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# BIOREMEDIACIÓN DE ARSENICO MEDIANTE LA FORMACION DE MINERALES DE SULFURO DE ARSENICO


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TFG REALIZADO EN PROGRAMA DE INTERCAMBIO

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TÍTULO: ARSENIC BIOREMEDIATION BY FORMATION OF ARSENIC-SULFIDE MINERALS

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## RESUMEN

La bioremediación de aguas contaminadas con arsénico mediante la formación de minerals de sulfuro de arsénico ha sido probada con éxito usando la reducción microbiana simultanea de arsenato y sulfato, pero solo bajo estrictas condiciones de pH < 7 y un ratio estequiométrico de S/As. El principal objetivo de esta investigación es evaluar la eficacia de este método usando ratios S/As alejados del valor estequiométrico y con un pH < 7.



## PALABRAS CLAVE

- ARSENICO
- SULFATO
- BIOREMEDIACIÓN
- TOXICIDAD
- BIOREACTOR

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## ABSTRACT

Arsenic (As) is a highly toxic and carcinogen metalloid present in the environment and found at high concentrations in groundwater and surface in numerous locations around the world. A variety of physico-chemical treatment processes have been developed for remediation of arsenic-contaminated water. However, the application of these processes is limited by their high maintenance and operational costs and problems related to the generation of arsenic residuals. Nowadays, new biological treatments are being studied as an alternative for the remediation of arsenic due to their environmental compatibility and possible cost-effectiveness.

The bioremediation of arsenic-contaminated groundwater by formation of arsenic sulfide minerals (ASM) has already been successfully demonstrated using simultaneous microbial reduction of arsenate (As(V)) and sulfate ($SO_4^{2-}$), but only under strict conditions (pH < 7 and stoichiometric S/As ratio required for $As_2S_3$ precipitation). The main objective of this research was to evaluate the effectiveness of this environmental biotechnology for aqueous streams containing concentrations of $SO_4^{2-}$ far above those meeting the stoichiometric S/As ratio of 3/2. Batch and continuous-flow bioreactor experiments were performed to study the microbial reduction of As(V) by anaerobic granular sludge and demonstrate the importance of the S/As ratio on the formation of ASM under anaerobic conditions. Complementary experiments were also conducted to study the aqueous solubility of several ASM minerals (commercial orpiment ($As_2S_3$), crushed orpiment, realgar ($\alpha$-$As_4S_4$), arsenopyrite (AsFeS)) under aerobic and anoxic conditions in order to understand the potential for ASM mobilization under shifting redox conditions.

A microbial anaerobic consortium obtained from a full-scale anaerobic upflow sludge bed (UASB) bioreactor was found capable to reduce As(V) to arsenite (As(III)) in anaerobic batch bioassays performed at 30°C using $H_2$ as electron donating substrate. The rate of reduction increased with increasing initial As(V) concentrations and the reaction followed first-order kinetics (rate constant = 0.864 $day^{-1}$) The maximum As(V) reduction rate determined at the highest concentration tested (187 mg/L) was 107.8 mg As/g VSS-day.

Furthermore, the results of anaerobic batch bioassays conducted in sulfate-free medium demonstrated that As(V) and As(III) are potent inhibitors of the methanogenic activity of anaerobic granular sludge. The 50% inhibitory concentrations determined for As(V) and As(III) were very close, 5.7 and 6.2 mg/L, respectively. In contrast, As(V) and As(III) were not inhibitory to As(V)-reducing microorganisms at concentrations as high as 180 mg/L.

Two continuous-flow anaerobic bioreactors were operated in parallel for 120 days at a hydraulic retention time of 24 h with a synthetic influent containing 65 µM As(V). One bioreactor received 0.2 mM $SO_4^{2-}$ (close to the stoichiometric S/As relation for precipitation of $As_2S_3$), and the other 1 mM $SO_4^{2-}$ (molar S/As = 15). The influent of both reactors was adjusted to pH values ranging 6.4-6.6 to promote the formation of ASM which dissolution is enhanced at pH values exceeding 7.0. Whereas the reactor fed with 0.2 mM $SO_4^{2-}$ removed an average of 79.6 % of the total As in the influent, the reactor fed with 1 mM $SO_4^{2-}$ only removed 33.1% of the total As. This decrease was associated with the formation of thioarsenites ($AsS_xO_{3-x}^{3-}$) as confirmed by ion chromatography analyses. In conclusion, these results demonstrated that the effectiveness of ASM precipitation by simultaneous microbial reduction of As(V) and $SO_4^{2-}$ is greatly reduced when there is a high excess of $SO_4^{2-}$, demonstrating that controlling the S/As ratio is critical for the success of this arsenic bioremediation process.

Long-term dissolution experiments demonstrated that the stability of ASM depends on the nature of the minerals and the medium pH. Orpiment ($As_2S_3$) presented a higher solubility than other common ASM, *i.e.,* realgar ($\alpha$-$As_4S_4$) and arsenopyrite (FeAsS), under aerobic and anoxic conditions at moderately alkaline pH (8.45), with an average of 14.5% of total arsenic solubilized after 50 days. The percentage of arsenic solubilized from realgar and arsenopyrite after the same time period averaged 1.15 % and1.9 %, respectively. The solubility of each ASM under aerobic and anaerobic conditions was very similar.

# 1. INTRODUCTION

# 1. Introduction

## 1.1. Arsenic overview

Arsenic (As) is a metalloid present in the environment which is characterized by its high toxic potential and carcinogenicity. Arsenic is a non-threshold class 1 carcinogen [6]. The maximum As concentration recommended concentration in drinking water by the World Health Organization and the US Environmental Protection Agency is 10 μg/L (WHO, 1993; US-EPA, 2001) Arsenic can be found almost everywhere in the environment and is the 20th most common element in the Earth's [7]. It is released into the environment in volcanic eruptions and it concentrates in the earth's crust, bedrocks and leaches gradually into groundwater and surface water [8, 9]. Arsenic can be found in his native state, or forming a wide variety of compounds, being more than 300 the total number of arsenic compounds identified to date [10].

Exposure to arsenic through consumption of contaminated food, water, air and even occupational exposure can lead to a range of medical complications with serious effects on human health. There is increasing evidence that exposure to low levels of As increases the risk of cancer and non-carcinogen diseases [11]. Also, there is an important medical complication termed as "arsenicosis" which is related with long term exposure to low doses of arsenic. The health consequences include skin problems , skin cancer, liver, kidney and lungs , blood vessel problems in the feet and legs. Other possible symptoms include diabetes , high blood pressure and reproductive problems.[12]

## 1.2. Properties

### *1.2.1. Atomic properties*

Arsenic is a chemical element characterized as a metalloid in the periodic table[7](Figure1.1).

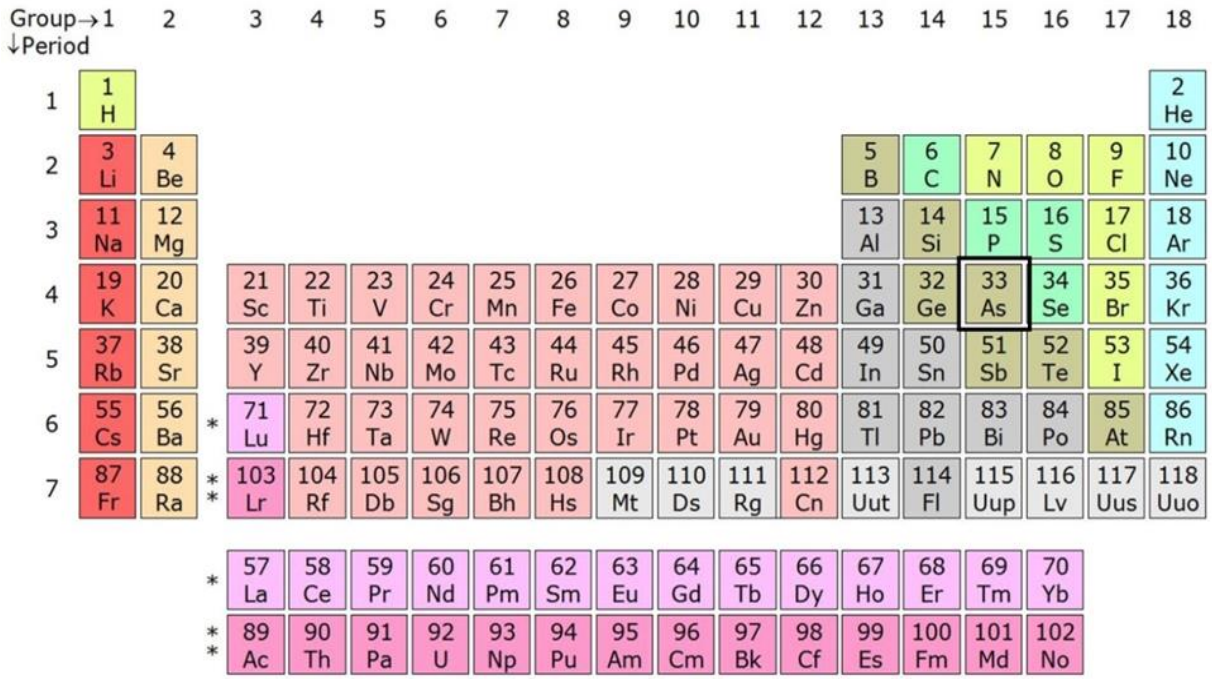


**Figure 1. 1.** Perodic table.

Arsenic has properties of metal and nonmetal, that is the reason why it is characterized as a metalloid. It can be found in group 15 of the periodic table (IUPAC notation). It has a molecular weight of 74.92 g and an atomic number of 33. The electron configuration is $1s^2$ $2s^2p^6$ $3s^2p^6d^{10}$ $4s^2p^3$ with the following electrons per energy level: 2, 8, 18, 5. The number of protons, electrons, and neutrons is 33, 33, and 42, respectively[7].

### *1.2.2. Chemical properties*

*Inorganic arsenic.* Arsenic has 4 oxidation states: (-3), (0), (+3) and (+5). The most common oxidation states are (+3) and (+5) because the most common arsenic compounds naturally formed are arsenite ($AsO_3^{3-}$), also known as As(III), and arsenate ($AsO_4^{3-}$), known as As(V)[7]. As(V) and As(III) have acid/base characteristics[8]. Their dissociation reactions and their corresponding dissociation are listed on Table 1.1. As(V) is the predominant arsenic species in oxidative environments and As(III) in reducing environments.

**Table 1.1.**Arsenic acid-basic chemistry [13]

| Arsenic specie | Dissociation reactions | pKa |
|---|---|---|
| | $H_3AsO_4 \rightarrow H_2AsO_4^- + H^+$ | 2.3 |
| As(V) reactions | $H_2AsO_4^- \rightarrow HAsO_4^{2-} + H^+$ | 6.8 |
| | $HAsO_4^{2-} \rightarrow AsO_4^{3-} + H^+$ | 11.6 |
| | $H_3AsO_3 \rightarrow H_2AsO_3^- + H^+$ | 9.2 |
| As(III) reactions | $H_2AsO_3^- \rightarrow HAsO_3^{2-} + H^+$ | 12.1 |
| | $HAsO_3^{2-} \rightarrow AsO_3^{3-} + H^+$ | 13.4 |

*Organic arsenicals.* Microorganisms can methylate As by aerobic and anaerobic conditions producing organic arsenic compounds. The most common are monomethylarsonous acid ($MMA^{III}$), dimethylarsinous acid ($DMA^{III}$), methylarsonic acid ($MMA^{v}$), trimethylarsine oxide ($TMAO^{v}$) and other more complex compounds like arsenobetaine (AsB) and asernocholine (AsC)[11].

*Volatile arsenicals.* There are also some volatile inorganic compounds of arsenic. One of the most common is arsine gas ($AsH_3$) which is formed with arsenic and hydrogen contained in water or acids. Exposure to arsine in sufficient quantities can be fatal[14]. In figure 1.2. some chemical structure of different As compounds are shown.

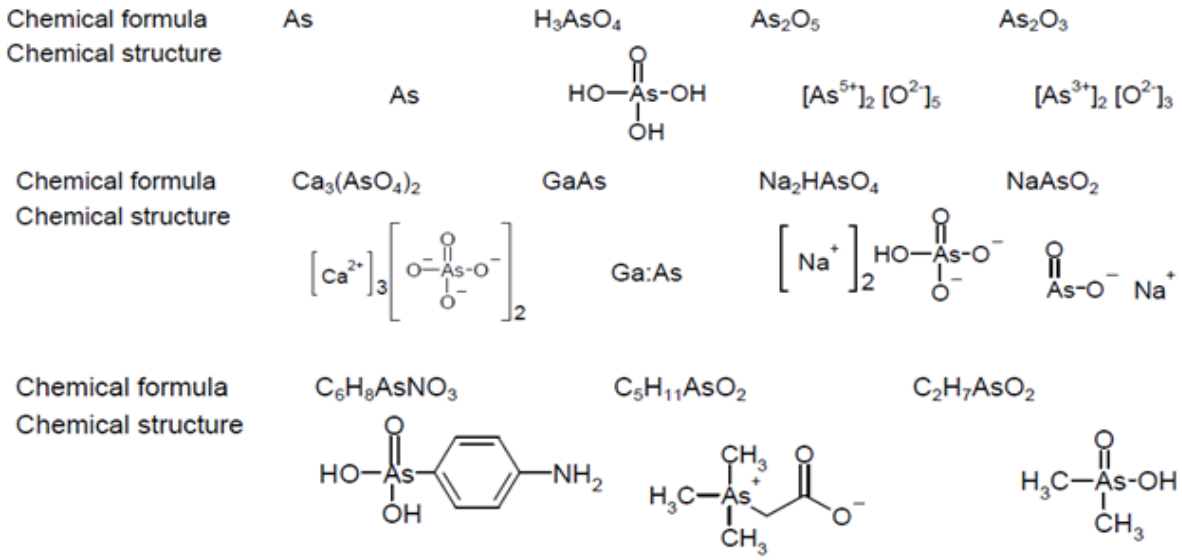


**Figure 1. 2.** Chemical structure of different As compounds.

### 1.3. Arsenic in the environment

#### *1.3.1. Arsenic minerals*

Over 300 compounds may be combined with arsenic, not all are combined in the same way [10]. It's important to know that As(III) and As(V) are species strongly absorbed by Fe(III), [15]. Hence in environments with Fe, As is absorbed onto Fe oxides and oxyhydroxides [16]. On the other hand, in environments where the amount of Fe is not relevant and there is S, the

solubility of As is controlled by the precipitation of As in ASM 'Arsenic Sulfide Minerals'[17]. The most common ASMs are orpiment ($As_2S_3$), realgar (AsS), and arsenopyrite (FeAsS) (Figure 1.3.)[18]. Making a global quantification of all compounds, 60% are arsenates, 20% are S-minerals, 10 % are oxides, and the other 10 % is formed of arsenates, arsenides, elemental As and metal alloys[10]. Most of the common arsenic compounds are showed in table 1.2.

**Table 1.2.** Most common arsenic compounds[10]

| Mineral | Formula | Mineral Group |
|---|---|---|
| Native arsenic | As | Native metalloid |
| Arsenopyrite | $Fe^{3+}As^{-}S^{2-}$ | Sulfide |
| Arsenical pyrite | $Fe^{2+}(S^{-},As^{-})_2$ | Sulfide |
| Loellingite | $Fe^{2+}As^{-}_2$ | Arsenide |
| Niccolite | $Ni^{3+}As^{3-}$ | Arsenide |
| Realgar | $As^{3+,1-}S^{2-}$ | Sulfide |
| Orpiment | $As^{3+}_2S^{2-}_3$ | Sulfide |
| Enargite | $Cu_3AsS^{2-}_4$ | Sulfosalt |
| Claudetite | $As^{3+}_2O_3$ | Oxide |
| Manganarsite | $Mn_3As^{3+}_2O_4(OH)_4$ | Oxyhydroxide |
| Scorodite | $Fe^{3+}As^{5+}O_4.H_2O$ | Fe-arsenate |
| Beudantite | $PbFe^{3+}_3(As^{5+}O_4)(S^{2-}O_4)(OH)_6$ | Fe sulfoarsenate |
| $As^{v}$-sorbed hydrous ferric oxide | $Fe^{3+}(OH)_3$ | Fe oxides/hydroxides |

**Figure 1.3.** From left to right: orpiment, realgar and arsenopyrite

### *1.3.2. Arsenic in water*

The highest As concentrations are found in in groundwater [5]. Arsenic levels in the environment are mainly affected by the weathering of the arsenic-containing natural minerals, while anthropogenic contributions include mining, smelting of nonferrous metals, burning of fossil fuels, arsenical pesticides, the leaching of wood preservatives, and disposal of industrial wastes [7]. Elevated concentrations of arsenic in drinking water resources (> 10 µg/L) is a problem affecting many regions in different countries includingBangladesh, Chile, Mexico, China, Argentina and USA, among others [19, 20]. An interesting place suffering from As contamination is Monolake (Figure 1.4) in California because it has really high As concentrations (3000 µg/L). This means that around 150 million people are still affected by this problem, so it has now turned to be a major environmental concern in different parts of the world (Figure 1.5) [5, 20].

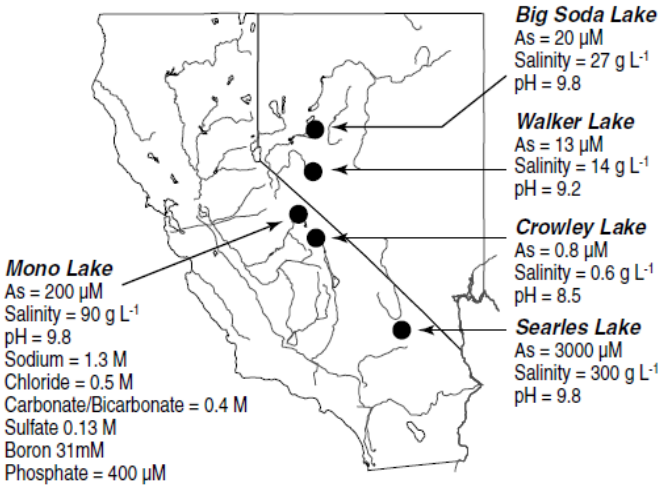


**Figure 1. 4.** Most critical points of As contamination in Monolake (California) [3].

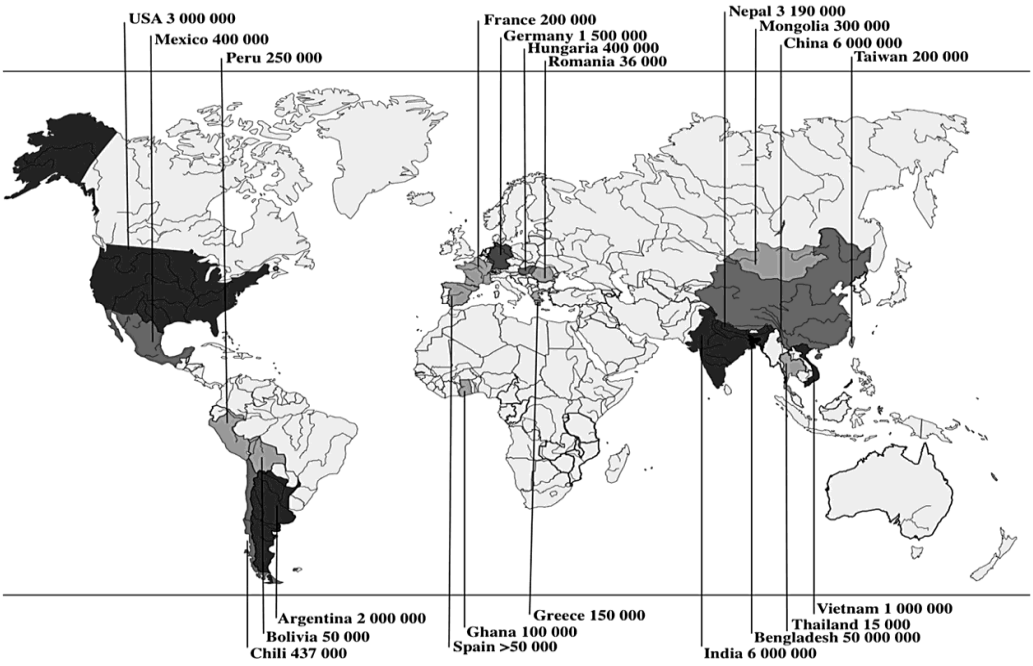


**Figure 1.5.** People affected by drinking water with an As concentration above 10 μg/L [5].

### 1.3. Toxicity

Arsenic is well documented as a highly toxic compound and a potent human carcinogen [6, 7, 9]. But several non-cancer diseases are also associated with chronic exposure to arsenic trough drinking water containing more than 10 μg As/L [18]. Arsenic toxicity in aquatic organisms is also an important point of discussion[21].

#### *1.3.1. Human toxicity: Carcinogenic effects*

As has been considered a human carcinogen since late 17$^{th}$ century, and has shown the ability to increase cancer risk in some parts of the human body. Exposure to low arsenic

concentrations has been associated with increased risk of skin, lung, bladder, prostate and liver cancer[7].(Figure 1.6).

### *1.3.2. Human toxicity: Non-carcinogenic effects*

Although the development of cancer is one of the main problems related to arsenic toxicity, there are many other non-carcinogenic effects that can result from chronic exposure to low arsenic levels. Some of the effects produced in people exposed to high As levels in drinking water are skin problems as hyperkeratosis and pigments changes, diabetes, reproductive disorders, hypertension, cirrhosis or bone marrow depression[2] .(Figure 1.6)

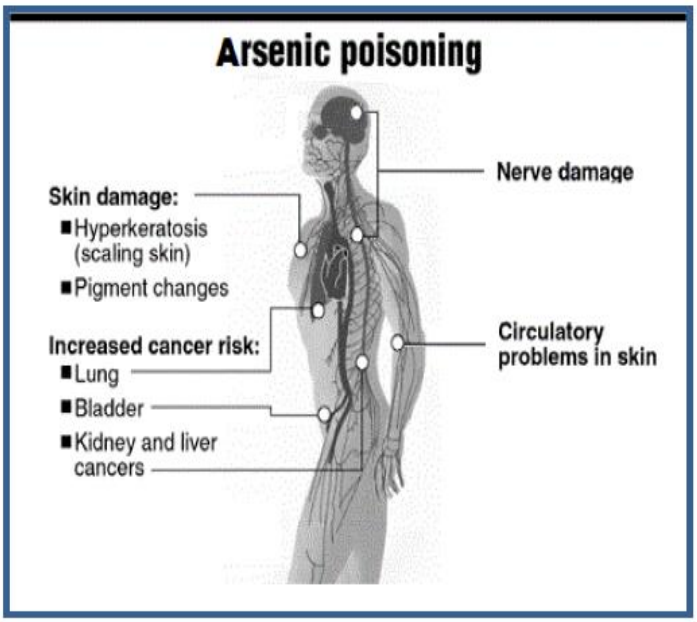


**Figure 1.6.** Arsenic poisoning in human body [2].

### *1.3.3. Arsenic toxicity to aquatic organisms*

Bad effects of arsenic in humans are well known and have been studied trough years, but aquatic organisms are also affected by the toxicity of arsenic in different ways. Some aquatic species (zebra fish embryo) are no affected by the toxicity, reporting that at concentrations lower than 500 μM of As(III) there were no significant zebrafish embryo mortality, and only 12.5-15.6 % of mortality after 24 h at 500 μM of As(III) [22]. However, another aquatic species (Daphnia) reported a $LC_{50}$ (lethal concentration for 50% of population) for As(III) of 33 μM and 50 μM for As(V), showing a higher effect of the As toxicity in them [23]. But above all , some studies report that aquatic microorganisms (non-exposed granular sludge) are the most affected by the toxicity of either As(III) or As(V), with values for $IC_{50}$ (half maximal inhibitory concentration) as low as 19 μM [24]. On the other hand, in response to this toxicity, some microorganisms have been able to develope mechanisms for arsenic resistance and evolve enzymes that oxidize As(III) to As(V) or reduce As(V) to As(III) [25].

## 1.4. Different arsenic treatments

### *1.4.1. Physico-chemical treatments*

Remediation of arsenic contaminated soils and groundwater is necessary for providing safe drinking water. There are lot physico-chemical arsenic removal technologies that can reduce

the final concentration of arsenic. Coagulation, membrane separation, and anion exchange are widely used to remove arsenic from water. Precipitation, adsorption and stabilization/solidification technologies are applied to retain arsenic from contaminated soils [2, 26]. Although these methods are able to eliminate arsenic, they are limited because of their high material costs, generation of sludge, high energy requirements, and problems related to disposal of arsenic-rich residuals[26]. Some new biological treatments are being studied as an alternative to these physico-chemical methods.

### *1.4.2. Biological treatments*

Bioremediation of arsenic contaminated soils and groundwater is a technique that involves the use of organisms to remove arsenic. This technique might be an alternative to physico-chemical treatment because it shows great advantages for future developments due to its environmental compatibility and possible cost-effectiveness[26]. Some of the most promising biological methods rely on microorganisms that are capable to precipitate soluble As(V) as arsenite-sulfide minerals (ASM) [17, 18, 27, 28].

There are also other arsenic bioremediation processes that depend on formation of iron oxides. Arsenic is remediated through coprecipitation with biogenically formed Fe(III) oxyhydroxides/oxides that is produced by the transfer of electrons from Fe(II) to a variety of electron acceptors, including oxygen and nitrate. This process also offers simple and potentially cost-effective solutions [29].

### *1.4.3. Arsenic bioremediation by formation of arsenic sulfide minerals (ASM)*

Some anaerobic microorganisms (*Pyrobaculum aerophilum, Alcaligenes faecalis, Hydrogenophaga Arthrobacter sp.,)* are able to obtain energy using As(V) as electron acceptor for anaerobic respiration using sucrose, molasses, methanol, acetate, ethanol, etc as an electron donor [26, 30]. The reaction is energetically favorable to join the oxidation of organic matter due to the value of the redox potential As(V)/As(III) (135mV). Actually, there are at least 16 species that are affected by this phenomenon. Some of these species are representatives of ε-Proteobacteria, low-GC Gram-positive bacteria, thermophilic Eubacteria, and Crenoarchaea[21]. Also, sulfate-reducing bacteria (SRB) can reduce sulfate ($SO_4^{2-}$) anaerobically to ($S^{2-}$) using $H_2$ or organic compounds as electron donors. Some of these SRB include thermophiles, psycrophiles, halophiles, alkaliphiles and acidophiles [31].Hence, combining the biogeochemical cycle of As and S (Figure 1.7), under reducing conditions and with the presence of an electron donor, ASM can be obtained once As(V) and $SO_4^{2-}$ have been microbially reduced to As(III) and $H_2S$, respectively [18]. The important reactions to consider in the microcosm studies are summarized in figure 1.8.

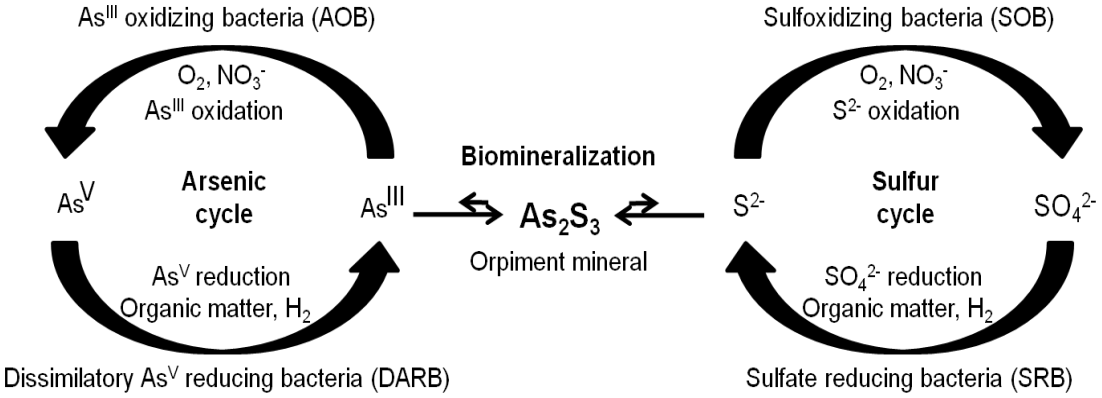


**Figure 1. 7.** Interaction between the As and S cycles to promote the precipitation of ASM [4].

**Ethanol acetogenesis:**

$CH_3CH_2OH + H_2O \rightarrow CH_3COO^- + 2H_2 + H^+$

**Acetoclastic methanogenesis:**

$CH_3COO^- + H^+ \rightarrow CH_4 + CO_2$

**Hydrogenotrophic methanogenesis:**

$4H_2 + CO_2 \rightarrow CH_4 + 2H_2O$

**Sulfate reduction coupled to $H_2$ oxidation:**

$SO4^{2-} + 4H_2 + 2H^+ \rightarrow H_2S + 4H_2O$

**Arsenate reduction coupled to $H_2$ oxidation:**

$H_2AsO_4^- + H_2 + H+ \rightarrow H_3AsO_3 + 2H_2O$

**Mineralization:**

$x\ H_3AsO_3 + yHS^- + (3x - y)\ H^+ \rightarrow As_xS_y(s) + 3xH_2O$

$x = y = 1$, realgar ($\alpha$-AsS) formation

$x = 2; y = 3$, orpiment ($As_2S_3$) formation

**Figure 1. 8.** Table summarize most important reactions in the As and S cycles [1]

The interaction between As and S cycles to promote the precipitation of ASM has been tested successfully in different experiments [1, 18, 32]. Anaerobic batch experiments were performed with As(V) (0.5 mM) and sulfate (0.75 mM) under pH conditions ranging from 6.1 to 7.2. The experiments demonstrated that precipitation of ASM is greatly enhanced at mildly acidic conditions[1]. (At pH values above 7 a decrease in ASM formation was observed that was attributed to the generation of soluble thioarsenite species ($AsO_{3-x}S_x^{3-}$; x= 1-3). Thioarsenite formation by reaction of sulfide and As(III) at pH above 7 was also reported in other recent

studies[33]. Successful arsenic remediation by formation of ASM was also shown in a continuous anaerobic bioreactor fed with $As^V$ (1 mM ) and sulfate (1.5 mM) at slightly acidic pH (6.25-6.50) [18]. However, previous treatment studies were set up using a stoichiometric As/S ratio of 2/3 (required for the formation of realgar, $As_2S_3$) and there is a general lack of information about the feasibility of the process to remove arsenic from aqueous streams under conditions which may be more typical in the environment such as a non-stoichiometric As/S ratios or different pH values. This is particularly important because excess sulfide has the potential to enhance the formation of soluble thioarsenite species even under low pH conditions. Hence, further study is important to better understand the feasibility of arsenic bioremediations processes relying on the interaction between As and S microbial cycles to promote the precipitation of ASM

# 2. OBJECTIVES

## 2. Objetives

- To study the inhibitory impact of As(V) and As(III) on the methanogenic activity of anaerobic granular sludge.

- To investigate the microbial reduction of As(V) by a methanogenic microbial consortium under anaerobic conditions.

- To study the role of the molar sulfate ($SO_4^{2-}$) to As(V) ratio on the effectiveness of the biological formation of arsenic-sulfide minerals (ASM) by microbial reduction of As(V) and sulfate ($SO_4^{2-}$) at pH < 7.

- To evaluate the solubility of different arsenic-sulfide minerals (commercial orpiment, crushed orpiment, arsenopyrite and realgar) under aerobic and anoxic conditions at moderately basic pH (8.45).

# 3. MATERIALS & METHODS

## 3. Materials and methods

### 3.1. Inoculum

An anaerobic granular sludge obtained from a full scale up-flow anaerobic sludge blanket (UASB) reactor at Mahou's (beer brewery in Guadalajara, Spain) wastewater treatment plant, was used as the source of inoculum. This biomass contained 0.0792 g volatile suspended solids (VSS)/g wet wt. The maximum methanogenic activities of the sludge were 565.8±63.8 mg COD-$CH_4$ per gram volatile suspended solids (VSS) per $day^{1}$ and 570.9±25.9 mg COD-$CH_4$/g VSS/day for the assays utilizing acetate and hydrogen as substrate, respectively. The sludge was stored under $N_2$ at 4°C.

### 3.2. Culture media batch bioassays

The composition of the culture media used in the batch experiments was the same, but the concentration of the medium was 1x for methanogenic assays, and 2x for reduction experiments. The concentrations of minerals and trace metals in the medium are provided in Tables 3.1 and 3.2, respectively. The pH of the medium was adjusted to 7.0 before adding the sodium bicarbonate, which acts as a buffer and a source of carbon for growth of autotrophic microorganisms.

**Table 3.1.** Basal medium 1

| | **Methanogenic assays** | **Reduction assays** |
|---|---|---|
| **Compounds** | mg/L | |
| $NH_4Cl$ | 350 | 560 |
| $NaHCO_3$ | 3750 | 6000 |
| $K_2HPO_4$ | 313 | 500 |
| $CaCl_2.2H_2O$ | 12.5 | 20 |
| $MgCl_2.6H_2O$ | 229 | 366 |
| Yeast extract | 125 | 200 |
| Trace elements | 1.3 | 2 |

**Table 3.2.** Basal medium 2

| **Trace element solution** | **mg/L** |
|---|---|
| $H_3BO_3$ | 50 |
| $FeCl_2.4H_2O$ | 2000 |
| $ZnCl_2$ | 50 |
| $MnCl_2.4H_2O$ | 50 |
| $(NH4)_6MO_7O_{24}.4H_2O$ | 50 |
| $AlCl_3.6H_2O$ | 90 |
| $CoCl_2.6H_2O$ | 2000 |
| $NiCl_2.6H_2O$ | 50 |
| $CuCl_2.2H_2O$ | 30 |
| $NaSeO_3.5H_2O$ | 100 |
| EDTA | 1000 |
| Resazurin | 200 |
| 36% HCl | 1 mL |

### 3.3. Bioreactor culture medium

The composition of the culture medium used in the bioreactor study was the same that in batch experiments, but it also included the compound $NaH_2PO_4$. The concentration of minerals in the basal medium is provided in Table 3.3. The concentration of the trace elements solution is provided in Table 3.2.

**Table 3.3.** Basal medium bioreactor

| **Compounds** | **mg/L** |
|---|---|
| $NH_4Cl$ | 560 |
| $NaH_2PO_4$ | 1798 |
| $NaHCO_3$ | 500 |
| $K_2HPO_4$ | 1200 |
| $CaCl_2.2H_2O$ | 20 |
| $MgCl_2.6H_2O$ | 166 |
| Yeast extract | 40 |
| Trace elements | 2 |

### 3.4. Batch bioassays

Static batch experiments were performed in 160 mL serum bottles supplied with 100 mL of basal medium. Each bottle (except solubility experiments) contained basal medium (Tables 3.1 and 3.2),arsenic and inoculum. All the experiments were conducted in duplicate and they included several controls and the different treatments. The specific composition of medium in the

different bottles will be described in each experiment. The headspace of the flasks was flushed with $N_2$:$CO_2$ gas (80:20, v/v) to maintain an anaerobic environment and $H_2$ gas was supplemented as electron donor by injecting $H_2$:$CO_2$ gas (80:20 v/v) to a pressure of 8 psi. All the bottles were incubated in an orbital shaker (100 rpm) at 30 ºC .

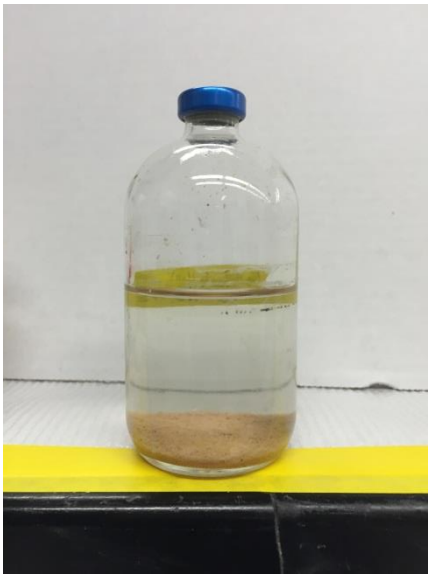

**Figure 3.1.** Bottles utilized in batch experiments.

### *3.4.1 Methanogenic toxicity bioassays with acetate and hydrogen as substrate*

The experiment included 13 bottles: three controls and five treatments. All concentrations of the bottles are summarized on Table X.4. The experiment with Arsenate was also set up with different concentrations: 0.005, 0.0125, 0.0250, 0.05, and 0.2 mM. This experiment was monitored measuring four times per day, during 4 days, the production of methane of each bottle, taking a sample of the gas phase.

**Table 3.4.** Protocol methanogenic toxicity bioassays

| | | DAY 0 | | DAY 1 | | |
|---|---|---|---|---|---|---|
| **Serum flask #** | **As final conc [mM]** | **Basal Medium [mL]** | **Wet sludge [mg]** | **As stock sol [mL]** | **MQ-Water [mL]** | **Total liquid Volume [mL]** |
| 1-control | 0 | 24 | 570 | 0 | 6 | 30 |
| 2-control | 0 | 24 | 570 | 0 | 6 | 30 |
| 3-control | 0 | 24 | 570 | 0 | 6 | 30 |
| 4 | 0.05 | 24 | 570 | 1.5 | 4.5 | 30 |
| 5 | 0.005 | 24 | 570 | 1.5 | 4.5 | 30 |
| 6 | 0.075 | 24 | 570 | 2.2 | 3.8 | 30 |
| 7 | 0.075 | 24 | 570 | 2.2 | 3.8 | 30 |
| 8 | 0.1 | 24 | 570 | 3 | 3 | 30 |
| 9 | 0.1 | 24 | 570 | 3 | 3 | 30 |
| 10 | 0.15 | 24 | 570 | 4.5 | 1.5 | 30 |
| 11 | 0.15 | 24 | 570 | 4.5 | 1.5 | 30 |
| 12 | 0.2 | 24 | 570 | 6 | 0 | 30 |
| 13 | 0.2 | 24 | 570 | 6 | 0 | 30 |

### *3.4.2 Microbial reduction of As(V)*

The experiment included ten bottles: five treatments with his duplicates. All the experiment was prepared in the same day. The composition of the medium in each bottle is provided in Table 3.5.This experiment was monitored taking two liquid samples of 1mL four times per day. Every sample was centrifuged at 13,000 rpm during 10 min. After that, the centrifuged sample were diluted and transferred to different vials to measure the amount of As(V) and total arsenic.

**Table 3.5.** Protocol microbial reduction of As(V)

| Serum flask # | As(V) final conc (mM) | Basal medium (mL) | Wet sludge (mg) | 5 mM As (V) stock solution (mL) | $H_2O$ (mL) |
|---|---|---|---|---|---|
| 1 | 0.25 | 50 | 1901 | 5 | 45 |
| 2 | 0.25 | 50 | 1901 | 5 | 45 |
| 3 | 1 | 50 | 1901 | 20 | 30 |
| 4 | 1 | 50 | 1901 | 20 | 30 |
| 5 | 1.5 | 50 | 1901 | 30 | 20 |
| 6 | 1.5 | 50 | 1901 | 30 | 20 |
| 4 | 2 | 50 | 1901 | 40 | 10 |
| 5 | 2 | 50 | 1901 | 40 | 10 |
| 6 | 2.5 | 50 | 1901 | 50 | 0 |
| 6 | 2.5 | 50 | 1901 | 50 | 0 |

### 3.5 Arsenic bioremediation by formation of arsenic sulfide minerals in a continuous-flow anaerobic

Two reactors of 270 mL, 41 mm of diameter and 360 mm of length were used for this experiment. Both reactors were operated in the same way except the concentration of sulfate, that was different in each reactor. Reactor 1 (R1) received an influent containing the basal medium and an inorganic sulfur (0.4 mM sulfate) and arsenic(V) (130 µM as $Na_2HAsO_4.7H_2O$). Reactor 2 (R2) was fed with an influent containing the same concentration of basal medium and As(V) as R1 and a significantly higher concentration of sulfate (2 mM), R1 and R2 also received an influent containing Ethanol (8.25 mM). Influents were stored in a fridge at 5°C to avoid ethanol

degradation. The influents were mixed before entering the reactors, obtaining a concentration of 0.2 mM of sulfate ,65 µM of arsenic, and 4.12 mM of ethanol in R1. For R2, the concentration of the mix contained the same concentration of As(V) and ethanol as R1 and a higher concentration of sulfate (1mM).The bioreactors were inoculated with 54.66 g of the anaerobic granular biofilm of Mahou., obtaining an equivalent concentration of 10 g sludge-VSS/L

Each reactor consisted of two glass parts (top and bottom) which were clamped together. For each column, the top section had three exits: one to lead the effluent to the container, other, which was plugged with a septum, to allow effluent samples were taken under anoxic conditions with a syringe, and the last to allow the output of gasses. Figure 3.2 & 3.3 illustrate the reactors.

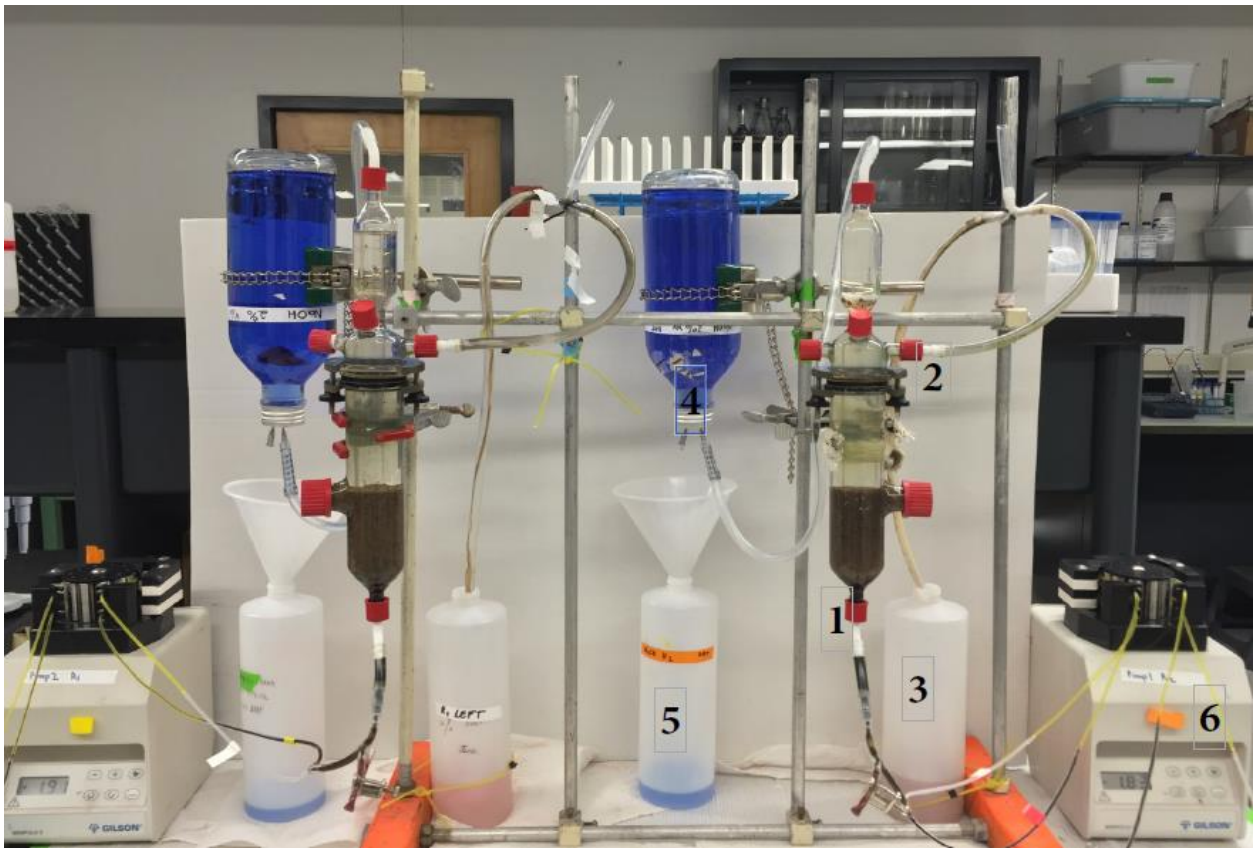


**Figure 3.2.** (**1**) Input Influent; (**2**) Output Effluent; (**3**) Collector Effluent; (**4**) NaOH dissolution to absorbe $CO_2$; (**5**) Collector of the NaOH dissolution displaced by CH4 produced in the bioreactor. (**6**) Pump.

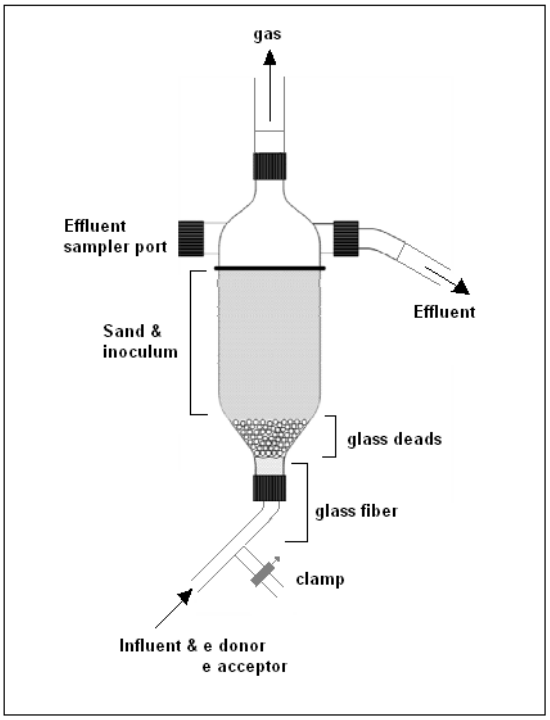


**Figure 3.3.** Representation of the reactors used in the experiment.

The effluent lines from each column were set to create a water lock in order to prevent air contact with the column contents and to prevent a level of liquid too high in the reactors. The bottom of the columns was packed with fiberglass and glass beads and then with the sludge. Glass fibers were tightly fitted into the bottom inlets of each column, and a particle volume of about 3 mL of glass beads was layered above the glass fiber to avoid that the sludge would clog the inlet tubing.

The composition of the mineral medium used is described in Table 3.3. The medium was flushed with $N_2/CO_2$ (80:20, v/v) to ensure anaerobic conditions. The $NaHCO_3$:$CO_2$ system was used to control the reactor pH between 6.4-6.6 in the influent (0.50-0.25 g/L NaHCO3). Basal media and electron donor were fed to each reactor using a peristaltic pump (Gilson Minipuls III, Middleton, WI, France). The influent samples were taken after the medium was driven by the

pump. Both reactors were operated at a hydraulic retention time (HRT) of 24 h at 22°C. In both reactors, three periods of operation can be distinguished:

Stage 1 (Day 0 to 40): This stage was intended to promote the growth of sulfate reducing bacteria in the inoculum using ethanol (8.25 mM) as e-donor and sulfate as electron acceptor (0.1 mM) sulfate for R1, and 1 mM sulfate for R2) at pH 6.4 . The ethanol solution and the sulfate solution were stored in the refrigerator (4 °C) in separate containers to minimize biotransformation during storage

Stage 2 (Day 40 to 74): This stage was intended to make sure that all the data was consistent

Stage 3 (Day 74 to 120): As(V) (130 µM was added to the container with basal medium and sulfate in both reactors, The final concentration of AS(V) entering to the reactors was 65 µM.

Flow rates, pH values, sulfide, sulfate, and ethanol, were measured in the influent and effluent of each reactor six times per week during 2 months before adding As(V). After adding arsenic, flow rates, pH values, sulfide, sulfate,ethanol, As(V) and total arsenic were measured in the influent and effluent of each reactor three times per week. Volumetric flows were calculated by weighing the effluent collected during a known time period. pH and $H_2S$ in influent and effluent samples were measured immediately after sampling. Samples for analysis of total dissolved As ($As_{Tot(aq)}$), As species, $SO_4^{2-}$, ethanol and acetate were centrifuged (10 min, 13,000 rpm) and stored at -20°C until analysis. $CH_4$ gas production was monitored by measuring the

volume of liquid displaced in an inverted bottle containing 2% NaOH (to scrub $CO_2$) connected to the gas effluent.

### *3.5.1 Synthesis of $Na_3As0_3S.7H_2O$*

Na3As03S. 7I-I:0 A 1.44-g amount of sulfur (0.045 mol S) was added to a mixture of 5.00 g of As20 3 (0.050 mol As) and 6.00 g of NaOH (0.150 mol Na) in 20 ml of water and the solution was heated to 100°C. After 2 h, the excess of sulfur was filtered off and the solution was cooled slowly to 4°C. Colorless needle-shaped crystals were obtained (yield of Na3AsO3S. 7H20:0.038 mol, 76%). The rest of the solvent was removed under vacuum.

### *3.5.2 Synthesis of thioarsenate mixture using $S^{2-}$ and As(III) at a $S^{2-}$/As(III) molar ratio of 10:1*

First of all, a stock solution of 50 mM $S^{2-}$ and 30 mM $As^{III}$ were prepared separately using boiled water and adding 93.5 mg NaH2PO4·H2O and 405 mg $K_2HPO_4$ in both stock solutions as a buffer. 30 mL $S^{2-}$ stock sol (50 mM) ,5 mL $As^{III}$ stock sol (30 mM) and 65 mL of boiled water were added in a Pyrex bottle. After that the headspace was flushed with $N_2$. The mixture had to stand on lab bench overnight before anaylisis.

### *3.5.3 Synthesis of thioarsenates mixture using polysulfide and As(III) at a polysulfide/As(III) molar ratio of 10:3*

A stock solution of 5 mM polysulfide was prepared adding 160 mg $S^0$ (500 mM) to 10 mL of $S^{2-}$ stock sol (50 mM) prepared in 3.5.2 section. After that, was mixed for 1 hour, filtered

to a close test tube trough a 0.45 um filter, clamped with an aluminum seal and flushed (only headspace) with pure $N_2$. this solution must be prepared 1.5 hours before the thioarsenate mixture. 0.91 mL of polysulfide stock solution, 5 mL $As^{III}$ stock sol (30 mM) prepared in 3.5.2 section and 94 mL of boiled water were added in a Pyrex bottle. After that the headspace was flushed with $N_2$. The mixture had to stand on lab bench overnight before analysis.

## 3.6. Microbial mobilization of arsenic from commercial orpiment, arsenopyrite, realgar and crushed orpiment with oxygen or nitrate as electron acceptor

### *3.6.1. Mineral preparation*

Orpiment, realgar and arsenopyrite should be powder, so they were ground and stored in different bottles before starting the experiment.

### *3.6.2. Chemical dissolution of orpiment, realgar and arsenopyrite under different redox conditions*

The experiment was conducted in duplicate and it included 20 bottles: four controls without minerals and sixteen treatments. Minerals were prepared in bottles supplemented with $O_2$ or nitrate as electron acceptor. All bottles had an initial concentration of 225 mg/L of total arsenic. The composition of each bottle and basal medium is provided in Tables 3.6, 3.7 and 3.8. The pH was adjusted to 8.45 adding NaHCO3. Bottles with O2 as an electron acceptor were prepared with water and NaHCO3 (Basal medium $O_2$). Bottles with nitrate as an electron acceptor were prepared with water, NaHCO3 and KNO3 (Basal medium $NO_3$). Finally, liquid

and gas phases of nitrate bottles were flushed with pure He to avoid the presence of O2. The headspace of the bottles using $O_2$ as electron acceptor was air.

This experiment was monitored by taking a liquid sample (1mL) every day. Samples were centrifuged at 13,000 rpm during 10 min. The supernatant was diluted as needed to measure the concentration of total arsenic

**Table 3.6.** Basal medium for chemical dissolutions of different ASM.

| **Basal medium ($O_2$)** | | **Basal medium (NO3)** | |
|---|---|---|---|
| NaHCO3 (mg/L) | 2000 | NaHCO3 (mg/L) | 2000 |
| Water | | KNO3 (mg/L) | 3791.37 |
| | | Water | |

**Table 3.7.** Protocol microbial mobilitation of different ASM.

| **O2** | | | |
|---|---|---|---|
| Commercial Orpiment (mg) | 36.91 | Realgar (mg) | 32.09 |
| Basal Medium 1 | 100 mL | Basal Medium 1 | 100 mL |
| | | | |
| Crushed Orpiment (mg) | 36.91 | Arsenopyrite (mg) | 24.42 |
| Basal medium ($O_2$) | 100 mL | Basal medium ($O_2$) | 100 mL |

| **Nitrate ($NO_3^-$)** | | | |
|---|---|---|---|
| Orpiment powder (mg) | 36.91 | Realgar (mg) | 32.09 |
| Basal medium (NO3) | 100 mL | Basal medium (NO3) | 100 mL |
| | | | |
| Crushed orpiment (mg) | 36.91 | Arsenopyrite (mg) | 24.42 |
| Basal medium (NO3 | 100 mL | Basal medium (NO3) | 100 mL |

## 3.7 Analysis

### *3.7.1. Total arsenic determination*

The total concentration of As was measured by using an inductively coupled plasma-optical emission spectrometry (ICP-OES, Optima 5100 DV, PerkinElmer Inc., Shelton, CT, USA)(Figure 3.4). Arsenic was monitored at wavelength 188.7 nm. The samples were diluted with nitric acid (2% v/v) to stay within the limits of the calibration line 0-5 mg/L. The detection limit for arsenic was 10 µg/L.

**Figure 3.4.** ICP-OES, Optima 5100 DV, PerkinElmer Inc., Shelton, CT, USA.

### *3.7.2. Arsenic (V) and sulfate determination*

Arsenate and sulfate were analyzed by suppressed conductivity ion chromatography using a Dionex IC-3000 system (Sunnyvale, CA, USA) (Figure 3.5) fitted with a Dionex IonPac AS11 analytical column (4 mm x 250 mm) and AG11 guard column (4 mm x 50 mm). The

injection eluent (KOH) was 40 mM for 20 min. Some analyses were performed using 20 mM KOH for 15 min, others using 40 mM KOH for 60 min.

**Figure 3.5.** Dionex IC-3000 system (Sunnyvale, CA, USA).

### *3.7.3. Arsenic speciation*

Some effluent samples were prepared for arsenic speciation analysis. Samples were exposed to the air to ensure oxidation of any thioarsenite species to thioarsenates[33](As(III), As(V), methylarsonic acid ($MMA^V$) and dimethylarsonious acid ($DMA^V$)) using a high pressure liquid chromatography system (Agilent 1100 HPLC, Agilent Technologies, Inc.) with a reverse-phase C18 column (Gemini 5µ C18 110A, 150 mm x 4.60 mm, Phenomenex, Torrance, CA) and guard cartridge. The mobile phase (pH 5.85) contained 4.7 mM tetrabutylammonium hydroxide, 2 mM malonic acid, and 4% (v/v) methanol at a flow rate of 1.2 mL/min. The column temperature was maintained at 50$^o$C and samples are kept at 4$^o$C in a thermally controlled autosampler. Following HPLC separation, As species were detected using an ICP-mass spectrometer (ICP-MS) (Agilent 7500ce) with a conical nebulizer (Glass Expansion). The

operating parameters were as follows: RF power 1500 watts, plasma gas flow 15 L/min, carrier flow ~ 0.9 L/min, makeup gas 0.15 L/min, and arsenic monitored at mass 75. The detection limits for As (in μg/L) were: 3 for As(III), 17 for As(V), 11 for DMA(V), 15 for $MMA^V$, and 10 for total As.

### *3.7.4. Ethanol and acetate determination*

Ethanol and acetate, were regularly measured by gas chromatography (GC). The equipment used was a 7890 A GC System and 7683 B Series Injector (Agilent Technologies, CA, USA)(Figure 3.6) fitted with a flame ionization detector (FID). The injected volume was 0.5 μL. Different samples were used to measure ethanol and acetate. The acetate samples were prepared by adding 10 μL of formic acid.

**Figure 3.6.** 7890 A GC System and 7683 B Series Injector.

### *3.7.5. Sulfide determination*

Dissolved sulfide was determined using the methylene blue method [34] and measured using a UV-visible spectrophotometer (Agilent 8453, Palo Alto, CA, USA)(Figure 3.9).The samples of both reactors in the effluent had to be diluted (6.25x for R1 and 50x for R2) because the detection limits for sulfide ranged from 0 to 1 mg/L

### *3.7.6. Methane analysis*

Methane was measured by gas chromatography (GC). The equipment used was a 7890 A GC System and 7683 B Series Injector (Agilent Technologies, CA, USA) fitted with an FID detector.

### *3.7.7. Other analysis*

The pH of liquid samples was measured using a pH electrode (VWR SympHony SB20)(Figure 3.8). Culture samples were analyzed immediately after sampling to prevent pH changes due to loss of dissolver $CO_2$. The VSS content in sludge samples was determined according to standard methods [35].


**Figure 3. 7.** VWR SympHony SB20.

### 3.8 Chemicals

Sodium arsenate dibasic 7-hydrate ($NaHAsSO_4.7H2O$) was purchased from J.T. Baker (Germany). Sodium arsenite ($NaAsO_2$) was purchased from Fisher Scientific (USA). Commercial Orpiment was purchased from Acros Organics (USA). Crushed orpiment was purchased from The Russian Stone (Toronto, Canada), Realgar was purchased from The Russian Stone (Toronto, Canada). Sodium sulfate (Na2SO4) was purchased from Sigma Aldrich (India).

# 4. RESULTS & DISCUSSION

### 4.1. Methanogenic toxicity of As(V) and As(III)

This experiment was performed to study the toxicity of arsenate (As(V)) and arsenite (As(III)) on the methanogenic activity of anaerobic granular sludge. The electron donating substrate in these bioassays was $H_2$. Both experiments were stopped when the control (without arsenic) reached the maximum expected methanogenic production (14%, v/v).

Figures 4.1A and 4.1B show the methane production as a function of time in methanogenic assays exposed to increasing concentrations of As(III) and As(V), respectively. The results obtained revealed a considerable decrease in the methane production with increasing concentrations of As(III), reaching the minimum value of 2±0.23%, v/v, for a As (III) concentration of 15 mg/L (0.2 mM) (Figure 4.1B). Similarly, the methane production also decreased when the sludge was exposed to increasing concentrations of As(V), reaching the minimum value (1.51±0.37%, v/v) at highest concentration tested (15 mg/L or 0.2 mM) (Figure 4.1B).

The concentrations that induced 20%, 50% and 80% inhibition ($IC_{20}$, $IC_{50}$ and $IC_{80}$) relative to the toxicant-free control were calculated in both experiments. Figure 4.2 shows the inhibitory activity of As(III) (Figure 4.2C) and As(V) (Figure 4.2D) towards $H_2$-utilizing microorganisms as a function of the concentration of As added in each case. The concentrations of As(III) and As(V) measured in the medium at the beginning and at the end of the experiments are listed in Figures 4.3E and 4.3F, respectively. On the average, 6.9% and 16.9% of the initially added arsenic was removed from solution at the end of the experiments (day 15) with As(III) experiment and As(V).

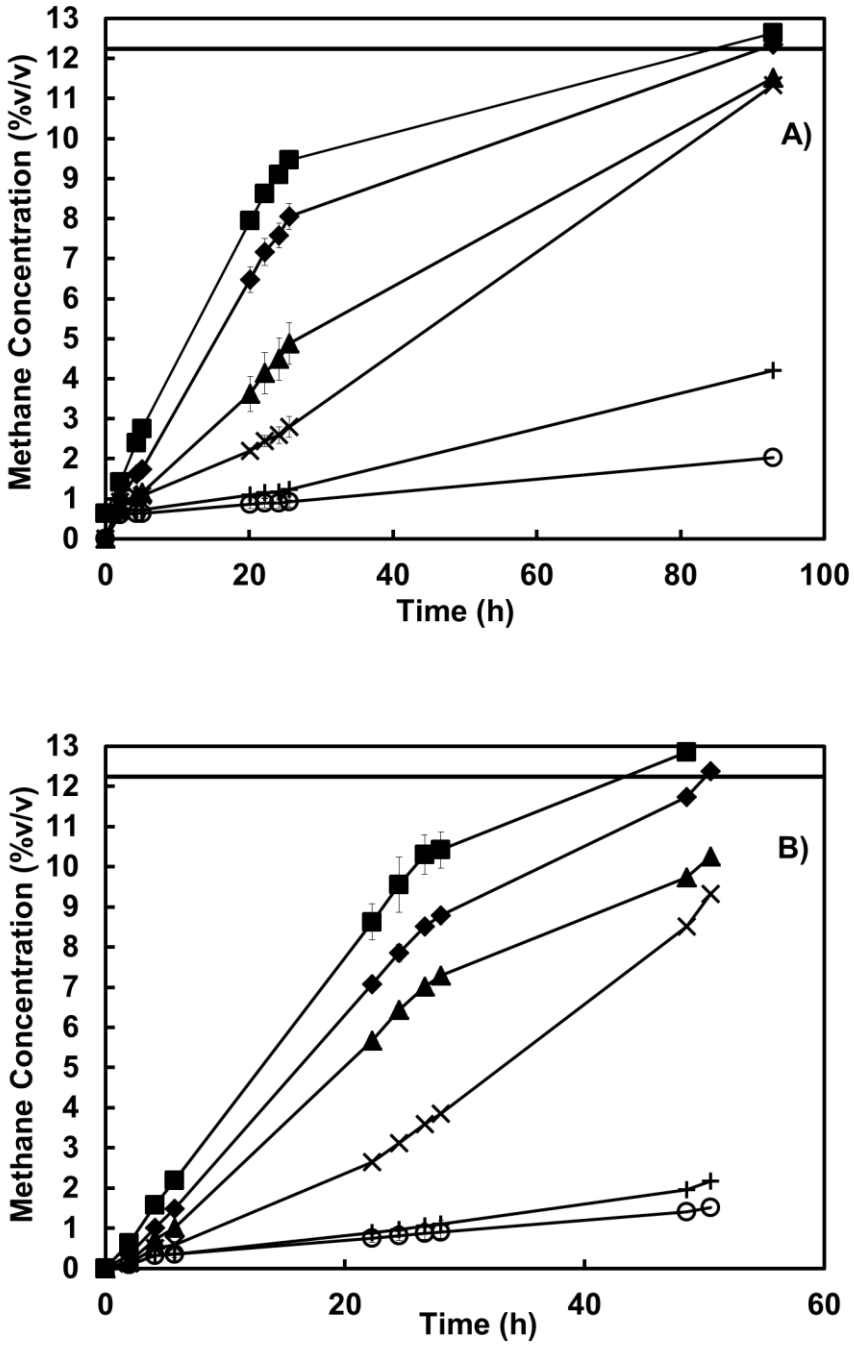


**Figure 4.1.** Methane ($CH_4$) production in the experiments assessing the toxicity of different concentrations (expressed in mg/L) of As(III) (**A**) and As(V) (**B**): 0 (■); 3.75 (♦); 5.62 (▲); 7.5 (x); 11.24 (+); and 15 (o). The horizontal black line represents the maximum theoretical concentration of $CH_4$ that can be produced. The error bars represent the standard deviation of duplicates assays, or triplicates in the case of the control.

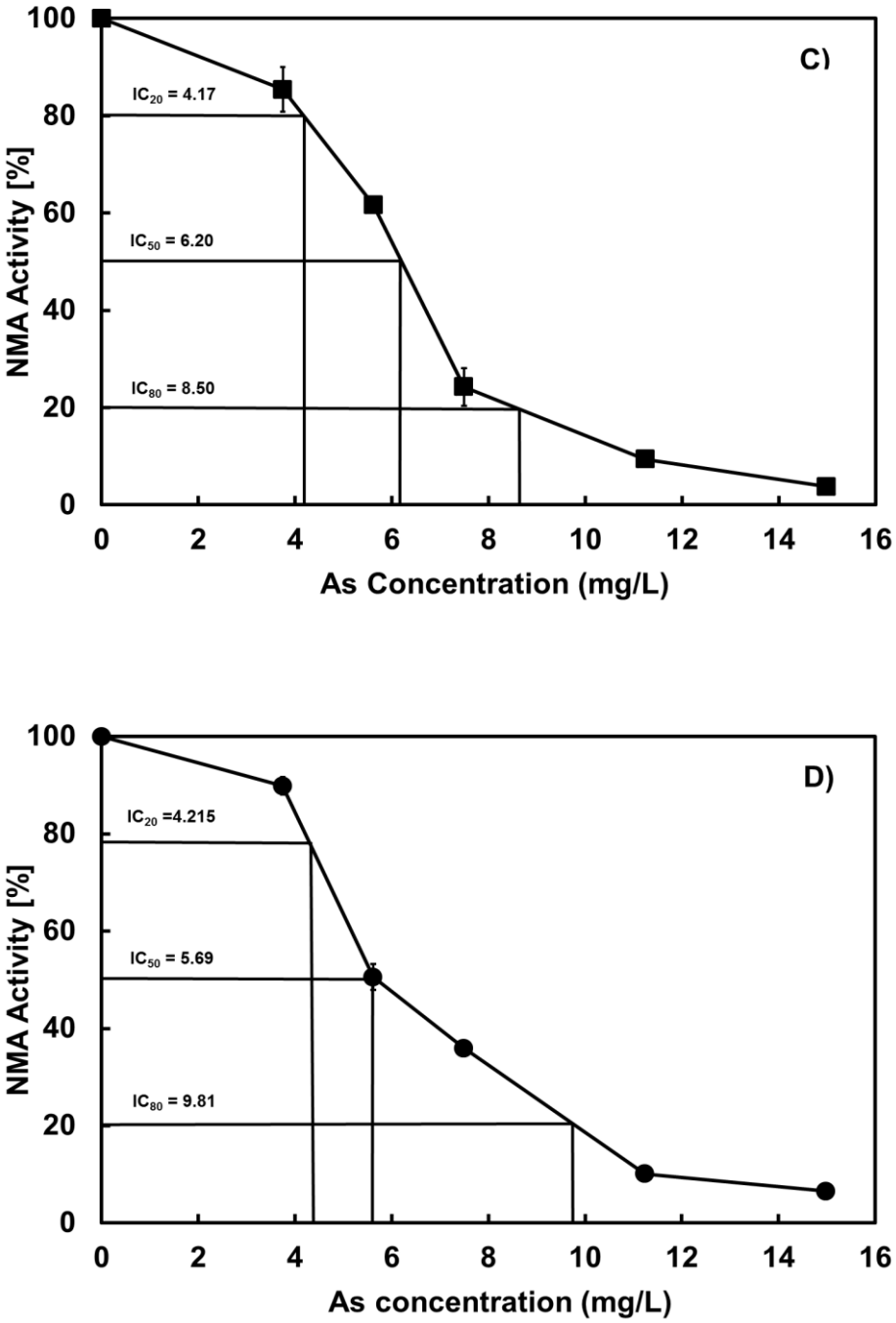


**Figure 4.2.** Normalized maximum methanogenic activity of the granular sludge as a function of the initial concentration of As(III) (**C**) and As(V) (**D**). The 20% ($IC_{20}$), 50% ($IC_{50}$) and 80% ($IC_{80}$) inhibitory concentrations are shown on the figures. The error bars represent the standard deviation of duplicates assays, or triplicates in the case of the control.

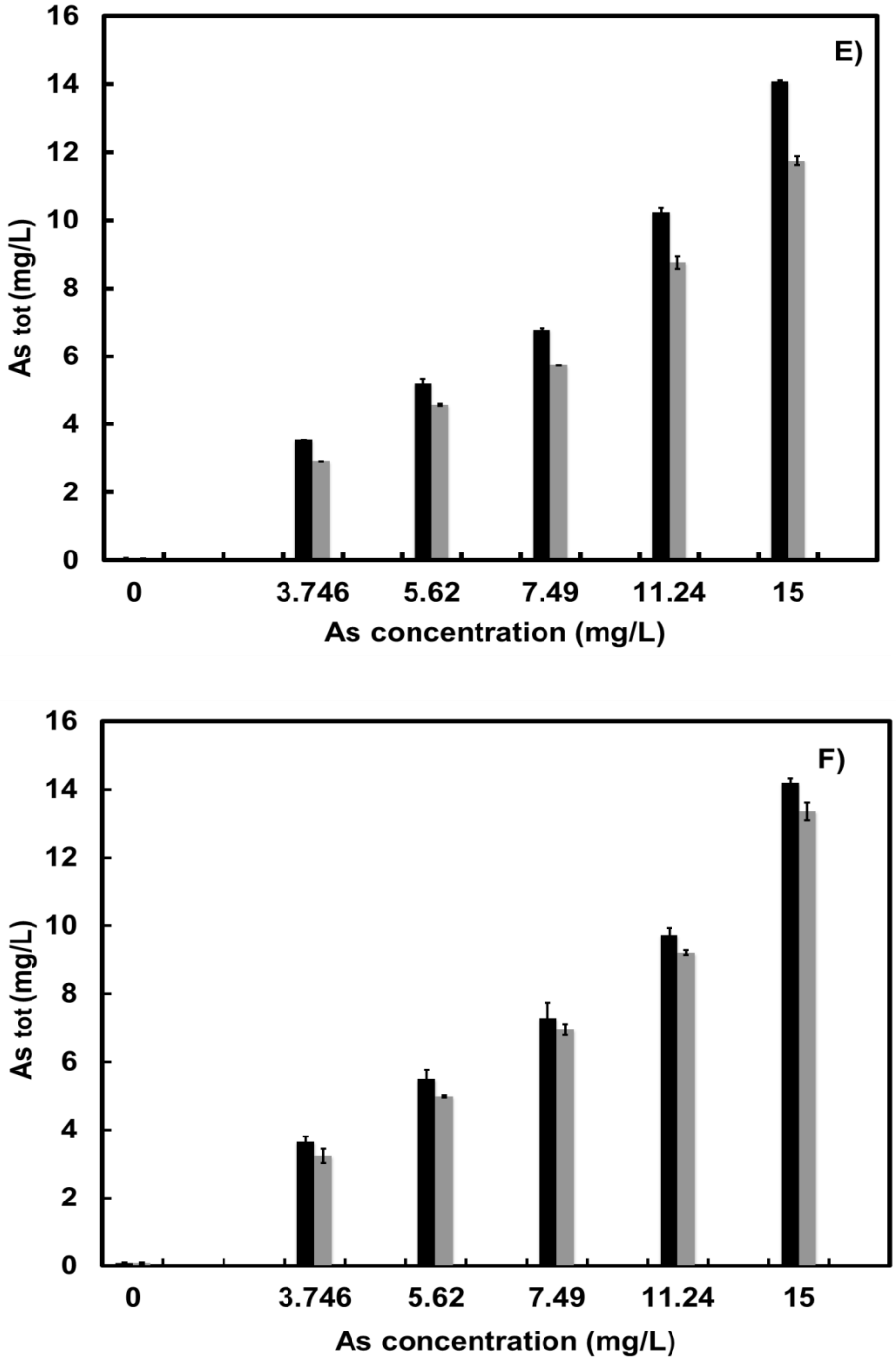


**Figure 4.3.** Total As(III) concentration (**E**) and total As(V) concentration (**F**) at the beginning and at the end of the experiments. Black bars represent the initial concentration. Grey bars represent the final concentration. The error bars represents the standard deviation of duplicates assays, or triplicates in the case of the control.

These experiments demonstrated that As(III) and As(V) are potent inhibitors of the methanogenic activity of anaerobic granular sludge. The toxicity of As(III) can be explained by its high affinity for sulfhydryl groups [24]. The affinity of As(III) with S causes its association with thiol groups on enzymes, impeding its normal function and expression. On the other hand, As(V), an analogue to phosphate, can replace phosphate in the oxidative phosphorylation disrupting ATP synthesis [4].

The $IC_{50}$ values determined for As(III) and As(V) in hydrogenotrophic methanogenic bioassays were 6.20 and 5.69 mg/L, respectively, showing that both compounds are almost equally toxic for granular anaerobic sludge. The microbial toxicity of different As species has also been proved in previous experiments [24, 36]. However, in a previous study with anaerobic sludge and using different electron donors (acetate and $H_2$), As(III) was reported in both cases to display a higher methanogenic inhibition than As(V), that did not cause a notorious methanogenic inhibition [36]. On the other hand, these previous experiments were performed with different granular anaerobic sludges.

The observed decrease in the dissolved concentration of arsenic present in the medium with incubation time might be related to the formation of insoluble arsenical compounds during the process. Some previous batch studies have proved that different arsenic sulfide minerals (ASM) can be formed under anaerobic conditions in presence of a pure culture [1]. However, this experiment utilized a sulfate-free medium. Hence the only possible source of sulfide would be endogenous decomposition of the granular sludge and reduction of sulfate released by sulfate-reducing formation leading to the formation of biogenic sulfide.

### 4.2. Microbial reduction of As (V)

The objective of this experiment was to study the microbial reduction of As(III) to As(V) by a mixed anaerobic consortium using $H_2$ as electron donor. Another goal of this experiment was to study the effect of the initial As(V) concentration on the rate of As(V) reduction. A stabilized methanogenic granular sludge (Mahou sludge) was used to perform this experiment.

Figure 4.4 illustrates the time course of As(V) reduction to As(III) for different initial concentrations of As(V) ranging from 18.7 to 187.3 mg/L. Complete reduction of As(V) was determined after 22.5 h in the bioassay spiked with 18.7 mg As(V)/L. Similarly, As(V) was completely reduced after 22.5 h in the bioassays with 74.9 and 112.4 mg/L As(V), after 43 h in the bioassays with initial As(V) concentrations of 149.8 mg/L and after 60 h in the bioassay with187.3 mg/L As(V). Figure 4.5 illustrates the effect of the initial As(V) concentration on the rate of As(V) conversion. The maximum reduction rates determined in the various assays are shown in Table 4.1. These results show that the rate of As(V) reduction increased linearly with initial increasing As(V) concentration, which indicates that the reaction followed first-order kinetics. The highest reduction rate (4.49 mg As/g VSS-h) was determined in the assay spiked with 187.3 mg As(V)/L.

The linear fitting of the data is represented in Figure 4.5. The equation obtained is:

$$y = 0.024x$$

where: “y” =Rate As conversion (mg As/g VSS-h) and “x” = As concentration (mg/L)

Hence, the rate of As conversion can be associated to a first-order rate with a reaction coefficient of 0.024 L/gVSS-h * 1.5 gVSS/L= 0.036 $h^{-1}$ with a $R^2$ coefficient of 0.96.

**Table 4.1.** Rate As(V) conversion with $H_2$ (8 psi) as electron donor.

| As(V) concentration | | As(V) reduction rate |
|---|---|---|
| **mM** | **mg/L** | **(mg As/g VSS-hour)** |
| 0.25 | 18.7 | 1.02 ± 0.70 |
| 1.0 | 74.9 | 1.81 ± 0.04 |
| 1.5 | 112.4 | 2.72 ± 0.15 |
| 2.0 | 149.8 | 3.52 ± 0.23 |
| 2.5 | 187.3 | 4.49 ± 0.01 |

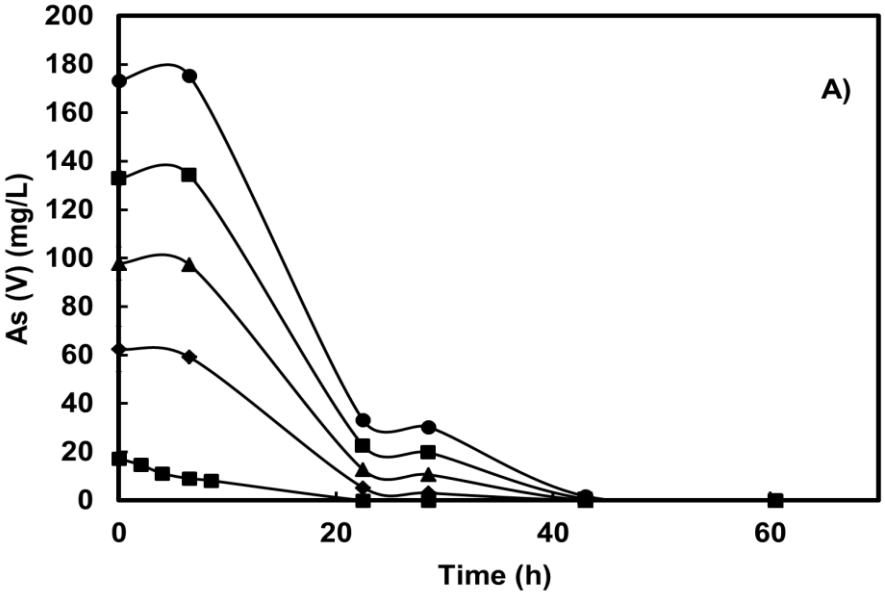


**Figure 4.4.** As(V) reduction to As(III) in Mahou anaerobic granular sludge using different initial As(V) concentrations (expressed in mg/L): 18.7 (■); 74.9 (♦); 112.4 (▲); 149.8 (■); and 187.3 (●). The error bars represent the standard deviation of duplicates assays.

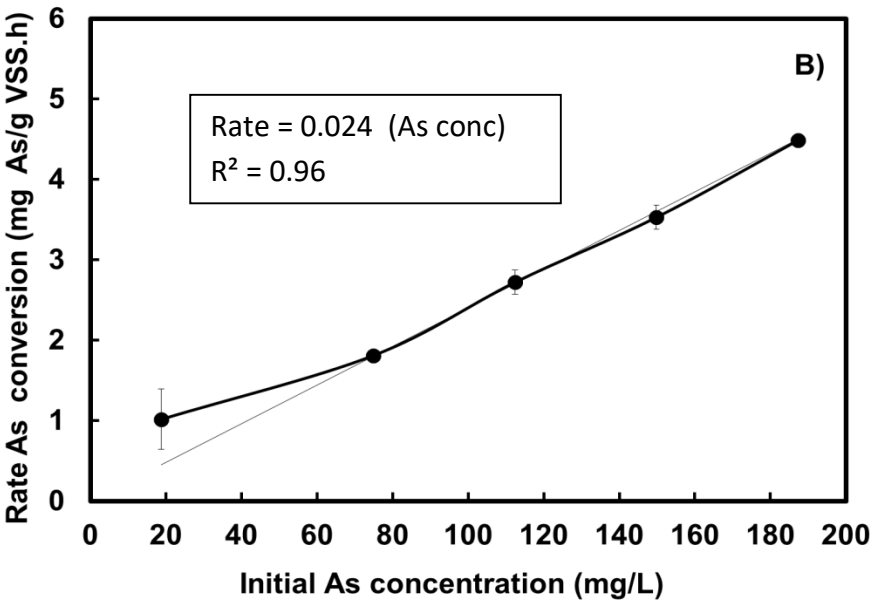


**Figure 4.5.** The rate of As(V) reduction to As(III) in Mahou anaerobic granular sludge incubated with at variable initial concentrations of As(V) with $H_2$ (8 psi) as electron donor. The error bars represent the standard deviation of duplicate assays. Linear fitting is represented in the graphic.

The present study demonstrates that As(V) is readily reduced to As(III) using methanogenic granular sludge. The linear increase in the reduction rate with increasing As(V) concentrations up to 187.3 mg/L (2.5 mM) indicates that As(V) and As(III) are not inhibitory to the microorganisms involved in the reduction. Conversion of As(V) to As(III) in anaerobic microbial communities has been already proved in previous experiments using different methanogenic granular sludge obtained from an industrial anaerobic treatment plants treating distillery wastewaters (2.5 g VSS/L, Nedalco sludge). As(V)-reducing microorganisms from Nedalco sludge have been shown to utilize different electron donors such as $H_2$, glucose, ethanol,

among others in bioassays performed at 30°C. In experiments with an initial As(V) concentration of 0.5 mM, $H_2$ was the most effective electron donating substrate with a reduction rate of 0.178 mg As/g VSS-h [37]. In another study, the maximum As(V) reduction rate determined in bioassays performed with a methanogenic granular sludge obtained from an anaerobic treatment plant treating recycle paper wastewater (3.2 g VSS/L, Eerbeek sludge) and using lactate (10 mM) as electron donor and arsenic concentrations similar to those used in the present study, was 1.49 mg As/g VSS-h [37-39]. Inhibition of As(V) reduction in Eerbeek sludge using lactate(10 mM) as electron donor was only particularly evident after 100 h of incubation (30°C) at high As concentrations (10 mM) [37].

### 4.3. Arsenic bioremediation by formation of arsenic sulfide minerals in a continuous-flow anaerobic

The microbial formation of arsenic sulfide minerals (ASM) for the bioremediation of As-groundwater has already been successfully demonstrated using simultaneous As(V) and $SO_4^{2-}$ reduction in bioreactors, but only under strict conditions (pH < 7 and stoichiometric S/As required for $As_2S_3$ precipitation) [18]. The main objective of this study was to demonstrate that the precipitation of ASM decreases when the amount of sulfate is much higher than the stoichiometric amount required for the formation of ASM (S/As ratio for $As_2S_3$ = 1.5). For this purpose, two anaerobic bioreactors were operated in parallel at an average hydraulic retention time (HRT) of 24.5 h with an influent containing ethanol and 65 µM As(V) and adjusted to moderately acid pH values (6.4-6.6). The concentration of sulfate ($SO_4^{2-}$) in the influent of the two reactors was different; whereas reactor 1 (R1) received 0.2 mM ($SO_4^{2-}$), corresponding to a

molar S/As ratio of 3, reactor 2 (R2) received 1 mM $SO_4^{2-}$, corresponding to a molar S/As ratio of 15.

Three periods of operation can be distinguished in the operation of the bioreactors:

Stage I (Day 0 to 40): This stage was intended to promote the growth of sulfate-reducing bacteria in the inoculum using ethanol (8.25 mM) as e-donor and sulfate as electron acceptor for R1 (0.1 mM sulfate), and R2 (1 mM sulfate) at pH 6.4.

Stage II (Day 40 to 74): This stage was intended to establish the performance of the bioreactors under pseudo steady-state conditions.

Stage III (Day 74 to 120): The influent of both reactors was supplemented with As(V) (65 µM).

### *4.3.1. Hydraulic retention time*

The hydraulic retention time (HRT) for both reactors is represented in Figure 4.6. The HRT was established in 24 h. The HRT for reactor 1 and reactor 2 (Figures 4.6A and 4.6B, respectively) was fairly constant during the three stages of operation. The average HRT during stage II and III was 25.4 ± 0.80 and 24.88 ± 1.1 h, respectively, for reactor 1, and 24.9 ± 0.73 and 24.09 ± 0.98 for reactor 2.

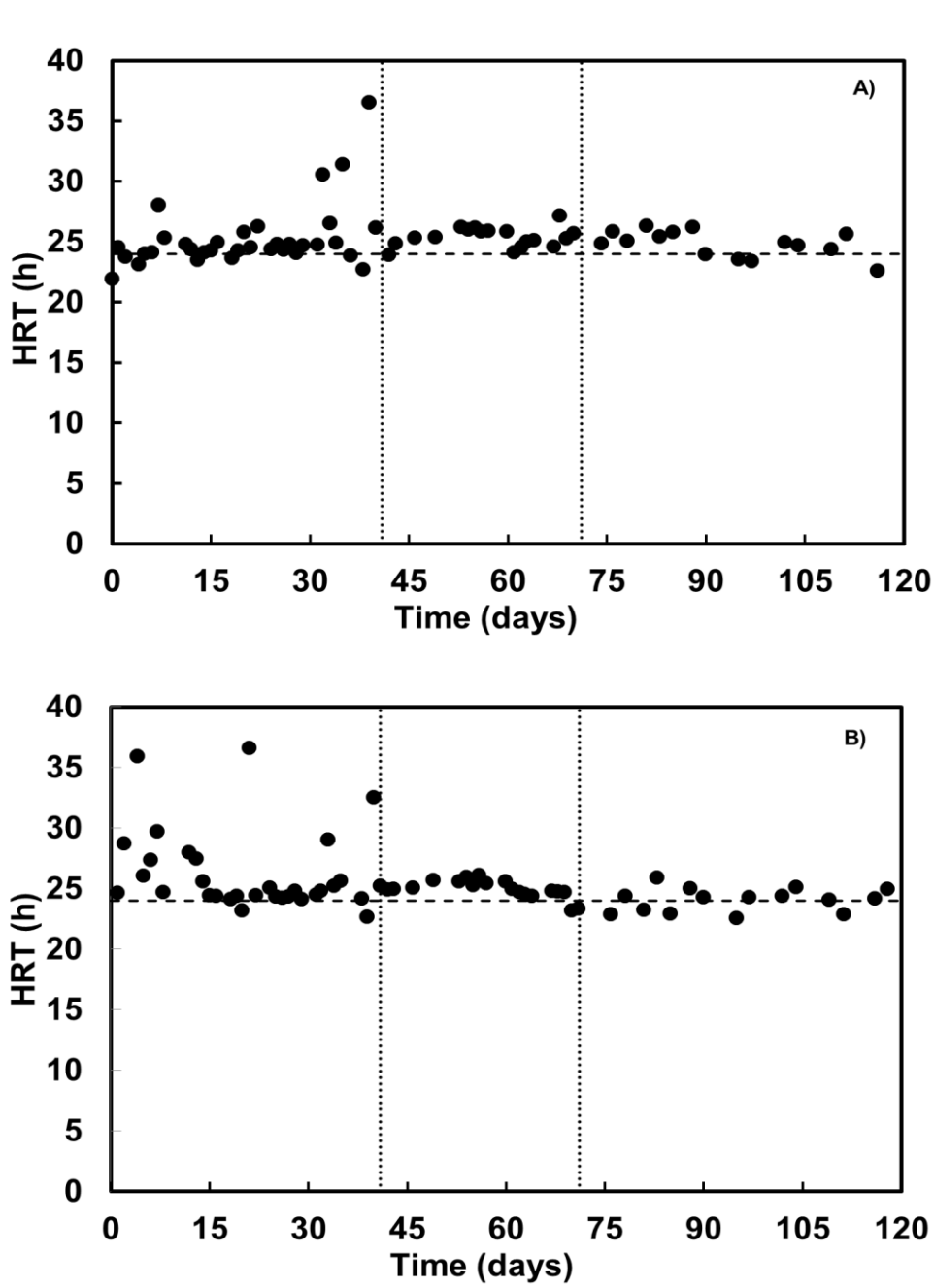


**Figure 4.6.** Hydraulic retention time (HRT) in reactor 1 (A) and reactor 2 (B). The horizontal black line represents the set point of the HRT (24 h). The vertical dashed lines indicate the separation between the three stages in the reactor.

### *4.3.2. pH*

The pH in the influent and in the effluent for both reactors is represented in Figure 4.7. The average pH values determined in the influent and effluent of R1 and R2 for each stage are represented in Tables 4.2 and 4.3, respectively.

**Table 4.2.** pH in the influent and effluent of reactor 1.

| | | pH | |
|---|---|---|---|
| **Stage** | **Period (days)** | **R1 INF** | **RI EFF** |
| II | 40-74 | 6.50 ± 0.07 | 6.58 ± 0.04 |
| III | 74-120 | 6.54 ± 0.07 | 6.62 ± 0.06 |

**Table 4.3.** pH in the influent and effluent of reactor 2.

| | | pH | |
|---|---|---|---|
| **Stage** | **Period (days)** | **R2 INF** | **R2 EFF** |
| II | 40-74 | 6.54 ± 0.07 | 6.74 ± 0.04 |
| III | 74-120 | 6.56 ± 0.13 | 6.74 ± 0.07 |

The average pH in the influent and in the effluent of R1 during the stage III was 6.54 and 6.62, respectively. The average pH in the influent and in the effluent of reactor 2 during the stage III was 6.56 and 6.74, respectively. Adequate pH control during stage III was critical because some previous studies demonstrated that the precipitation of ASM is greatly enhanced at pH < 7 [40]. Hence, the experiment worked under the pH expected. The small variations of the pH observed in Figure 4.7 were controlled changing the amount of $NaHCO_3$ added to each basal medium (0.5-0.3 g/L in R1, and 0.25-0.1 g/L in R2).

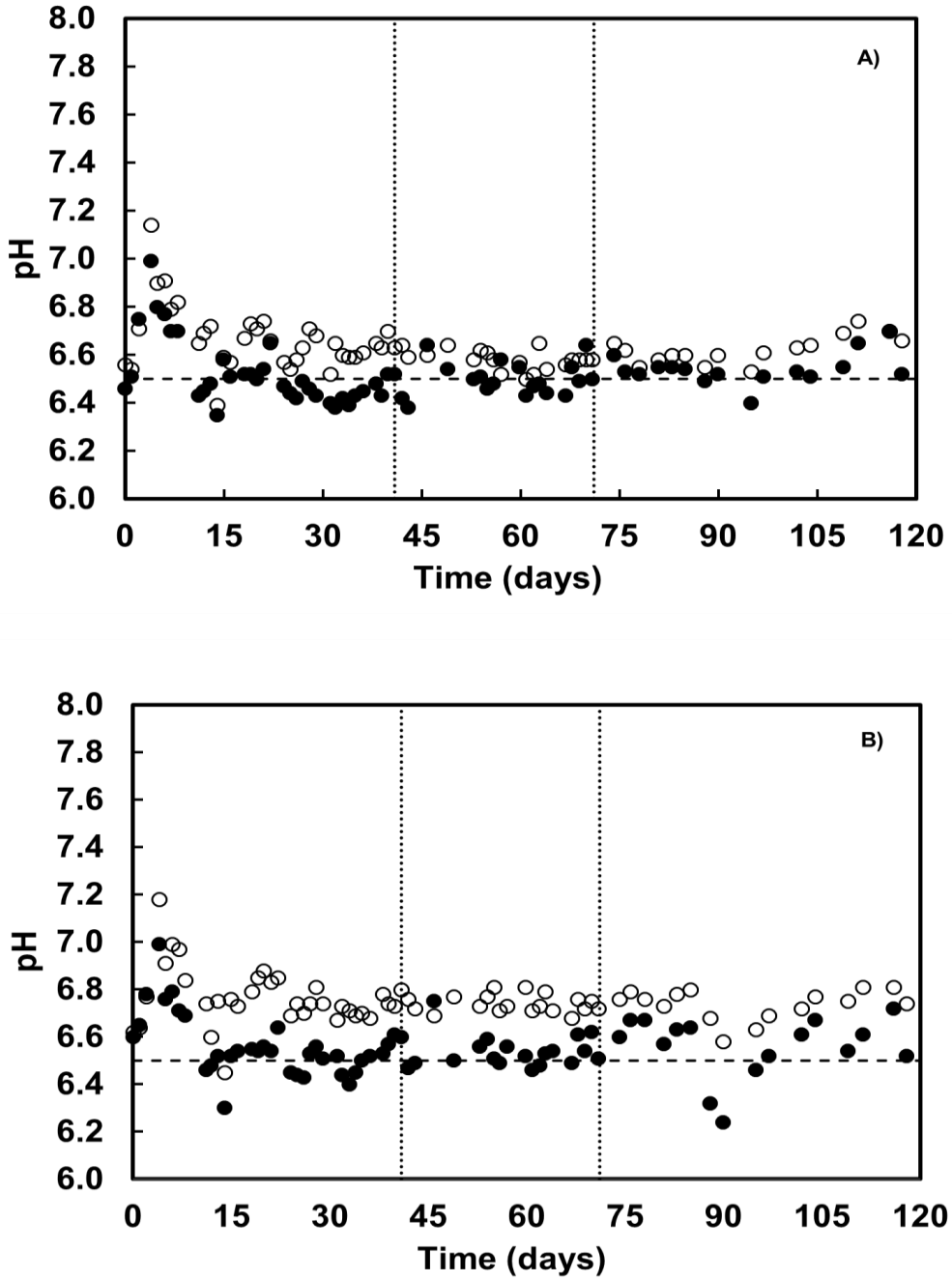


**Figure 4.7.** pH in the influent (●) and in the effluent (o) of reactor 1 (panel A) and reactor 2 (panel B). The horizontal black line represents the set point of the pH in the influent (6.5). The vertical dashed lines indicate the separation between the three stages in the reactor.

pH was the most critical point in the reactor. Most of studies using simultaneous As(V) and $SO_4^{2-}$ reduction achieved a high As removal rate only at pH values lower than 7 [1, 18, 32]. However, studies where the effluent pH exceeded 7 reported As removal efficiencies as low as 8% [1, 41, 42]. Hence, it is clear that pH has to be controlled every day to obtain the expected results.

### *4.3.3. Ethanol & acetate*

The ethanol and acetate concentrations in the influent and in the effluent of both reactors are represented in Figure 4.8. The average values measured in reactor 1 and 2 for each stage are shown on Tables 4.4 and 4.5, respectively.

**Table 4.4.** Ethanol and acetate concentrations (mM) in reactor 1.

| | | **Ethanol** | | **Acetate** | |
|---|---|---|---|---|---|
| **Stage** | **Period (days)** | **R1 INF** | **RI EFF** | **R1 INF** | **RI EFF** |
| II | 40-74 | 3.96 ± 0.69 | 0.01 ± 0.02 | 0 | 0 |
| III | 74-120 | 3.76 ± 0.18 | 0.03 ± 0.04 | 0 | 0 |

**Table 4.5.** Ethanol and acetate concentrations (mM) in reactor 2.

| | | **Ethanol** | | **Acetate** | |
|---|---|---|---|---|---|
| **Stage** | **Period (days)** | **R2 INF** | **R2 EFF** | **R2 INF** | **R2 EFF** |
| II | 40-74 | 3.80 ± 0.1.21 | 0.04 ± 0.04 | 0 | 0.04 ± 0.18 |
| III | 74-120 | 3.52 ± 1.10 | 0.03 ± 0.04 | 0 | 0.16 ± 0.20 |

During the first period of operation, the amount of ethanol in the influent of both reactors was 1.3x of the stoichiometric amount necessary to reduce all the sulfate to sulfide (2.68 mM).

However, in both reactors the amount of ethanol measured was lower than expected (Figure 4.8). This problem could be attributed to a possible degradation of ethanol during sample storage. They were diluted 2x. For that reason, after 15 days, the amount of ethanol entering to the reactors was changed to 2x of the stoichiometric (4.12 mM), equivalent to 396.1 mg COD/L, to make sure that there was enough ethanol to reduce the desired amount of sulfate to sulfide. Figure 4.8 shows a small increase in the data measured after day 15, but most of the data were also under the set point. At the beginning of stage two, samples of ethanol were prepared without any dilution and the data started to be close to the expected concentration until the end of the experiment (Tables 4.4 & 4.5). Figure 4.8 shows that all the ethanol was completely degraded in R1 and R2 since day 1 of operation. This means that the 396.1 mg COD/L entering each of the reactors was consumed.

There was no accumulation of acetate during any stage in reactor 1, indicating that there was no or only limited methanogenic inhibition after the addition of As(V) (Figure 4.8 A). There was a small accumulation of acetate at the beginning of stage III in reactor 2 (0.16 ± 20 mM), but after 15 days, acetate was effectively transformed to $CH_4$ (Figure 4.8B). In both cases, the concentration of As(V) added was very small compared with the amount of wet sludge added to each reactor (13.3 g VSS/L), so this could explain why there is no evidence of acetoclastic methanogenic toxicity.

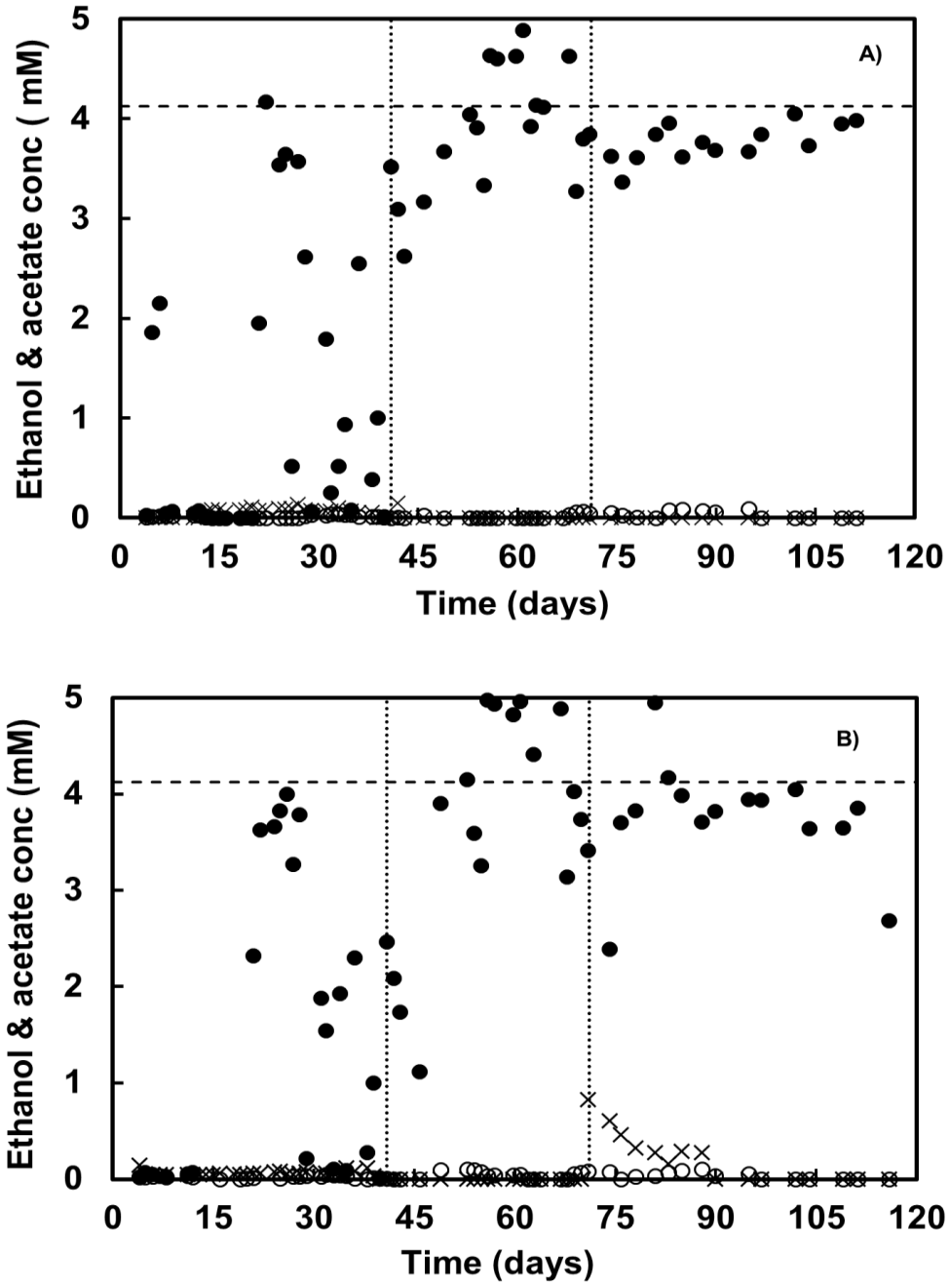


**Figure 4.8.** Transformation of ethanol and production of acetate in the reactors. (A) ethanol and acetate concentration in reactor 1; (B) ethanol and acetate concentration in reactor 2. (●) ethanol in the influent; (o) ethanol in the effluent; (x) acetate in the effluent. The horizontal black line represents the set point of the ethanol in the influent. The vertical dashed lines indicate the separation between the three stages in the reactor.

### *4.3.4. Methane production*

Methane production was only measured after day 80. The average concentrations of ethanol-COD in the influent of reactor 1 and 2 were 360.4 ± 91.7 mg COD/L and 360.4 ± 56.6 mg COD/L, respectively. This COD was used to reduce As(V) and $SO_4^{2-}$, and to produce methane. The theoretical percentages of COD used in each case were calculated before the measurement to make sure that it could be possible to measure methane (Table 4.6). The percentage of COD measured for the production of each reactor is represented in Table 4.7.

**Table 4.6.**Theoretical % COD used for the reduction of As(V), $SO_4^{2-}$ and formation of $CH_4$

| | **Theoretical % COD used** | |
|---|---|---|
| | **R1** | **R2** |
| As(V) reduction | 0.26 | 0.26 |
| $SO_4^{2-}$reduction | 3.23 | 16.16 |
| Methanogenesis /cell yield | 96.51 | 83.58 |

**Table 4.7.** Percentage of COD used for the formation of $CH_4$ in each reactor

| | **R1** | **R2** |
|---|---|---|
| % COD used for methanogenesis | 36.73 | 42.09 |

The results showed that 36.7% and 42.1% of the total COD consumed in R1 and R2, respectively, was converted to methane. The values are too small compared with the theoretical % COD used for the production of methane, but the system to measure methane (liquid

displacement) was not really appropriated to quantify the small production of methane that was expected. However, these results are enough to confirm that the production of methane was happening in both reactors and also that the toxicity of As(V) did not have an important role in the methanogenic production of the granular sludge.

### *4.3.5. Sulfate*

The $SO_4^{2-}$ reducing activity of the sludge was evaluated during the time course of both reactors. Figure 4.9 shows the reduction of $SO_4^{2-}$ and the total S in the effluent ($H_2S_{(aq)} + SO_4^{2-}$). The average values measured for reactor 1 and 2 in each stage are represented in Tables 4.8 and 4.9, respectively.

**Table 4.8 .** $SO_4^{2-}$ and $H_2S_{(aq)}$ concentrations (mM) in reactor 1.

| | | **$SO_4^{-2}$** | | **$H_2S_{(aq)}$** | **$H_2S_{(aq)} + SO_4^{-2}{}_{effluent}$** |
|---|---|---|---|---|---|
| **Stage** | **Period (days)** | **R1 INF** | **R1 EFF** | **R1 EFF** | **R1 EFF** |
| II | 40-74 | 0.20 ± 0.02 | 0.09 ± 0.02 | 0.10 ± 0.01 | 0.18 ± 0.03 |
| III | 74-120 | 0.19 ± 0.01 | 0.02 ± 0.02 | 0.09 ± 0.01 | 0.11 ± 0.03 |

**Table 4.9.** $SO_4^{2-}$ and $H_2S_{(aq)}$ concentrations (mM) in reactor 2.

| | | **$SO_4^{-2}$** | | **$H_2S_{(aq)}$** | **$H_2S_{(aq)} + SO_4^{-2}{}_{effluent}$** |
|---|---|---|---|---|---|
| **Stage** | **Period (days)** | **R2 INF** | **R2 EFF** | **R2 EFF** | **R2 EFF** |
| II | 40-74 | 0.98 ± 0.09 | 0.40± 0.03 | 0.66 ± 0.05 | 1.06 ± 0.05 |
| III | 74-120 | 0.99 ± 0.08 | 0.39 ± 0.02 | 0.79 ± 0.13 | 1.17 ± 0.13 |

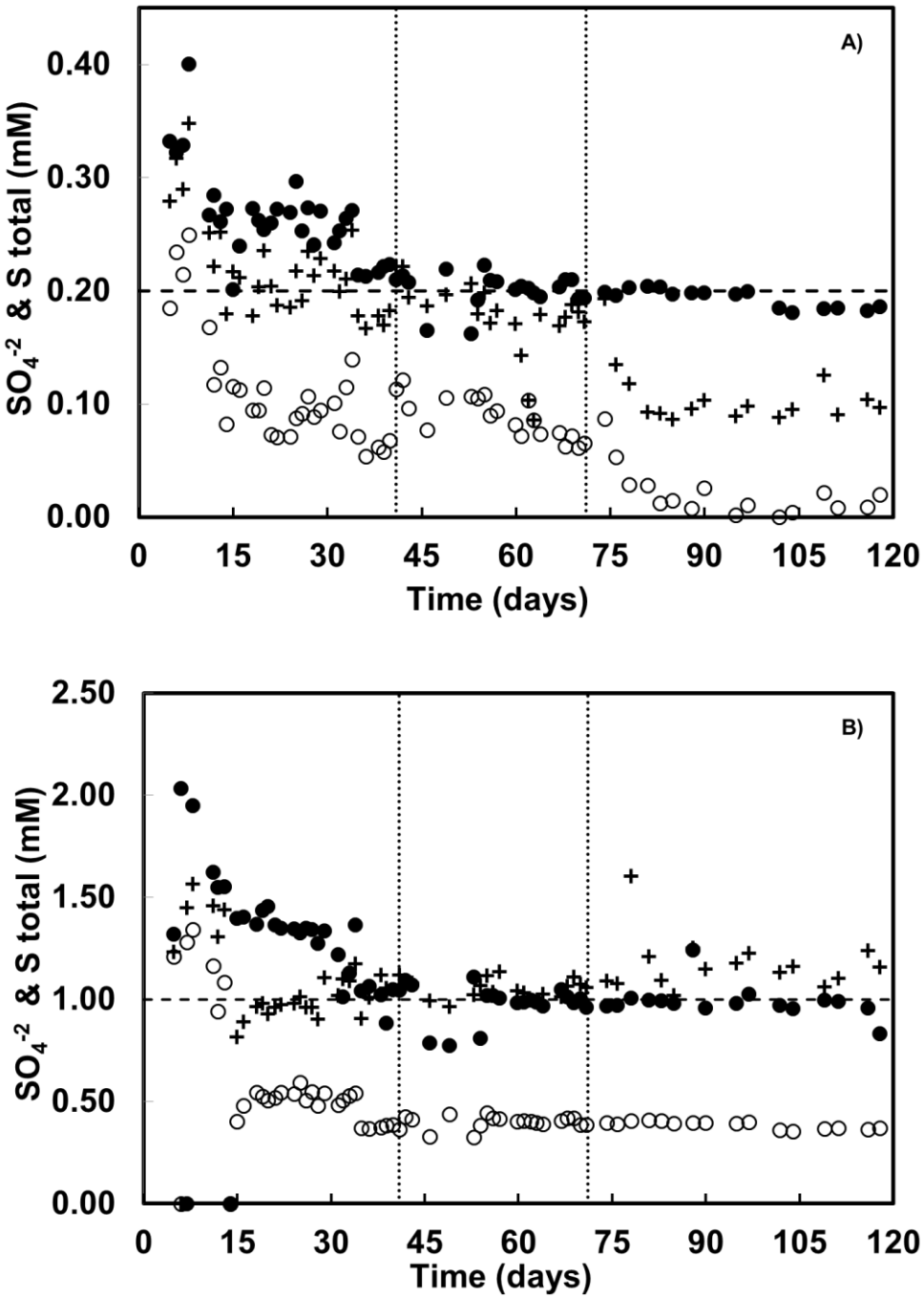


**Figure 4.9.** $SO_4^{2-}$ and total S concentration in reactor 1 (A) and reactor 2 (B). (●) $SO_4^{2-}$in the influent; (o) $SO_4^{2-}$ in the effluent; (+) S total in the influent; (+) As(V) in the effluent. The horizontal black line represent the set point of As total and As(V) in the effluent (65 **µ**M). The vertical dashed lines indicate the separation between the three stages in the reactor. $S_{total}$ = $(SO_4^{2-} + H_2S)_{EFF.}$

In reactor 1, sulfate was reduced partially during stage I and II (55.80 ± 9.42% of the initial value), but during stage III the removal of sulfate increased to 89.34 ± 11.11%. The increase in the sulfate reduction is indicating of enrichment of sulfate-reducing microorganisms in the bioreactor. Approximately 10% of the $SO_4^{2-}$ added was not reduced and remained in the influent, and 45% of the $SO_4^{2-}$ reduced corresponded to the concentration of $H_2S_{(aq)}$ in the effluent. The gap in the S balance in Figure 4.9A could be explained by the precipitation of the "missing" $H_2S$ with As(III) as ASM (Figure 4.10). Previous studies have also demonstrated that the total S concentration ($SO_4^{2-}$ + $H_2S$) in the effluent of a anaerobic bioreactor decreased after the influent was supplemented with As(V) and the decreased was related to the formation of ASM [18].

In reactor 2, sulfate was partially reduced during all stages (I, II, III) with an average of 60.68 ± 2.85% of the total sulfate in the influent (Figure 4.10). In this case, all sulfate removed was presented as $H_2S_{(aq)}$. The total amount of dissolved S in the influent and in the effluent was similar, indicating that sulfide was not precipitated as ASM (Figure 4.10). Reactor 1 was designed to enhance the precipitation of ASM and reactor 2 was designed to avoid the formation of ASM, hence the results obtained are logical. In the next section, a small formation of ASM will be appreciated in reactor 2, that disagree with the 0% of sulfate assigned to the formation of ASM obtained in reactor 2. Some of the sulfide could have been removed by stripping with the biogas ($CH_4$) explaining the small loss in S, but also the small concentrations of As(V) and the utilization of different techniques and instruments for the measurements could explain why any $H_2S$ formatted was associated as ASM formation.

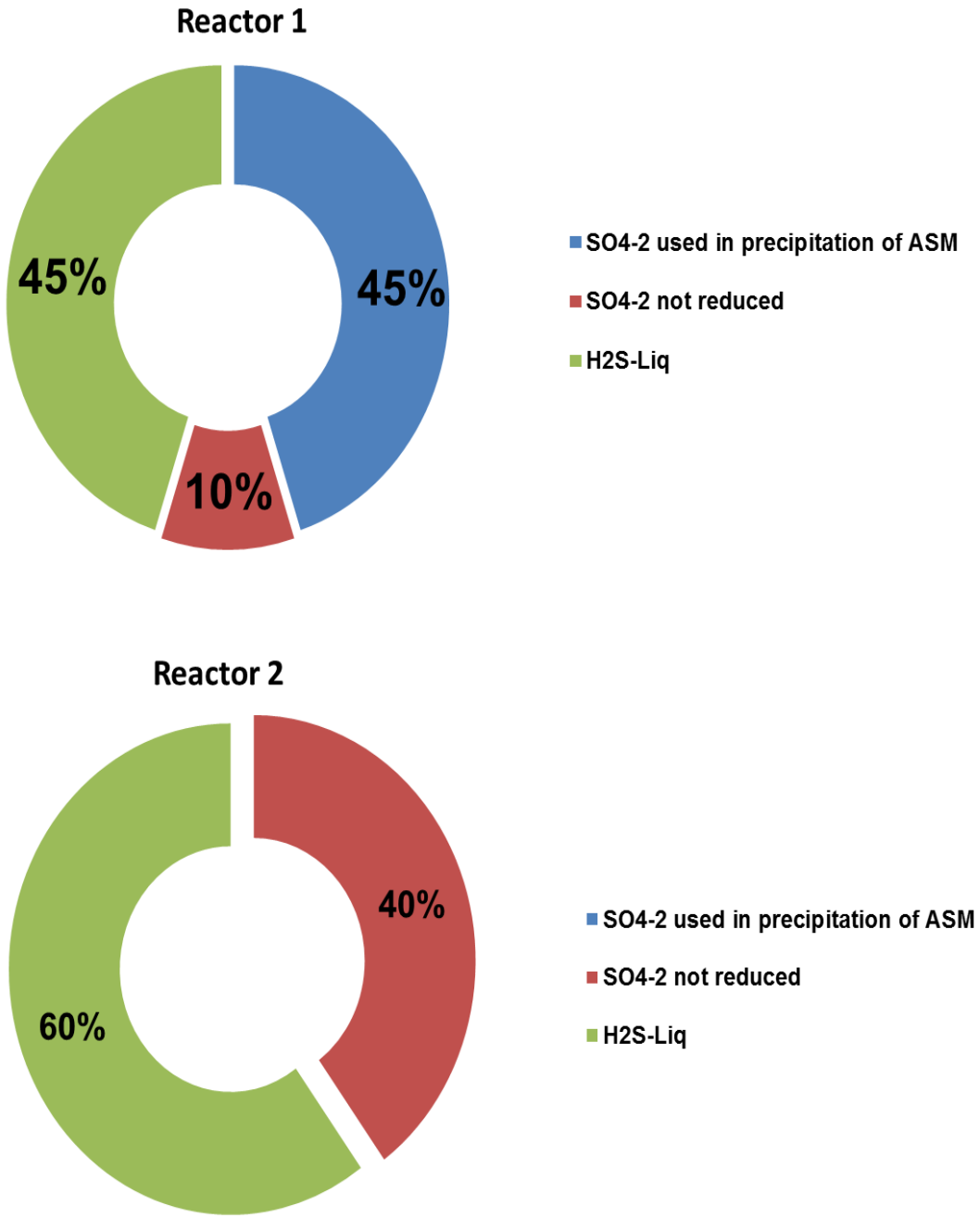


**Figure 4.10.** Percentages of $SO_4^{2-}$ transformation reached by the bioreactors operated in this study.

### *4.3.6. As(V) and Total arsenic*

The total concentrations of soluble arsenic and As(V) were evaluated in both reactors. Figure 4.11 shows the total As and As(V) concentration in the influent and in the effluent of each reactor. The average values removed and the percentage removed in R1 and R2 are represented in Tables 4.10 and 4.11, respectively.

Arsenic was added to both reactor as As(V), therefore, the As(V) and total soluble arsenic data in the influent are similar (Figure 4.11). The reduction of As(V) was high in both reactors, *i.e.*, 95.45 ± 1.98% of in R1 (Table 4.10) and 96.96 ± 1.8 % in R2 (Table 4.11). Figure 4.11A shows that a small amount of the total arsenic added remained in the effluent of R1 (13.14 ± 8.70 μM), corresponding to 79.60 ± 13.41% removal of total arsenic (Table 4.10). However, the total concentration of soluble arsenic remaining in the effluent of R2 was considerably higher (42.08 ± 12.30 μM) and only 33.13 ± 4.01% of the total arsenic was removed in that reactor (Table 4.11).

Figure 4.12 shows a simplified diagram illustrating the fate of arsenic in both reactors. Overall, the total As volumetric removal rate in R1 and R2 were 2.11 ± 0.32 μM/(L·h) and 0.86 ± 0.1 μM/(L·h), respectively. The ratio of sulfur loss to arsenic loss ($S_{loss}/As_{loss}$) was used to predict mineral phase precipitation based on the expectation that the S/As ratio of the ASM formed would be 1.5, corresponding to $As_2S_3$. The ratio obtained was 1.76 ± 0.53 for R1, and 0 for R2 (Tables 4.12 & 4.13).

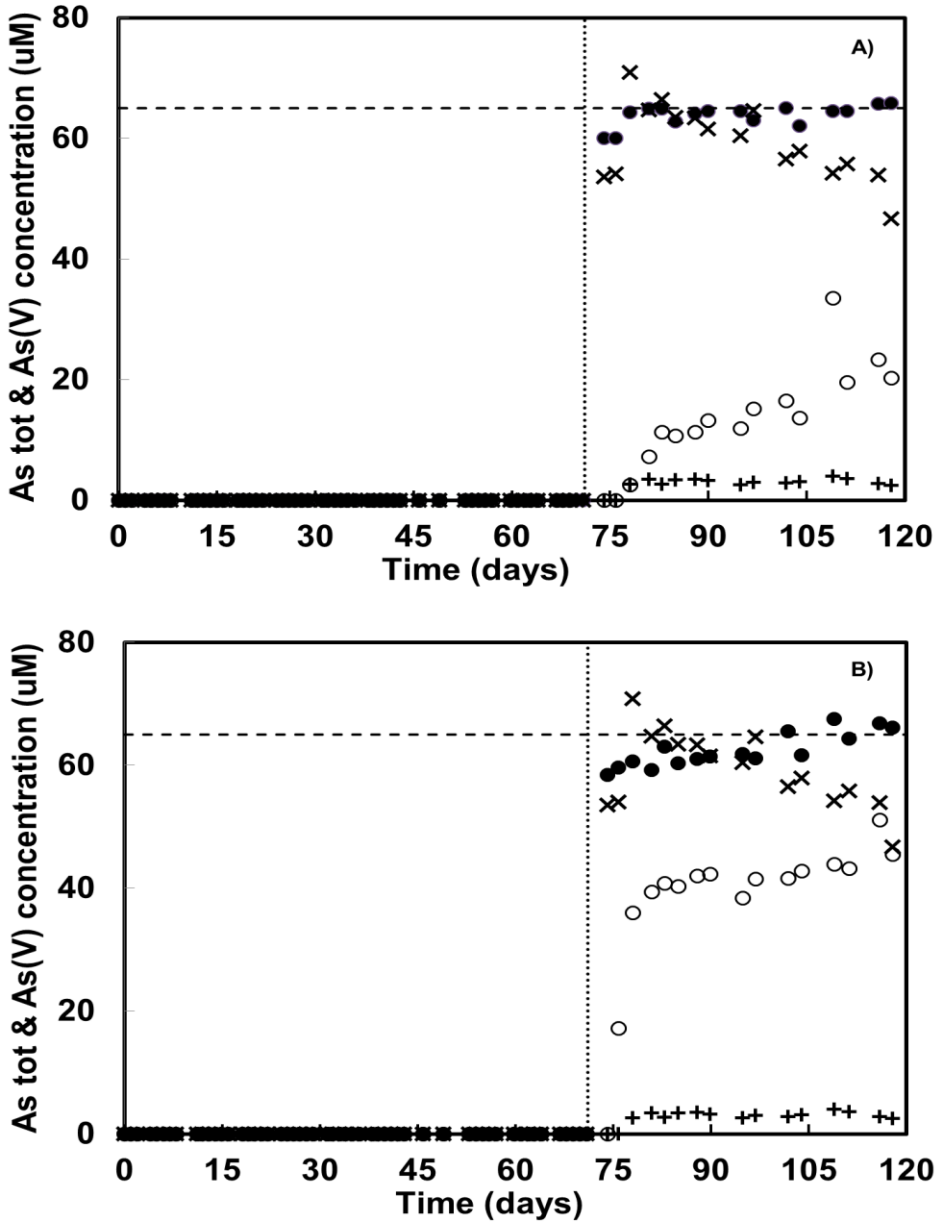


**Figure 4.11.** Total As and As(V) concentration in reactor 1 (A) and reactor 2 (B). (●) As total in the influent; (o) As total in the effluent; (x) As(V) in the influent; (+) As(V) in the effluent. The horizontal black line represent the set point of As total and As(V) in the effluent (65 µM). The vertical dashed lines indicate the beginning of the stage three in the reactor.

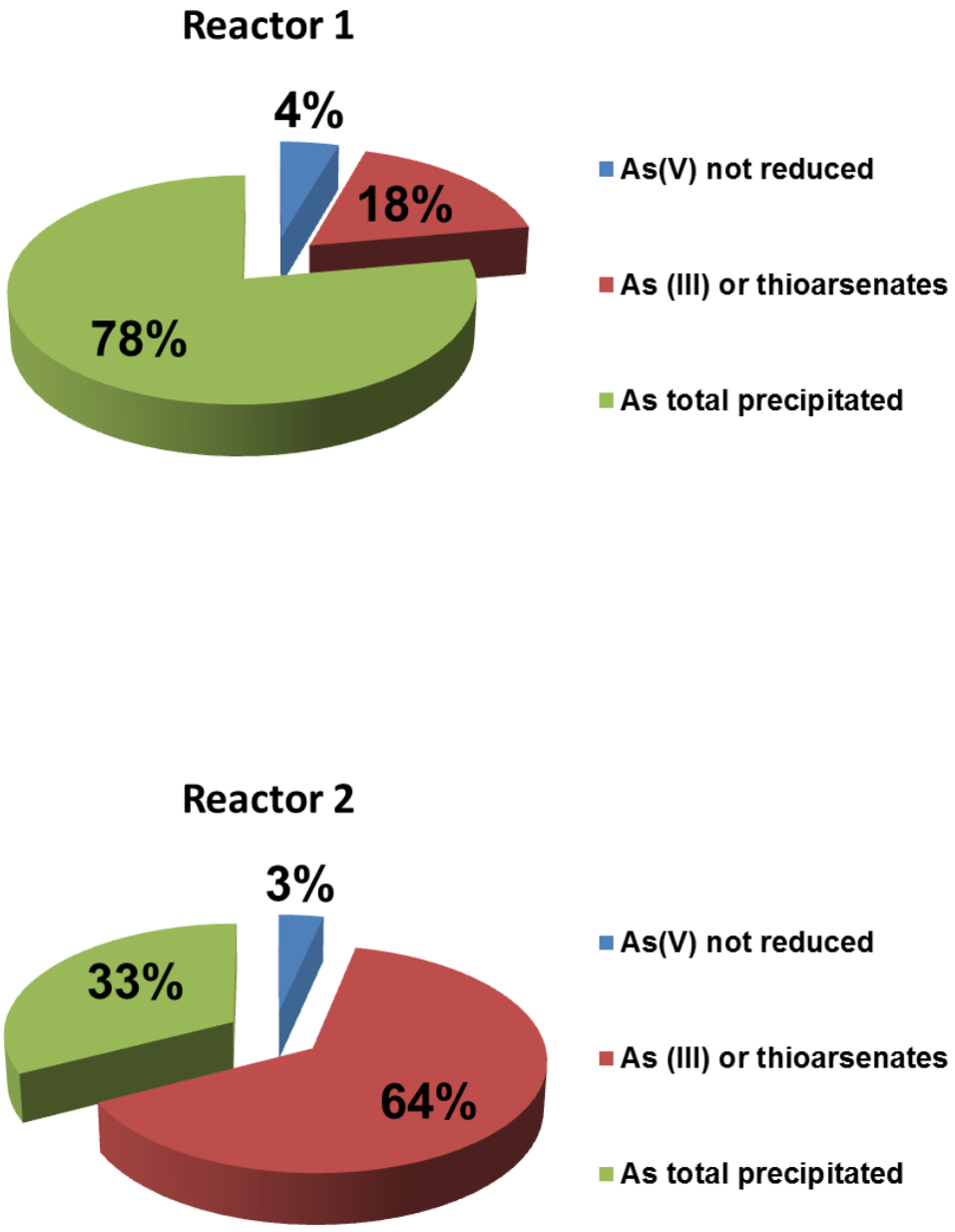


**Figure 4.12.** Arsenic speciation after treatment of an influent containing As(V) and different concentrations of sulfate in bioreactor 1 and 2.

**Table 4.10.**Total As removed and % total As removed in reactor 1.

| Stage | Period (days) | Total As removed (µM) | % Total As removed | As(V) removed (µM) | % As (V) removed |
|---|---|---|---|---|---|
| III | 74-120 | 50.64 ± 7.76 | 79.60 ± 13.41 | 56.56 ± 5.95 | 95.45 ± 1.98 |

**Table 4.11.**Total As removed and % total As removed in reactor 2.

| Stage | Period (days) | Total As removed (µM) | % Total As removed | As(V) removed (µM) | % As (V) removed |
|---|---|---|---|---|---|
| III | 74-120 | 20.81 ± 2.47 | 33.13 ± 4.01 | 60.40 ± 5.32 | 96.96 ± 1.88 |

**Table 4.12.** Ratio of inorganic S removed to arsenic removed ($S_{loss}/As_{loss}$) in reactor 1.

| $As_{loss}$ (uM) | $S_{loss}$ (µM) | $S_{loss}/A_{loss}$ |
|---|---|---|
| 50.64 ± 7.76 | 87.31 ± 27.14 | 1.76 ±0.53 |

$$S_{loss} = (SO_4^{2-} + H_2S)_{INF} - (SO_4^{2-} + H_2S)_{EF}$$

$$As_{loss} = (TotAs)_{INF} - (TotAs)_{EF}$$

**Table 4.13.** Ratio of inorganic S removed to arsenic removed ($S_{loss}/As_{loss}$) in reactor 2.

| $As_{loss}$ (µM) | $S_{loss}$ (µM) | $S_{loss}/A_{loss}$ |
|---|---|---|
| 20.81 ± 2.47 | 0 | 0 |

The results obtained shows the data expected, since reactor 1 was designed with a small amount of sulfate to enhance the precipitation of ASM, as other previous studied demonstrated that under stoichiometric conditions and a pH lower than 7, the ASM precipitation is enhanced. In that previous study, with an initial As concentration of 1 mM, 93.56 ± 3.99% of the total

arsenic was removed, obtaining a final concentration in the effluent of 64.4 µM, [18]. By comparison, the percentage of As removed in the current experiment was 14% lower. This could be explained because the small concentration of As added to the reactor was lower than the stoichiometric to make sure that the Mahou sludge was not inhibited by dissolved arsenic. Also the small concentration of As implies that any small change in parameters could have greatly affected the results obtained. Either way, the percentage of arsenic removed in R2 is lower than in R1, confirming the hypothesis that with a higher amount of sulfate, ASM formation is disfavored. The removal rate of As(V) obtained in R1 is lower than that obtained in a previous study (38.7 µM/(L·h)) performed in a continuous anaerobic bioreactor with an initial As(V) concentration of 1 mM and stoichiometric S/As ratio of 1.5 [18]. A higher As removal rate of 33.4 µM/(L·h) was also reported in a fixed-film sulfate-reducing bioreactor fed an acidic pH influent (2.7-5.0) containing 1.33 mM As(V) and using $H_2$ as e-donor [32]. The higher arsenic removal loading rates in these two reports are likely due to the considerably higher initial As(V) concentrations used compared to just 0.065 mM in the present study.

The molar S/As removal ratio obtained in R1(1.76 ± 0.53) was the expected in the formation of $As_2S_3$. A previous study demonstrated the formation of $As_2S_3$ and AsS in a continuous anaerobic bioreactor where As(V) and $SO_4^{2-}$ were simultaneously reduced (using EXAFS spectra and visual evidence) and reported an average S/As removal ratio of 1.20 [1]. The small ratio obtained in R2 was expected as a study reported the possibility of formation of other arsenic soluble compounds [18, 33, 42, 43]. The dissolution of $As_2S_3$ with high sulfide concentrations has been also reported in other experiments [42, 43]. Hence the decreasing in the effectiveness of this process with higher sulfate could be related to the formation of arsenic soluble compounds such as thioarsenites.

### *4.3.7 .Thioarsenates/Thioarsenites*

Thioarsenites ($AsO_{3-x}S_x^{3-}$) and thioarsenates ($AsO_{4-x}S_x^{3-}$) are arsenic soluble species. Thioarsenite formation has been observed in aqueous samples where arsenite and high concentrations of sulfide are present at pH > 7[33]. The existence of these compounds has been demonstrated in frozen samples collected in an anaerobic glove box using solid state spectroscopic techniques [18]. Thioarsenites are extremely reactive in aerobic environments and decompose readily in the presence of atmospheric oxygen resulting in the formation of thioarsenates [33]. Moreover, the highly alkaline conditions commonly utilized in the speciation of thioarsenites by ion chromatography also lead to their decomposition yielding thioarsenates. The detection of thioarsenates is generally accepted to provide indirect evidence for the presence of thioarsenites in a sample.

An experiment during the growth of a bacterium strain (haloalkaliphilic), using As(V) as electron donor and sulfide as electron acceptor, at pH 9.8 and low S/As ratios (0.4 and 0.82) reported formation of mixed arsenic-sulfur species under anaerobic conditions [33]. Also, a study developed with abiotic mixes of As(III) and sulfide at different ratios S/As, reported a high formation of soluble arsenic species at high S/As molar ratios (6), and a poor formation with low S/As ratios (2) [33]. Different techniques were used to detect the presence of some of these compounds.

*High performance liquid chromatography – Inductively coupled plasma – Mass spectrometry (HPLC-ICP-MS)*

First of all, a HPLC-ICP-MS technique was used to analyze a sample of the effluent of each reactor. Monothioarsenate, thioarsenates (generated by mixing As(III) with sulfide or polysulfide) were synthesized and analyzed by HPLC-ICP-MS. The simultaneous detection of As and S was also attempted. The procedures to synthesize monothioarsenate and prepare the mixtures of thioarsenates are detailed in the Materials and Methods section.

Unfortunately, the HPLC-ICP-MS determination of the thioarsenates did not work too well. The synthesized monothioarsenate showed two broad peaks indicative of compounds that are poorly separated at seven and nine minutes (Figure 4.13A). There was no clear evidence of thioarsenates in the effluent samples (Figure 4.13B). The analysis of sulfur did not yield valuable results but that was expected as the injected samples were relatively diluted and the isotopic abundance of $^{34}S$ is only 4.5% (of total S) and there was interference at *m/z* 32 from O dimer (16*2). However, there were some large "As" gaps (missing arsenic) in some samples that might be associated with the formation of thioarsenic species (Table 4.14).

**Table 4.14.**Total As measured in samples prepared. Red numbers shows the gap of total As that could be because of the formation of thioarsenates/thioarsenites species.

| | **Total As expected** | **Gap total As** |
|---|---|---|
| | **µg/L** | **µg/L** |
| Reactor 1 (S/As ratio= 3) | 21 | -4 |
| Reactor 2 (S/As ratio= 15) | 20 | **15** |
| Mix As(III) + Sulfide | 36 | **16** |
| Mix As(III) + Polysulfide | 36 | -7 |
| Synthesized monothioarsenate | 10 | 7 |

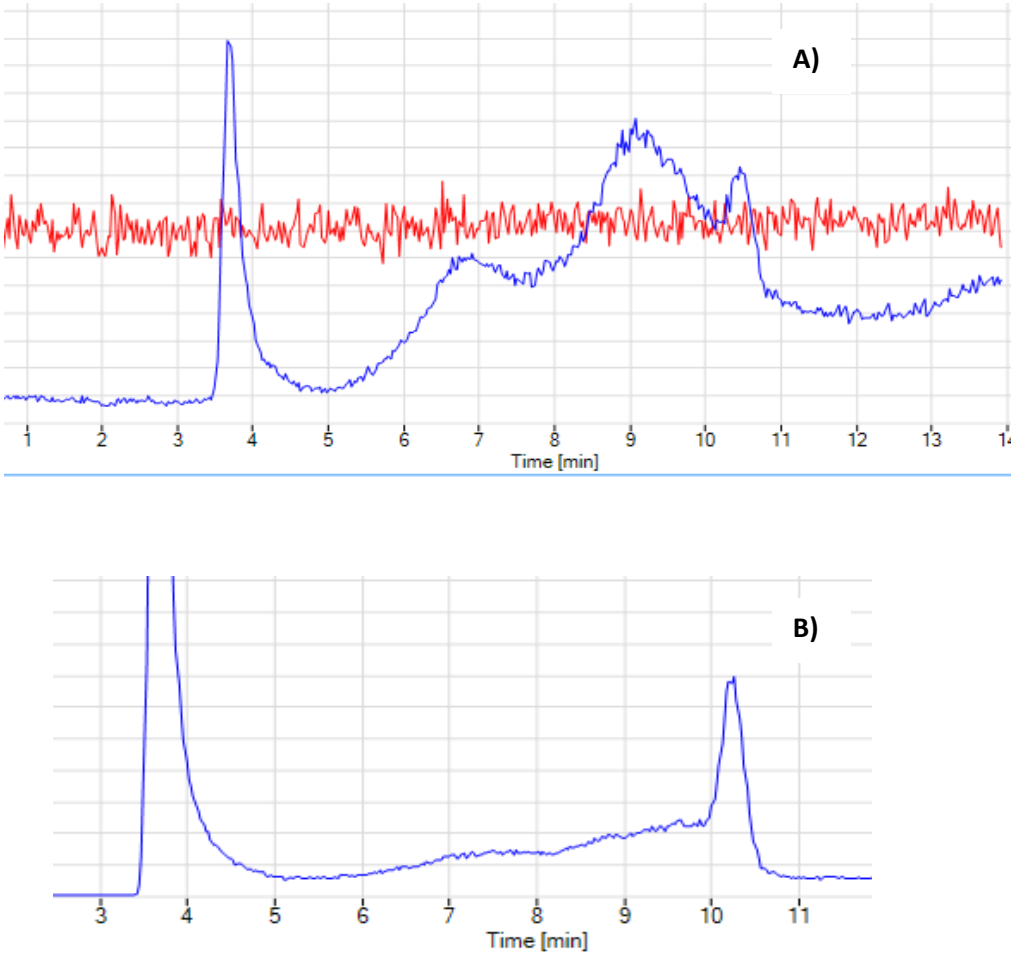


**Figure 4.13.** HPLC-ICP-MS chromatograms. (A) Monothioarsenate;(B) Effluent sample of reactor 2.

*Ion chromatography*

Once the previous method did not work as well as we expected, the detection of thioarsenate species was tried using ion chromatography with suppressed conductivity detection. The synthetized monothioarsenate was analyzed using a gradient eluent concentration of KOH and its retention time was determined (Figure 4.14). Also a mixture of thioarsenates (synthetized by mixing As(III) and sulfide) was analyzed (Figure 4.15). Monothioarsenate was clearly

detected at 16.13 min and there were some peaks that appeared after monothioarsenate that could be di-, tri- and tetra-thioarsenates (Figure 4.15).

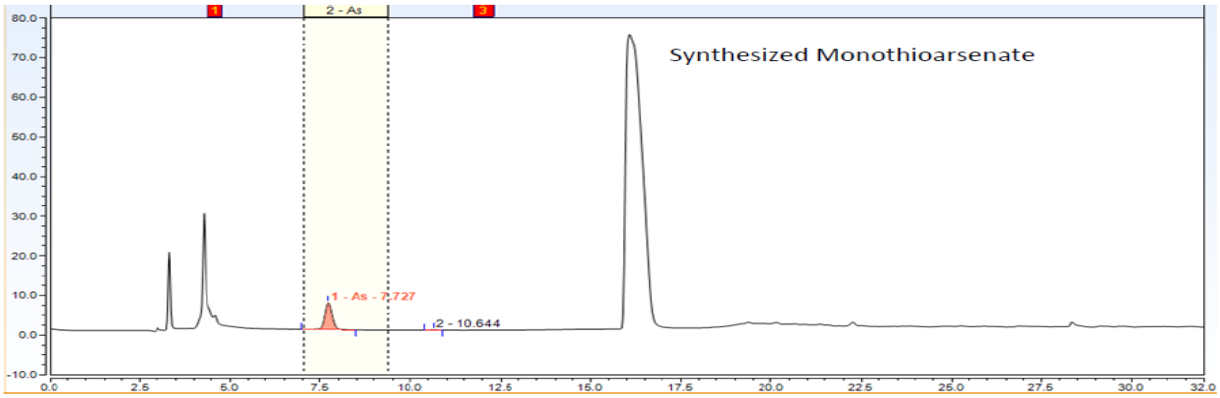


**Figure 4.14.** Monothioarsenate standard separation using ion chromatography. Axis Y (Intensity); Axis X (Time (min)).

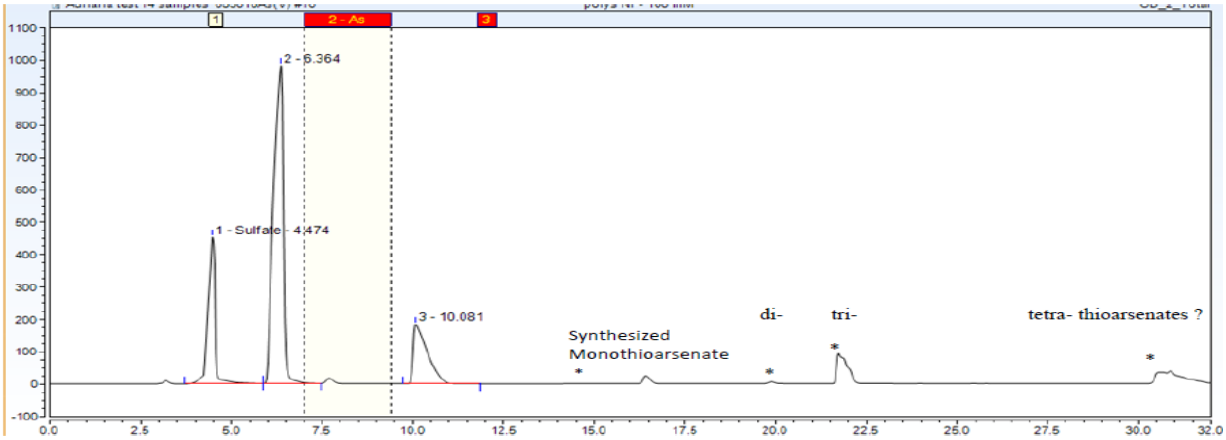


**Figure 4.15.** Separation of a mixture of thioarsenates (generated by mixing As(III) and sulfide) using ion chromatography. Axis Y (Intensity); Axis X (Time (min)).

The detection of thioarsenate species has been successfully demonstrated in previous experiments using ion chromatography (Figure 4.16) [33, 44]. On the other hand, the interference presented at *m/z* 32 from O dimer (16 x 2) when measurement of sulfur was attempted by LC-ICP-MS could be solved with an ICP-OES (inductively coupled plasma -

optical emission spectrometry). With ICP-OES, oxygen does cause any interference in optical emission spectrometry. Hence combining IC with ICP-OES (IC-ICP-OES) would be a good technique to determine these species.

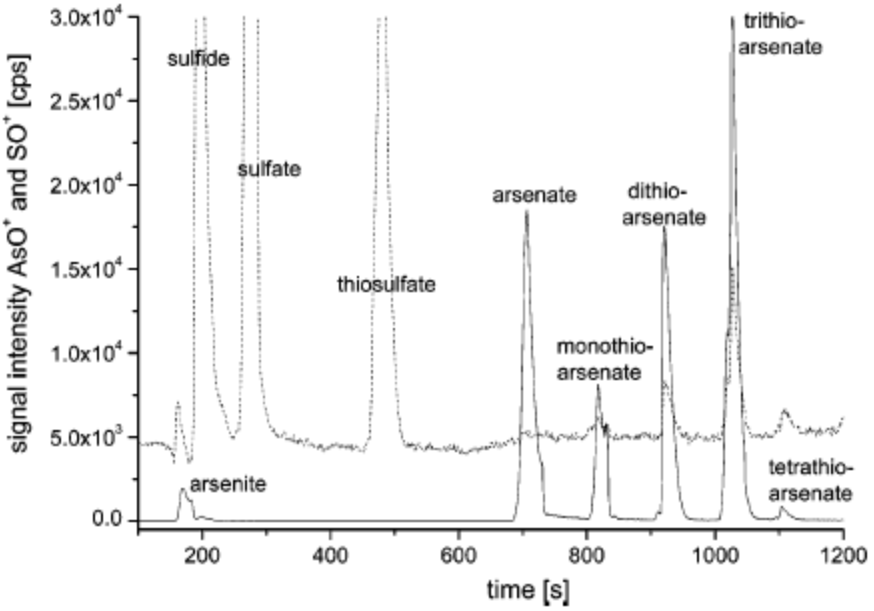


**Figure 4.16.** Chromatogram of an alkaline (pH 9, 90°C) geothermal water with high sulfide concentrations (3.7 mg/L) and a predominance of thioarsenates (solid line= $AsO^+$, dashed line = $SO^+$). This chromatogram was obtained in a liquid chromatography system coupled to an ICP-MS [15].

### 4.4 . Dissolution of arsenic from commercial orpiment, arsenopyrite, realgar and crushed orpiment under aerobic and anoxic conditions

This experiment was performed to study the solubility of commercial orpiment ($As_2S_3$), arsenopyrite (FeAsS), crushed orpiment ($As_2S_3$) and realgar (AsS) under aerobic conditions (using $O_2$ as electron acceptor) and under anoxic conditions (using nitrate ($NO_3^-$) as electron acceptor) with a moderately basic pH (8.45).

The dissolution of $As_2S_3$ (commercial and crushed), FeAsS, and AsS under aerobic conditions is represented in Figure 4.17A. After 55 days of incubation, the total As concentration released into solution was much higher for commercial orpiment, with 33.28 ± 11 mg/L (14.8 ± 5.1% of the total As supplied) than the others ASM (Table 4.15). The dissolution of the same ASM under anoxic conditions is represented in Figure 4.17B. After 55 days of incubation, the total As concentration released into solution under anoxic conditions was also higher for commercial orpiment (14.3 ± 1.9 % of the total As supplied) than the other ASM (Table 4.16).

**Table 4.15.** Total As dissolved (%) of the different ASM under aerobic conditions ($O_2$ as electron acceptor) at pH 8.45.

| ASM | As total concentration (mg/L) | As total dissolved (%) |
|---|---|---|
| Commercial orpiment | 33.3 ± 11.4 | 14.8 ± 5.1 |
| Crushed orpiment | 17.6 ± 7.3 | 7.8 ± 3.3 |
| Arsenopyrite | 5.7 ± 1.2 | 2.6 ± 0.2 |
| Realgar | 2.7 ± 0.5 | 1.2 ± 0.2 |

**Table 4.16.**Total As dissolved (%) of the different ASM under anaerobic conditions ($NO_3^-$ as electron acceptor) at pH 8.45.

| ASM | As tot concentration (mg/L) | As tot dissolved (%) |
|---|---|---|
| Commercial orpiment | 32.1 ± 4.2 | 14.3 ± 1.9 |
| Crushed orpiment | 5.0 ± 0.0 | 2.2 ± 0.0 |
| Arsenopyrite | 0.8 ± 0.4 | 1.2 ± 1.9 |
| Realgar | 2.4 ± 03 | 1.1 ± 0.1 |

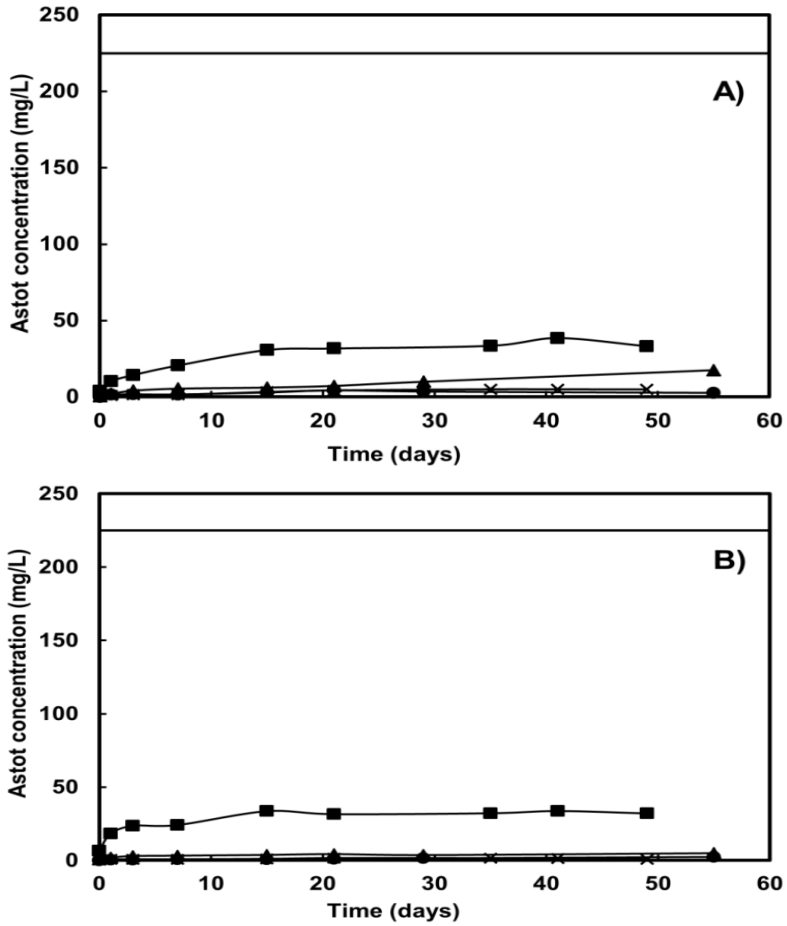


**Figure 4.17.** Dissolution of different ASM (225 mg/L as As)) under aerobic conditions (**A**), and under anaerobic conditions (**B)** in batch assays at pH 8.45: Commercial orpiment (■), crushed orpiment (▲), arsenopyrite (x), realgar (●). The horizontal black line represents the maximum theoretical concentration of As that can be dissolved.

Some previous experiments studied the dissolution of different ASM under aerobic or anoxic conditions [4, 45, 46]. There are several factors that can affect the As mobilization from ASM, among them redox conditions, pH and the presence of microorganisms are the main ones to consider [47].

Some studies have proven that the solubility of orpiment ($As_2S_3$) increase with increasing pH [48, 49]. One study reported that in aqueous solution, more orpiment is dissolved at pH 7 than at pH 4 [50]. In the abiotic experiments performed at pH 8.45, the highest solubility in aerobic and anoxic conditions was obtained for $As_2S_3$ (14% for aerobic and anoxic conditions). The mobilization of As from $As_2S_3$ under aerobic conditions was already described [45]. Some previous experiment also reported a high solubility of $As_2S_3$ (11.6 % of the total As (1 mM)) under aerobic conditions and at pH 7.1-7.2 [18]. However, that previous study reported a higher solubility of $As_2S_3$ in anoxic condition (87%) using $NO_3^-$ as electron acceptor [18]. The solubility obtained for arsenopyrite (FeAsS) in aerobic conditions (2.2%) is lower compared with the obtained in that previous study for arsenopyrite (9.6% of the total As) [18]. The solubility for arsenopyrite under anoxic conditions (1.2%) was also low. A previous study concluded that $NO_3^-$ does not oxidize arsenopyrite in acidic conditions (pH 2.0-4.5) [46]. Hence the influence of pH could not affect the arsenopyrite solubility.

The solubility of realgar (AsS) in the present study was low (1.1%). There are few studies reporting on the solubility of realgar. An electrochemical characterization of orpiment and realgar in the presence of a bacteria-free and acidic bacterial growth medium, reported similar electrochemical behavior of both species due to the proximity of their potentials [45]. Another study showed that realgar is appreciably soluble in alkaline solutions at high temperatures (75°C) under aerobic conditions [51].

It is important to note that the values of solubility for the two types of orpiment tested (commercial orpiment and crushed orpiment) vary in both cases (Tables 4.15 & 4.16) despite of being the same compound ($As_2S_3$). This difference could be explained because the crushed orpiment was purchased and after that crushed partially, whereas the commercial orpiment was

presented as a fine powder. The oxidation of amorphous $As_2S_3$ in neutral to alkaline pH has been studied [48]. Another study reported that the oxidation rate of $As_2S_3$ (amorphous) between pH 6.2-8.2 is higher than natural orpiment, suggesting that reaction mechanisms for oxidation of the two solids are likely different [52].

# 5. CONCLUSIONS

## 5. Conclusions

- Arsenate (As(V)) and arsenite (As(III)) cause severe inhibition of the microorganisms involved in the production of methane ($CH_4$) in anaerobic granular sludge. Both arsenic species displayed the same degree of toxicity towards $H_2$-utilizing methanogenic microorganisms, presenting similar values for the half maximal inhibitory concentration (6.2 and 5.7 mg/L for As(III) and As(V), respectively).

- As(V) at concentrations between 2-180 mg/L is effectively reduced to As(III) under anaerobic conditions by microorganisms in methanogenic sludge. The rate of microbial reduction increased with increasing initial As(V) concentrations and the reaction followed 1$^{st}$ order kinetics with a rate constant of 0.864 day$^{-1}$. Hence As(V) does not appear to be inhibitory to the microorganisms involved in its reduction.

- Arsenic was removed effectively in a continuous anaerobic bioreactor operated at pH 6.4-6.6 with a molar S/As ratio close to the stoichiometric requirement for $As_2S_3$ precipitation (1.5). Removal was due to microbial reduction of As(V) and sulfate ($SO_4^{2-}$) and precipitation of the As(III) formed with biogenic $H_2S$ as insoluble arsenic-sulfide minerals (ASM).

- In contrast, very poor arsenic removal efficiencies were attained by a continuous anaerobic bioreactor operated under the same conditions with a feed containing with much higher sulfate concentration (molar S/As ratio of 15) due to the formation of soluble thioarsenite species that remained in solution.

- Hence, effective removal of arsenic by formation of ASM in continuous sulfidogenic bioreactors requires operation at pH < 7 as well as tight control of the amount of $SO_4^{2-}$ present in the influent to values close to the stoichiometric requirement for $As_2S_3$ precipitation.

- Commercial orpiment ($As_2S_3$) presented a higher solubility than other common arsenic sulfide minerals, *i.e.*, realgar ($\alpha$-$As_4S_4$) and arsenopyrite (FeAsS), under aerobic and anoxic conditions at moderately alkaline pH (8.45), with an average of 14.5% of total arsenic solubilized after 50 days. The percent of arsenic solubilized from realgar and arsenopyrite after the same time period averaged 1.15 % and 1.9 %respectively. The solubility of each ASM under aerobic and anaerobic conditions was very similar.

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# APPENDIX

# APPENDIX I: MATERIAL SAFETY DATA SHEETS

# Material Safety Data Sheet
## Sodium arsenite, powder, certified

ACC# 21360

## Section 1 - Chemical Product and Company Identification

**MSDS Name:** Sodium arsenite, powder, certified
**Catalog Numbers:** S225I-100, S225I-500
**Synonyms:** Arsenenous acid, sodium salt; Sodium metaarsenite; Sodium arsenite; Sodium dioxoarsenate.
**Company Identification:**

Fisher Scientific
1 Reagent Lane
Fair Lawn, NJ 07410

**For information, call:** 201-796-7100
**Emergency Number:** 201-796-7100
**For CHEMTREC assistance, call:** 800-424-9300
**For International CHEMTREC assistance, call:** 703-527-3887

## Section 2 - Composition, Information on Ingredients

| CAS# | Chemical Name | Percent | EINECS/ELINCS |
|---|---|---|---|
| 7784-46-5 | Sodium arsenite | 100 | 232-070-5 |

## Section 3 - Hazards Identification

### EMERGENCY OVERVIEW

Appearance: white to gray white solid.
**Danger!** May be fatal if swallowed. May be fatal if absorbed through the skin. Harmful if inhaled. Contains inorganic arsenic. Causes eye, skin, and respiratory tract irritation. Cancer hazard.
**Target Organs:** Lungs, skin.

### Potential Health Effects

**Eye:** Causes eye irritation.
**Skin:** Causes skin irritation. May be fatal if absorbed through the skin. Exposure to arsenic compounds may produce hyperpigmentation of the skin and hyperkeratoses of plantar and palmar surfaces as well as both primary irritation and sensitization types.
**Ingestion:** May be fatal if swallowed. Poison by ingestion. Causes gastrointestinal irritation with nausea, vomiting and diarrhea. All soluble arsenic (As) compounds are considered to be poisonous to humans. Inorganic arsenic is more toxic than organic arsenic. Organic arsenic is excreted more rapidly than inorganic arsenic. Arsenic 5+ is excreted more rapidly than arsenic 3+.
**Inhalation:** Causes respiratory tract irritation. Inhalation of arsenic compounds may lead to irritation of the respiratory tract and to possible nasal perforation. Long-term exposure to arsenic compounds may produce impairment of peripheral circulation.
**Chronic:** Prolonged or repeated skin contact may cause dermatitis. Inorganic arsenic compounds may cause skin and lung cancers in humans.

## Section 4 - First Aid Measures

**Eyes:** In case of contact, immediately flush eyes with plenty of water for a t least 15 minutes. Get medical aid.
**Skin:** In case of contact, immediately flush skin with plenty of water for at least 15 minutes while removing contaminated clothing and shoes. Get medical aid immediately. Wash clothing before reuse.
**Ingestion:** Call a poison control center. If swallowed, do not induce vomiting unless directed to do so by medical personnel. Never give anything by mouth to an unconscious person. Get medical aid.
**Inhalation:** If inhaled, remove to fresh air. If not breathing, give artificial respiration. If breathing is difficult, give oxygen. Get medical aid.
**Notes to Physician:** Treat symptomatically and supportively.

## Section 5 - Fire Fighting Measures

**General Information:** As in any fire, wear a self-contained breathing apparatus in pressure-demand, MSHA/NIOSH (approved or equivalent), and full protective gear. Substance is noncombustible.
**Extinguishing Media:** Use agent most appropriate to extinguish fire.
**Flash Point:** Not applicable.
**Autoignition Temperature:** Not available.
**Explosion Limits, Lower:**N/A
**Upper:** N/A
**NFPA Rating:** (estimated) Health: 3; Flammability: 0; Instability: 0

## Section 6 - Accidental Release Measures

**General Information:** Use proper personal protective equipment as indicated in Section 8.
**Spills/Leaks:** Vacuum or sweep up material and place into a suitable disposal container. Clean up spills immediately, observing precautions in the Protective Equipment section. Wear a self contained breathing apparatus and appropriate personal protection. (See Exposure Controls, Personal Protection section). Avoid generating dusty conditions. Provide ventilation. Evacuate unnecessary personnel. U.S. regulations require reporting spills and releases to soil, water and air in excess of reportable quantities. Do not let this chemical enter the environment.

## Section 7 - Handling and Storage

**Handling:** Wash thoroughly after handling. Wash thoroughly after handling. Remove contaminated clothing and wash before reuse. Do not get in eyes, on skin, or on clothing. Do not breathe dust. Use only with adequate ventilation or respiratory protection. Change contaminated clothing promptly. Do not take working clothes home.
**Storage:** Store in a cool, dry place. Store in a tightly closed container. Store protected from moisture.

## Section 8 - Exposure Controls, Personal Protection

**Engineering Controls:** Facilities storing or utilizing this material should be equipped with an eyewash facility and a safety shower. Use adequate general or local exhaust ventilation to keep airborne concentrations below the permissible exposure limits. See 29CFR 1910.1018 for regulatory requirements pertaining to all occupational exposures to inorganic arsenic.
**Exposure Limits**

| Chemical Name | ACGIH | NIOSH | OSHA - Final PELs |
|---|---|---|---|
| | | | 10 æg/m3 TWA (as As) (listed under Arsenic, |

| | | | |
|---|---|---|---|
| Sodium arsenite | 0.01 mg/m3 TWA (as As) (listed under Arsenic, inorganic compounds). | 5 mg/m3 IDLH (as As) (listed under Arsenic, inorganic compounds). | inorganic compounds).5 æg/m3 Action Level (as As); 10 æg/m3 TWA (as As, Cancer hazard - see 29 CFR 19 10.1018, except Arsine) (listed under Arsenic, inorganic compounds). |

**OSHA Vacated PELs:** Sodium arsenite: No OSHA Vacated PELs are listed for this chemical.
**Personal Protective Equipment**
**Eyes:** Wear appropriate protective eyeglasses or chemical safety goggles as described by OSHA's eye and face protection regulations in 29 CFR 1910.133 or European Standard EN166.
**Skin:** Wear appropriate protective gloves to prevent skin exposure.
**Clothing:** Wear appropriate protective clothing to prevent skin exposure.
**Respirators:** Follow the OSHA respirator regulations found in 29 CFR 1910.134 or European Standard EN 149. Use a NIOSH/MSHA or European Standard EN 149 approved respirator if exposure limits are exceeded or if irritation or other symptoms are experienced.

## Section 9 - Physical and Chemical Properties

**Physical State:** Solid
**Appearance:** white to gray white
**Odor:** odorless
**pH:** basic in solution
**Vapor Pressure:** Not applicable.
**Vapor Density:** Not applicable.
**Evaporation Rate:**Not applicable.
**Viscosity:** Not applicable.
**Boiling Point:** Decomposes
**Freezing/Melting Point:**615 deg C
**Decomposition Temperature:**Not available.
**Solubility:** Soluble.
**Specific Gravity/Density:**1.87
**Molecular Formula:**NaAsO2
**Molecular Weight:**129.91

## Section 10 - Stability and Reactivity

**Chemical Stability:** Stable. Absorbs carbon dioxide from the air.
**Conditions to Avoid:** Moisture, extreme temperatures.
**Incompatibilities with Other Materials:** Strong acids.
**Hazardous Decomposition Products:** Oxides of arsenic.
**Hazardous Polymerization:** Has not been reported

## Section 11 - Toxicological Information

**RTECS#:**
**CAS#** 7784-46-5: CG3675000
**LD50/LC50:**
CAS# 7784-46-5:
Oral, rat: LD50 = 41 mg/kg;
Skin, rat: LD50 = 150 mg/kg;
.

**Carcinogenicity:**
CAS# 7784-46-5:

- **ACGIH:** A1 - Confirmed Human Carcinogen (listed as 'Arsenic, inorganic compounds').
- **California:** carcinogen, initial date 2/27/87 (listed as Arsenic, inorganic compounds).
- **NTP:** Known carcinogen (listed as Arsenic, inorganic compounds).
- **IARC:** Group 1 carcinogen

**Epidemiology:** Epidemiological studies have demonstrated evidence of a causal relationship between environmental, occupational, and medicinal exposure of humans to inorganic arsenic and cancer of the skin and lungs.
**Teratogenicity:** Has caused teratogenic effects in animal studies.
**Reproductive Effects:** Can cause reproductive effects in animal studies.
**Mutagenicity:** See actual entry in RTECS for complete information.
**Neurotoxicity:** See actual entry in RTECS for complete information.
**Other Studies:**

## Section 12 - Ecological Information

**Ecotoxicity:** No data available. Arsenic compounds tend to be accumulated by oysters and other shellfish.
**Environmental:** No information available.
**Physical:** No information available.
**Other:** May be toxic to aquatic organisms; May cause long-term adverse effects in the aquatic environment.

## Section 13 - Disposal Considerations

Chemical waste generators must determine whether a discarded chemical is classified as a hazardous waste. US EPA guidelines for the classification determination are listed in 40 CFR Parts 261.3. Additionally, waste generators must consult state and local hazardous waste regulations to ensure complete and accurate classification.
**RCRA P-Series:** None listed.
**RCRA U-Series:** None listed.

## Section 14 - Transport Information

| | US DOT | Canada TDG |
|---|---|---|
| **Shipping Name:** | SODIUM ARSENITE, SOLID | SODIUM ARSENITE, SOLID |
| **Hazard Class:** | 6.1 | 6.1 |
| **UN Number:** | UN2027 | UN2027 |
| **Packing Group:** | II | II |

## Section 15 - Regulatory Information

### US FEDERAL

**TSCA**
CAS# 7784-46-5 is listed on the TSCA inventory.
**Health & Safety Reporting List**

None of the chemicals are on the Health & Safety Reporting List.

**Chemical Test Rules**

None of the chemicals in this product are under a Chemical Test Rule.

**Section 12b**

None of the chemicals are listed under TSCA Section 12b.

**TSCA Significant New Use Rule**

None of the chemicals in this material have a SNUR under TSCA.

**CERCLA Hazardous Substances and corresponding RQs**

CAS# 7784-46-5: 1 lb final RQ; 0.454 kg final RQ

**SARA Section 302 Extremely Hazardous Substances**

CAS# 7784-46-5: 500 lb lower threshold TPQ; 10000 lb upper threshold T PQ

**SARA Codes**

CAS # 7784-46-5: immediate, delayed.

**Section 313**

This material contains Sodium arsenite (listed as Arsenic, inorganic compounds), 100%, (CAS# 7784-46-5) which is subject to the reporting requirements of Section 313 of SARA Title III and 40 CFR Part 373.

**Clean Air Act:**

CAS# 7784-46-5 (listed as Arsenic, inorganic compounds) is listed as a hazardous air pollutant (HAP).

This material does not contain any Class 1 Ozone depletors.

This material does not contain any Class 2 Ozone depletors.

**Clean Water Act:**

CAS# 7784-46-5 is listed as a Hazardous Substance under the CWA.

None of the chemicals in this product are listed as Priority Pollutants under the CWA. CAS# 7784-46-5 is listed as a Toxic Pollutant under the Clean Water Act.

**OSHA:**

None of the chemicals in this product are considered highly hazardous by OSHA.

**STATE**

CAS# 7784-46-5 can be found on the following state right to know lists: California, New Jersey, Pennsylvania, Minnesota, (listed as Arsenic, inorganic compounds), Massachusetts.

**California Prop 65**

**The following statement(s) is(are) made in order to comply with the California Safe Drinking Water Act:**

WARNING: This product contains Sodium arsenite, listed as `Arsenic, inorganic compounds', a chemical known to the state of California to cause cancer.

California No Significant Risk Level: None of the chemicals in this product are listed.

## European/International Regulations

**European Labeling in Accordance with EC Directives**

**Hazard Symbols:**

T N

**Risk Phrases:**

R 23/25 Toxic by inhalation and if swallowed.
R 45 May cause cancer.
R 50/53 Very toxic to aquatic organisms, may cause long-term adverse effects in the aquatic environment.

**Safety Phrases:**

S 20/21 When using do not eat, drink or smoke.
S 28 After contact with skin, wash immediately with...
S 45 In case of accident or if you feel unwell, seek medical advice immediately (show the label where possible).
S 60 This material and its container must be disposed of as hazardou s waste.
S 61 Avoid release to the environment. Refer to special instructions /safety data sheets.

**WGK (Water Danger/Protection)**

CAS# 7784-46-5: 3

**Canada - DSL/NDSL**

CAS# 7784-46-5 is listed on Canada's DSL List.

**Canada - WHMIS**

This product has a WHMIS classification of D1A, D2A, D2B.

This product has been classified in accordance with the hazard criteria of the Controlled Products Regulations and the MSDS contains all of the information required by those regulations.

**Canadian Ingredient Disclosure List**

CAS# 7784-46-5 is listed on the Canadian Ingredient Disclosure List.

## Section 16 - Additional Information

**MSDS Creation Date:** 8/05/1998
**Revision #9 Date:** 2/13/2008

*The information above is believed to be accurate and represents the best information currently available to us. However, we make no warranty of merchantability or any other warranty, express or implied, with respect to such information, and we assume no liability resulting from its use. Users should make their own investigations to determine the suitability of the information for their particular purposes. In no event shall Fisher be liable for any claims, losses, or damages of any third party or for lost profits or any special, indirect, incidental, consequential or exemplary damages, howsoever arising, even if Fisher has been advised of the possibility of such damages.*

MSDS Number: **S2858** * * * * * *Effective Date:* ***11/02/01*** * * * * * *Supercedes:* ***11/17/99***

**MSDS** Material Safety Data Sheet

*From*: Mallinckrodt Baker, Inc.
222 Red School Lane
Phillipsburg, NJ 08865

J.T.Baker ®

24 Hour Emergency Telephone: 908-859-2151
CHEMTREC: 1-800-424-9300

National Response in Canada
CANUTEC: 613-996-6666

Outside U.S. And Canada
Chemtrec: 703-527-3887

NOTE: CHEMTREC, CANUTEC and National Response Center emergency numbers to be used only in the event of chemical emergencies involving a spill, leak, fire, exposure or accident involving chemicals.

All non-emergency questions should be directed to Customer Service (1-800-582-2537) for assistance.

# SODIUM ARSENATE HEPTAHYDRATE

## 1. Product Identification

**Synonyms:** Arsenic acid, disodium salt, heptahydrate; sodium acid arsenate heptahydrate; Disodium arsenate, heptahydrate; Sodium arsenate, dibasic, 7-hydrate
**CAS No.:** 7778-43-0 (Anhydrous)
**Molecular Weight:** 312.01
**Chemical Formula:** Na2HAsO4 7H2O
**Product Codes:** 3486

## 2. Composition/Information on Ingredients

| Ingredient | CAS No | Percent | Hazardous |
|---|---|---|---|
| Arsenic Acid, Disodium Salt | 7778-43-0 | 98 - 100% | Yes |

## 3. Hazards Identification

**Emergency Overview**
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**DANGER! MAY BE FATAL IF SWALLOWED OR INHALED. CANCER HAZARD. CONTAINS INORGANIC ARSENIC WHICH CAN CAUSE CANCER. Risk of cancer depends on duration and level of exposure. CAUSES IRRITATION TO SKIN, EYES AND RESPIRATORY TRACT. MAY CAUSE LIVER AND KIDNEY DAMAGE. USE ONLY WITH ADEQUATE VENTILATION AND RESPIRATORY EQUIPMENT.**

**J.T. Baker SAF-T-DATA(tm)** Ratings (Provided here for your convenience)
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Health Rating: 4 - Extreme (Cancer Causing)
Flammability Rating: 0 - None
Reactivity Rating: 1 - Slight
Contact Rating: 1 - Slight
Lab Protective Equip: GOGGLES; LAB COAT; VENT HOOD; PROPER GLOVES
Storage Color Code: Blue (Health)
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**Potential Health Effects**
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**Inhalation:**
Arsenic may cause inflammation of the mucous membranes with cough and foamy sputum, restlessness, dyspnea, cyanosis, and rales. Symptoms like those from ingestion exposure may follow. May cause pulmonary edema.
**Ingestion:**
Arsenic is highly toxic! May cause burning in esophagus, vomiting, and bloody diarrhea. Symptoms of cold and clammy skin, low blood pressure, weakness, headache, cramps, convulsions, and coma may follow. May cause damage to liver and kidneys. A suspected fetal toxin. Death may occur from circulatory failure. Estimated lethal dose 120 milligrams.
**Skin Contact:**
May cause irritation, symptoms including redness, itching, and pain.
**Eye Contact:**
May cause irritation with itching, burning, watering of eyes; may cause conjunctiva damage.
**Chronic Exposure:**
Arsenic on repeated or prolonged skin contact may cause bronzing of the skin, edema, dermatitis, and lesions. Repeated or prolonged inhalation of dust may cause damage to the nasal septum. Chronic exposure from inhalation or ingestion may cause hair and weight loss, a garlic odor to the breath and perspiration, excessive salivation and perspiration, central nervous system damage, hepatitis, gastrointestinal disturbances, cardiovascular damage, and kidney and liver damage. Arsenic compounds are known human carcinogens and may be teratogenic based on effects in laboratory animals.
**Aggravation of Pre-existing Conditions:**
No information found.

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# 4. First Aid Measures

**Inhalation:**
Remove to fresh air. If not breathing, give artificial respiration. If breathing is difficult, give oxygen. Get medical attention.

**Ingestion:**
Induce vomiting immediately as directed by medical personnel. Never give anything by mouth to an unconscious person. Get medical attention immediately.
**Skin Contact:**
Immediately flush skin with plenty of water for at least 15 minutes while removing contaminated clothing and shoes. Get medical attention immediately. Wash clothing before reuse. Thoroughly clean shoes before reuse. Contaminated work clothes should be laundered by individuals who have been informed of the hazards of exposure to this substance.
**Eye Contact:**
Immediately flush eyes with plenty of water for at least 15 minutes, lifting lower and upper eyelids occasionally. Get medical attention immediately.

**Note to Physician:**
If emesis if unsuccessful after two doses of Ipecac, consider gastric lavage. Monitor urine arsenic level. Alkalization of urine may help prevent disposition of red cell breakdown products in renal tubular cells. If acute exposure is significant, maintain high urine output and monitor volume status, preferably with central venous pressure line. Abdominal X-rays should be done routinely for all ingestions. Chelation therapy with BAL, followed by n-penicillamine is recommended, but specific dosing guidelines are not clearly established.

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# 5. Fire Fighting Measures

**Fire:**
Not considered to be a fire hazard.
**Explosion:**
Not considered to be an explosion hazard.
**Fire Extinguishing Media:**
Use any means suitable for extinguishing surrounding fire.
**Special Information:**
In the event of a fire, wear full protective clothing and NIOSH-approved self-contained breathing apparatus with full facepiece operated in the pressure demand or other positive pressure mode.

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# 6. Accidental Release Measures

Ventilate area of leak or spill. Wear appropriate personal protective equipment as specified in Section 8. Spills: Sweep up and containerize for reclamation or disposal. Vacuuming or wet sweeping may be used to avoid dust dispersal.

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# 7. Handling and Storage

Keep in a tightly closed container, stored in a cool, dry, ventilated area. Protect against physical damage. Isolate from incompatible substances. Wear special protective equipment (Sec. 8) for maintenance break-in or where

exposures may exceed established exposure levels. Wash hands, face, forearms and neck when exiting restricted areas. Shower, dispose of outer clothing, change to clean garments at the end of the day. Avoid cross-contamination of street clothes. Wash hands before eating and do not eat, drink, or smoke in workplace. Containers of this material may be hazardous when empty since they retain product residues (dust, solids); observe all warnings and precautions listed for the product.

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# 8. Exposure Controls/Personal Protection

**Airborne Exposure Limits:**
-OSHA Permissible Exposure Limit (PEL):
10 ug(As)/m3 ppm (TWA)
-ACGIH Threshold Limit Value (TLV):
0.01 mg(As)/m3 (TWA),
listed as A1, confirmed human carcinogen.
**Ventilation System:**
A system of local and/or general exhaust is recommended to keep employee exposures below the Airborne Exposure Limits. Local exhaust ventilation is generally preferred because it can control the emissions of the contaminant at its source, preventing dispersion of it into the general work area. Please refer to the ACGIH document, *Industrial Ventilation, A Manual of Recommended Practices*, most recent edition, for details.
**Personal Respirators (NIOSH Approved):**
If the exposure limit is exceeded and engineering controls are not feasible, a half-face high efficiency particulate respirator (NIOSH type N100 filter) may be worn for up to ten times the exposure limit or the maximum use concentration specified by the appropriate regulatory agency or respirator supplier, whichever is lowest. A full-face piece high efficiency particulate respirator (NIOSH type N100 filter) may be worn up to 50 times the exposure limit, or the maximum use concentration specified by the appropriate regulatory agency or respirator supplier, whichever is lowest. If oil particles (e.g. lubricants, cutting fluids, glycerine, etc.) are present, use a NIOSH type R or P filter. For emergencies or instances where the exposure levels are not known, use a full-facepiece positive-pressure, air-supplied respirator. WARNING: Air-purifying respirators do not protect workers in oxygen-deficient atmospheres.
**Skin Protection:**
Wear impervious protective clothing, including boots, gloves, lab coat, apron or coveralls, as appropriate, to prevent skin contact.
**Eye Protection:**
Use chemical safety goggles and/or full face shield where dusting or splashing of solutions is possible. Maintain eye wash fountain and quick-drench facilities in work area.
**Other Control Measures:**
Any area where inorganic arsenic is stored, handled, used, etc., must be established as a 'Regulated Area' with controlled access, limited to authorized persons. Containers of inorganic arsenic and Regulated Areas must be labeled to show a CANCER SUSPECT AGENT is present. Eating, drinking, and smoking should not be permitted in areas where solids or liquids containing arsenic or lead compounds are handled, processed, or stored. See OSHA substance-specific standard for more information on personal protective equipment, engineering and work practice controls, medical surveillance, record keeping, and reporting requirements. (arsenic: 29 CFR 1910 .1018; lead: 29 CFR 1910.1025).

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# 9. Physical and Chemical Properties

**Appearance:**
Colorless crystals.
**Odor:**
Odorless.
**Solubility:**
61g/100ml water @ 15C.
**Density:**
1.87
**pH:**
Aqueous soln. is alkaline to litmus.
**% Volatiles by volume @ 21C (70F):**
0
**Boiling Point:**
150C (302F) Decomposes.
**Melting Point:**
57C (135F) If heated rapidly, becomes anhydrous at 100C (212F) .
**Vapor Density (Air=1):**
No information found.
**Vapor Pressure (mm Hg):**
No information found.
**Evaporation Rate (BuAc=1):**
No information found.

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# 10. Stability and Reactivity

**Stability:**
Stable under ordinary conditions of use and storage.
**Hazardous Decomposition Products:**
Emits toxic fumes of arsenic when heated to decomposition.
**Hazardous Polymerization:**
Will not occur.
**Incompatibilities:**
Acids; iron, aluminum, and zinc in the presence of water. Strong reducing agents.
**Conditions to Avoid:**
Incompatibles.

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# 11. Toxicological Information

No LD50/LC50 information found relating to normal routes of occupational exposure. Investigated as a mutagen.

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--------\Cancer Lists\------------------------------------------------------
                                    ---NTP Carcinogen---
Ingredient                          Known    Anticipated    IARC Category
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| | | | |
|---|---|---|---|
| Arsenic Acid, Disodium Salt (7778-43-0) | Yes | No | 1 |

# 12. Ecological Information

**Environmental Fate:**
No information found.
**Environmental Toxicity:**
No information found.

# 13. Disposal Considerations

Whatever cannot be saved for recovery or recycling should be handled as hazardous waste and sent to a RCRA approved waste facility. Processing, use or contamination of this product may change the waste management options. State and local disposal regulations may differ from federal disposal regulations. Dispose of container and unused contents in accordance with federal, state and local requirements.

# 14. Transport Information

**Domestic (Land, D.O.T.)**
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**Proper Shipping Name:** RQ, SODIUM ARSENATE
**Hazard Class:** 6.1
**UN/NA:** UN1685
Packing Group: II
**Information reported for product/size:** 500G

**International (Water, I.M.O.)**
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**Proper Shipping Name:** SODIUM ARSENATE
**Hazard Class:** 6.1
**UN/NA:** UN1685
Packing Group: II
**Information reported for product/size:** 500G

**International (Air, I.C.A.O.)**
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**Proper Shipping Name:** SODIUM ARSENATE
**Hazard Class:** 6.1
**UN/NA:** UN1685
Packing Group: II
**Information reported for product/size:** 500G

# 15. Regulatory Information

--------\Chemical Inventory Status - Part 1\---------------------------------

| Ingredient | TSCA | EC | Japan | Australia |
|---|---|---|---|---|
| Arsenic Acid, Disodium Salt (7778-43-0) | Yes | Yes | Yes | Yes |

--------\Chemical Inventory Status - Part 2\---------------------------------

| Ingredient | Korea | --Canada-- DSL | NDSL | Phil. |
|---|---|---|---|---|
| Arsenic Acid, Disodium Salt (7778-43-0) | Yes | Yes | No | No |

--------\Federal, State & International Regulations - Part 1\----------------

| Ingredient | -SARA 302- RQ | TPQ | ------SARA 313------ List | Chemical Catg. |
|---|---|---|---|---|
| Arsenic Acid, Disodium Salt (7778-43-0) | No | No | No | Arsenic comp |

--------\Federal, State & International Regulations - Part 2\----------------

| Ingredient | CERCLA | -RCRA- 261.33 | -TSCA- 8(d) |
|---|---|---|---|
| Arsenic Acid, Disodium Salt (7778-43-0) | No | No | No |

Chemical Weapons Convention: No TSCA 12(b): No CDTA: No
SARA 311/312: Acute: Yes Chronic: Yes Fire: No Pressure: No
Reactivity: No (Mixture / Solid)

**WARNING:**
THIS PRODUCT CONTAINS A CHEMICAL(S) KNOWN TO THE STATE OF CALIFORNIA TO CAUSE CANCER.

**Australian Hazchem Code:** 2X
**Poison Schedule:** None allocated.
**WHMIS:**
This MSDS has been prepared according to the hazard criteria of the Controlled Products Regulations (CPR) and the MSDS contains all of the information required by the CPR.

# 16. Other Information

**NFPA Ratings:** Health: **3** Flammability: **0** Reactivity: **0**
**Label Hazard Warning:**
DANGER! MAY BE FATAL IF SWALLOWED OR INHALED. CANCER HAZARD. CONTAINS INORGANIC ARSENIC WHICH CAN CAUSE CANCER. Risk of cancer depends on duration and level of exposure. CAUSES IRRITATION TO SKIN, EYES AND RESPIRATORY TRACT. MAY CAUSE LIVER

AND KIDNEY DAMAGE. USE ONLY WITH ADEQUATE VENTILATION AND RESPIRATORY EQUIPMENT.

**Label Precautions:**

Do not get in eyes, on skin, or on clothing.
Do not breathe dust.
Keep container closed.
Use only with adequate ventilation.
Wash thoroughly after handling.

**Label First Aid:**

If swallowed, induce vomiting immediately as directed by medical personnel. Never give anything by mouth to an unconscious person. Get medical attention immediately. If inhaled, remove to fresh air. If not breathing, give artificial respiration. If breathing is difficult, give oxygen. In case of contact, immediately flush eyes or skin with plenty of water for at least 15 minutes. Remove contaminated clothing and shoes. Wash clothing before reuse. In all cases, get medical attention.

**Product Use:**

Laboratory Reagent.

**Revision Information:**

MSDS Section(s) changed since last revision of document include: 8.

**Disclaimer:**

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