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As(III) Oxidation and Cr(VI) Reduction Insight in an Indigenous Mixed Culture of Anaerobic Bacteria from a Local Environment

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Transition metal and metalloid oxyanions such as Cr-compound (CrO_4^{-2}) and As-compound (H_3AsO_3) are common pollutants routinely detected in effluent waste streams from the mining industry around the world. Biological treatment strategies commonly applied for a specific metallic pollutant may not be successful due to a dual toxic effect imposed by the metallic component. In the current study, Cr(VI) reduction linked to As(III) oxidation was observed in a mixed culture of anaerobic bacteria isolated from cow dip in Tzaneen (Limpopo Province), and activated sludge from Brits Wastewater Treatment Works (NW Province), both in South Africa. The cultures were acclimatized for 264 h, and utilized bicarbonate as a carbon source. In an experiment containing 30 to100 mg/L of Cr(VI) at a constant 120 mg/L As(III) concentrations, near complete Cr (VI) reduction at initial concentrations from 30 to70 mg/L was observed Lower removal efficiency was seen when Cr(VI) concentration was increased to 100 mg/L. In a different experiment, As(III) concentrations ranging from 50 to 400 mg/L at a constant 70 mg/L Cr(VI) concentrations was also investigated. Results show that Cr(VI) removal efficiency increases as As(III) concentration was increased from 150 to 400 mg/L. Low Cr(VI) removal rate was seen as As(III) concentration decreased from100 to 50 mg/L. These experiments suggest that Cr(VI) reduction in the presence of As(III) was not inhibited at higher As(III) concentration, rather As(III) enhanced the redox process by acting as inorganic electron donor for Cr(VI) reduction. Abiotic control shows no insignificant reduction in Cr(VI) or oxidation of As(III) in the absence of these isolates.

1. Introduction

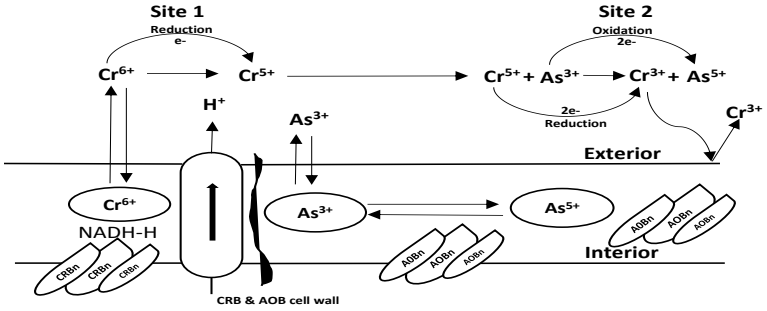
Various industrial development and mining processes have had a substantial impact on the quality of surface and ground water catchment due to discharge of untreated heavy metallic waste (Cheng et al., 2009). The discharge of untreated metallic waste into the ecological system has become a serious public health concern and has ecological implications (Ahemad, 2014). Toxic metalloids pose a serious biological threat due to their common ecological occurrence and carcinogenic properties (Federal Register, 2004). Their presence in the ecosystem can be attributed to their use in industrial applications and mining processes such as in chrome plating and leather tanning (Molokwane et al., 2008), and in the manufacture of dyes, paints, pharmaceuticals, and insecticides (Dong et al., 2014). When As (III) and Cr(VI) are introduced into the environment through direct a release, they may leach into groundwater aquifers where they can accumulate to concentrations that will pose health risk.

It is possible to bio-catalytically reduce Cr(VI) to Cr(III) and oxidize As(III) to As(V) which are much less toxic (Igboamalu and Chirwa, 2016). Current methods for As(III) oxidation and Cr(VI) reduction are expensive and produce a second hand waste which will require further chemical treatment before disposal (Igboamalu and Chirwa, 2014). The biochemical oxidation of As(III) and reduction of Cr(VI) is an enzymatic catalytic process which involves microbially induced redox process between As(III) and Cr(VI) to generate As(V) and Cr(III). Compared to expensive chemical or membrane treatment, this process proposes a relatively inexpensive and environmentally friendly solution if it can be implemented in a large scale continuous method. Several studies on As(III) and Cr(VI) simultaneous removal from waste water with facultative microbes utilizing nitrate (NO_3^-) (Sun et al., 2008) or chlorate (ClO_3^-) as terminal electron acceptors while conserving energy for cell growth are

already reported (Sun et al., 2010). As(III) oxidation was also achieved by facultative microbes utilizing bicarbonate as sole carbon source, while generating -256 kJ/mol of energy (Aniruddha and Wang, 2010). Under acidic condition, simultaneous Cr(VI) reduction and As(III) oxidation by *Bacillus firmus* TE7 was reported by Bachate et al. (2013).

1.1 Bio-detoxification mechanism

As reported on our previous report, bio-detoxification mechanism involves stepwise reduction of Cr(VI) to Cr(VI) followed by redox conversion of Cr(V) and As(III) (Figure 1). Cr(VI) infiltrates into the cell membrane, reduces to Cr(V) by accepting 1 proton (H^+) from the NADH-H (Cervantes et al., 2001). In the presence of As(III), Cr(V) accepts 2 protons ($2H^+$) from As(III) for mutual beneficial reduction of Cr(V) to Cr(III) and oxidation of As(III) and As(V). The redox reaction is an exothermic process, generating reasonable amounts of energy for cell growth and metabolism (Igboamalu and Chirwa, 2014).



$$As^{3+} + Cr^{5+} \rightarrow As^{5+} + Cr^{3+} (\Delta G = -256\ kJ/mol)$$

Figure 1: Proposed induced bio-catalytic detoxification mechanism of As^{3+} and Cr^{6+} in aqueous solution

In contrast to previous study, the current study investigates As(III) resistant anaerobic bacteria isolated from cow dip in South Africa. The mixed culture together with previous isolated Cr(VI) bacteria were checked for Cr(VI) reduction linked to As(III) oxidation at pH 7 while utilizing HCO_3^- as sole carbon source. The first As(III) oxidizing bacteria was isolated in South Africa by Green (1918). From my knowledge, this is the second time As(III) oxidizing bacteria was isolated from cow dip in South Africa. However, an in-depth understanding of As(III) conversion was checked through As(III) and As(V) quantification. This was necessary to ensure if As(III) was indeed a beneficial inorganic metalloids for Cr(VI) reduction. The feasibility of this study is based on the fact that Cr(VI) and As(III) predominate at close pH range from 6-9 (Park et al., 2005) Eq(1-2).

$$HCrO_4^- \overset{k_{a2}}{\Leftrightarrow} CrO_4^{2-} + H^+ \qquad K_{a2} = 10^{-5.6} \qquad pH = -\log [K_{a2}] = 5.6 \qquad (1)$$

$$H_3AsO_3^o \overset{k_{a4}}{\Leftrightarrow} H_2AsO_3^- + H^+ \qquad K_{a4} = 10^{-9.2} \qquad pH = -\log [K_{a4}] = 9.2 \qquad (2)$$

2. Material and methods

2.1 Culture and media

Water samples from an old cow dip in Tzaneen Limpopo and dried sludge samples from sand drying beds at Brits Wastewater Treatment Work were used as inoculum for the mixed culture of bacteria used. The water sample from the cow dip consists of insecticide which is normally used for insect control on cows. This product has been reported to consist of a reasonable amount of arsenic compounds. Dry sludge is also from a treatment plant located near an abandoned chrome processing facility, which periodically received flows from it. The water and sludge samples were cultivated for 24 h in 100 mL of sterile nutrient broth amended with 70 mg/L of Cr(VI) and 120 mg/L As(III) under shaking at 120 rpm in a rotary Environmental Shaker (Labotech, Gauteng, South Africa). Anaerobic cultures were grown in 200 mL bottle purged with nitrogen gas (99 %) for 5 min, closed with silicone rubber, aluminium stoppers and covered with aluminium foil to prevent light penetration. The 16S rRNA genes analysis was used for characterization and resulting sequences were matched to known bacteria in the GenBank using a basic BLAST search of the National Centre for Biotechnology Information (NCBI, Bethesda, MD).

2.2 Batch experiment investigation

In a 200 mL bottle covered with aluminium foil containing 200 mL nutrient broth, cultures were grown anaerobically for 24 h. Cells were collected, and centrifuged for 10 min at 6,000 rpm at 4 ^oC after 24 h. The supernatant was decanted and the remaining pellet was washed three times in a sterile saline solution (0.85 %

NaCl. Anaerobic As(III) oxidation and Cr(VI) reduction experiments were conducted in 200 mL bottles using cells harvested after 24 hours incubation. Anaerobic condition was achieved by purging 99 % N_2 gas in the bottle containing harvested cell. Prior inoculating the bottles with harvested cells, 1 mL of the sample was initially withdrawn from the bottle to determine the As(III) and As(V) concentration, and absorbance of Cr(VI) before reintroducing the cells in each bottle. In 200 mL basal mineral medium, the cell was re-suspended before adding Cr(VI) and As(III). As(III) and Cr(III) stock solutions were added to give a final required concentration. The experiments were conducted at 30±2 ^{o}C over 264 h at 120 rpm on the orbital shaker (Labotec, Gauteng, South Africa). The samples withdrawn in serum bottles over time were centrifuged using a 2-5 mL. Eppendorf tube at 6,000 rpm for 10 min in a Minispin® Microcentrifuge (Eppendorf, Hambury, Germany). The supernatant was used for As(III), As(V) and Cr(VI) analysis.

2.3 Analytical Methods

As(III) and As(V) were measured using ion chromatography equipped with 944 professional UV/Vis detector and 856 professional conductivity detector (Metrohm, South Africa). Cr(VI) on the other hand was measured using the UV/Vis spectrophotometer (WPA, light wave II, Labotech, South Africa). The presence of Cr(VI) in the sample was visualized by the change of the colour after adding 1, 5-diphenyl carbazide (APHA, 2005).

3. Results and discussion

3.1 Microbial analysis

Microbial performance of 12 isolated cultures in the water and dried sludge samples from local environment was evaluated for microbial induced As(III) oxidation and Cr(VI) reduction under anaerobic condition at pH of 7.3. Individual performance of these isolates (Y1-Y12) were investigated at initial Cr(VI) and As(III) concentrations of 70 mg/L and 120 mg/L. In Figures 2 and 3 results showed a near complete Cr(VI) reduction within 168 hours of incubation with Cr(VI) removal efficiency close to 99 %. The tendency for these isolates to achieve near complete Cr(VI) reduction suggest the existence of Cr(VI) reducing and As(III) oxidizing enzymes. These were indicated by observed removal rates as shown in Figure 2. High Cr(VI) removal rate was achieved in samples (Y2, Y5, Y8, Y10, and Y11) when compared to other isolates. The performance of these isolates could be associated with their acclimatization to Cr(VI) and As(III) concentration, and the diverse means of detoxification, suggesting the beneficial use to enhance redox conversion of Cr(VI) to Cr(III), and oxidation of As(III) to As(V). The strain identification was based on the ± 700 bp partial sequence of the 16S rRNA gene of the organism. The sequences were compared against the GenBank of the National Centre for Biotechnology in the United States of America using a basic BLAST search and it showed 100 % sequence identity with Bacilli sp, Exiquobacterium profundum and Staphylococcus sp. (Figure 4).

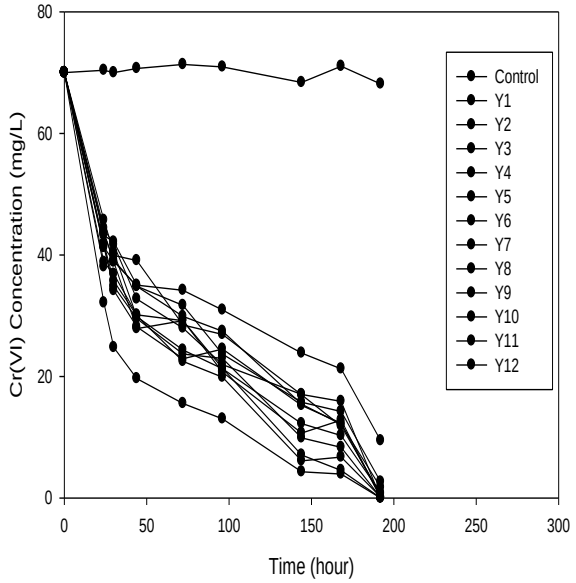


Figure 2: Cr(VI) removal efficiency of individual isolates

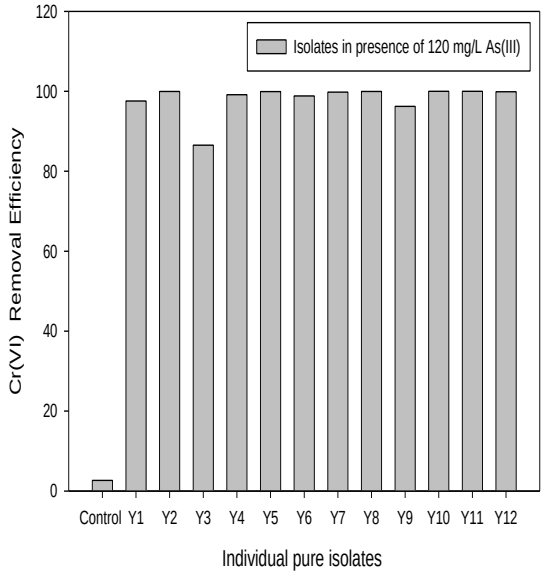


Figure 3: Performance of individual isolate in 70 mg/L Cr(VI) and 120 mg/L As(III)

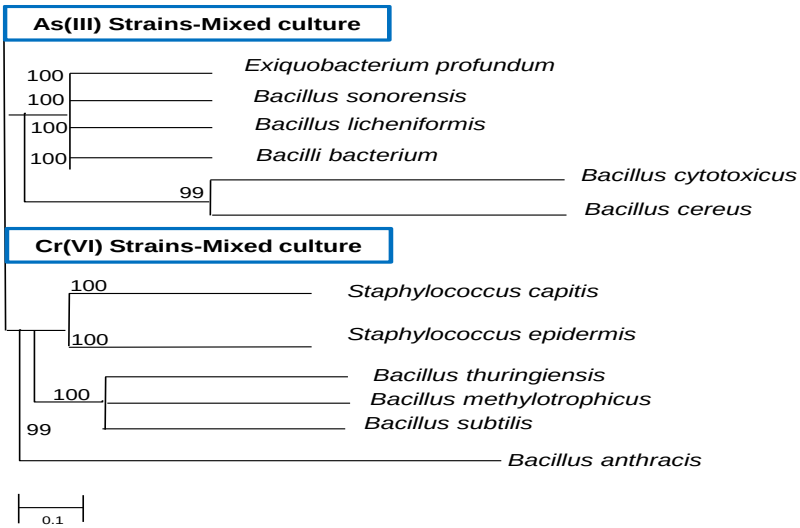


Figure 4: Phylogenetic tree of persistent bacterial cells in inoculated batch reactor after operation derived from the 16S rRNA gene sequence.

3.2 Individual culture at different Cr(VI) concentration in the presence of As(III)

Figures 6, 7 and 8 show selected individual isolates Y2, Y5, Y8, Y10, and Y11 at initial Cr(VI) concentrations ranging from 50 mg/L to 100 mg/L at constant 120 mg/L As(III) concentration .Results showed a near complete Cr(VI) reduction achieved by the individual strains, with removal efficiency of ≥ 99 % from (50-120)h of incubation at 50 mg/L Cr(VI) concentrations as shown in Figures 5 and 6. 86-97 % of C(VI) removal efficiency was achieved by the individual strains when Cr(VI) concentration was increased to 70 mg/L at 120 mg/L As(III) concentration in Figure 8. 28 - 59 % Cr(VI) removal efficiency was achieved when Cr(VI) concentration was further increased to 100 mg/L at 120 mg/L As(III) concentration as shown in Figure 7. Increasing Cr(VI) concentration above 100 mg/L at constant 120 mg/L As(III) concentration, inhibition effect was observed. Considering the corresponding 120 mg/L As(III) concentration, the result is not shown. At initial Cr(VI) concentration ranging from 30 mg/L to 70 mg/L, 80-99 % As(III) oxidation efficiency was achieved in all the strains in Cr(VI) concentration ranging from 30-70 mg/L.

3.3 Simultaneous As(III) oxidation and Cr(VI)reduction under anaerobic condition

Simultaneous oxidation of As(III) and Cr(VI) reduction was investigated in a batch experiment under varying As(III) concentration ranging from 50 mg/L to 400 mg/L at constant 70 mg/L Cr(VI) concentration in an anaerobic mixed culture of isolated bacteria. The strains used in this experiment were previously checked for toxic resistance and ability to induce redox conversion of Cr(VI) and As(III). Results showed that mixed culture achieved near complete Cr(VI) reduction as As(III) concentrations increases from 50 mg/L to 400 mg/L. However, much higher Cr(VI) removal efficiency was seen when As(III) concentrations was increased from 150 mg/L to 400 mg/L see in Figure 9. Furthermore, Cr(VI) reduction was seen as a function of As(III) concentration and microbial cells, as Cr(VI) removal efficiency increases from 7 % to 99 %. This suggests that microbes play a vital role during redox conversion of Cr(VI) to Cr(III). Catalytic induced stepwise reduction of Cr(VI) to Cr(III) started after 1-2h of incubation at high As(III) concentrations, Slow Cr(VI) reduction rate was observed as As(III) concentration decreases from 80 to 50 mg/L. At 50 mg/L As(III) concentration, only 7 % of Cr(VI) concentration was reduced in Figure 9. Clearly, this is not the case of an inhibition effect, but it could be attributed to low Cr(VI) to As(III) mole ratio. As(III) oxidation on the other hand was investigated after 2 - 3 h, and near completed oxidation was achieved after 72 h to 216 h at lower As(III) concentrations in Figure 10. As(III) oxidation was enhanced at 70 mg Cr(VI)/L as As(III) concentration increases from 50 mg/L to 120 mg/L with corresponding increase in As(V) concentration. Slow oxidation rate was seen as As(III) concentration increased to ≥ 250 mg/L. This suggest that low oxidation rate at high As(III) concentration may be as a result of complete reduction of intermediate form of Cr(VI), i.e., (Cr(V)). Further oxidation of As(III) at this concentration could suggests that As(III) oxidation was also facilitated by enzymatic reactions.

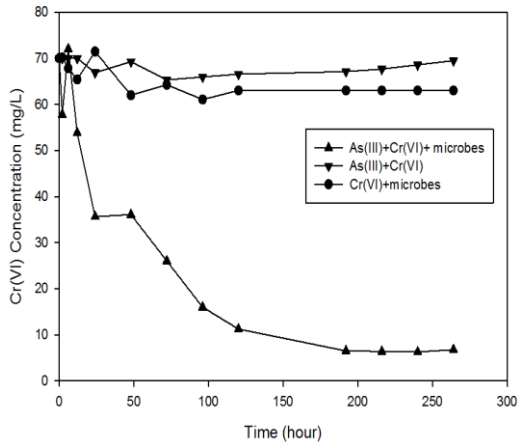


Figure 5: Abiotic control versus Cr(VI) reduction

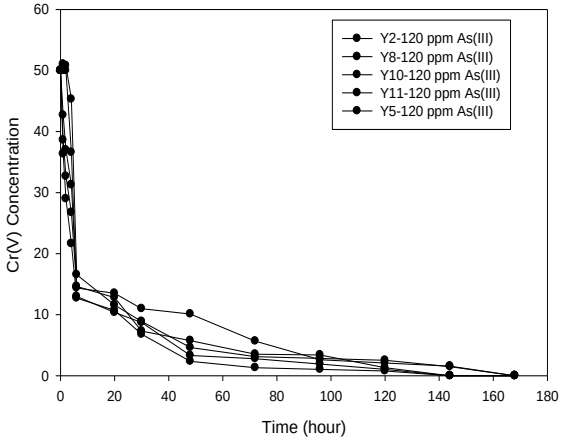


Figure 6: 50 mg/L Cr(VI) and 120 mg/L of Arsenic

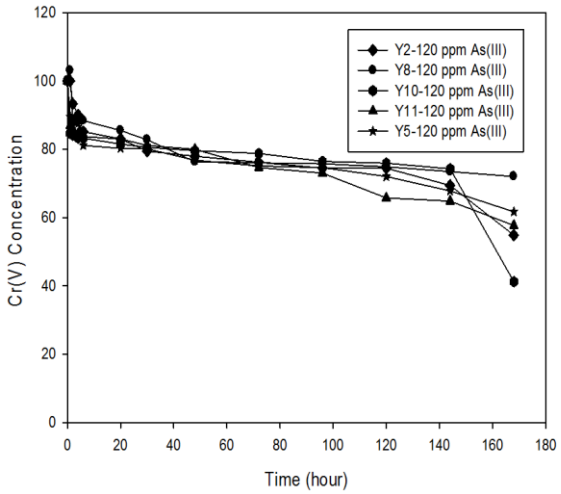


Figure 7: 100 mg/L Cr(VI) and 120 mg/L of Arsenic

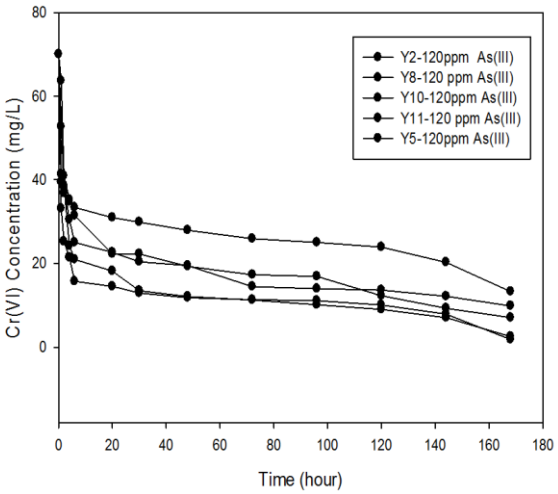


Figure 8: 70 mg/L Cr(VI) and 120 mg/L of Arsenic

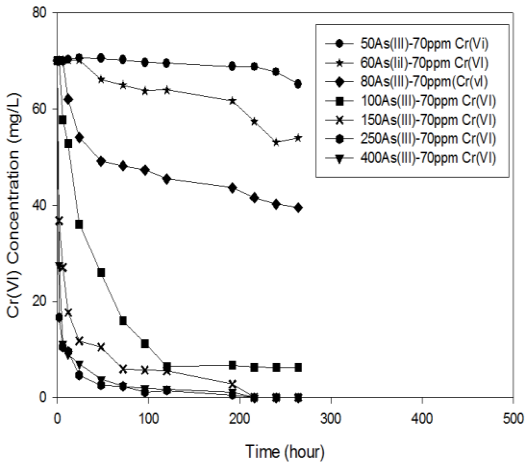


Figure 9: Cr(VI) reduction at As(III) ranging from ranging 50 - 400 mg/L

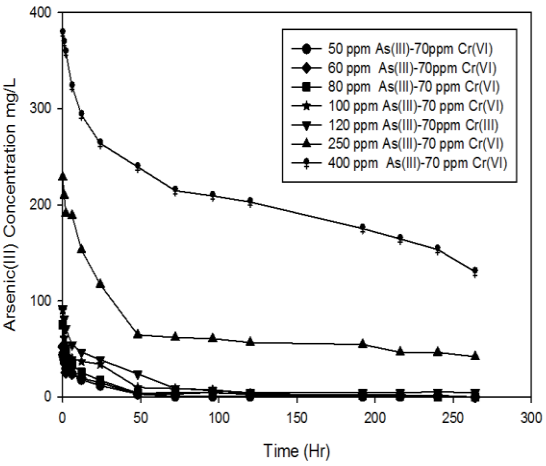


Figure 10: As(III) oxidation at different concentration from 50 - 200 in presence of 70 mg/L Cr(VI)

3.4 Control study investigation

A control experiment was run to check if indeed the redox conversation of Cr(VI) and As(III) was actually induced by enzymatic process. Figure 5 above shows different batch experiment with 70 mg/L and 100 mg/L As(III) concentration. The first set of control experiments was As(III) and Cr(VI) with living strains, the second set of experiments was As(III) and Cr(VI) with heat killed strains and lastly was experiment with Cr(VI) and living strains. The experiment was thoroughly checked and the strains were grown under anaerobic condition, utilizing bicarbonate as sole carbon source. Cr(VI) reduction was seen in an experiment amended with As(III) and Cr(VI) with heat killed strains. When compared to the experiment amended with heat killed strains or without As(III), Cr(VI) reduction was insignificant. It was seen that Cr(VI) reduction simultaneously depends on microbes and the concentrations of As(III) in the aqueous solution This suggests that the presence of microbes and Cr(VI) influences redox conversion of As(III) and Cr(VI), However, in the absence of strains and As(III) concentration the reaction is highly inhibited.

4. Conclusions

Microbial induced redox conversion of As(III) and Cr(VI) was successfully demonstrated under anaerobic conditions at different As(III) and Cr(III) concentrations at neutral pH. The overall performance of the isolates shows the existence of enzymatic ability to induce redox conversion of As(III) and Cr(III) under threshold concentration. This experiment shows a promising economical alternative to most biological treatment of toxic heavy metals. This is because the catalyst (bacteria) is self-regulatory naturally regenerative and can be achieved under natural aquatic conditions and can induce the conversion of As(III) and Cr(VI) by utilizing bicarbonate as sole carbon source.

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