Bioprecipitation of Arsenic from Water under Sulfidogenic Conditions Promoted by Elemental Iron

Zacarias Estrada OL.,* Ballinas Casarrubias L., ** Orrantia Borunda E.,* Luna Velasco A.*
* Department of Environment and Energy, Research Center in Advanced Materials, SC (E-mail: cimav.edu.mx)
** Faculty of Chemical Sciences, Autonomous University of Chihuahua. (E-mail: uach.mx)

Abstract

Arsenic (As) is a pollutant distributed worldwide that importantly impacts natural water. The removal of arsenic from water through its precipitation with biogenic sulfide could attain safe health and environmental As levels. The aim of this study was to evaluate the removal of As from water by its precipitation with biogenic sulfide, using elemental iron (ZVI) as an electron donor in the sulfidogenic process. The effect of arsenic concentration on sulphate reducing activity of anaerobic sludges was also studied. Batch experiments inoculated with non pre-activated and pre-activated SRB in anaerobic sludge, added with ZVI and with different concentrations of pentavalent As (As^{V}) were performed to evaluate sulphate (SO_4^{-2}) reduction and arsenic removal. The As_{tot}, sulfide (S^{-2}) and sulphate (SO_4^{-2}) concentration were monitored in assays. High percentages of As removal (>98%) and low quantifications of S^{-2} (≤50%) were observed in treatments dosed with up to 5 mgL^{-1}As, with pre-activated and non pre-activate sludges that used ZVI as electron donor, indicating that precipitation of metalloid could de mediated by binding with biogenic sulfide to form ASM. The pre-activated anaerobic sludge was significantly more tolerant to As, maintaining the sulfidogenic activity up to 5mgL^{-1}added with As. SEM analysis of the pre- activated sludge showed granules of BSR more compact compared to non pre-activated sludge, which can be related to tolerance and activity of the BSR to As. Sulphate reduction was not observed in controls without ZVI, supporting that ZVI was the unique electron donor used by sulfate reducing bacteria in the experiments. The results suggest that the use of BSR with ZVI is a viable and feasible technology for removing contaminants from water such as As.

Keywords.
Arsenic, Sulfate Reducing Bacteria, bioremediation.

INTRODUCTION.

The presence of As in groundwater around the world is due to natural and anthropogenic sources, being the former the main source, due to this metalloid is part of rocks and sediments. (Welch et al., 2000). The three natural arsenic sulfide mineral forms are mainly, arsenopyrite (FeAsS), realgar (AsS) and orpiment (As_2S_3) (O'Day et al., 2004). When these minerals are washed up on the aquifers, arsenic is released to groundwater principally in the species of arsenates (As^{V}, $H_2AsO_4^-$ and $HAsO_4^{2-}$) or arsenites (As^{III}, H_3AsO_3), depending on the redox conditions. As^{V} is the predominant specie in oxidant environments whereas As^{III} predominates in reducing environments. The mobilization of As from rocks or sediments to groundwater normally elevates the concentration of this metalloid in aquifers creating health and environmental problems (Duruibe et al., 2007; Hashim et al., 2011). The United States Environmental Protection Agency (US-EPA) has set the As standard in drinking water at 0.01 mgL^{-1} and in Mexico the standard value is of 0.025 mgL^{-1} (NOM-127-SSAI-1994). The concentration of As in groundwater and drinking water frequently exceeds those limits in many locations across the world (US-EPA, 2001). In Chihuahua, México, As levels from 0.022 to 0.22 mgL^{-1} were recently reported in groundwater (Reyes-Gomez *et al*., 2013). Therefore, the removal of As from water is necessary to get safe values for drinking water. Biological methods are one of the emerging technologies for As remediation, including plants, cells or bio-compounds (Ma et al., 2001; Zouboulis and Katsoyiannis., 2005; Huang et al., 2004). Sulphate reducing bacteria has been used in arsenic

bioremediation studies, due to they can reduce sulphate to sulfide, giving the conditions for the formation of As-bearing sulfide minerals (ASM). The sulfidogenic activity of SRB used in As removal treatments has been stimulated with organic compounds such as lactate, acetate, methanol, ethanol, sucrose, etc. as electron donors for the reduction of sulphate (Teclu et al., 2008; Onstott et al., 2011; Rodriguez-Freire et al., 2014). According to those studies, the precipitation of ASM could occur by the adsorption or incorporation of As in the iron sulfides mackinawite FeS or pyrite FeS_2, resulting the formation of As_2S_3 and AsS by the reduction of As and S. Some studies have coupled ZVI with organic electron donors for sulphate reduction to improve the removal of metals and Arsenic from groundwater (Onstott et al., 2011; Beaulieu and Ramirez, 2013). The ZVI can act supplying a source of reductants like Fe^{2+} and hydrogen gas (H_2). The cathodic H_2 is generated by the reaction of Fe^0 with H_2O (Eq. 1). Hydrogen serves as an electron donor for autotrophic bacteria involved in the reducing process of pollutants under anaerobic conditions. BSR associated with ZVI can use the H_2 formed in the above reaction to reduce sulphate (Van Noothen et al., 2007).

$$Fe^0 + 2H_2O \rightarrow Fe^{2+} + 2OH^- + H_2 \qquad \text{(Eq.1)}$$

The use of ZVI in the absence of SRB for the removal of arsenic was also reported in several studies (Biterna et al., 2010; Rahmani et al., 2011, Eljamal et al., 2011). The main mechanisms reported for As removal are adsorption, precipitation and redox transformation onto surface iron corrosion products (eg. (oxy)hydroxides and green rust). However, those forms of As immobilization have shown low stability, mainly by the reductive dissolution of arsenic from iron hydroxides. In contrast, iron sulfides are more stable sink for arsenic in groundwater and the ASM generated have also shown to be stable at changes of redox conditions (Onsttot et al. 2011).

To the best of our knowledge, there are no reports using ZVI as unique electron donor for SRB cultures involved in arsenic removal from water. The use of inorganic electron donors in water decontamination helps to reduce organic residuals in water and they are slow released during the treatment. The proposal of this paper was to test the ZVI as an exogenous electron donor by SRB in the sulfidogenic process to promote the removal of As from water by forming arsenic sulfides.

MATERIALS AND METHODS.

Microorganisms source and culture medium

The anaerobic sludge was obtained from anaerobic digester of a wastewater treatment plant (Chihuahua, Chih, Mexico). The sludge was examined for As content in acid digested samples and As was not detected at the detectable limit of 0.000337 mgL^{-1} in atomic absorption spectrometry (AAS) coupled to a hydride generator (HG-AAS). Anaerobic sludge was stored at 4 ºC (non-preactivated). The pre-activation of SRB in anaerobic sludge was achieved by continuously feeding glucose and sulphate in a ratio of 0.67 mol (SO_4: glucose) for a period of 36 weeks. In order to spend the remaining glucose in sludge used as inoculum, it was overnight incubated in culture medium free of glucose and ZVI. The basal medium (ABM-2) used in batch assays contained (in mg L^{-1}): NHCl (280); $NaHCO_3$ (5000), K_2PO_4 (600); NaH_2PO_4. $2H_2O$ (796), CaCl $2H_2O$ (10), MgCl.6 H_2O (100), Na_2SO_4 (500), yeast extract (20), and 1 mL of trace element solution, according to Karri et al., 2005. The medium pH was adjusted to 7.

Batch assays.

The sulfidogenic activity and arsenic removal were performed in glass bottles of 120 ml containing 80

mL of liquid medium, inoculated with 10% v/v of non pre-activated or pre-activated SRB in anaerobic sludge, added with ZVI (0.31 g, size of 40 um) as electron donor and dosed with arsenic concentrations from 0.1 to 5 mg L^{-1}. All bottles were quickly sealed with rubber septa and aluminum crimp seal. The headspace was flushed with a gas mixture of N_2:CO_2 80:20% v/v for 5 min to displace oxygen and get anaerobic conditions. Then, bottles were incubated at 30-32 °C under stirring at 80 rpm. Control treatments non-inoculated, without As and without ZVI were parallel run in triplicates. Arsenic was dosed by adding the required volume of a stock solution of 1000 mgL^{-1} As obtained from sodium pentavalent arsenate heptahydrate salt (Na_2HAsO_4-$7H_2O$- Sigma Aldrich). Liquid samples were taken with a syringe at different incubation times to measure sulphate, sulfide, iron and As_{tot}.
The use of ZVI as electron donor to support sulphate reduction in anaerobic sludge was tested in assays free of As and treatments added with acetate as electron donor were parallel run to compare the sulfidogenic activity of ZVI with that of a simple organic compound.

Analytical methods.

Iron, sulfide and sulphate determination.

Iron and sulfide were determined according to standardized methods (APHA 1998). Samples were taken with a syringe (500-1000 μL) and quickly acidified to measure the ion Fe^{2+} using the method based on phenanthroline, which forms a reddish orange complex with Fe^{2+} that absorbs at a wavelength of 510 nm. The sulfide was quantified in fresh samples through the colorimetric method of methylene blue (670nm).Sulphate was determined in fresh samples by precipitating it with barium chloride crystals according to the manufacturer kit, Hanna HI 9675. Sulphate concentration is directly proportional to the turbidity of the precipitated sample, measured at a wavelength of 466 nm.

Arsenic determination.

Total arsenic was determined in 1 mL of samples from cultures exposed to As and from controls without As. The samples were centrifuged and stored in 0.02% HNO_3 at -80^0C until analysis. Samples were taken at days 0, 3, 6, 9, 15 and 18 after incubation. The concentration of arsenic in the samples was determined by atomic absorption spectrometry (AAS) coupled to a hydride generator (HG-AAS) using an AAS spectrometer Analyst 400 of Perkin Elmer. All samples were mixed with potassium iodide 10% as reducing agent, in a total volume of 10 mL. After 45 min of mixture, As measurements were performed using sodium borohydride ($NaBH_4$) 0.2%, NaOH 0.05% and HCl 10% as carrying solutions. The generated volatile hydrides (arsines) were carried with argon gas into a heated quartz cell at 900 °C, using air as the oxidant. Samples, blanks and standards were treated under the same conditions. The percentage of As removal in was calculated according to Eq. 2, which denotes the change in arsenic concentration Δ[As]versus change of time Δ(t).

$$\% \text{ As Removal Rate} = \Delta (\% \text{ As removal}) / \Delta (t) \qquad \text{(Eq.2)}$$

Preparation of biological samples for SEM.

Representative samples of sludge were prepared following the methodology described by Scott et al., 2011. Briefly, 0.5 mL aliquots of sludge: pre-activated, non-pre-activated and pre-activated exposed to As, were incubated in 3% glutaraldehyde for 13 h. Then, samples were washed with buffer Milloning and dehydrated with different ethanol gradients (30, 50, 70, 90 and 100 %). Prepared samples were analyzed in a JSM7401f Scanning Electron Mycroscope of JEOL to determine morphological differences.

RESULTS.

ZVI as electron donor in sulfidogenic process.

The use of ZVI as electron donor to support sulphate reduction in anaerobic sludge (non pre-activated) was first tested in batch assays. The efficiency of ZVI as electron donor for sulphate reduction was obtained by comparing the sulfate reduction rates of ZVI with the obtained for the simple organic electron donor, acetate. Table 1 shows the rate values of SO_4^{-2} reduction for both, ZVI and acetate. Noting that rate values for ZVI and acetate are very similar, which demonstrates the good efficiency of ZVI as an electron donor for SRB in the anaerobic sludge. This is in accordance with a previous report that showed the use of ZVI as electron donor for sulphate reduction by BSR present in an anaerobic sludge (Karri et al., 2005). Chemical corrosion of ZVI under anaerobic conditions can supply cathodic hydrogen (H_2) (eq. 1) and previous studies observed that SRB are able to use H_2 in reductive processes (Van Nooten et al., 2007).

Tabla 1. Sulphate redution rates in anaerobic sludge with ZVI and acetate.

Treatment	sulphate reduction rate (mmol day^{-1})
ZVI	0.2231
Acetate	0.2198

Sulfidogenic activity and As removal

Sulfidogenic activity and As removal were assayed in batch experiments inoculated (10%) with pre-activated and non pre-activated sludge and exposed to different concentrations of arsenic (0.1-5 mgL^{-1}). Controls without arsenic and without ZVI were performed in triplicate. SO_4^{-2} consumption, generation of S^{-2}, Fe^{2+} and removal of As_{tot} were evaluated along 18 days of incubation. First will be presented data for assays with pre-activated sludge. Figure 1A shows sulphate consumption behavior at different As concentrations. Treatments exposed to As concentrations of 0.1 and 0.5 mgL^{-1} had very similar sulphate reduction rates compared with controls without As. Whereas in treatments exposed to As concentrations of 1 and 5 mg L^{-1}, the sulphate consumption was lower compared with control treatments without As. Showing a decrease of almost two times in reduction rates at those As concentrations (0.18 and 0.15 mmol day^{-1}, respectively) based on controls (0.37 mmol day^{-1}). These results agree with those reported by Teclu et al., 2009, for an enriched culture of SRB from anaerobic sediments, which presented a decrease in growth and activity (25, 40 and about 75%) at the end of 14 days of incubation with As^V at concentrations of 1, 5 and 20 mg L^{-1}, respectively. SRB cultures exposed to As^{III} showed somewhat less growth and activity. Therefore, the decrease in sulphate reduction by exposition of high concentration of both As species was attributed to the low percentage of growth. In the other hand, sulphate reduction was not observed in controls without ZVI, supporting that ZVI was used by sulfate reducing bacteria in anaerobic sludge. Figure 2B shows the generation of S^{-2} in treatments with different concentrations of As and controls without As. The maximum amount of sulfide produced in controls was the expected based on the stoichiometric equation for sulphate reduction (eq. 3). Whereas, in all treatments exposed to arsenic the maximum amount of sulfide produced during the incubation time was almost half lower than the stoichiometric amount expected. The low amount of S^{-2} in treatments exposed to As could be due to their interaction with iron ion (Fe^{2+})

and As (Onstott et al., 2011, Rodriguez-Freire et al., 2014). However, the amount of Fe^{2+} had the same trend between controls and treatments exposed to As (Fig 2C), observing a small decrease of Fe^{2+} during the first 5 days of incubation and after that it was maintained constant. Thus, the shrinkage of S^{-2} could be more due to its interaction with arsenic, immobilizing it from aqueous phase. In Figure 2D is indicated the arsenic removal from aqueous phase. The arsenic was 100% removed in treatments added with 0.1 y 0.5 mg L^{-1} of the metalloid and most arsenic was removed in treatments with high As concentrations of 1 y 5 mg L^{-1} (99 y 98%, respectively). The high removal of As in treatments is attributable to the formation of arsenic sulfides, since biogenic S^{-2} produced was higher than the stoichiometric amount required (Eq. 4) to achieve the removal of all arsenic added and this correlates with the decrease of S^{-2} measured in those treatments. The precipitation of As with biogenic sulfides has been previously demonstrated as the As removal process from water using organic electron donors in sulfate reduction (Neuman et al., 1997; Teclu et al., 2008; Xu et al., 2011; Battaglia-Brunet et al, 2012). Additionally, the redox (-0.27 -0.22 mV) and pH conditions (6-8-7.2) in which treatments were developed favor the precipitation of As with sulfide, according to the prediction analysis with Hydra Medusa sofware. The mineral forms generated under these conditions could be orpiment (As_2S_3) and realgar (AsS) (Onstott et al., 2011; Rodríguez-Freire et al,, 2014), which are natural arsenic minerals present in environments containing As and BSR (Demergasso et al., 2007; Saunders et al., 2008).

$$4Fe^{o} + SO_4^{-2} + 9H^{+} \xrightarrow{\textbf{SRB}} HS^{-} + 2Fe^{+2} + H_2 + 2OH^{-} + 2H_2O \qquad \text{(Eq. 3)}$$

$$2H_2AsO_3^{-} + 3HS^{-} + 5H^{+} \longrightarrow As_2S_3 + 6H_2O \qquad \text{(Eq. 4)}$$

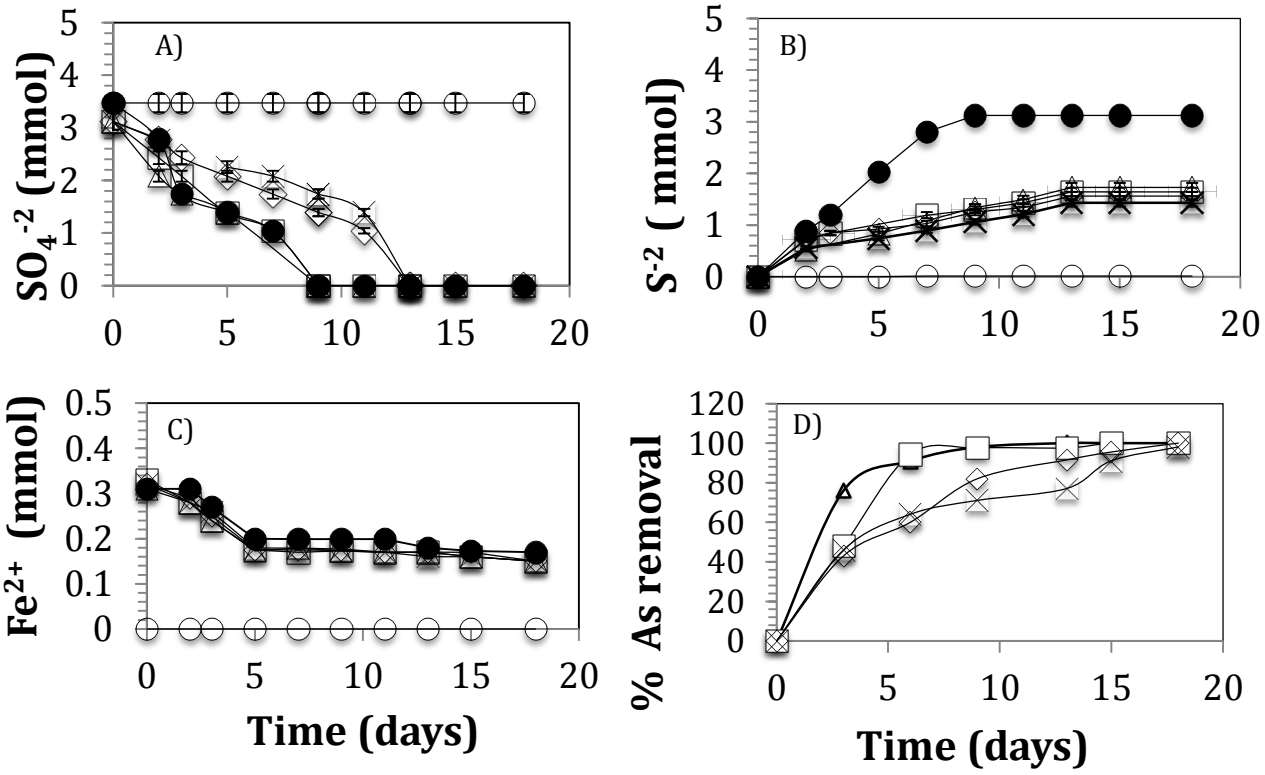

Fig 1. Time-course of sulfate consumption (A), generation of sulfide (B) and Fe^{2+} (C), and arsenic removal (D) in treatments with pre-activated anaerobic sludge dosed with different concentrations of As (in mgL^{-1}): 0 (●), 0.1 (△) 0.5 (□), 1 (◇), 5 (×) and control without ZVI and As (○). The initial

and end pH was of 7 and 6.3-6.5, respectively. Bars represent the standard deviation of duplicates or triplicates.

In order to determine if the pre-activation of BSR in anaerobic sludge improves its tolerance to As exposure, non-pre-activated anaerobic sludge was exposed to same concentrations of As and under same incubation conditions used in assays with pre-activated sludge. The results obtained for sulfate consumption showed low sulfidogenic activity in controls without ZVI until day 5 of incubation (data not shown), which could be due to the presence of residual organic matter in non pre-activated anaerobic sludge providing it endogenous activity. The rate values of sulphate consumption, sulfide generation and arsenic removal in treatments with non pre-activated sludge in comparison to treatments with pre-activated sludge, are presented in table 2. The sulfate reduction rates and sulfide generation in non pre-activated anaerobic sludge were somewhat lower than rates observed in pre-activated anaerobic sludge. The low activity of sulfate reduction in that sludge could be due to low SRB cell proportion. In relation to As removal, the removal rates were slightly higher in non pre-activated anaerobic sludge exposed to high As concentrations (1 and 5 mgL^{-1}). That behavior could be associated to the interaction and/or formation of complexes of the metalloid with residual organic matter present in anaerobic sludge (Utgikar et al., 2001), as was noted above for sulfate reduction. The exposure of non pre-activated sludge to those high As concentrations (1 and 5 mg L^{-1}), diminished the sulfate reduction activity and around day 10 of incubation it was fully inhibited, which did not occurs in pre-activated anaerobic sludge. Even when sulphate reduction was lowered or inhibited, again, the biogenic sulfide produced was stoichiometrically sufficient to remove most of the As dosed in treatments. As removal of 100% was obtained in treatments with 0.1 and 0.5 mgL^{-1}, whereas in those with 1 and 5 mgL^{-1}, the removal of As was below and slightly above reference standards, respectively.
Taking into account the sulfidogenic and As removal rates of both assays, inoculated with non pre-activated and pre-activated anaerobic sludge, it was clearly showed that BSR in pre-activated sludge had higher tolerance to As and the activity was maintained at 5 mg L^{-1} of As.

Table 2. Rate values of SO_4^{-2} consumption (mmol day^{-1}), S^{-2} generation (mmol day^{-1}) and As removal (mgL^{-1} day^{-1}) in treatments with non pre-activated sludge in comparison to treatments with pre-activated sludge.

Treatment	**Rate values (mmol day^{-1})**					
As (mgL^{-1})	**Non pre-activated**			**Pre-activated**		
	SO_4^{-2}	**S^{-2}**	**As**	**SO_4^{-2}**	**S^{-2}**	**As**
*0	0.23	0.41	0	0.37	0.4	0
0.1	0.19	0.08	0.002	0.29	0.11	0.002
0.5	0.19	0.07	0.02	0.3	0.08	0.03
1	0.11	0.05	0.04	0.18	0.07	0.07
5	0.11	0.05	0.22	0.15	0.07	0.19

Sludge characterization.

The sludge samples from the treatment (pre-activated and non pre-activated) were analyzed using SEM in order to characterize the morphology and organization of the BSR. Figure 2 shows SEM images of analyzed samples taken at lower and corresponding higher magnification for more cell details. Figure 2A and B illustrate granules of non pre-activated sludge, which are poor integrated and compacted and they are conformed of a mixture of cell morphology, including *rods*, *bacilli* and *ovoid*. Whereas

granules in pre-activated sludge (Fig.2 C and D) are more compact and bigger than those present in non pre-activated sludge. In these granules predominate cells with *vibrio* morphology and there are some *bacilli*. The *vibrio* morphology is characteristic of SRB (Fitchel et al., 2012) and its abundance corresponds with the purpose of pre-activation of SRB in anaerobic sludge.
In addition to the treatments exposed to As (0.5 and recently to 1 mg L^{-1}), although they are active and the BSR granules stay compact, changes are observed in the morphology of the cell membrane after 37 days of exposure, as well as a decrease in size (Fig. 2 E and F). The differences with respect to morphology and organization of BSR can be related as shown in Fig.1 with respect to consumption of SO_4^{-2} in treatments exposed to high concentrations of As in pre-activated and non pre-activated treatments.

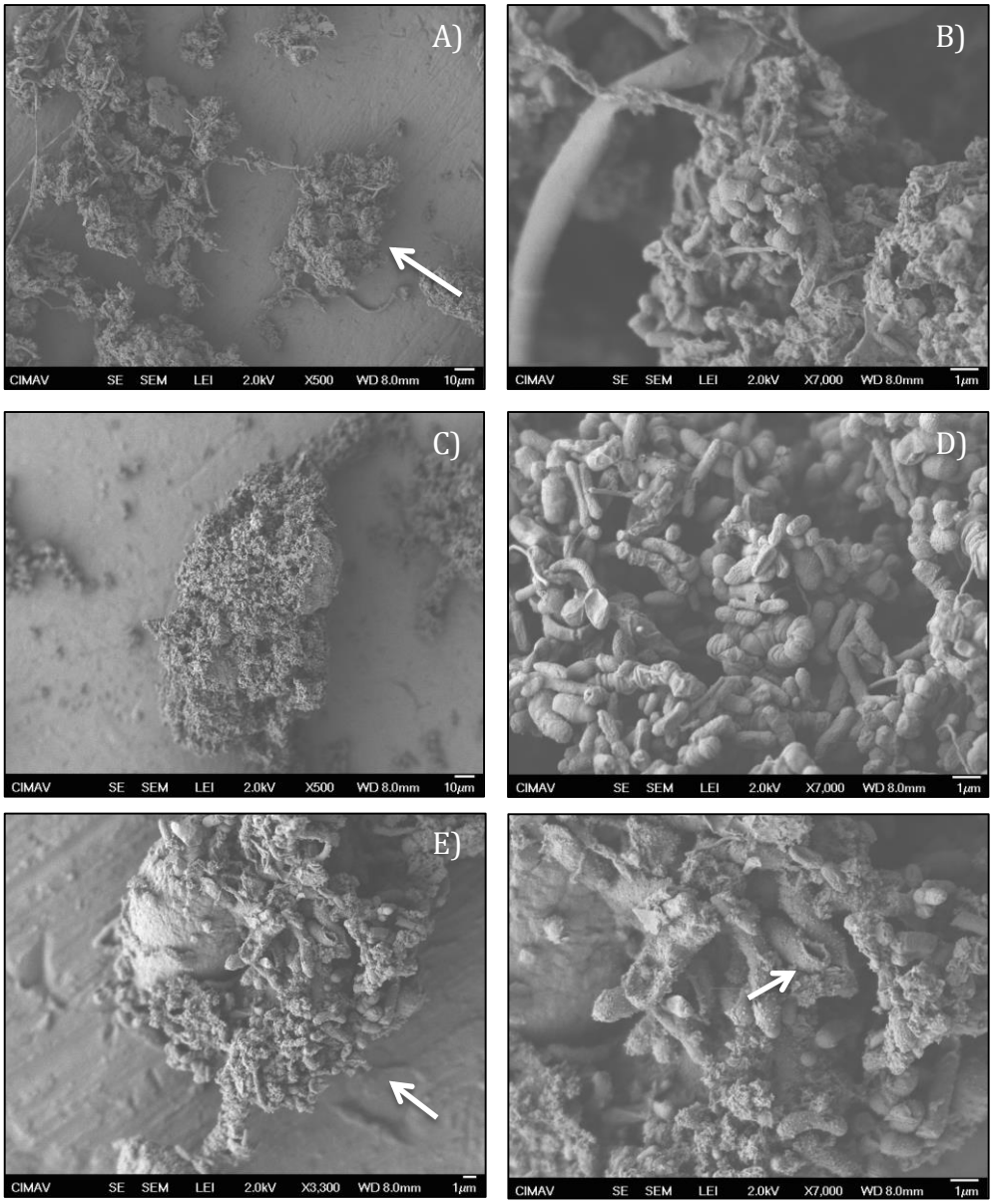

Fig 2. SEM images of sludge samples from non pre-activated (A and B), pre-activated (C and D) and Pre-activated and exposed to As (E and F) at 240, 183 and 37 days of incubation. The arrow in figure A, C an E indicate the granules and in figure F the possible damage in the cell membrane.
183 days37 days

CONCLUSIONS.

SRB in anaerobic sludge were able to utilize ZVI as an electron donor in sulfidogenic activity and it was not affected by arsenic concentrations comparable to those found in groundwater (as in Chihuahua Mexico). The removal of the arsenic from water was associated to its precipitation with biogenic sulfide. The overall results demonstrate the potential use of ZVI to promote sulfidogenic activity in anaerobic sludge and bio-precipitation of As from water, exceeding international standards, promising this As bioremediation process as inexpensive, sustainable and effective to solve As contamination problems in water.

ACKNOWLEDGMENTS.

The present work was supported by CONACYT support scholarships for the PhD in science and technology Environmental of the Research Center in advanced materials (CIMAV). We thank to the Faculty of Chemical Sciences of the University Autonomy of Chihuahua for the support in arsenic determinations. Similarly to the technicians of CIMAV for the SEM-EDS analysis.

REFERENCES.

Battaglia-Brunet, F., Crouzet, C., Burnol, A., Coulon, S., Morin, D., & Joulian, C. (2012). Precipitation of arsenic sulphide from acidic water in a fixed-film bioreactor. Water Research, 46(12), 3923-3933.

Biterna, M., Antonoglou, L., Lazou, E., & Voutsa, D. (2010). Arsenite removal from waters by zero valent iron: Batch and column tests. Chemosphere, 78(1), 7-12.

Demergasso, C. S., Chong D, G., Escudero G, L., Pueyo Mur, J. J., & Pedros-Alio, C. (2007). Microbial precipitation of arsenic sulfides in Andean salt flats. Geomicrobiology Journal, 24(2), 111-123.

Duruibe, J. O., Ogwuegbu, M. O. C., & Egwurugwu, J. N. (2007). Heavy metal pollution and human biotoxic effects. International Journal of Physical Sciences, 2(5), 112-118.

Eljamal, O., Sasaki, K., & Hirajima, T. (2011). Numerical simulation for reactive solute transport of arsenic in permeable reactive barrier column including zero-valent iron. Applied Mathematical Modelling, 35(10), 5198-5207.

Eljamal, O., Sasaki, K., Tsuruyama, S., & Hirajima, T. (2011). Kinetic Model of Arsenic Sorption onto Zero-Valent Iron (ZVI). Water Quality Exposure and Health, 2(3-4), 125-132.

Fichtel, K., Mathes, F., Koenneke, M., Cypionka, H., & Engelen, B. (2012). Isolation of sulfate-reducing bacteria from sediments above the deep-subseafloor aquifer. Frontiers in Microbiology, 3

Hashim, M. A., Mukhopadhyay, S., Sahu, J. N., & Sengupta, B. (2011). Remediation technologies for heavy metal contaminated groundwater. Journal of Environmental Management, 92(10), 2355-2388.

Huang, JW., Poynton, CY., Kochian, LV., Elless, MP. (2004): Phytofiltration of Arsenic from Drinking Water Using Arsenic-Hyperaccumulating Ferns. Environ Sci Technol 38, 3412–3417.

Karri, S., Sierra-Alvarez, R., & Field, J. A. (2005). Zero valent iron as an electron-donor for methanogenesis and sulfate reduction in anaerobic sludge. Biotechnology and Bioengineering, 92(7), 810-819.

Lee, K.-J., Lee, Y., Yoon, J., Kamala-Kannan, S., Park, S.-M., & Oh, B.-T. (2009a). Assessment of zero-valent iron as a permeable reactive barrier for long-term removal of arsenic compounds from synthetic water. Environmental Technology, 30(13), 1425-1434.

Lee, K.-J., Lee, Y., Yoon, J., Kamala-Kannan, S., Park, S.-M., & Oh, B.-T. (2009b). Assessment of zero-valent iron as a permeable reactive barrier for long-term removal of arsenic compounds from synthetic water. Environmental Technology, 30(13), 1425-1434.

Ma, QL., Komar, KM., Tu, C., Zhang, W., Cai, Y., Kennelley, ED. (2001). Afern that hyperaccumulates arsenic. Nature 409, 579.

O'Day, P. A., Vlassopoulos, D., Root, R., & Rivera, N. (2004). The influence of sulfur and iron on dissolved arsenic concentrations in the shallow subsurface under changing redox conditions. Proceedings of the National Academy of Sciences of the United States of America, 101(38), 13703-13708.

Onstott, T. C., Chan, E., Polizzotto, M. L., Lanzon, J., & DeFlaun, M. F. (2011). Precipitation of arsenic under sulfate reducing conditions and subsequent leaching under aerobic conditions. Applied Geochemistry, 26(3), 269-285.

Rahmani, A. R., Ghaffari, H. R., & Samadi, M. T. (2011a). A COMPARATIVE STUDY ON ARSENIC (III) REMOVAL FROM AQUEOUS SOLUTION USING NANO AND MICRO SIZED ZERO-VALENT IRON. Iranian Journal of Environmental Health Science & Engineering, 8(2), 175-180.

Rahmani, A. R., Ghaffari, H. R., & Samadi, M. T. (2011b). A COMPARATIVE STUDY ON ARSENIC (III) REMOVAL FROM AQUEOUS SOLUTION USING NANO AND MICRO SIZED ZERO-VALENT IRON. Iranian Journal of Environmental Health Science & Engineering, 8(2), 175-180.

Reyes-Gomez, V. M., Teresa Alarcon-Herrera, M., Gutierrez, M., & Nunez Lopez, D. (2013). Fluoride and Arsenic in an Alluvial Aquifer System in Chihuahua, Mexico: Contaminant Levels, Potential Sources, and Co-occurrence. Water Air and Soil Pollution, 224(2).

Rodriguez-Freire, L., Sierra-Alvarez, R., Root, R., Chorover, J., & Field, J. A. (2014). Biomineralization of arsenate to arsenic sulfides is greatly enhanced at mildly acidic conditions. Water Research, 66, 242-253.

Saunders, J. A., Lee, M. K., Shamsudduha, M., Dhakal, P., Uddin, A., Chowdury, M. T., et al. (2008). Geochemistry and mineralogy of arsenic in (natural) anaerobic groundwaters. Applied Geochemistry, 23(11), 3205-3214.

Scott, A. Thomasson and Joseph R. Thomasson. (2011). A comparison of CPD (critical point drying) and HMDS (hexamethyldisilazane) in the preparation of *Corallorhiza* spp. *rhizomes* and asociated mycorrhizae for SEM (scanning electron microscopy). Kansas Academy of Science, 114 (1-2), 129-134).

Teclu, D., Tivchev, G., Laing, M., & Wallis, M. (2008). Bioremoval of arsenic species from contaminated waters by sulphate-reducing bacteria. Water Research, 42(19), 4885-4893.

Teclu, D., Tivchev, G., Laing, M., & Wallis, M. (2009). Determination of the elemental composition of molasses and its suitability as carbon source for growth of sulphate-reducing bacteria. Journal of Hazardous Materials, 161(2-3), 1157-1165.

US-EPA, 2001. National Primary Drinking Water Regulations. Arsenic and Clarifications to Compliance and New Source Contaminants Monitoring.

Utgikar, V. P., Harmon, S. M., Chaudhary, N., Tabak, H. H., Govind, R., & Haines, J. R. (2002). Inhibition of sulfate-reducing bacteria by metal sulfide formation in bioremediation of acid mine drainage. Environmental Toxicology, 17(1), 40-48.

Van Nooten, T., Lieben, F., Dries, J., Pirard, E., Springael, D., & Bastiaens, L. (2007). Impact of microbial activities on the mineralogy and performance of column-scale permeable reactive iron barriers operated under two different redox conditions. Environmental Science & Technology, 41(16), 5724-5730.

Welch, A.H., Westjohn, D.B., Helsel, D.R., Wanty, R.B., (2000). Arsenic in ground water of the United States: occurrence and geochemistry. Ground Water 38 (4), 589e604.

Xu, L., Zhao, Z., Wang, S., Pan, R., & Jia, Y. (2011). Transformation of arsenic in offshore sediment under the impact of anaerobic microbial activities. Water Research, 45(20), 6781-6788.

Zouboulis, AI., Katsoyiannis, IA., (2005). Recent advances in the bioremediation of arsenic-

contaminated groundwaters. Environ Int 31(2) 213–219.