously immobilized sub-surface As(V) into water in newly drilled wells. It is possible that microbial metabolism (arsenite oxidase coupled with precipitation in mineral deposits) can be harnessed for feasible bioremediation of wastewater or drinking water contaminated with arsenic. Under aerobic conditions, it uses arsenate for its growth and produces arsenite. It shows auto-induction metabolism to produce the arsenate-reducing enzyme. Some other metals, like lead and bismuth, were also able to induce the arsenate reductase enzyme. Induction of the arsenate-reducing enzyme was also observed when bacteria were cultured anaerobically. Different culture conditions were tested, and amongst them, biofilm formation was the most potent condition to induce arsenate reductase enzyme. Enzymatic treatment to separate cells so that they can release more enzymes in biofilm gave more promising results than mechanical treatment. The bacterial isolate can be exploited for the study of the mechanism of arsenic metabolism and subsequently, for the possible bioremediation of arsenic-containing water.

Authors' Contributions: MNJA performed the experiments, analyzed data, and wrote the article; SS, SMM, NA, MZI, USZ, and MSR designed the research, analyzed data, and wrote the article.

Conflict of Interest: The authors declare that they have no conflicts of interest.

Acknowledgment: This work was supported in part by a research grant from Jahangirnagar University to Md. Zahidul Islam.

REFERENCES

- Afkar, E., Lisak, J., Saltikov, C., Basu, P., Oremland, R.S. and Stolz, J.F. 2003. The respiratory arsenate reductase from *Bacillus selenitireducens* strain MLS10, *FEMS Microbiol Lett.* **226**(1):107-12.
- Ahmad, S.A., Sayed, M.H., Barua, S., Khan, M.H., Faruquee, M.H., Jalil, A., Hadi, S.A. and Talukder, H.K. 2001. Arsenic in drinking water and pregnancy outcomes. *Environmental health perspectives.* **109**(6): 629.
- Arbi, M.N.J., Thahsin, N., Nurjahan, A., Sharmin, S., Islam, M.Z., Zohora, U.S., *et al.* 2022. Homology Modelling and Docking Studies of Arsenate Reductase of *Bacillus megaterium*. *J Appl Biotechnol Rep.*; **9**(3):707-18.
- Dhar, R.K., Biswas, B.K., Samanta, G., Mandal, B.K., Chakraborti, D., Roy, S., Jafar, A., Islam, A., Ara, G., Kabir, S. and Khan, A.W. 1997. Groundwater arsenic calamity in Bangladesh. *Current Science.* **73**(1); 48-59.
- Ferreccio, C., González, C., Milosavljevic, V., Marshall, G., Sancha, A.M. and Smith, A.H. 2000. Lung cancer and arsenic concentrations in drinking water in Chile. *Epidemiology.* **11**(6):673-679.
- Glasser, N.R., Oyala, P.H., Osborne, T.H., Santini, J.M. and Newman, D.K. 2018. Structural and mechanistic analysis of the arsenate respiratory reductase provides insight into environmental arsenic transformations. *Proceedings of the National Academy of Sciences.* **115**(37): E8614-E8623.
- Hopenhayn-Rich, C., Browning, S.R., Hertz-Picciotto, I., Ferreccio, C., Peralta, C. and Gibb, H. 2000. Chronic arsenic exposure and risk of infant mortality in two areas of Chile. *Environmental health perspectives.* **108**(7): 667.
- IARC, Working Group on the Evaluation of Carcinogenic Risks to Humans, 2004. World Health Organization and International Agency for Research on Cancer. *Some drinking-water disinfectants and contaminants, including arsenic.* (Vol. **84**). IARC.
- Islam, M.Z., Arbi, M.N.J., Moni, R., Zohora, U.S., Nishat, F.Z., Khanam, R. and Rahman, M.S. 2019. Reduction of Arsenate in a new isolate of *Bacillus megaterium*. *Jahangirnagar University Journal of Biological Sciences.* **8**(2): 47-59.
- Jain, C.K., and Singh, R.D. 2012. Technological options for the removal of arsenic with special reference to South East Asia. *Journal of environmental management.* **107**: 1-18.
- Khanam, R., Moni, R., Islam, M.Z., Billah, M.M., Zohora, U.S., Sabrin, F., Rahman, M.S. 2019. Study of an Arsenic Metabolizing Bacteria from Arsenic Contaminated Soil of Chandpur District Bangladesh. *Jahangirnagar University Journal of Biological Sciences.* **8**(1):57-65.
- Lee-Feldstein, A. 1989. A comparison of several measures of exposure to arsenic: matched case-control study of copper smelter employees. *American journal of epidemiology.* **129**(1):112-124.

- McCarty, K.M., Chen, Y.C., Quamruzzaman, Q., Rahman, M., Mahiuddin, G., Hsueh, Y.M., Su, L., Smith, T., Ryan, L. and Christiani, D.C. 2007. Arsenic methylation, GSTT1, GSTM1, GSTP1 polymorphisms, and skin lesions. *Environmental health perspectives*. **115**(3):341.
- Mandal, S.M, Mondal, K.C., Dey, S. and Pati, B.R. 2007. A rapid colony screening method for the detection of arsenate-reducing bacteria. *Indian journal of microbiology*. **47**(2): 167-169.
- Mukhopadhyay, R., Rosen, B.P., Phung, .LT. and Silver, S. 2002. Microbial arsenic: from geocycles to genes and enzymes. *FEMS microbiology reviews*. **26**(3): 311-325.
- Pi, J., Yamauchi, H., Kumagai, Y., Sun, G., Yoshida, T., Aikawa, H., Hopenhayn-Rich, C. and Shimojo, N. 2002. Evidence for induction of oxidative stress caused by chronic exposure of Chinese residents to arsenic contained in drinking water. *Environmental health perspectives*. **110**(4): 331.
- Pu, Y.S., Yang, S.M., Huang, Y.K., Chung, C.J., Huang, S.K., Chiu, A.W.H., Yang, M.H., Chen, C.J. and Hsueh, Y.M. 2007. Urinary arsenic profile affects the risk of urothelial carcinoma even at low arsenic exposure. *Toxicology and applied pharmacology*. **218**(2):99-106.
- Safiuddin, M. and Karim, M.M. 2001. Groundwater arsenic contamination in Bangladesh: causes, effects and remediation. In *Proceedings of the 1st IEB International Conference and 7th Annual Paper Meet on Civil Engineering*. 2-3.
- Saltikov, C.W., Wildman Jr., R.A. and Newman, D.K. 2005. Expression dynamics of arsenic respiration and detoxification in *Shewanella* sp. strain ANA-3. *Journal of bacteriology*. **187**(21):7390-7396.
- Smith, A.H., Goycolea, M., Haque, R. and Biggs, M.L. 1998. Marked increase in bladder and lung cancer mortality in a region of Northern Chile due to arsenic in drinking water. *American journal of epidemiology*. **147**(7): 660-669.
- Smith, A.H., Lingas, E.O. and Rahman, M. 2000. Contamination of drinking-water by arsenic in Bangladesh: a public health emergency. *Bulletin of the World Health Organization*. **78**(9): 1093-1103.
- Xu, W., Jin, Y. and Zeng, G. 2024. Introduction of heavy metals contamination in the water and soil: a review on source, toxicity and remediation methods. *Green Chemistry Letters and Reviews*. **17**(1): 2404235
- Zohora, U.S., Rahman, M.S. and Takashi, A.N.O. 2009. Biofilm formation and lipopeptide antibiotic iturin A production in different peptone media. *Journal of Environmental Sciences*. **21**: S24-S27.