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REMOVAL OF ARSENIC FROM WATER USING TOLERANT BACTERIA

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Abstract

The deadly metalloid arsenic is abundantly found in nature. Under oxic conditions, it typically exists as arsenate, however under reducing conditions, arsenite predominates. The main anthropogenic and natural sources of arsenic pollution in the environment are aquifers and natural sources. Arsenite salts are known to be more hazardous than arsenate because they attach to nearby thiols in pyruvate dehydrogenase, whereas arsenate hinders the process of oxidative phosphorylation. Phosphate transporters, aquaglyceroporins, and the active extrusion system all take up arsenic, which is then reduced by arsenate reductases through a dissimilatory reduction mechanism. Arsenic oxyanions are used by some kinds of microorganisms that are both autotrophic and heterotrophic for energy renewal. Arsenate can be used as a nutrient by some types of microbes during the respiratory process. Microorganisms frequently exhibit arsenic resistance through detoxification operons. Consequently, using bioremediation could be a practical and cost-efficient strategy to lessen this environmental pollutant. The present study aimed to determining their biosorption ability, calculate the Minimum Inhibitory Concentration (MIC) of arsenic that prevents the growth of 3b bacteria. 0.5gm pellet of 3b was able to remove 79.2% (100-20.88) arsenic from 10mg/L arsenic solution within 1 hour.

Keywords:

Arsenic reducing bacteria; Biochemical test , Biosorption Molybdenum blue

1. INTRODUCTION

One of the deadliest metalloids, arsenic can be found in more than 200 various mineral forms, with arsenates typically making up 60% of them, followed by sulphosalts and sulphides at 20%, and arsenite, oxides, arsenide, silicates, and elemental arsenic at 20% [1, 2]. Arsenopyrite was created as a result of the intrusion

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of orogenesis and granitic magma [1]. Albertus Magnus made the initial discovery of arsenic in 1250 [3]. When the earth was in its original state, arsenic cycled at the surface where the disintegration of rocks created arsenic trioxide from arsenic sulphides [2, 4]. Arsenic is known to exist in a variety of oxidation states in aquatic environments, where it can be found in either organic or inorganic compounds [5, 6]. According to Root et al. [8] and Zobrist et al. [7], redox mechanisms, precipitation, sorption, and dissolution processes influence the mobility of arsenic inorganic compound in contaminated aquatic and sediment environment. The sorption of dissolved arsenate in oxic groundwater is reported to be aided by ferric iron phase [8]. In the meantime, microbial activity, which includes detoxifying and metabolic pathways, frequently mediates the decrease of arsenate into arsenite in the transition from aerobic to anoxic pore fluids [8]. Saalfield and Bostick [9] made the hypothesis in a different investigation that the presence of calcium and bicarbonate, which are waste products of biological activities, will accelerate the release of arsenic, and correlations between calcium and bicarbonate and arsenic were then found.

As3 (arsine), As (arsenic), As+3 (arsenite), and As+5 (arsenate) are the four common oxidation states of as[4,10]. Arsenic is typically found in soil environments in two oxidation states, As+3 (arsenite) and As+5 (arsenate), and is typically present in air as a mixture of As+3 (arsenite) and As+5 (arsenate) [2]. The primary species of the two oxidation states linked with soil arsenic contaminations is arsenate, which is frequently written as AsO4 3 and is quite similar to phosphate [11, 12].

Certain biomass naturally engages in biosorption, a physiochemical process that enables it to passively concentrate and bind pollutants onto its cellular structure. The ability of biological materials to absorb heavy metals from wastewater via metabolically mediated or physico-chemical uptake mechanisms is known as biosorption. Scientists and engineers are expecting that this phenomena may offer a cost-effective option for extracting harmful heavy metals from industrial effluent and help with environmental rehabilitation, even though biomass has been used in environmental cleaning for some time.

High arsenic concentrations may be handled quite well, and studies of bacterial growth at high arsenic-phosphorus ratios have shown that it can be implicated in important cellular processes [13]. With the help of two distinct kinds of arsenate

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reductases, Corynebacterium glutamicum can withstand arsenic stress. The single-cysteine monomeric enzymes Cg-ArsC1 and Cg-ArsC2 are connected to the mycothiol/mycoredoxin redox pathway utilising a mycothiol transferase mechanism, whereas Cg-ArsC1' is a homodimer that contains three cysteines and employs a reduction mechanism connected to the thioredoxin route .

2. MATERIALS AND METHODS

2.1. To study arsenic resistance of bacteria

We used a particular bacterial stain present in research laboratory . Bacterial strain designated as '3b' was cultured with the increasing doses of Arsenic, in the Nutrient agar and Nutrient broth medium, to determine the arsenic resistance of the bacteria.

Dosing of toxicants.

A master stock of arsenic solution was prepared, with the concentration of 10gm in 1000ml. 2gm of Sodium Arsenite dissolved in 200ml of distilled water.(Stock Solution-2gm in 200ml)Initial Arsenic dose exposure was set between range of 0.02gm/L to 2gm/L. The bacteria which were able to survive upto 2gm/L were later subjected to MIC distribution at doses ranging from 1-8gm/L. The concentrations of 1g/l to 8g/l were prepared in 200ml volume of nutrient broth with 0.5ml of inoculum and optical density noted at 600nm for three consecutive days. To determine the MIC (minimum inhibitory concentration) of arsenic to inhibit the growth of bacteria: Due to increasing concentration of arsenic, the broths containing low doses showed turbidity or growth sooner than the broths containing higher doses.

Therefore, optical density was noted for three days, first at 24 hours of inoculation, second at 48 hours and third at 72 hours.

2.2. Isolation & characterisation of the bacteria

The given bacterial strain 3b is cultured and sub-cultured several times using single continuous streaking technique to obtain pure cultures. The colony morphology showed isolated and discrete colony formations. One of these colonies were isolated and stained to identify the microscopic characters of the given strain. The smears were prepared from isolated colonies or from broth cultures and Gram staining was done. Biochemical tests were applied for the characterization of the 3b bacterial strain

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like Indole test, Urease test, Simmon's Citrate test, Triple Sugar iron agar test, Oxidase test and Catalase test

2.3 Biosorption of the arsenic by the bacteria

In the set experiment of biosorption nutrient broth medium was prepared in 100ml volume in a 500ml flask. After 24 hours, its optical density was noted and cell count was done. After growth, pellets were prepared. This was done by taking 10ml of 3b bacterial strain in different 15ml centrifuge tubes and centrifuged at 4500-5000 rpm for 10 minutes.

Arsenic solution (10mg/L) prepared in deionised autoclaved water was added to all the pellets of 3b bacterial strain in the volume of 10ml in each, and then they are transferred to the flasks and kept in shaker for 1 hour at 100rpm at 30˚C. After 1 hour, these pellets were transferred to centrifuge tubes and centrifuged at 5000 rpm for 10 minutes to make pellets and supernatant.

In the obtained supernatant 'Molybdenum blue method' is performed. Molybdenum blue method is for the analysis of arsenic, from this we can analyse the arsenic content in the 3b pellets.

Molybdenum blue method for analysis of arsenic

1ml sample is taken in a 10ml volumetric flask in which 0.2ml hydrogen peroxide, 1ml Sodium Molybdate (3%), 1ml sulphuric acid (50%) and 0.5ml ascorbic acid (2%) is added. Then this solution is boiled at 90-100˚C for 10 minutes. The volume of this solution is made 10ml by adding deionised water. Optical density noted at 840nm wavelength. Also optical density of the control without pellet is noted. From these values calculate the final concentration of arsenic in arsenic water exposed to 3b bacterial cells.

3. RESULTS

3.1 To study arsenic resistance of the bacteria

From the above observation, it was determined that MIC (minimum inhibitory concentration) of arsenic to inhibit or stop the growth of this bacterium was 8gm/L, having the optical density of 0.037 at 600nm.The optical density of 3b at 48 hours was observed in increasing concentration of arsenic.

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Table: 1 Measuring arsenic resistance of the bacteria

Dose of Arsenic in increasing concentration (10gm/L)	Volume of Nutrient broth (ml)	Amount of inoculum (ml)	Optical density at λ=600 nm
0.02gm/L	20ml	0.1ml	0.891
0.04gm/L	20ml	0.1ml	1.782
0.06gm/L	20ml	0.1ml	1.99
0.08gm/L	20ml	0.1ml	1.885
0.1gm/L	20ml	0.1ml	1.467
0.12gm/L	20ml	0.1ml	1.856
0.14gm/L	20ml	0.1ml	1.910
0.16gm/L	20ml	0.1ml	1.605
0.18gm/L	20ml	0.1ml	1.852

Graph: 1 determining arsenic resistance of the bacteria

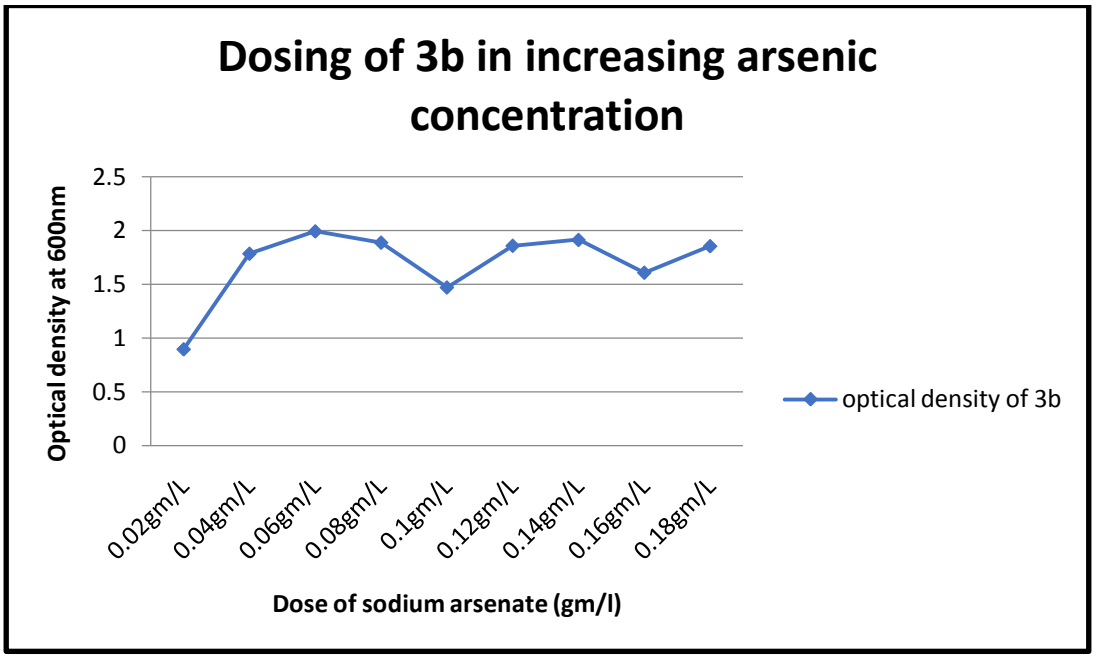

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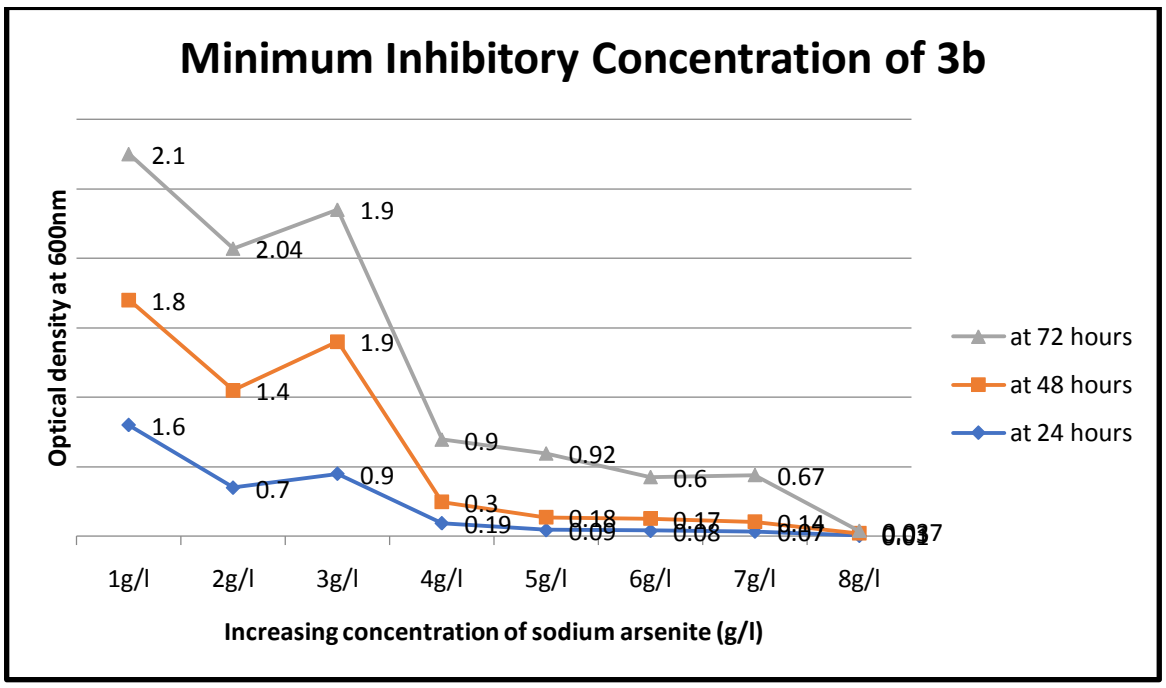
Table: 2 Measuring minimum inhibitory concentration of arsenic

Concentration of Arsenic solution (gm/L)	Optical density noted at 600nm		
	At 24 hours	At 48hours	At 72hours
1g/l	1.602	1.856	2.110
2g/l	0.775	1.419	2.044
3g/l	0.987	1.975	1.951
4g/l	0.19	0.380	0.908
5g/l	0.093	0.187	0.926
6g/l	0.088	0.176	0.698
7g/l	0.071	0.142	0.671
8g/l	0.016	0.033	0.037

Graph: 2 minimum inhibitory concentration of arsenic



3.2 Characterization of the bacteria

The 3b was found out to be gram positive having small rod shaped cells upon microscopic examination. Biochemical reactions of 3b determined that 3b decomposes urea to ammonia and possess oxidase enzyme, but it does not ferment sugars, not uses carbon as sole source of energy and do not decomposes tryptophan to indole. Also it does not possess Catalase enzyme

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Table: 3 Characterization of 3b bacterial strain

S.No.	Characteristics	Test results
1	Minimum inhibitory concentration	8g/l
2	Colony morphology	Off white to cream colored colonies with smooth margins
3	Gram's staining	Color- purple (positive) Shape- small rod shaped Arrangement- scattered
4	Indole test	Negative
5	Urease test	Positive
6	Citrate test	Negative
7	TSI test	Negative
8	Oxidase test	Positive
9	Catalase test	Negative

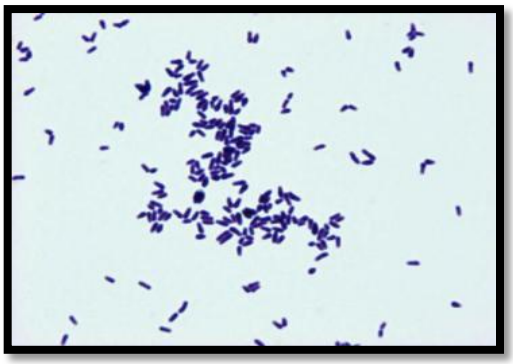

Fig: 1 showing gram positive rod shaped cells
Fig: 2 Indole reaction with in the smear of 3b cells

(Yellow rings on top depicts negative test)

Fig: 3 Urease reaction with the control negative and blue colour depicts positive test)
Fig: 4 Citrate reaction with the control (Color change of media from yellow to pink (Green color media depicts depicts positive test)

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Fig: 5 TSI reaction with the control

(No color change, no cracks or bubbles and no black ppt means negative test)

Fig: 6 Oxidase test of 3b

Fig: 7 Catalase test of 3b

3.3 Biosorption of the bacteria

At 24 hours Optical density of 3b bacterial strain was 1.472 (at 600nm),Cell count of 3b bacterial strain was 24000/mm3 and Pellet sizes 3b bacterial strain was 0.3gm, 0.4gm and 0.5gm.

Table:4 Optical density of arsenic water containing 3b bacterial strain

Pellet size	Arsenic concentration	Optical density
0.3gm	10ml of 10gm/L	0.046
0.4gm	10ml of 10gm/L	0.286
0.5gm	10ml of 10mg/L	0.685

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Optical density of control without pellet at 840nm:

Total 10mg/L = 0.985

0.985 has 10mg of arsenic

Table: 5 Arsenic concentrations in water after exposure to bacterial pellets

Pellet size of 3b	Initial concentration of arsenic	Final concentration of arsenic	Percentage of Arsenic
0.3gm	10	0.186	1.86%
0.4gm	10	1.451	14.51%
0.5gm	10	2.088	20.88%

Table: 6 Determination of percentage of arsenic removed by 3b bacterial strain

Pellet sizes of 3b bacterial strain	Percentage of arsenic removed after biosorption (%)
0.3gm	98.14%
0.4gm	85.49%
0.5gm	79.2%

4. DISCUSSION

The primary purpose of this study was to determine the Minimum inhibitory concentration (MIC) of arsenic that inhibits the growth of 3b bacteria, along with this characterization of the bacteria and finding out the Biosorption capacity of the given bacteria. In the previous study suggested that four isolates, ORAs1, ORAs2, ORAs5 and ORAs6, showed minimum inhibitory concentration values equal or superior to 16·68 mmol l−1 and 133·47 mmol l−1 in the presence of As(III) and As(V), respectively [14]. Our results revealed that minimum inhibitory concentration value was equal to 8g/l in the arsenic dosage of 2gm in 200ml (10%) that was used in increasing concentration.

Isolated arsenic resistant bacteria by standard plate count method on the basis of viable growth on plate count agar amended with arsenate was ranging from 0, 0.5, 10, 40, 80 to 160 milligram per litre (mg/l) [15]. In our results 3b was cultured with increasing concentration of arsenic from 1g/L to8g/L in the arsenic dosage of 2gm in 200ml (10%).

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In our study morphological and biochemical tests are use to identify that the given 3b bacterium was gram positive, indole, citrate, TSI negative and urease positive; also it was catalase negative and oxidase positive.

In the previous study of Biosorption tests, The maximum biosorption capacity of living cells of *B. cereus* for arsenic (III) was found to be 32.42 mg/g at pH 7.5, at optimum conditions of contact time of 30 min, biomass dosage of 6 g/L, and temperature of 30 ± 2 °C [17]. The biosorption capacity of the biomass for As(+3) and As(+5) was found to be 74.91 mg/g (pH 7.0) and 81.63 mg/g (pH 3.0), respectively using 1 g/L biomass with a contact time of 30 min at 28 degrees C [18]. In our results, the biosorption capacity of the biomass of 3b for Arsenic (V) was found to be 98.14%, 85.49% and 79.2% respectively using 0.3g/L, 0.4g/L and 0.5g/L biomass with the contact time of 1 hour at 30˚C.

5. Conclusion

From the above experiments it can be concluded at last that arsenic-tolerant bacterial strains can be employed to remove arsenic from water and water sources. Our findings included the minimal inhibitory concentration, characterisation, and biosorption capability of the bacterial strain. Based on these findings, additional research can be done to examine the kinetics of the bacterial biosorption mechanism. The aforementioned tests were also conducted using deionized water, but as water from natural sources contains a variety of minerals and salts, research can also be done to ascertain how these elements affect the bacteria's ability to absorb nutrients. For future aspect Analysis of 16S rRNA gene sequences can used to reveal that the organism 3b is grouped to which specific genera .

REFERENCES

[1] A. A. L. S. Duarte, S. J. A. Cardoso, and A. J. Alcada, “Emerging and innovative techniques for arsenic removal applied to a small water supply system,”Sustainability, vol. 1, pp. 1288–1304, 2009.

[2] B. K. Mandal and K. T. Suzuki, “Arsenic round the world: areview,” Talanta, vol. 58, no. 1, pp. 201–235, 2002.

[3] B. P. Rosen, “Families of arsenic transporters,” Trends in Microbiology, vol. 7 no. 5, pp. 207–212, 1999.

ISSN: 0975-3583,0976-2833 VOL 3, ISSUE 04, 2012

[4] R. S. Oremland and J. F. Stolz, "Arsenic, microbes and contaminated aquifers," Trends in Microbiology, vol. 13, no. 2, pp. 45–49, 2005.

[5] J. G. Sanders, "Arsenic cycling in marine systems," Marine Environmental Research, vol. 3, no. 4, pp. 257–266, 1980.

[6] S. M. Tareq, S. Safiullah, H. M. Anawar, M. M. Rahman, and T.Ishizuka, "Arsenic pollution in groundwater: a self-organizingcomplex geochemical process in the deltaic sedimentary environment, Bangladesh," Science of the Total Environment, vol. 313,no. 1–3, pp. 213–226, 2003.

[7] J. Zobrist, P. R. Dowdle, J. A. Davis, and R. S. Oremland, "Microbial arsenate reduction vs arsenate sorption: experiments with ferrihydrite suspensions," Minerological Magazine A, vol. 62, pp. 1707–1708, 1998.

[8] R. A. Root, D. Vlassopoulos, N. A. Rivera, M. T. Rafferty, C. Andrews, and P. A. O'Day, "Speciation and natural attenuation of arsenic and iron in a tidally influenced shallow aquifer," Geochimica et Cosmochimica Acta, vol. 73, no. 19, pp. 5528–5553, 2009.

[9] S. L. Saalfield and B. C. Bostick, "Synergistic effect of calcium and bicarbonate in enhancing arsenate release from ferrihydrite," Geochimica et Cosmochimica Acta, vol. 74, no. 18, pp. 5171–5186, 2010.

[10] E. Afkar, "Localization of the dissimilatory arsenate reductase in Sulfurospirillum barnesii strain SeS-3," American Journal of Agriculture and Biological Sciences, vol. 7, pp. 97–105, 2012.

[11] J. F. Artiola, D. Zabcik, and S. H. Johnson, "In situ treatment of arsenic contaminated soil from a hazardous industrial site:laboratory studies," Waste Management, vol. 10, no. 1, pp. 73–78, 1990.

[12] S. Silver and L. T. Phung, "Genes and enzymes involved in bacterial oxidation and reduction of inorganic arsenic," Applied and Environmental Microbiology, vol. 71, no. 2, pp. 599–608,2005.

[13]. Nandal, N. Impact of product innovation on the financial performance of the selected organizations: A study in indian context. Psychol. Educ. J. 2021, 58, 5152–5163.

ISSN: 0975-3583,0976-2833 VOL 3, ISSUE 04, 2012

[14]. Jora, Neetu and Nandal, Naveen. (2020),Atittude of the millenial generation towards cryptocurrency, Efflatounia, 5 (2), pp. 275 – 285.

[15].Aarushi, Naveen Nandal and Anuradha, Satyam Computers Scam- Pre and Post Analysis, International Journal of Psychosocial Rehabilitation, Volume 24, Issue 6, pp. 1817-1824.

[16].B. P. Rosen, A. A. Ajees, and T. R. Mcdermott, "Life and death with arsenic," BioEssays, vol. 33, no. 5, pp. 350–357, 2011.

[17]M. Pepi, M. Volterrani, M. Renzi, M. Marvasi, S. Gasperini, E. Franchi, S.E. Focardi, First published: 5 July 2007

[18]. S.Shakya, B Pradhan, L Smith, J.Shrestha, S.Tuladhar, Journal of Environmental Management, Volume 95, Supplement, March 2012, Pages S250-S255