Vol-07 Issue 07, July-2023 ISSN: 2456-9348
Impact Factor: 6.736

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International Journal of Engineering Technology Research & Management
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HALOPHILIC ACTINOMYCETE MEDIATED BIODEGRADATION OF ARSENIC IN WASTES WATER COLLECTED FROM DIFFERENT SOURCES OF CHIDAMBARAM, TAMIL NADU

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Abstract
Introduction: Heavy metal toxicity is one of the vital problem in developing countries. Countries with no problem drainage system for releasing of industrial waste, household sewage, agricultural waste and other waste into water bodies is one of the reason of ground water pollution and toxicity. Therefore the heavy metal toxicity is one of the major health concern of today's world. Biodegradation is a process to reduce toxic heavy-metals, such as arsenic, in the environment using microorganisms.
Materials and Method: This study aimed to isolate arsenic biodegrading *Actinomycetes* strain from marine sediments and evaluate its arsenic bioremediation potential. Then morphological and biochemical characterization of the arsenic-resistant isolate was carried out. The isolate was further evaluated for its biodegradation potential against various concentration of arsenic.
Result: The results revealed that, the arsenic-resistant isolates was found to be an *Actinomycetes species* and it exhibited significant arsenic biodegradation activity. The highest bioremediation was found to be 75% after incubation with arsenic from different sources for 24 hours at 37 °C.
Conclusion: The results obtained from this study will greatly benefit in eliminating arsenic from contaminated sites and thereby reducing environmental pollutants of heavy metal using the isolated *Actinomycetes* strain.

Keywords: Biodegrdation, Arsenic, Microorganisms, Toxicity

1. Introduction

Heavy metal toxicity is one of the vital problem in developing countries. Countries with no problem drainage system for releasing of industrial waste, household sewage, agricultural waste and other waste into water bodies is one of the reason of ground water pollution and toxicity. The third world countries are the worst victim of underground water pollution with heavy metal toxicity. The heavy metal pollution in ground water includes presence of heavy metals like- Cadmium, arsenic, chloride, fluoride, Mercury, lead etc. Exposure of a human body to this toxicity had added up to the chronic health issues both in children as well as in adults. Persistence of this heavy metal on ground water as well as on soil has greatly influence the adverse effect on the water flora and fauna and in soil micro flora. The naturally degradation of heavy metal takes years and years due to its complex chemical structure. Presence of such heavy metals on the soil not only changes the micro flora of phyllosphere but also of rhizosphere.
Due to growing population in the world and countries like India there is a tremendous demand in conversion of agricultural land to residential complexes. The farmers are now forced to shift from traditional way of farming to chemical based farming. Due to the chemical based farming there is an increase rate of using this

The waste accumulation has become a major concern of this decade due to lack in proper waste management. It has led to a high level of pollution especially in underdeveloped countries. The industrial waste and their effluents have further complicated the situation . The major consequences of environmental pollution, such as

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synthetic based chemical and plastics, household waste, sewage etc have now become a perturbing danger that affects the hydrosphere. Pollution by heavy metals, into water bodies, surface run-off from agricultural fields containing chemical based fertilizers ,combustions from petroleum hydrocarbons, nuclear waste, etc.), is currently an important toxicological factor affecting marine organisms. The harmful threat of this heavy metals pollution has gradually increases the effect on various life forms on marine food chain.[1]. To mitigate the problem of heavy metal toxicity various techniques are currently being used for their removal – phytoremediation, bio-augmentation etc. There are a lot of disadvantage with this techniques as heavy metal concentration, its solubility also plays a significant role in its degradation. Largely there are a lot of problem the researchers are now facing in remediating the heavy metal waste due to the metal concentrations presence in trace amount. [3, 4].

Arsenic (As) being a toxic metalloid released into the environment by activities of weathering of rocks, volcanic eruptions or by man –made activities such as mining, smelting, and combustion of fossil fuels [5,6]. The various oxidative states of arsenic includes +5 (arsenate), +3 (arsenite), 0 (elemental arsenic), and −3 (arsine), which are found naturally. As (III) is more toxic than others [7,8]. Incidences on persistent arsenic poisoning among the general population has been widely reported in many developing countries of the world [8]. Certain report have claims that the elevated arsenic levels in ground water worldwide possessed a serious health concerned. The consumable arsenic percentage in drinking water, should not exceed beyond the arsenic concentration limit recommended by World Health Organization (WHO) is 0.01 mg/l [8,9]. High levels of arsenic in drinking water resulted in affecting human health with various types of toxicity, cancer and all sort of lethal diseases - skin lesions (e.g., hyperkeratosis, hyper pigmentation, desquamation and hair loss), cancer of various organs, such as skin, excretory organs, pulmonary organs, hypertension, disease of the legs and feet, blood vessels, and reproductive disorders [9,10]. Arsenic is a potent heavy metal which is used by some bacteria as electron donors, electron acceptors, or possess arsenic detoxification mechanisms [6,7]. Bioremediation is the process for reduction of environmental pollutants using microorganisms. Arsenic Bioremediation by microbial community involves oxidation, reduction, and methylation and intracellular bioaccumulation of these compounds [11,12]. The *actinobacteria* are widely involved in production of commercially important enzymes, bioremediation of industrial waste, and the production of recombinant proteins [13]. Several groups of *actinobacteria* are able to remove heavy metals from polluted environments and study on such microorganism will greatly help us in the bioremediation process.[14-16]. Bio-augmentation on such waste site has greatly increases the resistance among Actinobacter. [17,18]. Streptomyces are the most common actinobacteria isolates from polluted water sites. [19] [20]. *Streptomyces* can survive in heavy metal contaminated environments most likely due to their remarkable resistance to extreme environmental conditions and various pollutants. [21].

2. MATERIALS AND METHODSs

2.1. Sampling

Water samples were collected from eight different geographical locations of Cuddlore, Tamil Nadu, India. These water samples were then transferred to the laboratory, passed through 2 mm sieve and used for physical-chemical and microbial analysis (Nwuche and ugoji, 2008). The water sample collected was based on the amount of contaminants present especially from the heavily populated site within and around Chidambaram.

2.2. Collection of marine sediments and isolation of marine *actinomycetes*

Deep sea sediment sample was collected from the Pichavaram mangrove area, Chidambaram, Tamil Nadu. The sediment collector was utilized for the sediment collection.

The collected sediment sample was pre-treated with 60 ºC in a water bath for 5 min before serial dilution.(1 gm of the sample that was mixed with 9 ml of NS, and serial dilution was carried out . 0.1 ml of the sediment sample was transferred to starch casein agar and spread with sterile glass rod and incubated at 37 °C for about 7 days. After 7 days of growth, pure colonies were selected and maintained in SCA slants.

[22] to demonstrate the characters of the isolate.

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2.3. Morphological and biochemical characteristics

2.3.1. Gram staining

A smear of the isolated species was made on a clean glass slide to determine the Gram's nature of the isolated species by performing Gram staining.

2.3.2. Biochemical tests

The biochemical tests such as Indole test, Methyl Red test, Voges-Proskauer test, Citrate utilization test, Triple-sugar iron test, Catalase test, Oxidase test, Gelatin test and Nitrate reduction test were carried out to determine the characteristics of the isolated strain [24].

2.4. Growth curve of isolated strains

The isolated *Actinomycete* strain was grown on Starch Casein Agar for 6, 12, 24, 48 and 72 h at 37 °C. Post incubation, the cellular growth was measured spectrophotometrically at 600 nm at each time period. The growth curve was made based on the increase in the absorbance values with incubation time.

2.5. Arsenic measurement

In this study, arsenic was measured spectrophotometrically using a reagent dye called Leuco malachite green (LMG) at 617nm. In this method the arsenic present in trace amount can be detected by using this method. Here in this method the arsenic acted with Potassium iodate (KIO3) in the acidic environment to release iodine . Released iodine then undergoes oxidation and converts LMG to MG and changes its colour. Detection range of arsenic concentration in this method is 0.09-0.9 µg/ml. The MG dye shows maximum absorption at 617 nm [25].

2.6. Arsenite measurement (As III)

The measurement of arsenite from the water sample was done by following the method given by (Ghodsi ,2011)[30]

2.7. Arsenate measurement As (V)

After filtration of the samples a known volume was removed, then 0.5 ml of 5% KI and 5M HCl was added to the samples. All of the available arsenate was reduced to arsenite. To removed extra iodine in the solution ascorbic acid was added drop wise [26]. The amount of total arsenic in samples was measured .

2.9. Bioremediation of arsenic by the isolated strain

To check the arsenic accumulated by the isolate, the *Actinomycete* strain was allowed to grow with different concentrations of arsenic for 6, 12, 24, 48 and 72 h at 37 °C. Post incubation, the cellular growth and residual arsenic (%) was measured spectrophotometrically at 617 nm, respectively.

3. Results

3.1. Isolation and characterization of arsenic-resistant *Actinomycete* strain

The data from the morphological and biochemical experiments showed that the isolated strain belonged *Actinomycete* genera (Figure 1) (Table 1 and 2). Further, the growth of the isolated *Actinomycete* was observed to increase with time up to 18 h and then gradually declined after 24 h of incubation (Figure 3).

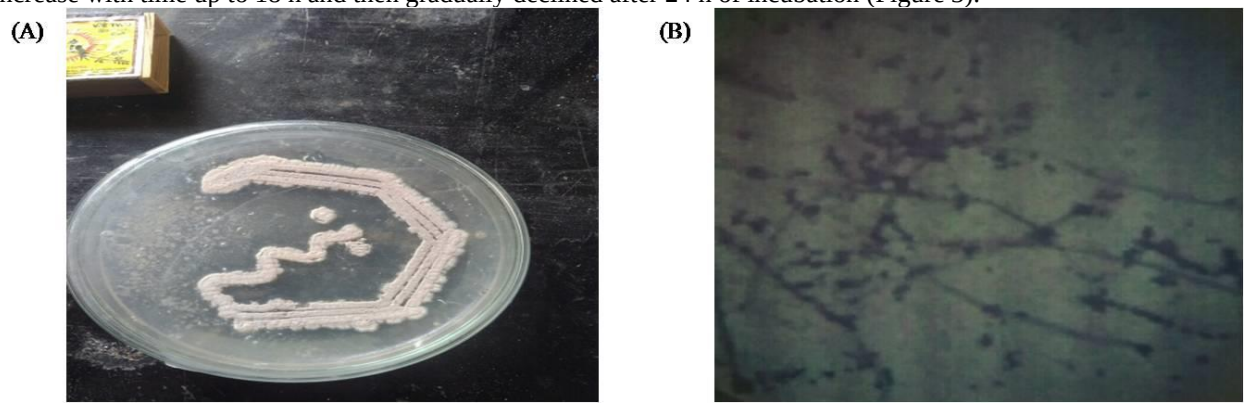

Figure 1. (A) Pure culture and (B) gram staining of the isolated arsenic resistant isolate.

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Table 1. Morphological characteristics of the isolated arsenic resistant isolate.

Characteristics	Results
Motility	Non-motile
Gram's nature	
Shape	Filamentous rod
Organism	Gram positive

Table 2. Preliminary observations of the isolated arsenic resistant isolate.

Sample	Dilution	Isolation	Media	Colony characters	Observation	Inference
Pichavaram mangrove area	10^{-3}, 10^{-2}, 10^{-5}	Spread plate	Starch Casein Agar (SCA agar)	White to grey spherical with entire margin Colonies displaying Typical characteristics of actinomycetes	Gram positive Branched Filamentous	May be genera of *Actinomycetes*

Figure 2. Result of biochemical tests performed on the arsenic resistant isolates

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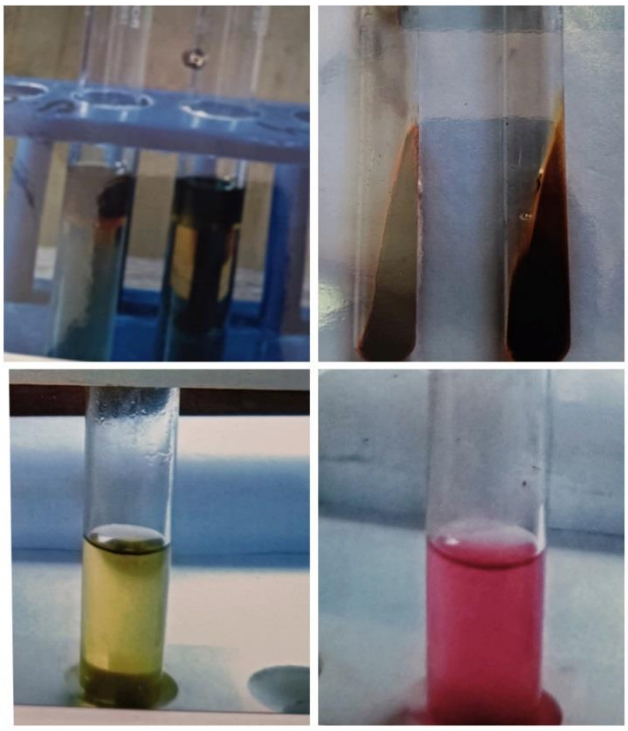

Table 3. Biochemical characterization of the arsenic resistant isolate.

S. No.	Name of the test	Result
1.	Indole Test	+
2.	MR Test	-
3	VP Test	-
4.	Citrate utilization Test	-
5.	TSI / H_2S Production	-
6.	Urea Test	+
7.	Gelatin Test	+
8.	Nitrate reduction Test	+
9.	Catalase Test	-
10.	Oxidase Test	-

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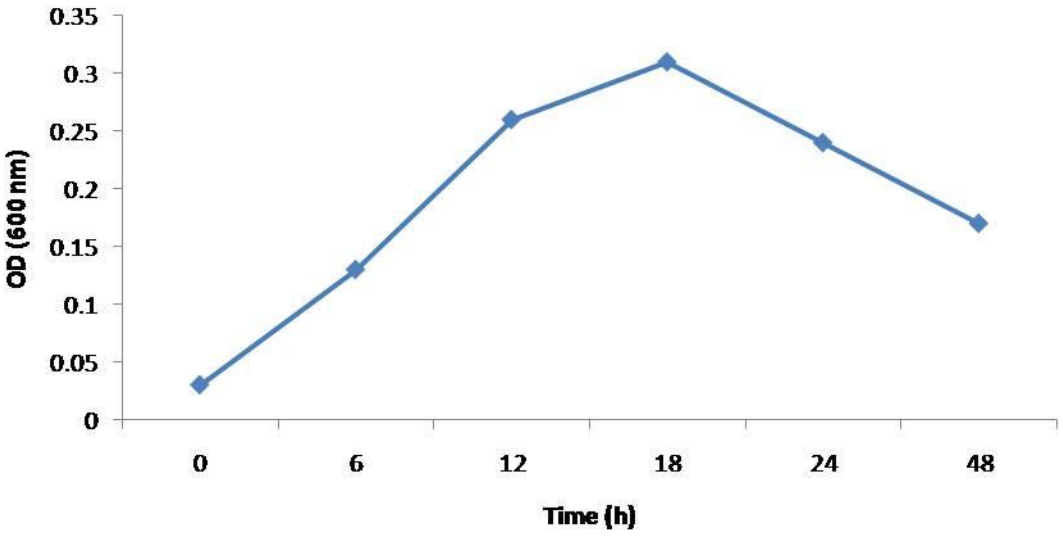

Figure 3. Growth curve of the isolated arsenic resistant *Actinomycetes*.

3.2. Arsenic concentration in water samples

We observed that the concentration of arsenic was dependent on the source of the water sample collected. A higher level of arsenic concentration was detected in the water sample (WS-8) collected from highly polluted source (Figure 4).

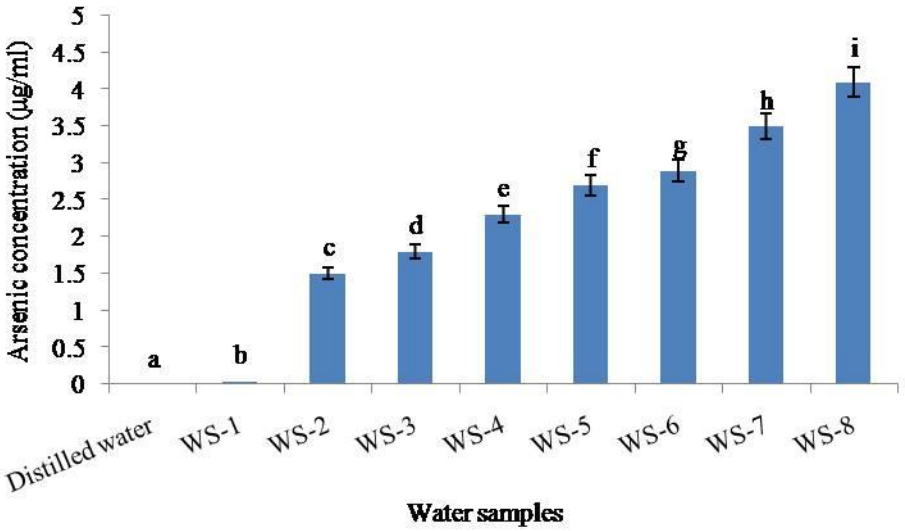

Figure 4. Arsenic concentration in different water samples collected from different sources.

3.3. Arsenite concentration in water samples

We observed that the concentration of arsenite was dependent on the source of the water sample collected. A higher level of arsenite concentration was detected in the water sample (WS-8) collected from highly polluted source (Figure 5).

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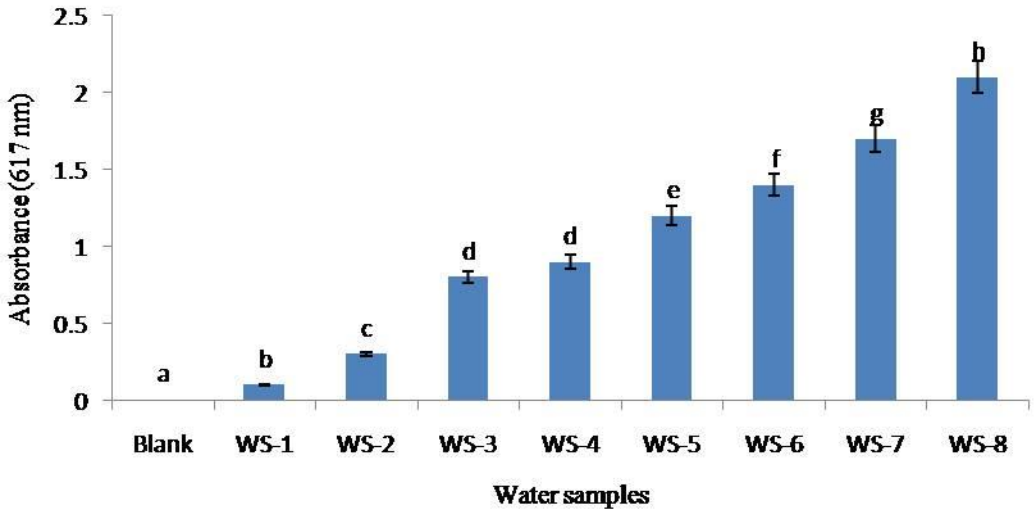

Figure 5. Arsenite concentration in different water samples collected from different sources.

3.4. Arsenite concentration in water samples

We observed that the concentration of arsenate was dependent on the source of the water sample collected. A higher level of arsenate concentration was detected in the water sample (WS-8) collected from highly polluted source (Figure 6).

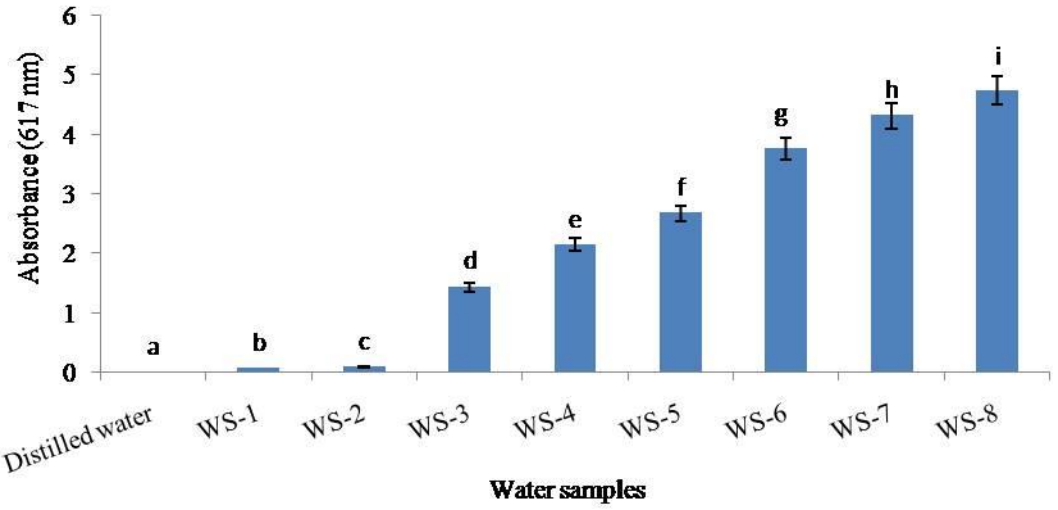

Figure 6. Arsenate concentration in different water samples collected from different sources.

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3.6. Bioremediation of arsenic with isolated *Actinomycete* strain

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We observed a significant arsenic biodegradation potential of the isolated Actinomycete strain. The highest level of biodegradation was shown as 75 % at an OD $_{600}$ of 0.37. The level of biodegradation was measured as the concentration of arsenic after different time points of bacterial growth (Figure 8).

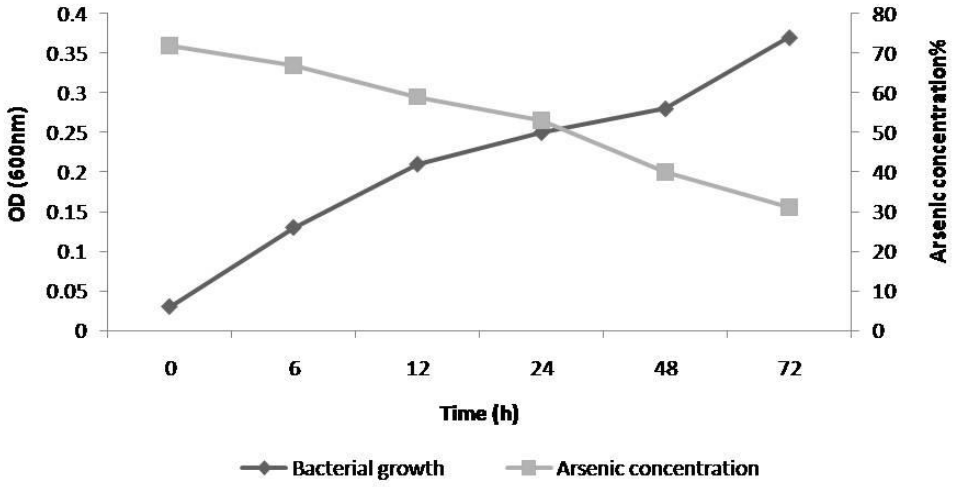

Figure 8. Biodegradation curve of arsenic with the isolated Actinomycete strain.

4. Discussion

Presence of arsenic in potable drinking water possesses a serious health threats . The soil samples basically existed as a mixed waste with other heavy metals along with arsenic- bismuth, iron, nickel and sulfur. However arsenic is a potent poison which is also used in autopsy of dead bodies. Chromated copper arsenate (CCA) is a compound used in treating woods for construction of wooden structures and houses. In India, West Bengal, the arsenic concentration has not reached the catastrophic level although it has been found associated to have dermal effect as well as certain internal effects based on literature review earlier [27].

The results of the experiments have shown that the isolate *Actinomycete* strain show significant resistance to different arsenic concentrations. The growth curve showed that it grows preferably in tryptone casein agar at 35 ˚C by observing its growth at the mentioned time period and conditions. The results from the bioremediation assays revealed that the isolated strain of *Actinomycete i*s well qualified for the bioremediation of arsenic in water samples. This was determined by its growth pattern with time in media supplemented with different concentrations of arsenic. The morphology of the arsenic adapted *Actinomycete* strain was noticed and its gram staining revealed that it displayed a Gram-positive nature. When this strain was exposed to the different toxic concentrations of arsenic, it adapted itself to these concentrations may be due to a change in its cell morphology.

Conclusion

The present study focused on the isolation and evaluation of its arsenic biodegradation potential in different sources. The results of this current investigation investigated the preliminary qualitative character of the isolate. However, further analysis of this *Actinomycete* strain needs to be carried out in order to understand better about the mechanisms employed by this organism in the arsenic biodegradation.

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