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Cr(VI) Remediation With Inorganic As(III) As An Electron Sink Immobilized In A Ceramic Bead Packed Bed Biofilm Reactor

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Ceramic bead media Biofilm Reactor Performance was evaluated for Cr(VI) reduction utilizing As(III) as electron source with mixed culture of chemoautotrophic bacteria. The inoculant used was isolated from cow dip in Tzaneen (Limpopo Province) and WWTW Brits Northwest South Africa. Preliminary studies show that these bacteria can remove Cr(VI) up to 99 %, and oxidizing As(III) up to 90 %. A bench-scale, ceramic bead media biofilm reactor was operated until steady-state conditions was approached under a range of influent Cr(VI) concentrations ranging from (30-200) mg/L and As(III) concentration ranging from (50-340) mg/L in the ratio of 1:1.7. Hydraulic detention time of 5 hour and 17 hours was observed for optimum Cr(VI) reduction and As(III) oxidation. Result suggests that As(III) was indeed utilized as an electron donor for complete Cr(VI) removal. Parameters such as pH, ORP, temperature and dissolved oxygen concentration was seen optimal at average of 7.1, -156 mV, 31 ^{o}C and 0.92 mg/O_2L. The steady-state Cr(VI) reduction efficiency was affected by the influent Cr(VI) and As(III) concentration and hydraulic detention time. Higher Cr(VI) concentration was seen in the effluent when the influent concentration was increase above 100 mg/Cr(VI)L and 170 mg/As(III)L. Similarly, at lower hydraulic detention time, Cr(VI) reduction As(III)oxidation were affected. The bioreactor showed strong resilience by recovering from Cr(VI) and As(III) overloading through reduction in influent Cr(VI) and As(III) concentrations.

1. Introduction

The focus site is located in South Africa at a facility that actively receives flow from an abandoned chrome facility, and cow dip facility used by local farmers. Unrelated historic land-use activities resulted in ground and surface water contamination with hexavalent chromium (Cr(VI)) and As(III). Chromium is one of the most widely used metals in industry in South Africa accounting about 40 % world use. It is used in activities such as metal finishing, petroleum, power plants and nuclear facilities resulting in large quantities being discharged into the environment (Chirwa and Molokwane, 2011). In mining processes, there may be tendencies to co-discharged Cr(VI) and As(III) or lead, CN, etc. (Igboamalu and Chirwa, 2014). Its toxicity depends on its speciation, since different species have different effects (Oliveira, 2012). Cr(VI) is more toxic and mobile than Cr(III), while Cr(III) could be regarded as a health benefit at certain concentrations. As(III) and As(V) on the other hand are the most stable form of Arsenic (Smedley and Kinniburgh, 2002). Aqueous speciation of arsenic species shows that arsenate As(V) dominate at pH ≤ 2.2, whereas arsenite As(III) dominate at pH ≤ 9.2 (Oremland and Stolz, 2003). As(III) is more toxic and mobile than As(V). However, biological treatment of this metalloid could involve oxidation of toxic As(III) to less toxic As(V) and reduction of Cr(VI) to Cr(III), which could serve as a pathway for reduction of other contaminants (Igboamalu and Chirwa, 2014). As(III) in the system serve as an inorganic electron donor and be oxidized to less toxic and immobile As(V) (Igboamalu and Chirwa, 2018), whereas Cr(VI) is reduced to Cr(III) by accepting electron from As(III) (Igboamalu and Chirwa, 2017).
Most used treatment technologies for removing chromium from industrial waste include ion-exchange, electrodepositing, and chemical reduction with iron- and sulfur containing solutions (FeSO4, Na2SO3, NaHSO3, and Na2S2O5), followed by precipitation at a high pH (Zhao and Duncan, 1997). These methods, although effective, can be quite costly, requiring high energy input or large quantities of chemical reagents, and can create other secondary waste with their own unique environmental concerns which could be detrimental to the

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environment (Igboamalu and Chirwa, 2016). A packed-bed biofilm reactor is a biological treatment process which utilises a porous media with an attached biofilm and are generally used for aerobic and anaerobic treatment (Rittman, 2001). The biggest advantage of the packed bed biofilm reactor over the other reactors is the capacity to withstand higher substrate loading rate due to the presence of strong attachment force between the cells and the surface (Aniruddha and Wang, 2010). Secondly, attached biomass system is anticipated to enhance biofilm growth which provides protection of useful microorganisms against toxicity through mass transport resistance. However, bacteria grown in suspension is known to be highly susceptible to toxic compounds such as Cr(VI) (Nkhalambayausi-Chirwa and Wang 2001). This research forms part of the project in which an in-situ bioremediation process is being developed to contain Cr(VI) and As(III) pollution at a contaminated site in South Africa. It addresses the need to develop cleanup pilot scale technologies for chromium-6 and Arsenic-3 contaminated sites.

2. Material and Methods

2.1 Culture and Media

Consortium of Cr(VI) reducing and As(III) bacteria was isolated from a cow dip in Tzaneen Limpopo and dried sludge samples from sand drying beds at Brits Wastewater Treatment Work (Igboamalu and Chriwa, 2017). When tested in suspended growth systems, the mixed cultures achieved 100 % Cr(VI) removal under initial concentrations up to 70 mg/L and As(III) concentration up to 500 mg/L. Basal mineral medium (BMM) include 10 mM NH_4l, 30 mM Na_2HPO_4, 20 mM KH_2PO_4, 0.8 mM Na_2SO_4, 0.2 mM $MgSO_4$, 50 µM $CaCl_2$, 25 µM $FeSO_4$, 0.1 µM $ZnCl_2$, 0.2 µM $CuCl_2$, 0.1 µM NaBr, 0.05 µM Na_2MoO_2, 0.1 µM $MnCl_2$, 0.1 µM KI, 0.2 µM H_3BO_3, 0.1 µM $CoCl_2$, and 0.1 µM $NiCl_2$ into 1 L of distilled water, amended with 1.5 g of bicarbonate. The prepared medium was sterilized before use by autoclaving at 121°C at 115 kg/cm^2 for 15 min. Growth media used includes; Luria-Bettani (LB) broth, Luria-Bettani (LB) agar, and Soy broth (Merck, Johannesburg, South Africa).

2.2 Up-Flow Bioreactor set-up and start-up

The continuous flow reactor (ceramic bead packed bed Reactor) was constructed from a Pyrex glass column (height: 70 ± 0.01 cm, internal diameter: 10.0 ± 0.01 cm) packed with 7,840, 5 mm spherical ceramic bead Figure 1. The volume and surface area of the reactor is 1980 cm^3 and 50 cm^2. The peristaltic pumps control valves and connecting tubing were cleaned, whereas control valves and connecting tubing were further autoclaved at 121°C for 15 min. For a working reactor volume of 5500 cm^3, distilled water was used to pre-calibrate peristaltic pumps to achieve the desired volumetric flow rate. The reactor was operated in an up-flow mode to ensure near completely submerged condition. The reactor was designed to operate continuously at hydraulic retention time 5 -17 h under volumetric flow 0.088 cm^3/s.

2.3 Ceramic bead media biofilm reactor performance

Prior to start up the interior of the reactor was rinsed in 95 % ethanol and dried. The reactor was operated in an up-flow mode for a period of 150 days over a range of influent Cr(VI) concentrations (30-200 mg/L) and As(III) concentrations in the ratio of 1:1.7 (51 - 340 mg/L). The reactor was designed to operate continuously at hydraulic retention time 5 -17 h under volumetric flow 0.088 cm3/s. Stored harvested cells used in the batch experiment were used as inoculum for the continuous biofilm reactor study. The reactor was inoculated with 60 mL overnight grown anaerobic mixed culture, mixed with LB broth medium, and incubated for 24 h at 32 ± 0.2 oC. After 24 h incubation, the reactor was operated under air tight condition for more than 14 days until visible cell attachment was observed on the ceramic beads and column of the reactor.

2.4 Sampling and analysis

Cr(VI) 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 colour change after introducing 1, 5-diphenyl carbazide following the method described in the Standard Methods for the Examination of Water and Wastewater (Chirwa and Wang,1997).

3. Result and Discussion

3.1 Simultaneous Cr(VI) reduction and As(III) oxidation profile and shock load effect

The bioreactor system was operated continuously for a period of 150 days over a range of influent Cr(VI) concentrations (30-200 mg/L) and As(III) concentrations in the ratio of 1:1.7 (51 - 340 mg/L) at liquid detention times (5-17 h). The data in Figure 2 show influent and effluent Cr(VI) concentrations throughout the course of this study. Cr(VI) breakthrough was observed in phase III when the influent Cr(VI) concentration was increased from 40 to 50 mg/L in the presence of As(III) concentration of 85 mg/L, after 68 days of operation. Except for phases II, IV and VI, Cr(VI) reduction was near completion. Same pattern of system response was seen when considering Cr(VI) reduction across the longitudinal column Figure 5. In general, effluent Cr(VI) concentration

began to increase soon after Cr(VI) and As(III) influent concentration was increased. This trend was observed whenever the influent Cr(VI) and As(III) concentration was increased to a higher value in all phases. Cr(VI) reduction in the reactor decreased with concomitant decrease in As(III) utilization when influent Cr(VI) and As(III) concentration was increased up to 100 mg/L and 200 mg/L in phase IV and VI. The rapid decrease in effluent Cr(VI) concentration after reducing influent Cr(VI) and As(III) concentrations from 100 to 40 mg/L Cr, and 180 to 68 mg/L As(III) in phase V or 200 to 30 mg/L Cr, and 340 to 51 mg/Las(III) in phase VII indicated rapid recovery of metabolic activity in the bioreactor. This trend suggests that higher metabolic activity in the bioreactor may result in higher rate of Cr(VI) reduction. The elevated Cr(VI) concentration in the effluent indicated that biological activity was inhibited by dual toxic effect of Cr(VI) and As(III). However, biological activity recovered is an evident by a rapid decrease in Cr(VI) concentration in phase V and VII. The pattern of As(III) utilization corresponded to Cr(VI) reduction in the reactor (data not shown). As(III) concentration in the reactors was oxidized with the achieved maximum efficiency approached 99 %, which accompanied by near complete removal of Cr(VI) in phases I-VI.

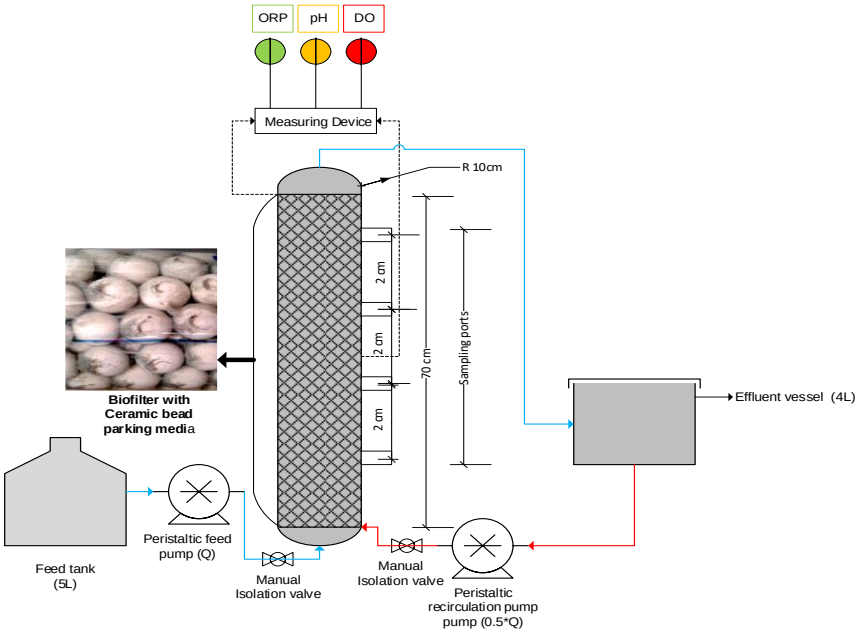

Figure 1: Laboratory set-up of a ceramic media biofilm reactor

3.2 Mass transport along the longitudinal column

Cr(VI) reduction efficiency across the longitudinal reactor column was evaluated at different Cr(VI) concentrations of (30 mg/L, 50 mg/L 100 mg/L, and 200 mg/L) and As(III) concentration in the mole ratio of 1:1.7 Figure 3 and 4. The reason for this investigation was to evaluate Cr(VI) profile along the reactor column utilizing As(III) as an inorganic electron source. As Cr(VI) and As(III) mass load simultaneously travels along the column of distance {x = 70 cm}, Cr(VI) reduction rate increase along the reactor longitudinal column. Result showed that the rate of Cr(VI) removal increases significantly over distance travelled across the column. A significant increase in Cr(VI) reduction efficiency was observed, for instance, in phase I at 30 mg/L and 51 mg/L influent concentration of Cr(VI) and As(III), Cr(VI)reduction efficiency along distance of 20, 30, 40 and 60 cm, was ≤ 65 %, ≤ 69 %, ≤ 70 % and ≤ 75 % Figure 3a. However, there was increase in Cr(VI) reduction efficiency along the column at phase (III) when influent Cr(VI) and As(III)concentration was increased to 50 mg//L and 85 mg//L Figure 3b. for example at distance x along the reactor column (x = 20, 30, 40 and 60 cm), ≤ 90 %, ≤ 91 %, ≤ 93 % and ≤ 97 % of Cr(VI) reduction efficiency was achieved. Effect of double shock load along the reactor column was investigated, this was evaluated by increasing the influent Cr(VI) and As(III) concentration from 1000 mg//L and 170 mg/L to 200 mg//L and 340 mg//L Figure 4a and b. Increasing reactor loading up to 200 mg/L Cr(VI) and 340 mg//L As(III) concentration, resulted to a significant decrease in Cr(VI) reduction efficiency; for instance, at reactor distance (x = 20, 30, 40 and 60 cm), ≤ 29 %, ≤ 37 %, ≤ 40 % and ≤ 44 % Cr(VI) reduction efficiency was achieved Figure 4b. These however, suggest the effect of shock load along the reactor column at higher Cr(VI) and As(III) concentration, which could be attributed to low metabolic activities. However, reduction of Cr(VI) across a vertical longitudinal reactor was seen to be directly proportional to height of the reactor, correlating to the low concentration of Cr(VI) at different column height (H_1-H_4) Figure 5. However, inhibitory

effect observed was attributed loss of cell reducing capacity, and dual toxic effect of Cr(VI) and As(III)along the reactor column.

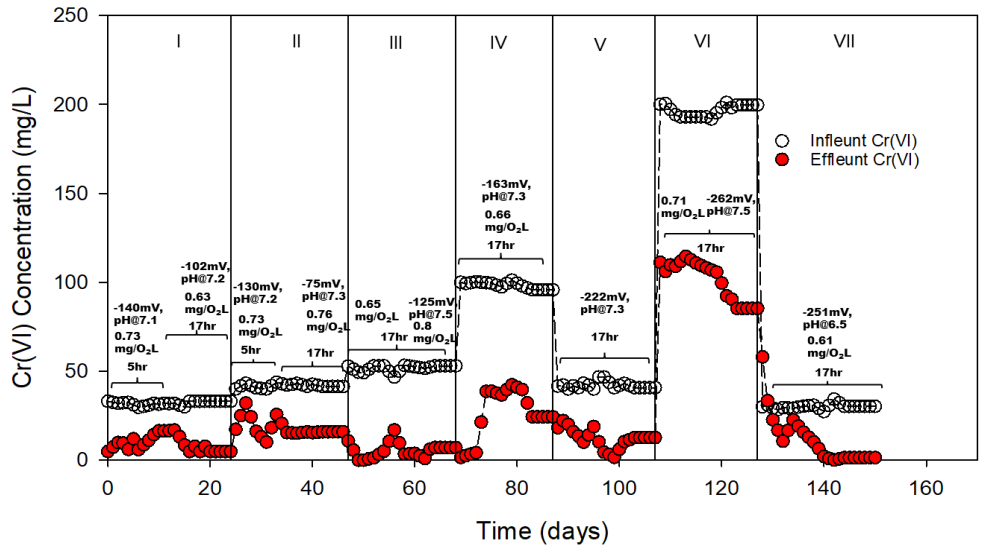

Figure 2: Cr(VI) Reduction in a Ceramic Bead Packed Bed Biofilm Reactor for 150 days operation

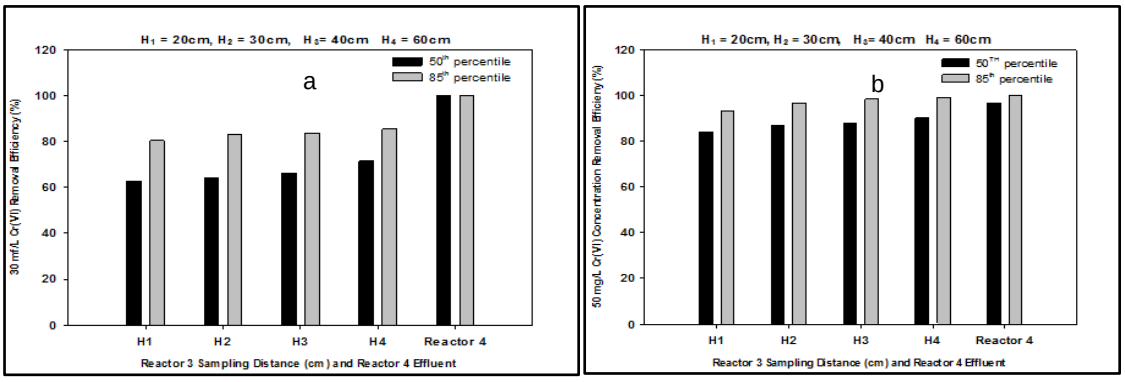

Figure 3: Mass transport along the longitudinal column at different concentrations (a) 30 mg/L, and (b) 50 mg/L

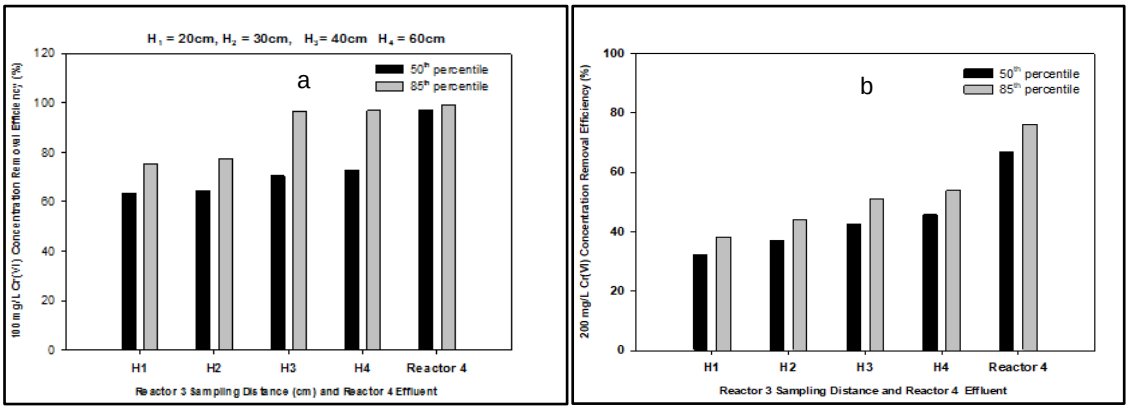

Figure 4: Mass transport along the longitudinal column at different concentrations (a) 1000 mg/L, and (b)200 mg/L

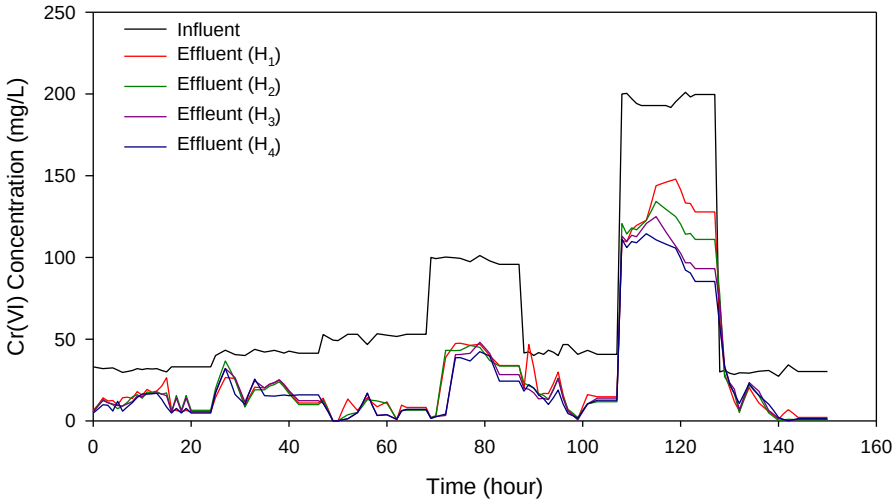

Figure 5: Cr(VI) removal profile along longitudinal column (distance {x} = 70 cm)

3.3 Effect of DO, pH and Oxidation-reduction potential

The steady-state dissolved oxygen (DO), pH, and oxidation reduction potential (ORP) level did not differ significantly between different operating conditions, varying from 0.032 to 1.3 mg/L, 6.36 to 7.38 and -385 to -5.7 mV Figure 6. DO, pH, and ORP optimum operating condition was seen at average of 0.83 mg/L, 7.1 and -164 mV. Anaerobic condition was observed I the all phase of operation Figure 6a, except in phase IV where DO level increases above 0.9 mg/L. The discrepancy observed might occur because of experimental errors at the point of sampling, or as a result of changes in consortium composition under different Cr(VI) and As(III) loading. In addition, there was no significant change in the pH of the reactors Figure 6b, except in phase VII where the pH drops to 6.4. However, the overall pH condition was recorded at 7.2, this corresponds to the observation made from the batch experiment, where optimum Cr(VI) reduction and As(III) oxidation occur at neutral pH condition (Igboamalu and Chirwa, 2014). Secondly, overall ORP was seen at the average of -156 mV for Figure 6b. This however depicts oxidation-reduction potential or strength the reactors throughout the operational phases (I-VII) with corresponding variation in the pH of the reactor Figure 6b. A fairly constant temperature was observed throughout the operational phases (I-VII) recorded at average temperature of 32.8^{o}C at minimum and maximum values recorded at 25^{o}C and 34.5^{o}C Figure 6a

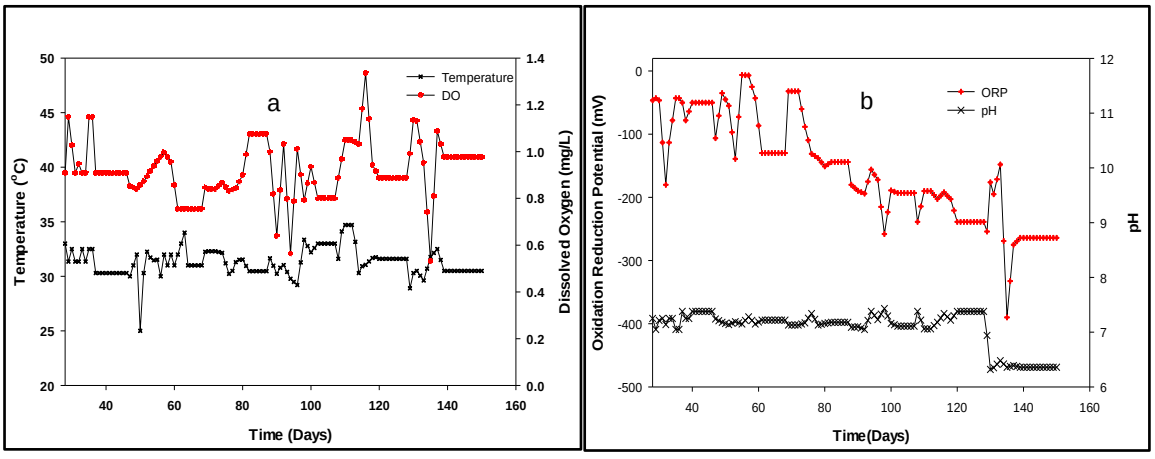

Figure 6: Response of Biofilm reactor in terms of (a) Effect of temperature and Dissolved oxygen(b)Effect of pH and ORP

4. Conclusion

The feasibility of utilizing promising Cr(VI) reducing and As(III) oxidizing bacteria in a small-scale continuous flow biofilm pilot study for bioremediation of chromium-arsenic contaminated wastewater was demonstrated by better performance of Cr(VI) reduction with As(III) as electron donor. A successful reduction of Cr(VI) at much higher Cr(VI) and As(III) concentration up 200 mg/L and 380 mg/L was achieved in all phase (I-IV) after 150 days of continuous operation. The reactor made a speedy recovery after double shock load effect at 100 and 200 mg/L of Cr(VI) and 170 and 380 mg/L As(III), at optimum dissolved oxygen (DO), temperature, pH and ORP of 0.83 mg/L, 32.8^{o}C, 7.1 and -164 mV. unlike the batch system in the previous study (Igboamalu and Chirwa. 2014), inhibitory effects were observed at much higher concentration of both metalloids which was attributed to hydraulic wash off, reducing attached microbial population. The system shows resilience by responding quickly to high or low loading. Secondly, Cr(VI) reduction efficiency across the longitudinal reactor column showed that Cr(VI) reduction or diffusion is proportional to the height of the column travelled with lower effluent Cr(VI) concentrations. This study gave a promising insight toward bioremediation of As(III) and Cr(VI) in a continuous flow system which could be a potential or ideal strategy for bioremediation of Cr(VI) and As(III) containing waste since it can be achieved under anaerobic, neutral pH.

References

Aniruddha D., Wang Y., 2010, Kinetics of arsenite oxidation by chemoautotrophic Thiomonas arsenivorans strain b6 in a continuous stirred tank reactor, J. Environ. Eng. 136(10), 1119-1127.

Chirwa, E.M., Molokwane, P.E., 2011, Biological Cr(VI) reduction: Microbial diversity, kinetics and biotechnological solutions to pollution, In A. Sofo (Ed.), Biodiversity, InTech Online Publishers, United Kingdom. Chapter 5, pp. 75-100.

Chirwa, E.M.N., Wang, Y.T., 2000, Simultaneous Cr(VI) reduction and phenol degradation in an anaerobic consortium of bacteria, Water Res, 34(8), 2376-2384.

Chirwa, E.M.N., Wang, Y.T., 1997b, Hexavalent chromium reduction by Bacillus sp. In a packed-bed bioreactor. Environ, Sci. and Tech, 31(5),1446-1451.

Igboamalu T., Chirwa E., 2016, Kinetic study of cr(vi) reduction in an indigenous mixed culture of bacteria in the presence of as(iii), Chemical Engineering Transactions, 49, 439-444 DOI: 10.3303/CET1649074.

Igboamalu T.E., Chirwa E., 2018, As(iii) oxidation and electron mass transfer kinetic in an enriched mixed culture of *Bacillus sp*., and *Exiguobacterium sp*., isolated from cow dip in south Africa, Chemical Engineering Transactions, 64, 505-510. DOI: 10.3303/CET1864085.

Igboamalu T.E., Chirwa E.M.N., 2014, Cr^{6+} reduction in an indigenous mixed culture of bacteria in the presence of As^{3+}, Chemical Engineering Transactions, 39, 1237-1242. DOI: 10.3303/CET1439207.

Igboamalu T E., Chirwa E.M.N., 2017, As(iii) oxidation and Cr(VI) reduction insight in an indigenous mixed culture of anaerobic bacteria from a local environment, Chemical Engineering Transactions, 61, 259-264. DOI:10.3303/CET1761041.

Oremland, R.S., Stolz, J.F., 2003, The Ecology of Arsenic. Science., 300(5621), 939 - 944.

Rittmann, B.E., McCarty, P.L., 2001, Environmental Biotechnology: Principles and Application, McGraw-Hill, New York.

Smedley, P.L., Kinniburgh, D.G., 2002, A review of the source, behaviour and distribution of arsenic in natural waters, Appl. Geochem. 17, 517-568.

Zhao, M. and Duncan, J.R., 1997, Column sorption and desorption of hexavalent chromium from aqueous solution and electroplating effluent using *Azolla filiculoides*, Resource and Environ. Biotech, 2(1),51-64.