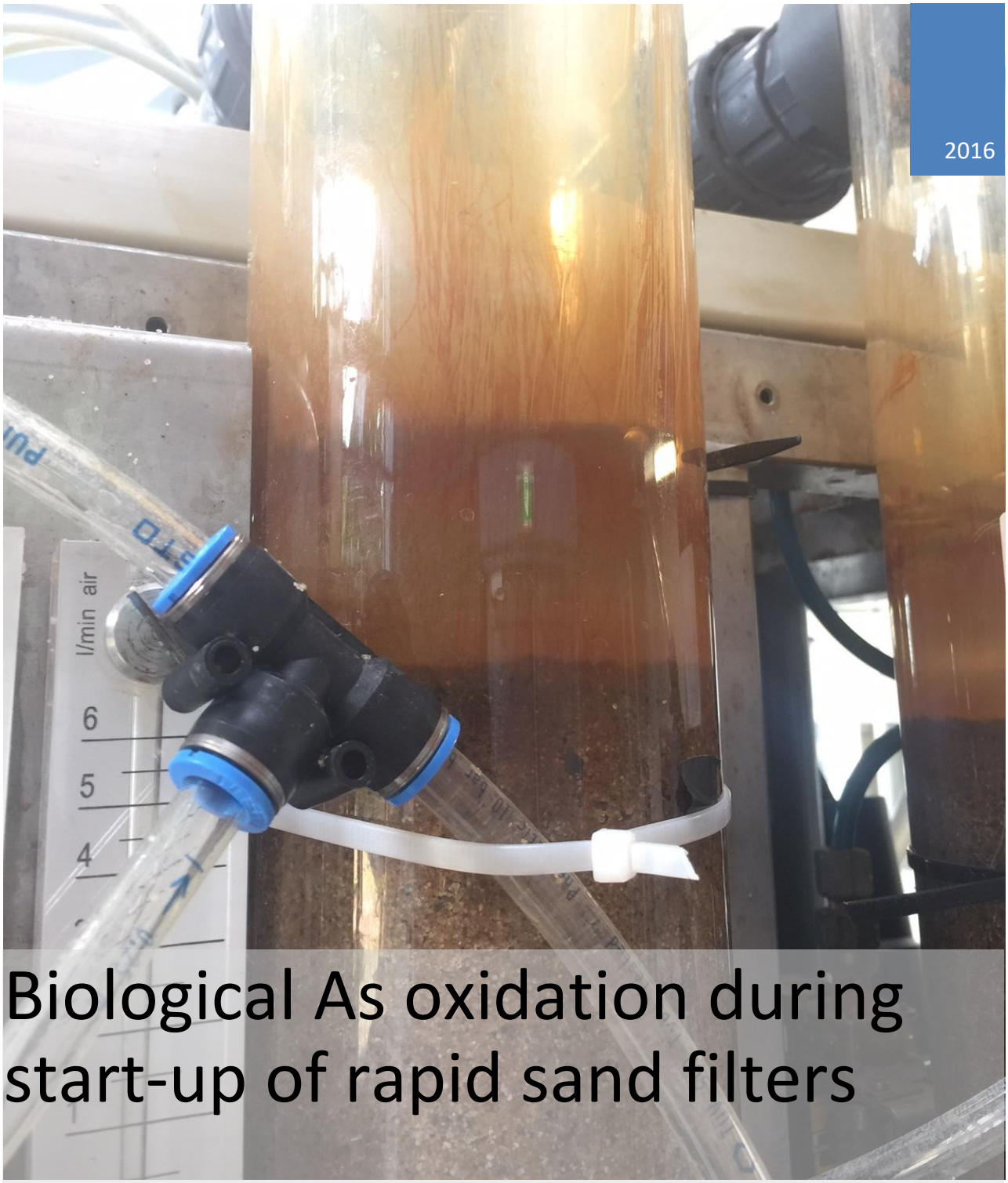
2016



Biological As oxidation during start-up of rapid sand filters

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Abstract

Due to more stringent regulation of the concentration of arsenic in drinking water in the Netherlands, Dutch water companies treating (ground)water containing arsenite (As(III)) need to re-evaluate and extend their treatment process. While As(III) is difficult to remove in conventional treatment (aeration, rapid sand filtration), oxidation to negatively charged arsenate (As(V)) is crucial for sufficient removal. This study aims to investigate the oxidation processes during start-up of rapid sand filters. For this purpose, several column experiments were done with drinking water (tap water) and raw groundwater, containing As(III) to identify the oxidation processes related to As(V) formation. Four columns were pre-loaded with tap water (1), tap water spiked with As(III) (2), tap water spiked with NH_4^+ (3) and tap water spiked with Mn(II) (4). In these columns bacteria were accumulated based on their specific influent and eventually tested on As(III) oxidation abilities. The columns pre-loaded with tap water only and tap water spiked with NH_4^+ experiments were repeated, after full nitrification these columns were loaded with As(III) for 21 days. Finally two sets of columns with fresh sand (1) and As(III) pre-loaded sand (2) were fed with raw groundwater for 50 days.

Results showed that As(III) can be oxidized for 97%, in 38 days in columns filled with virgin filter sand, with only tap water and As(III) as influent. Columns fed with tap water only and tap water spiked with Mn(II) did not show As(III) oxidizing results. Tap water spiked with NH_4^+ showed oxidation after 24 hours of As(III) loading. Pre-loaded columns with tap water spiked with ammonium (NH_4^+) and tap water only were both able to oxidize the As(III) concentration more than 90% in 14 and 21 days respectively.

In columns with virgin sand fed with raw groundwater was observed that after 14 days As(III) was oxidized to more than 90%, while NH_4^+oxidation took 26 days for >90% oxidation and manganese removal was not complete at the end of the experiment (50 days). In these raw groundwater experiments iron and phosphate were removed immediately for 99% and >80% respectively. Columns pre-loaded with As(III) immediately and continuously oxidized more than 97% of As(III), whereas iron, phosphate and NH_4^+ removal efficiencies were similar to virgin filter sand fed with raw groundwater. Only nitrite removal completed 7 days earlier in As(III) pre-loaded columns, compared to columns with virgin filter sand.

The general conclusion is, that during start-up of rapid sand filters As(III) oxidation is rapid, biotic and starts before the other microbial processes tested in this thesis: NH_4^+, nitrite and manganese oxidation. It is hypothesized that specific As(III) oxidizing bacteria are responsible for the oxidation of As(III).

Contents

Glossary

Accumulating biomass	: Increasing amount of bacteria (in column) by feeding specific substrate (NH_4^+, Mn(II), As(III))
Filtrate/effluent	: Name for water coming out of the filter column
Oxidase:	: "Any of a group of enzymes that bring about biological oxidation" ("Collins English Dictionary - Complete & Unabridged 10th Edition," 2017)
Pre-loading	: Period of dosing a certain chemical to create optimal column conditions, prior to the real goal of the experiment (As(III) dosing)
Spiked with …	: Chemical added to main water flow
Supernatant water	: Name for water in the top part of the column, above the filter sand
Tap water	: Water from the tap: drinking water
Virgin filter sand	: Sand directly out of sand bag, no (intentional) pre-treatment

Preface and acknowledgement

With pleasure I present to you my final version of my thesis report. After spending nearly a year on this very interesting but challenging topic, I'm quite satisfied with the results and findings. After finishing my HBO in Amsterdam, I didn't quite have the feeling I actually understood much of water treatment and water management in general. It didn't take a lot of time to decide that TU Delft would be the perfect place to at least learn something specific about water and water treatment. Looking back now, this was probably one of the best decisions I have made thus far.

During my thesis I have worked shortly at the TU, getting to know my experimental set up, mainly at Brabant Water's water treatment plant Dorst or Breda office and finally in Delft again for finishing the report. Especially the beginning of the thesis, reading a lot of unknown literature, getting used to terms frequently used, and finding a good direction of literature to focus on, were challenges for me. Once the experiments got more concrete and we could start the experiments, time seemed to fly as weeks passed by quickly. During the experiments I constantly had the feeling things could go wrong any minute, leaking pipes, failing pumps, missing results, crashing dropbox servers, but with all the experiments we've performed, there were only minor obstacles.

One of the things I have learned from my supervisors is that measured values, even from a professional lab, always need interpretation and common sense to be understood fully. This was critical in cases of confirmation of expected results, but even more in cases of unexpected results. Moreover, I've learned that academic research is not only criticizing your own data, but also other academic research.

I want to thank my committee for the guidenance and support. Jink Gude, I want to thank you for all the phone calls, contagious enthusiasm and answers to all the questions I could think of. Tim van Dijk, I want to thank you and Brabant Water for the opportunity to do all my experiments at Dorst and desk work at the Breda office. You facilitated everything I needed and stimulated my progress in Dorst. Doris van Halem, I want to thank you for linking me to Jink at the beginning of my thesis and convincing me that arsenic is a very interesting topic to explore (starting with Nicaragua). Furthermore I want to thank you for the support and encouragements during the thesis. To conclude, I want to thank Luuk Rietveld for the positive feedback and overall guidance of my thesis.

To everyone who takes the effort to read our findings, I hope you enjoy reading it!

1. Introduction

Currently, groundwater is typically treated with an aeration and rapid (sand) filtration concept in the Netherlands. Nevertheless, more and more activated carbon filters and dosages of strong oxidants like $KMnO_4$ are implemented in the groundwater treatment scheme to remove emerging contaminants, such as organic micro-pollutants and trace inorganic contaminants. One of the trace elements which has recently gained much interest, globally and in the Netherlands, is arsenic (As). Globally a limit of 10 µg/L has been set by the World Health Organisation (WHO) for maximum As concentration, a maximum which should be reachable by conventional treatment (WHO, 2011). In the Netherlands awareness has been created about possible adverse health effects even with low As levels in drinking water between 1 – 10 µg/L (van Halem et al., 2009). For this purpose, with consensus of all Dutch drinking water companies, a new 'VEWIN guideline' of 1 µg/L is implemented. This change in policy results in new challenges for several water companies in the Netherlands, especially those who extract groundwater with traces of As, and who will need to update their current treatment systems to reach this new target value.

1.1 Arsenic

As is a known toxic element, classified by the International Agency for Research on Cancer (IARC) as a group 1 human carcinogen (IARC, 2004). In the past As was used as medicine and poison, but currently it is identified as contamination in groundwater and as a major threat to human health (IARC, 2004; Nriagu, 2002; WHO, 2011). Trivalent As(III) and pentavalent As(V) inorganic As is most commonly found in natural waters and the main concern of toxicologists is about As in drinking water sources (Hughes, 2002; Smedley and Kinniburgh, 2002). The carcinogenic effect results in skin-, lung- and bladder cancer, milder symptoms are skin lesions and diabetes (Cebrian et al., 1983; Tseng et al., 1968; WHO, 2011).

As(III) oxidation is imperative for complete As(III) removal during conventional groundwater treatment consisting of aeration and rapid filtration. At neutral pH As(III) is not charged and more difficult to remove compared to negatively charged As(V), which can be adsorbed in rapid sand filters (van Halem et al., 2009).

1.2 Processes in a rapid sand filter

It was found that in typical groundwater treatment plants As is oxidized and removed in the rapid sand filtration step, where aeration did not add significantly to As(III) oxidation (Cherry et al., 1979; Gude et al., 2016). Within a rapid sand filter iron (Fe), manganese (Mn) are removed, chemically and/or biotically, where NH_4^+ removal is commonly a biological process (Stembal et al., 2004). As interacts with these elements and products formed within the sand filter, described in the rest of this section.

Fe is the most ubiquitous element in groundwater, its interaction with As(III and V) has been studied in great detail and can play an important role in As control after formation of Fe oxides (HFOs) from oxidation of Fe(II), already present in groundwater (Benefield and Morgan, 1990). As(III) and As(V) can both be adsorbed onto, among others, amorphous HFOs (Pierce and Moore, 1982; Wilkie and Hering, 1996), Goethite (Manning et al., 1998) and Ferrihydrite (Raven et al., 1998). Trace elements like phosphate (PO_4^{3-} (Gao and Mucci, 2001), silica and bicarbonate (Appelo et al., 2002; Meng et al., 2000) compete with As for sorption sites, where the first is the most effective competitor.

Another metal which is frequently found in groundwater is Mn, which is removed by chemical and/or biological oxidation, although the precise pathway is not known (Bruins et al., 2015). Mn oxides can be formed according to different pathways, producing different (intermediate) complexes including Birnessite and Hausmannite (Stumm and Morgan, 1996). These oxides can function as As oxidizers (Oscarson et al., 1981) and while abiotical Mn oxides can oxidize As(III), the oxidizing effect decreases within 3 days (Driehaus et al., 1995). After this reduction in oxidation capacity, bacteria enhance the oxidization after a period of 10 days (Driehaus et al., 1995). The latter research shows that As oxidation with Mn oxides is also a biotical process. It was found by Jones et al. (2012) that microbes in combination with Mn oxides provide the quickest oxidation of As(III).

NH_4^+ in groundwater treatment plants is biologically removed in two steps, first NH_4^+ is oxidized to nitrite (NO_2^-) via ammonia-oxidizing prokaryotes which includes ammonia-oxidizing bacteria and archaea (AOB and AOA) (Van Der Wielen et al., 2009). Once NO_2^- is formed it is oxidized to nitrate (NO_3^-) by NO_2^- oxidizing bacteria (NOB). Both types of oxidation use oxygen as their electron acceptor (Metcalf et al., 2004). Generally nitrification is a result of bacteria classified as β- and γ-Proteobacteria (Norton et al., 2002). Throughout this report microbiological terms will be mentioned, a short explanation of some of the key principles relevant to this thesis are shown in box 1.

Intermezzo for non-microbiologists

Taxonomy of bacteria.

To differentiate between bacteria they have been classified and identified in order to collect or separate them for scientists (Baron, 1996). There are three domains of life distinguished: Bacteria, Archaea and Eukarya. Bacteria and archaea can also be called Prokarya; Eukarya consist of Plantae, Animalia, Protista and Fungi (so called kingdoms). In the domain of bacteria further details are added with each new layer, as bacteria are further specified. Classification from general to very specific is as follows: Domain, Kingdom, Phylum, Class, Order, Family, Genus and finally Species. In this thesis terms like Proteobacteria and γ-Proteobacteria will be mentioned, they belong to the Phylum and Class taxonomy respectively.

Metabolism.

Microorganisms can use different kinds of metabolism. Bacteria can have differences in energy source, electron donor type and preferred carbon source. An overview of the different forms is given in table 1.

Table 1: Overview of different types of metabolisms

Type	Energy source	Electron donor	Carbon source	
Chemo-	Redox reaction			-Troph = eater
Photo-	Sun light			
Litho-		Inorganic compound		
Organo-		Organic compound		
Auto-			Inorganic carbon (f.e. CO_2)	
Hetero-			Organic carbon	

To characterize a type of bacteria properly, combinations can be made such as chemolithoautotrophic bacteria. Indication that these bacteria have a redox reaction as energy source with inorganic compounds as electron donor, and an inorganic carbon source

Box 1: Selection of important microbiological terms and principles explained

The importance of biological activity in sand filters has been the topic of several pilot- and full scale studies. A case study in a Greek water treatment plant (WTP) showed that during biological oxidation most of the As(III) was oxidized, simultaneous with Mn and NH_4^+ (Katsoyiannis et al., 2008). As(V) was removed in subsequent coagulation and filtration stages. In research done by Yang et al. (2015) a Mn removing filter bed was created, by a start-up period with Mn(II) and tap water as influent, was used to oxidize As concentrations up to 2.5 mg/L down to just 10 µg/L. It was suggested that As(III) was mainly oxidized by bacteria present in the media, after analysis of the filter sand in batch experiments. It was shown that on sterilized sand As(III) was considerably less oxidized than on non-sterilized sand (5.47% against 60.97%).

Yang et al. (2015) did research with high concentrations (400-2500 µg/L), where other studies did comparable research with low concentrations (Katsoyiannis et al., 2004; Lytle et al., 2007). Low levels of As

(34-42 µg/L) were studied in an upflow column experiment. Stated was that all the As(III) was oxidized biologically (Katsoyiannis et al., 2004). The researchers suggest that oxidation of As(III) was catalysed by the same bacteria responsible for Mn(II) oxidation. It is proposed that Leptothrix ochracea (responsible for oxidation of Mn(II)) releases a catalase, acting as H_2O_2 which in its turn oxidizes As(III). Another study (Katsoyiannis and Zouboulis, 2004) with groundwater, spiked with 40-200 µg/L As(III), has suggested that the microorganisms Gallionella ferruginea and Leptothrix ochracea are the microorganisms responsible for the biological As(III) oxidation and As removal.

1.3 As oxidation in nature

Lami et al. (2013) found that a soil fraction contaminated with As was capable of biologically oxidizing a continuous As dosing. Increased rates of oxidation were found when organic material (carbon source) was added to the influent, the latter was also found in a study done with isolated strains (Santini et al., 2002). In a research done by Macur et al. (2004), similar to Lami et al. (2013), bacteria in As contaminated soil were analysed on their As oxidation potential. Sterile controls and bacteria-free columns did not show any oxidation, where columns inoculated with As-oxidizing bacteria soil oxidized 75 and 200 µM As(III). Dosing As in 'living soil' results in a shift to microorganisms capable of detoxifying their surroundings, rather than using As as a source of energy (Lami et al., 2013; Macur et al., 2004).

1.4 As(III) oxidation by bacteria

In the above mentioned studies the important role of the microbiology in groundwater treatment plants, and nature in general, is described. This paragraph describes bacteria capable of oxidizing As, arsenic oxidizing bacteria (AsOB), and the mechanisms and principles of interest.

1.4.1 Aerobic As(III) oxidation

In general there are two types of AsOB's: heterotrophic As(III) oxidizing bacteria (HAOs) (Ike et al., 2008; Muller et al., 2003; Wan et al., 2010) and chemolithoautotrophic As(III) oxidizers (CAOs) (Battaglia-Brunet et al., 2002; Rhine et al., 2005; Wan et al., 2010). HAOs oxidize As(III) mainly as a detoxification mechanism (Huang, 2014; Tsai et al., 2009; Vanden Hoven and Santini, 2004). CAOs can use As(III) in their redox reaction as electron donor, in their reaction with CO_2 and O_2 (Santini et al., 2000). Both are believed to gain energy from As(III) oxidation (Santini and Vanden Hoven, 2004; Vanden Hoven and Santini, 2004).

CAOs use inorganic carbon (CO_2, $NaHCO_3$) as their source of carbon and for their metabolism less nutrition elements are required, compared to HAOs. Since organic compounds are scarce in water (order of magnitude mg/L's), it is more likely to come across CAOs as they can easier adapt in water (Wan et al., 2010). Different microorganisms can catalyze the oxidation of As, such as Alcaligenes faecalis, hydrogenophage sp., A. ferrooxidans, T. aquaticus and T. thermopohilus (Gihring and Banfield, 2001; Stolz et al., 2006; Zhang et al., 2007). Over 10 different AsOB from various sources have been reported (Suttigarn and Wang, 2005).

As interactions with bacteria

By excluding As(III) from the cell or binding them in the cell, As(III) is detoxified. It is hypothesized that the cell has both intra- and extra-cellular interactions with As(III) (Huang, 2014). Biosorption of As(III) and As(V) on the extracellular cell surface is the result of the electrostatic reactions with hydroxyl, amide and amino groups on those cell surfaces (Giri et al., 2013; Yan et al., 2010). Another method for detoxification is bioaccumulation of As intracellularly, in the cytoplasm and membrane of the cell (Takeuchi et al., 2007; Xie et al., 2013). Inside the cells As(III) complexes are formed with proteins and peptides consisting of thiol groups (Huang, 2014). These and other mechanisms are shown in figure 1.

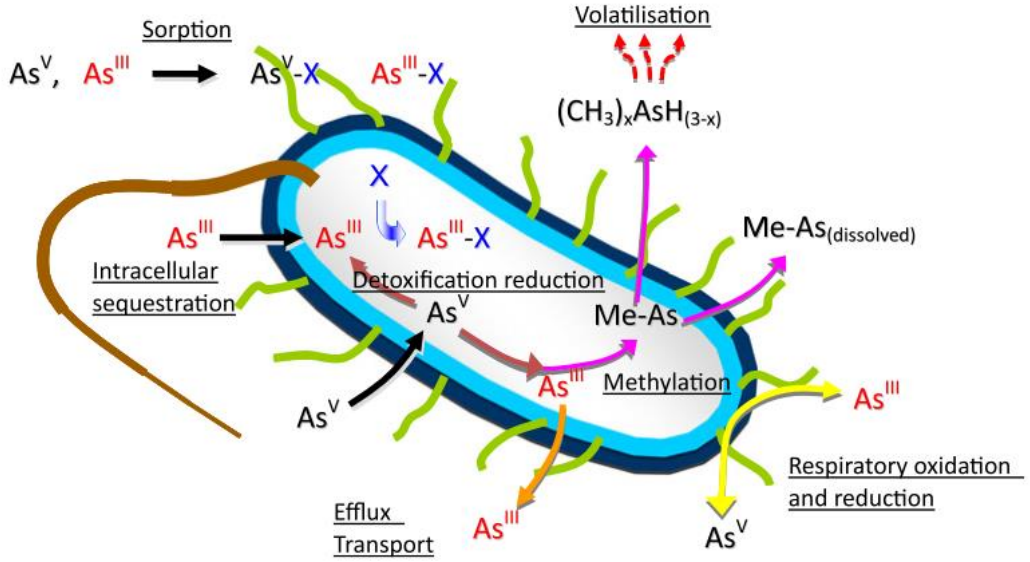

Figure 1: Schematic representation of the different interaction between As species and a microorganism (Huang, 2014)

Intracellular uptake of As(V) in the cells of bacteria goes via already present transportation pathways, due to the similarity of As(V) with PO_4^{3-} (Tsai et al., 2009). As(III) on the other hand is similar to glycerol, therefore glycerol transporters and aquaporin transporters are responsible for As(III) uptake into the cell (Huang, 2014; Tsai et al., 2009).

Genes and presence

Bacteria use the As(III) oxidase enzyme for oxidation of As(III) (Ellis et al., 2001). The As(III) oxidation takes place within the outer surface of the bacterial membrane (Anderson et al., 1992). In both CAOs and HAOs (Muller et al., 2003; Santini et al., 2000) As(III) oxidase was found, containing two subunits: large subunit (AioA) and a small subunit (AioB) (Lett et al., 2012). AioA encoding genes have been found in several strains, where the majority of the mesophilic branches (microorganisms that prefer median temperatures, 15-40 ^oC, in which they are most active) can be divided into two groups: (I) α-Proteobacteria and (II) β- and γ-Proteobacteria (Yamamura and Amachi, 2014).

Genes similar to AioA have been found in environments with both abundance of As (order of magnitude: mM's): geothermal environments (Hamamura et al., 2009), As contaminated soil (Sultana et al., 2012) and river (Quéméneur et al., 2010); as well as relatively low amounts (order of magnitude: µM's) of As (Lami et al., 2013; Yamamura et al., 2014). This suggests that these similar genes can be present in the environment independently of As contamination, based on isolates from known As contamination and from environments with low concentrations of As.

1.4.2 Anaerobic oxidation

From anaerobic oxidation less is known, as there are only several cases observed and investigated so far (Yamamura and Amachi, 2014). It has been researched that during denitrification As is oxidized as well. In a natural environment NO_3^- influences the presence of As heavily due to its oxidation capacity of ferrous iron and the resulting As adsorbing oxides (Senn and Hemond, 2002). Besides promoting the creation of adsorbents, it was found that addition of NO_3^- stimulates the microbial oxidation of As(III) and Fe(II) in the

denitrification process in anaerobic environments (Sun et al., 2009). It was shown by Sun et al. (2010) that NO_3^- can be used as an elector acceptor instead of oxygen, where denitrifying bacteria oxidize As(III).

1.5 Knowledge gap:

The presence of microorganisms in a filter bed of a natural aeration and rapid sand filtration process is an undisputed fact (Bruins et al., 2015; de Vet et al., 2011; Katsoyiannis and Zouboulis, 2004). However, since HFO and MnO_2 are present is has not been established what role microorganisms play in As(III) oxidation in the filter bed. This study is done to investigate whether microorganisms common in a rapid sand filter can oxidise As(III). And more specifically whether AsOBs are solely responsible for oxidation or whether other (nitrifying and Mn oxidising) bacteria or processes can co-oxidise As(III).

For this purpose bacterial communities are accumulated in a sand filter using tap water (1) without addition, (2) spiked with As(III), (3) spiked with NH_4^+ and (4) spiked with Mn(II) and tested on their ability to oxidise As(III) within 24h and >24h. The columns with tap water only and spiked with NH_4^+ were subsequently spiked with As(III) for several weeks, to test their As(III) oxidizing ability. Finally, As(III) was pre-loaded on fresh sand for several weeks to form an As(III) oxidizing biomass, followed by several weeks of raw ground water. Another set of columns with fresh sand received raw groundwater for the same period as the pre-loaded As(III) columns. From both types of raw water columns samples were taken to follow As(III) oxidation, As(V) removal and a selection of water quality parameters. In all experiments the start-up (ripening) of the filter was monitored, in aerobic conditions and with an empty bed contact time of approximately 30 minutes.

2. Methods and materials

2.1 Installation and basic settings

The dimensions of the 12 identical columns were 1 m in height and 90 mm in diameter. The columns were filled with 0.5m (+/- 2%) quarts sand (0.4 - 0.8 mm) obtained from 'Aqua Techniek' which is typical filter sand used in rapid sand filters. Before starting the experiment the columns were extensively backwashed with tap water until the supernatant was visually clear. The flowrate used for all experiments performed was set to 105 ml/min resulting in a filtration velocity of 1 m/h (+/- 20%). Supernatant water level was adjustable and the columns were continuously fed throughout the experiment. For the purpose of accumulating the desired biomass in the columns, the following chemicals were added to the supernatant water: tap water (1), tap water spiked with either As(III) (2), NH_4^+ (3) and Mn(II) (4). The 4 settings were executed in triplicate. The peristatic dosing pumps (Cole-Parmer Masterflex L/S) were able to deliver the chemicals in a continuous flow of 1 ml/min. All the columns were covered to block sunlight. The column setup is depicted in Figure 2.

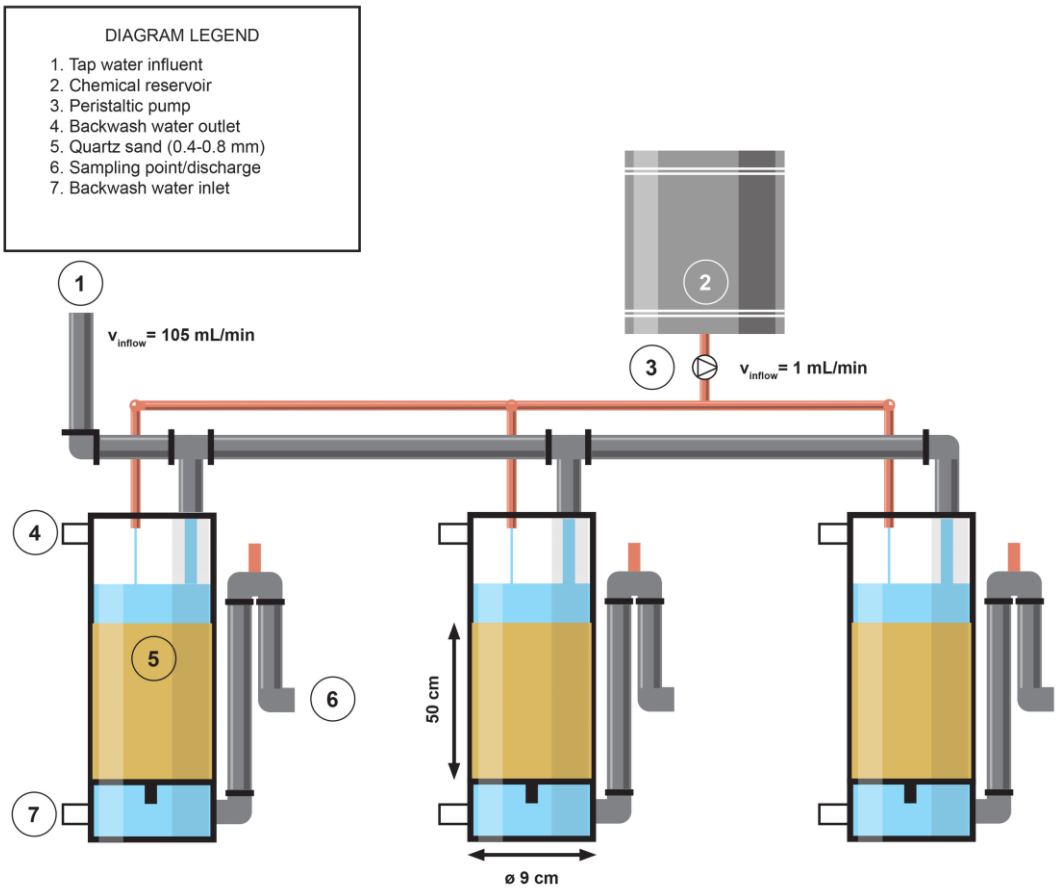

Figure 2: Schematic overview of filter columns

2.2 Chemicals, addition and concentrations

The As(III), Mn(II) and NH_4^+ dosing solutions are prepared from the following reagent grade chemicals: As(III)Cl_3 (Aldrich chemistry, 99.99% trace metals basis), Mn(II)Cl_2, (Aldrich chemistry, 98% beads) and NH_4Cl (Emsure, 99.8%). The chemicals were diluted in tap water to 10.6, 212 and 106 mg/L respectively. To prevent oxidation in the dosing vessels (25L), the vessels containing As(III) and Mn(II) were acidified to pH 3-4 by adding 8-12 ml 5M HNO_3. Columns were cleaned with hypochlorite tablets. The chemicals were continuously pumped into the supernatant water resulting in an influent concentration of 100 µg/L As(III), 2 mg/L Mn(II) and 1 mg/L NH_4^+. Prior to tests with As, homogeneous As(III) oxidation with O_2 was tested with As(III) solution in Dorst' tap water (pH= 7.8). Directly after preparing the solution, 1 h after and 24 h after As(III and V) samples were taken. Resulting in 9, 7 and 14 % As(III) oxidation respectively. Fluctuation of the resin can be observed in the first two measurements and slow oxidation with air must be the reason of increase of As(V) (slow oxidation) after 24h.

2.3 Water quality

The experiments were performed at WTP Dorst (Brabant Water). Both the quality of the tap water and the groundwater is shown in table 2. Groundwater in this treatment plant is treated with $NaMnO_4$, $FeCl_3$, aerated and filtered in rapid sand filters. Tap water is taken from the distribution network within the WTP, while groundwater is taken directly from the raw groundwater influent pipes (prior to any treatment step). Water temperature of tap water was measured between 16-17 ^oC in the supernatant, warming up a degree during filtration. Raw groundwater was between 13-18 ^oC, also warming up slightly during filtration.

Table 2: Tap and groundwater quality at WTP Dorst, tap water quality based on internal report from water company Brabant Water, raw groundwater from data previously published (Gude et al., 2016) *(n.m. is not measured).*

Water quality parameters	***Units***	***Tap water***	***Raw groundwater***
pH	*[-]*	*7.81*	*7.54*
O_2	*mg/L*	*9.07*	*-*
HCO_3	*mg/L*	*238*	*246*
Conductivity	*mS/m*	*39.13*	*440*
ORP	*mV*	*n.m.*	*-134*
As	*µg/L*	*< 1.15*	*13.2*
As(III)	*µg/L*	*n.m.*	*12.7*
Fe	*mg/L*	*<0.010*	*1.40*
Mn	*mg/L*	*<0.010*	*0.036*
NH_4^+	*mg/L*	*<0.03*	*0.42*
NO_3^-	*mg/L*	*2.1*	*n.m.*
PO_4^{3-}	*mg/L*	*0.05*	*0.15*
TOC	*mg/L*	*2.1*	*2.22*
SO_4^{2-}	*mg/L*	*2*	*0*

2.4 Sample and analytical methods

Samples from supernatant water and filtrate were collected in 500 ml bottles and further distributed in individual bottles for transport to a water laboratory for analysis. Supernatant water was directly subtracted from the filter columns. Filtrate was tapped from the discharge tube after the overflow to prevent changing the filtration rate and prevent the filter to run dry. To monitor changes in the columns pH, electrical conductivity (EC) and O_2 were measured with WTW electrodes (SenTix 940, TerraCon 925 and FDO925).

As, Fe and Mn were analysed with Inductively Coupled Plasma Mass Spectrometry (ICP-MS) (Thermo X2-series), NH_4^+, NO_2^-, NO_3^- and PO_4^{2-} were analysed by a discrete analyser spectophotometry (Aquakem 250, company: Thermo Scientific) and As speciation was done according the Clifford (2006) method where the As concentration is measured after contact with an anion resin (Amberlite® IRA-400 chlorite form) letting only the uncharged As(III) species through. As(V) was calculated by subtracting As(III) from the measured total As concentration. The speciation was performed by letting 150 mL of sample water pass through a syringe of 100 mL filled with 80 mL resin. The first 50 mL was discarded, the remaining 100 mL was collected and analysed. The Clifford method is a robust method, however it unavoidably retains 14% of As(III) on average (min=7%, max=23%; n=24).

The biomass accumulation stages were finalised by removing the filter sand from the columns and analysed on adenine triphosphate (ATP) for the purpose of quantifying the active biomass after the different experiments. From a completely filled sampling bottle (100 ml) 1 gram of sand was weighted and 9 ml of ATP free water was added. This sample was shaken shortly where after it was ultrasonically vibrated for 10 minutes in an ultrasonic “Bransson” bath. After this the sample rested for 5 minutes before ATP measurement. Samples were processed by the ATP meter “Centro XS3 LB960” (company: Berthold) using a chemical kit from “BioTHema”. The kit contains an extractant to release ATP, an ATP reagent for the

measurement and an ATP standard to make a reference line. To check the outcome, the lab used an own low and high biological control.

2.5 Microbial community profiling

For microbial community analysis sand samples were harvested and stored at -80 ^{o}C. These samples were used for DNA extraction (UltraClean microbial DNA isolation kit, MO Bio, USA) and the DNA was subjected to QC (on agarosegel, with QuBit and BioAnalyzer) after which bacterial (V3-V4) 16S rRNA genes were amplified and subjected to high throughput sequencing using the Illumina MiSeq platform (BaseClear, Leiden, the Netherlands). Reads were generated using the Illumina Casava pipeline (version 1.8.3), checked using Illumina Chastity filtering plus an in-house protocol (Baseclear) and final assessment was made using the FASTQC quality control tool (version 0.10.0). QIIME workflows were used to generate taxonomic summaries.

2.6 Timeline experiment

The accumulation of biomass to convert substrate of measurable and water treatment significant quantities takes weeks. The first biomass accumulation stage on the specific substrate (tap water, As(III), NH_4^+ and Mn(II)) lasted for 34 days. Without altering substrate concentrations, As(III) was dosed in the tap water influent to increase the As(III) concentration in all columns to 20 µg/L for 6 h for the purpose of testing the four different accumulated biomasses towards their potential ability to oxidise As(III). As(III) and As(V) were measured 2 and 4 hours after dosing. Hereafter the sand was removed from the As(III) columns, put on hold for 8 days before they were disinfected with hypochlorite and filled with fresh filter sand. Mn(II), NH_4^+ and tap water only pre-loaded columns continued for 46 days. The As(III) pre-loaded columns put on hold, continued with a second bioaccumulation stage where As(III) oxidation was closely monitored and after 38 days an exact replication of As(III) dosing after the first bioaccumulation stage was performed. Then all columns were tested on As(III) oxidation at the following intervals: 2, 4, 6 and 24 h. After 24 h As(III) dosing all columns were emptied and filter sand was analysed on ATP. Data of first 24h As(III) dosing session was not shown, since second time dosing of As(III) showed the same and more precise data.

9 out of the 12 columns were used for the third bioaccumulation stage, where, without disinfection, the tap water, As(III) and NH_4^+ loaded columns were commissioned again in triplicate with fresh filter sand. After 39 days the NH_4^+ was completely oxidised to NO_3^- and influent of tap water only and NH_4^+ loaded columns were spiked with 100 µg/L As(III), thereby testing the ability of both types of biomass to oxidise As(III) within 21 days. After 21 days the experiment was ended by empting the columns.

Three As(III) pre-loaded columns (28 days of ripening time and three days outflow of As delay) and three fresh sand column were used for experiments on aerated groundwater (table 2), for the purpose of comparing oxidation of As(III) containing groundwater on fresh sand and on the accumulated biomass from a third As(III) biomass accumulation stage. During the aerated groundwater experiments the raw water quality was measured weekly and the filtrate of the three columns containing fresh sand and the three columns pre-loaded with As(III) oxidising biomass were extensively analysed on As(III), As, Fe, NH_4^+, NO_2^-, Mn, PO_4^{3-}, pH for 50 days. After 50 days the filter sand was extracted and analysed on ATP, following procedure as described above. To give an overview of all setting a timeline was drafted and is depicted in Figure 3.

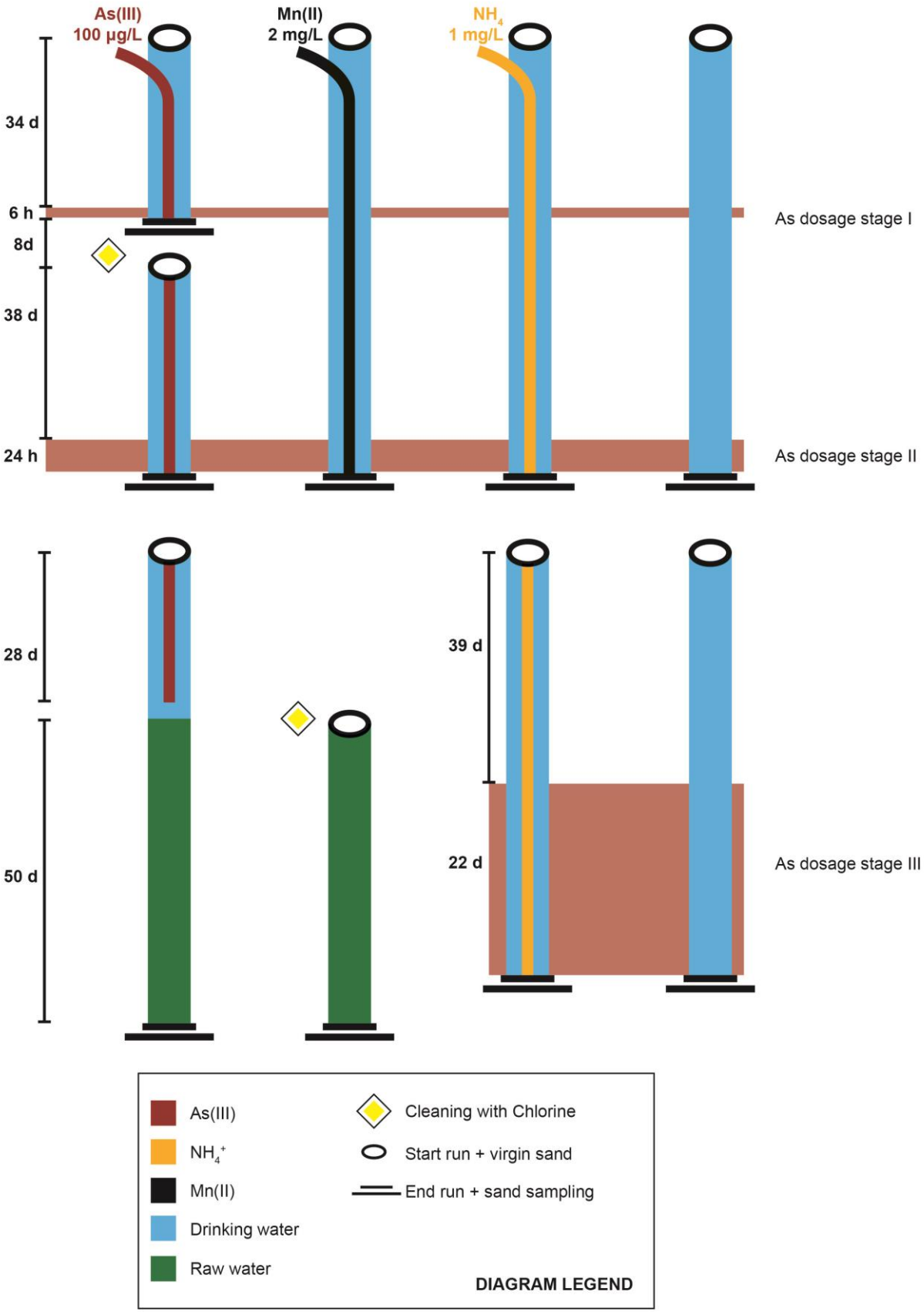

Figure 3: Timeline of biomass accumulation stages and As(III) dosing periods.

3. Results and discussion

The results and discussion section is divided in three elements: accumulating biomass with tap water and specific substrate to observe As(III) oxidation capacity with 24h of 20 µg As(III)/L (I), accumulating biomass with tap water and specific substrate to observe As(III) oxidation capacity with 100 µg As(III)/L for 21 days(II) and monitoring processes in pre-loaded As(III) columns and fresh sand columns with raw groundwater (III). Each of these elements is described in section 3.1, 3.2 and 3.3 respectively.

3.1 Exploring As(III) oxidation mechanisms with tap water

This section describes the obtained results from experiments done with tap water. Columns fed with tap water and As(III) pre-loaded columns are mentioned, followed by NH_4^+ pre-loaded columns, tap water only columns and finally Mn(II) pre-loaded columns. To start, the ATP concentrations of all column experiments are shown, showing the ATP concentrations at the end of the experiments up until As dosage stage II (see figure 3).

3.1.1 ATP results combined

In figure 4 the results of the ATP measurements are shown over the height, of all columns combined. The tap water only, NH_4^+ and Mn(II) pre-loaded columns had the same loading time, while only the second As(III) pre-loaded column experiment is shown here (see figure 3).

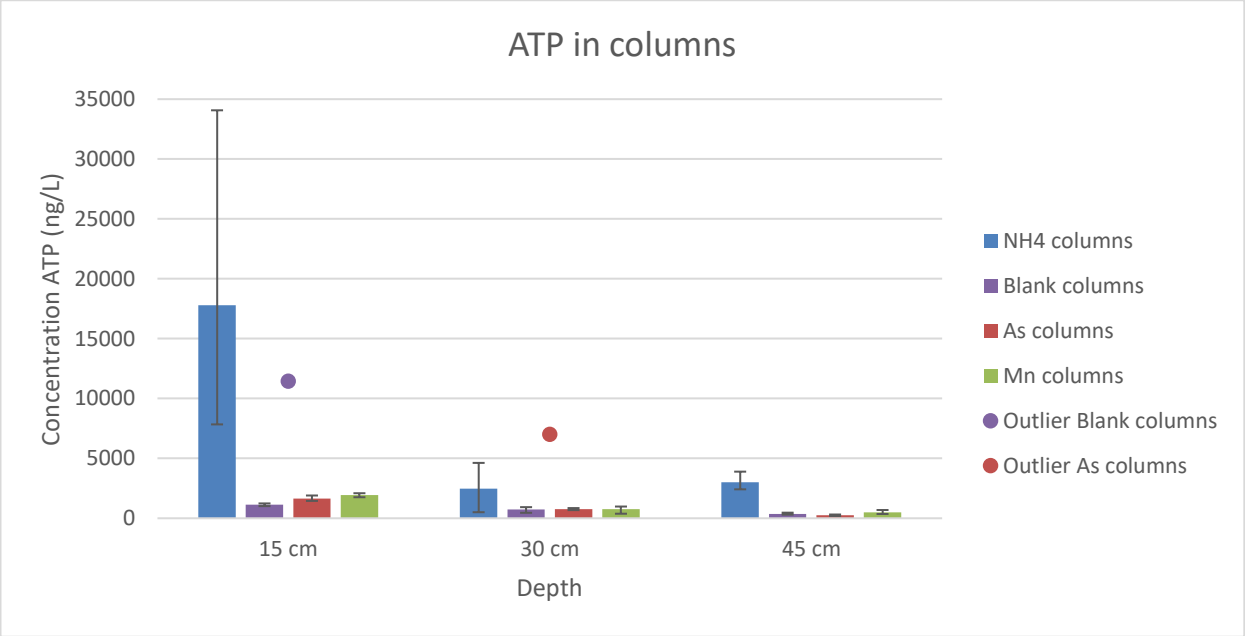

Figure 4: ATP results of all column types, sorted per height

Combining all ATP results showed that in the NH_4^+ pre-loaded columns most bacteria have been found in all the layers, compared to the other columns. In general can be said that all the ATP concentrations found in the top 15 cm were higher than the 30 cm samples, which were subsequently higher than the 45 cm samples. This is support by the general view that most (microbial) activity takes place in the top layer of the bed. In Jessen et al. (2005) it was found that in the top 20 cm of the filter bed 80% of the As(III) was oxidized, and the majority of Fe, Mn and NH_4^+ was removed in the top 20 cm in a pilot scale test done by Lee et al. (2014). Looking at the ATP results, it can be concluded that in the first 15 cm the majority of the bacterial activity was found.

Sand taken straight out of the sand bag was also analysed in triplicate, showing an average ATP concentration of 71 ng/L. Mn(II), As(III) and tap water pre-loaded have respectively average ATP concentrations of 1948, 1648, 1129 ng/L. The difference between ATP concentrations of these three types of columns decreases over depth, 30 cm and 45 cm depth ATP concentrations are similar. The lowest

concentrations measured at 45 cm depth was 246 ng/L (As pre-loaded), an increased concentration of more than 3 times with respect to the sand straight out of the sand bag.

Two measurement errors were encountered and indicated as a purple and red dot, for the tap water and As(III) pre-loaded columns respectively. As each height was sampled in triplicate, it can be observed that both the tap water pre-loaded and As(III) pre-loaded measurement points are far higher than the average of the two other measurements. No statistical analysis was done, but these two points were assumed to be outliers.

3.1.2 Loading columns with As(III) spiked tap water

Start-up of As oxidation

In some studies a bacterial inoculum is used to start an As oxidizing column. In this experiment we tested the possibilities of accumulating a biomass on fresh sand, by simply adding tap water spiked with 100 µg/L As(III) for a period of 38 days. In figure 5 the results of the triplicate can be seen.

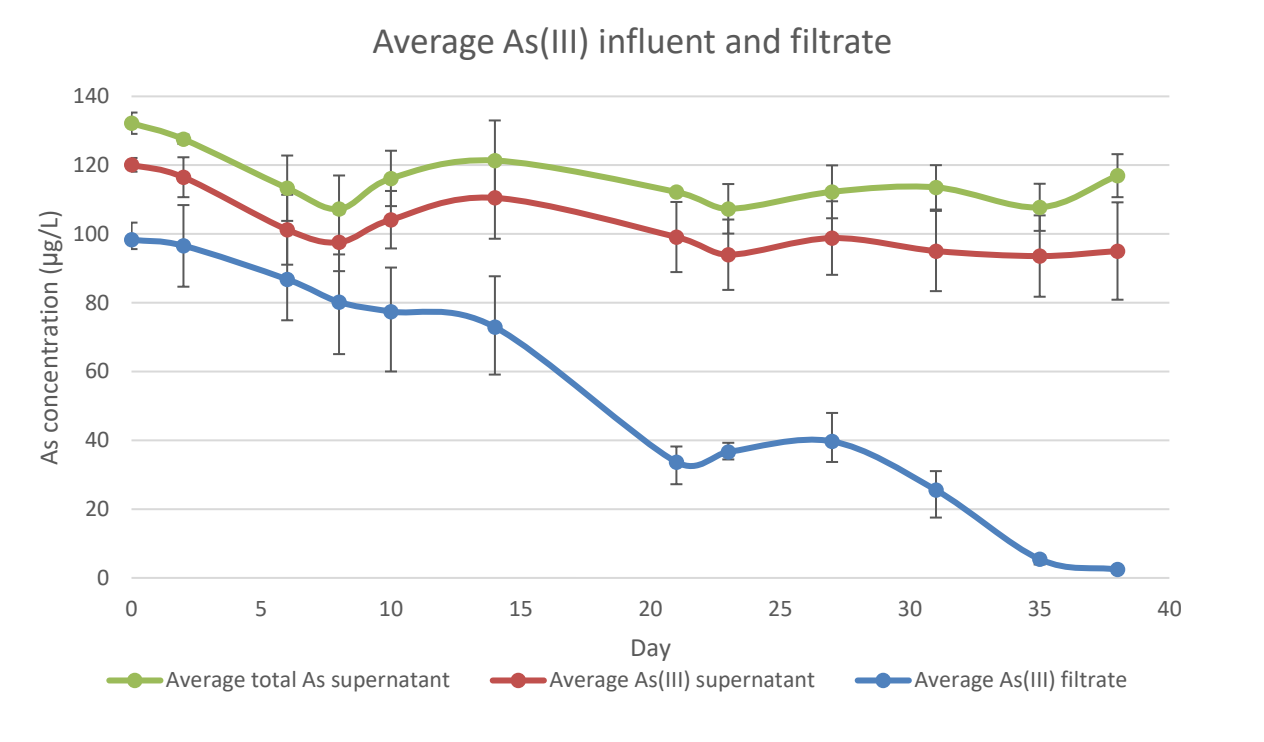

Figure 5: Average As(III) concentrations of supernatant water and filtrate over time

In the graph can be seen that As(III) concentration of the effluent of the filter (filtrate) decreased during the course of the experiment, while As(III) in the supernatant water stayed constant between 95 and 120 µg/L. In the graph the total As in the supernatant water is shown, from which can be observed that mainly As(III) flowed into the columns. In the graph can be seen that the effluent of the columns (filtrate) followed the shape of the supernatant As(III) concentration, during the first 9 days of the experiment. After this the influent concentration fluctuated around 100 µg As(III)/L, where the effluent concentration started to decrease. The sharp decrease between day 15 and 22 is difficult to explain, with only two measurement points. Similarly, the short increase of As in the effluent between day 22 and 28 was not fully understood. Both periods might be a result of the instability of bacteria or (resin-) measurements inaccuracy. Eventually, after 38 days the columns reached a state where As(III) is fully oxidized to As(V).

Microbial profiling

To investigate the microbes present in the As(III) pre-loaded columns, three samples have been chosen for further detailed analysis. Due to time limitation only three samples have been send for extensive DNA analysis, other sand samples will be published in future research. The sand samples do not belong to the experiment shown in graphs so far, but to a previous experiment. In this experiment the columns received

approximately 100 µg/L As for a period of 34 days. The results shown so far are the results of the same experiment, however As(III) influent concentrations were less stable, resulting in a combination of As(III) and As(V) in the influent.

Samples from the As(III) pre-loaded columns were done in triplicate, named 7_15, 8_15 and 9_15 for column 7 till 9 where the top layers (15 cm from the top) were analysed with DNA extraction to determine the major bacterial groups present. The distribution on Phylum level is shown in figure 6.

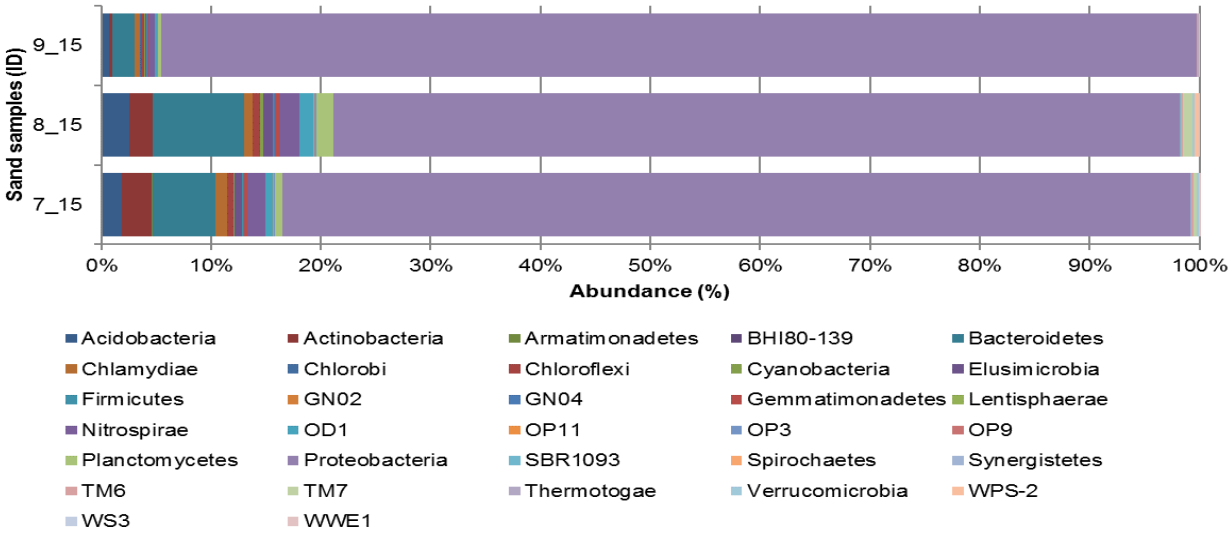

Figure 6: Bacterial population determined by DNA extraction of As columns (Phylum)

From the results in figure 6 can be concluded that mainly Proteobacteria were found in the top layer. Distribution of bacteria in column 7 and 8 seem to be similar, with 15-22% of different bacteria found. Column 9 contains even more Proteobacteria, here only 5% of the bacteria found are different types of bacteria.

These Proteobacteria can be subdivided in several types, based on the data gathered we will limit to α-, β-, and γ-Proteobacteria. The spread of each of these types can be seen in figure 7.

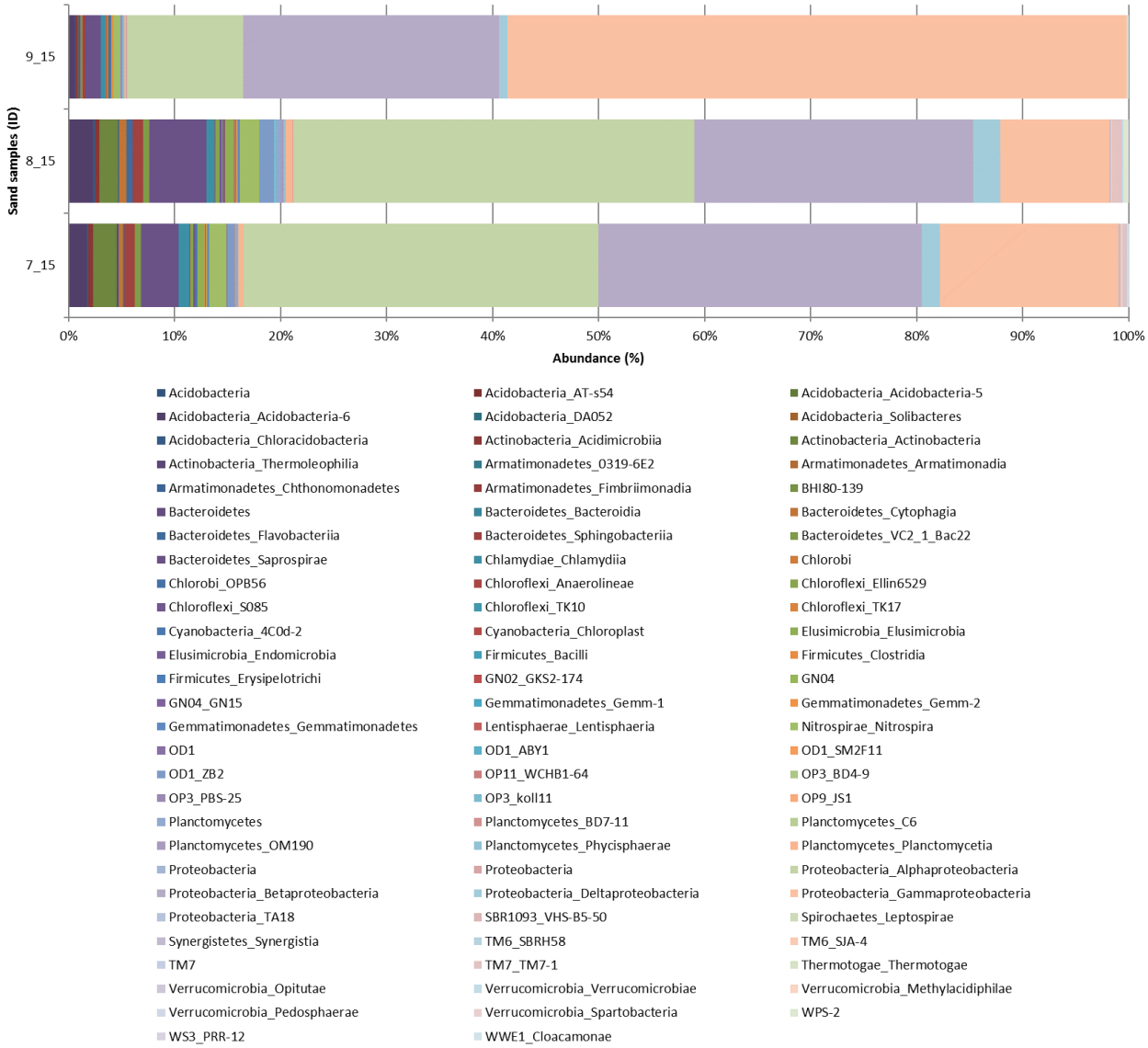

Figure 7: Detailed classification of bacteria (Class)

From figure 7 can be observed that columns 7 and 8 show very similar distributions of α- (green), β-(purple) and γ- (orange) Proteobacteria. In the top layer of column 9 the amount of β-Proteobacteria seem equal to the other two columns, where α - and γ -Proteobacteria are clearly under- and overrepresented in column 9 respectively. Variation in spread of different types of Proteobacteria between the three columns might be the result of random growth, since all conditions (influent concentration, type of sand, flow rate) were almost identical. All three columns reached a similar low As(III) effluent concentration (< 3% As(III) in effluent).

In Bachate et al. (2012) was found that As contaminated groundwater contained γ-Proteobacteria (Dissolved Oxygen 3.4 mg/L), while surface water contaminated with As contained both β - and γ- Proteobacteria and minor traces of α-Proteobacteria. In Fazi et al. (2016) β- and δ-Proteobacteria were found in ground and surface waters, while mainly β- and γ-Proteobacteria were found in thermal waters.

Besides Proteobacteria found in As contaminated waters, these types of bacteria are also found in pilot scale research. Our results seem to be consistent with the microbial analysis done by previous researchers. Li et al. (2016) developed a bioreactor capable of completely oxidizing 1100 µg/L As(III) in 10 minutes, inoculating their bioreactor with AsOB from a mine. After 140 days the microbial community was analysed, and Bacteroidetes and Proteobacteria were the dominant species found. In inoculated columns (50 cm in height, 3 cm diameter) fed with raw water and additional synthetic feed of As(III), the majority of bacterial population was classified as Proteobacteria (Bai et al., 2016).

All of the above mentioned results strongly point in the direction of (As oxidizing) bacteria responsible for the oxidation of As(III). Many researchers have investigated isolated As oxidizing bacteria from contaminated soil, and studied their characteristics and As(III) oxidizing potential (Bahar et al., 2016; Phillips and Taylor, 1976), whereas fewer studies looked at inoculating these bacteria in pilot scale columns or reactors (Macur et al., 2004; Wan et al., 2010). Surprisingly however, simply feeding fresh sand with just 100 µg/L of As(III) creates a biomass capable of oxidizing As(III) without any (intentional) inoculum required. This might be explained by the small amount of bacteria present on the sand already or traces of bacteria in the tap water still present after treatment. Similarly as observed in (Lami et al., 2013), the microbes probably formed or activated As(III) oxidizing genes.

As(III) in As(III) pre-loaded columns

Before ATP and DNA extraction, the As(III) pre-loaded columns received 20 µg/L for 24h to test their ability on oxidizing the different influent As concentration. In figure 8 the results of this experiment are shown.

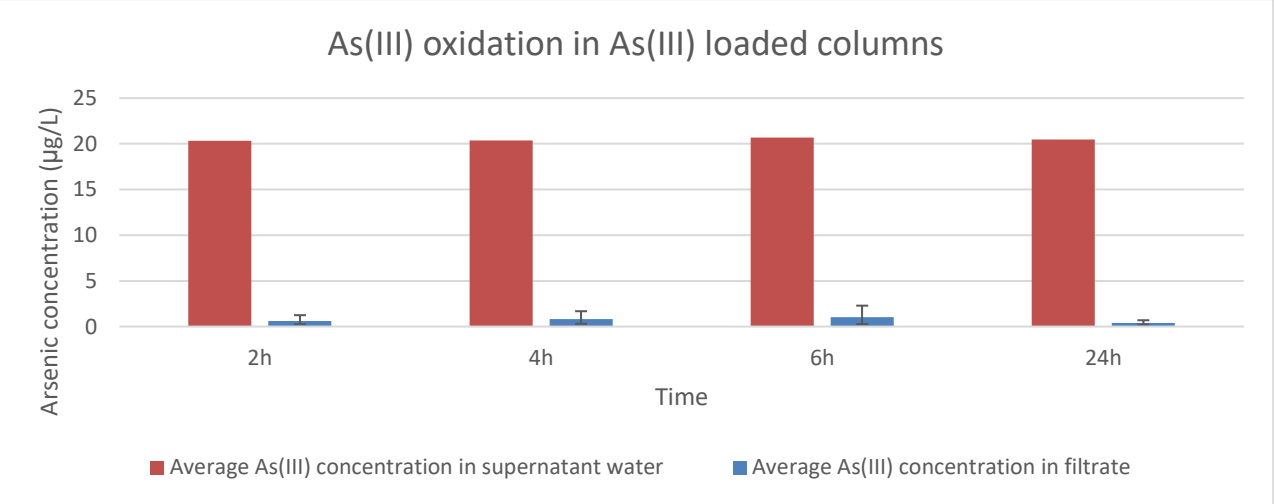

Figure 8: As(III) oxidation during 20 ug/L As(III) dosing, in As(III) pre-loaded columns

In the graph can be observed that the majority of the 20 µg/L As(III) was oxidized by the columns fed originally with 100 µg/L As(III). This comes as no surprise, considering the columns were already able to oxidize 100 µg/L of As(III) to below 3 µg/L As(III).

While measuring supernatant and filtrate As speciation, unexpected results were encountered worth mentioning. Results of As(V) and As(III) concentrations of supernatant and filtrate are shown in figure 9.

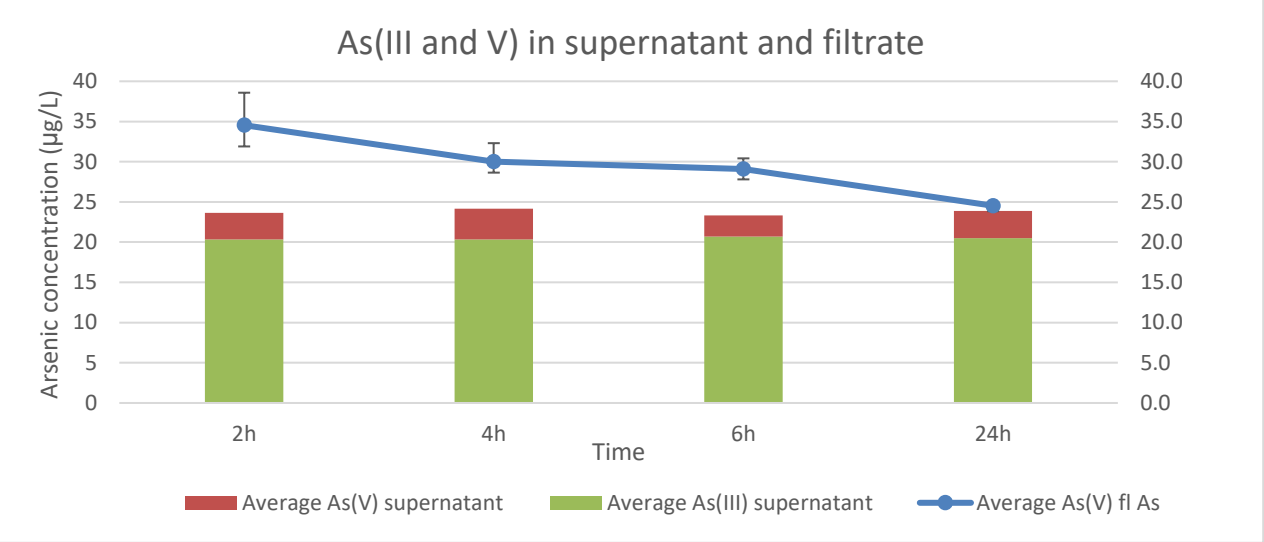

Figure 9: As speciation in supernatant and filtrate of As columns

The stacked bars show the As(III) and As(V) in respectively green and red in the supernatant water, which flows into the columns. The blue line is the As(V) concentration coming out of the columns (filtrate). What can be observed is that more As(V) flows out during the first 6 hours than total As flows into the columns. As(III) concentrations of filtrate are not shown, since their concentrations are equal or below 1 µg/L. After 24h the filtrate concentrations are similar to supernatant concentrations.

The elevated As(V) might be due to residue of the 100 µg/L dosing. This theory has been tested with a small scale salt test. Salt concentrations from supernatant water and filtrate were taken to test the residence time in the columns. It was found that the salt concentrations in influent and effluent were equal after 80-100 minutes, longer than the EBCT due to diffusion and dispersion processes in the column after the sudden concentration change of salt (Annex 1). This is an indication that after 2 hours changes in the system should be processed. Also, only As(V) is measured in the effluent, this indicates that it is probably not coming from the old (100 µg As(III)/L) dosing of As(III).

This leads to two other hypotheses: the bacteria present in the columns release stored As(V) from their cell bodies into the bulk fluid or release from As(V) adsorbed on minerals in the column. It is expected that the bacteria, after weeks of 100 µg/L As(III) in their environment, have established an equilibrium, once this is changed the bacteria can release some of the stored As(V). Release of elements was seen with phosphorus in bacteria, which are also capable of releasing stored P (Jansson, 1987). The other hypothesis is release of part of the adsorbed As(V) on the filter sand. During the 30 days ripening was observed that the influent is slightly higher compared to the effluent, which might be explained by adsorption. Changing the As(III) concentration with 80% to 20 µg/L might have led to desorption of stored As(V). Similar behaviour was observed in (Jessen et al., 2005), where changing influent concentrations to lower As levels led to an increase of As (presumably As(V)) in the effluent.

3.1.3 Loading column with NH_4^+ spiked tap water and its interactions with As(III)

Start-up of NH_4^+ removal

Similarly to accumulating biomass with As(III) as influent, in the next experiment biomass was created by feeding columns with NH_4^+. Total (<0.015 mg/L NH_4^+ in effluent) NH_4^+ removal was achieved after 28 days. While measurements taken at day 34 showed presence of NO_2^- and NO_3^-. This indicates that full conversion to NO_3^- was not yet reached. Measurements were taken again the day before As(III) dosing started (weeks later), assuming that without changing the influent, bacteria would eventually remove all the NO_2^-. Both

NH_4^+ (<0.015 mg/L) and NO_2^- (<0.003 mg/L) were completely removed, and only NO_3^- was found in the effluent. Data of the column ripening is shown in annex 2. Finally As(III) (20 µg/L) was dosed in the columns.

As(III) in NH_4^+ pre-loaded columns

To observe the behaviour of As(III) in NH_4^+ removing columns, approximately 20 µg/L As(III) was dosed for two days, and sampled 2, 4, 6 and 24 hours after introducing As(III) into the system (concentration NH_4^+ = 0.012 mg/L and concentration NO_2^- = 0.003 mg/L in effluent). Results of the As(III) dosing are shown in figure 10.

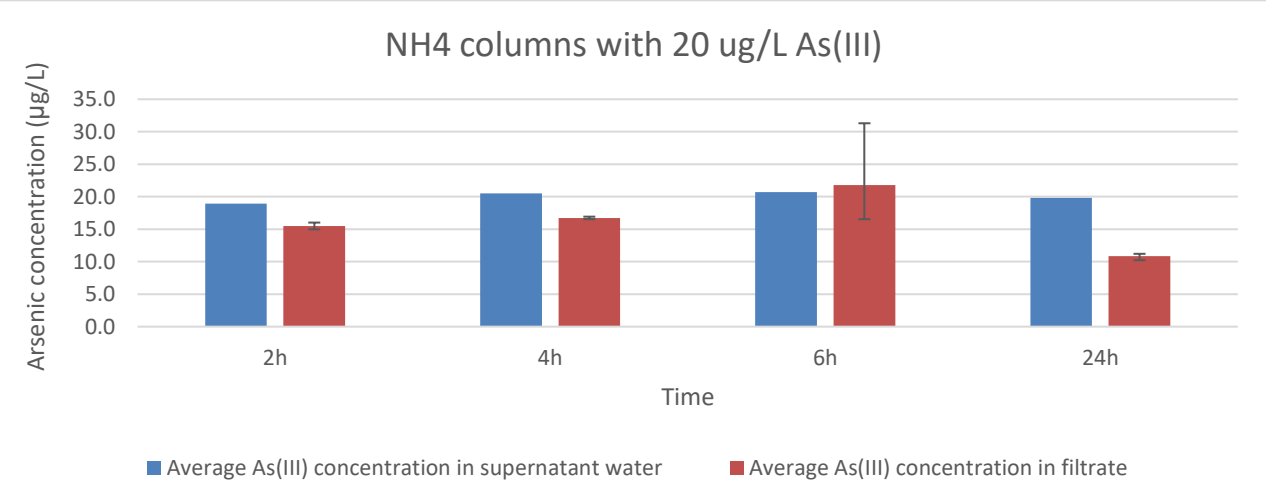

Figure 10: As(III) measured before and after column filtration, of the NH_4^+ pre-loaded columns

The first four hours seem very similar, where the As(III) in the effluent was slightly lower compared to As(III) in the influent. This was possibly due to inaccuracy with the Clifford method and/or retention of As in the column. The results from 6 hours show a large deviation compared to the other results. This is also observed in the large error bar. A measurement error is expected and no further hypothesis was proposed.

The last measurement taken 24h after the start of As(III) dosing, shows a remarkable drop of As(III) in the effluent. This behaviour cannot be seen as a measurement error due to the small error bar. More than half of As(III) was oxidized, which hints towards a hypothesis about nitrifying bacteria (or a biomass in general) which need an adjustment time before oxidation of As(III) is possible. It was described earlier that AsOBs can oxidize As(III) to create a less toxic environment for themselves, this might be (one of) the reasons why oxidation took place in these columns. Although it is known that under anoxic conditions denitrifying bacteria oxidize As (Sun et al., 2009), these conditions are not met here as the oxygen in the effluent was rich in dissolved oxygen. No literature has been encountered suggesting As(III) oxidizing capabilities of nitrifying bacteria. To better understand and explain the results, a follow-up experiment was done, described and discussed in section 3.2.

Influence of As dosing on NH_4^+ and NO_2^- removal.

During the two 20 µg/L sessions NO_2^- and NO_3^- formation was monitored. No inhibitory effect for NH_4^+ and NO_2^- removing bacteria was found after introducing As(III) to the system, as NO_2^- removal and NO_3^- production remained constant over a period of 24h (see annex 3). Also the inhibitory effect will be discussed in 3.2.

3.1.4 Loading columns with tap water only and its interactions with As(III)

Start-up of tap water columns

For 83 days the tap water columns have solely received tap water, with a minor exception of 20 µg/L of As(III) for 6h during As dosing stage I.

As(III) in tap water columns

To check whether a randomly grown biomass had any As(III) oxidizing capacity, 20 µg/L As(III) was dosed for a period of 24h.

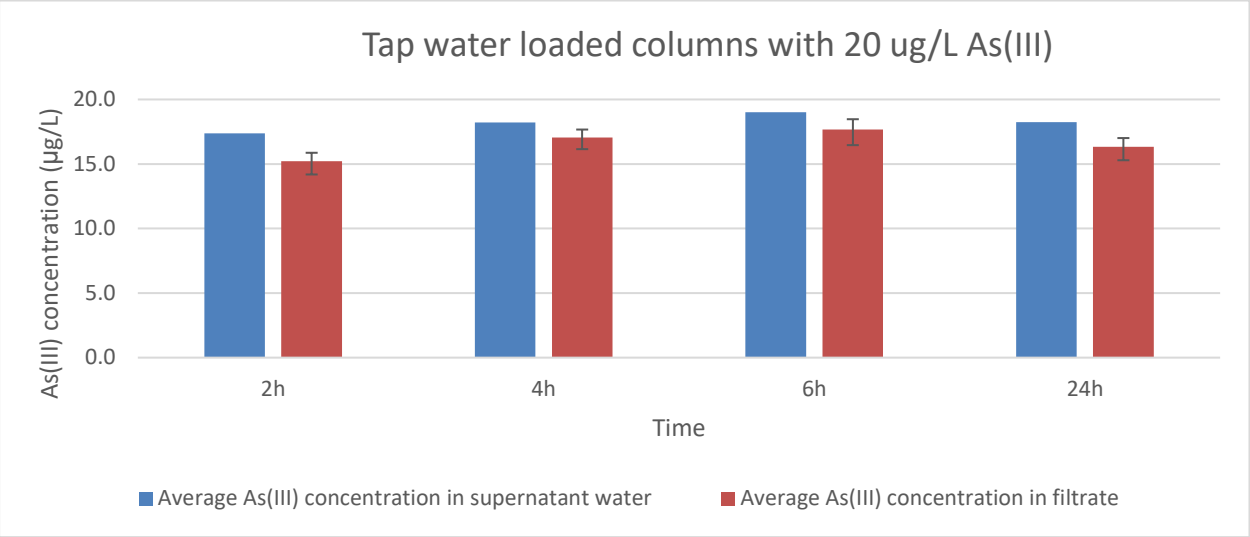

Figure 11: As(III) measured before and after column filtration, of the tap water pre-loaded columns

From figure 11 can be seen that there was a small difference between influent and effluent, but there was no indication that after 24h the biomass already oxidized As(III) as seen in the NH_4^+ pre-loaded columns. The fluctuation of measured values over time can be attributed by the margin of error in the resin and possible retention of As in the column, by biomass or adsorptive material (see also discussion in 4.1.1). As can be expected, the oxidation capacity was the lowest compared to the results of the other column types.

3.1.5 Loading columns with Mn(II) spiked tap water and its interactions with As(III)

Start-up of Mn removal

During 83 days three columns were fed with 1.5-2.0 mg/L Mn(II), in an attempt to create an environment which removes Mn(II). Figure 12 shows the results of this attempt.

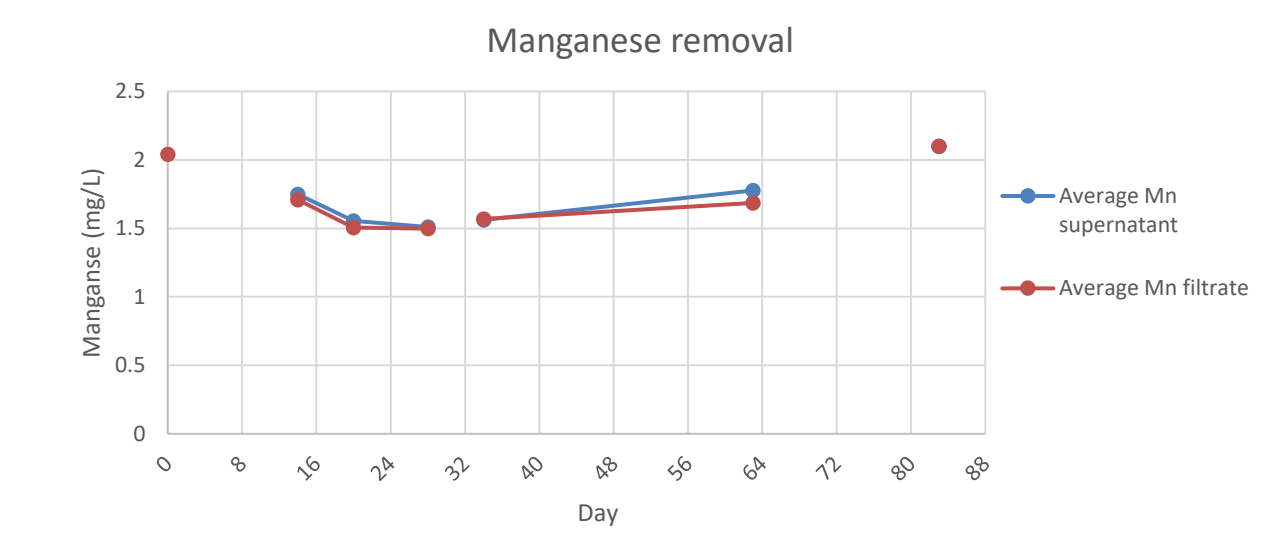

Figure 12: Concentration before and after filtration, measured in the Mn(II) pre-loaded columns

It can be observed that there is a small (or none at all) difference between the influent and filtrate, where the measurements on day 0 and day 83 showed almost identical concentrations that only the red points are visible. The decrease in concentration from 2 mg/L to 1.5 mg/L was probably due to oxidation in the reservoir. Brown flocks were visible on the bottom of the reservoir. Once observed the solution in the reservoir was acidified to pH between 3 and 4, which kept the Mn(II) in solution.

From personal communication with operators of WTP Dorst was learned that Mn removal starts in regular (full scale sand filters) after 6-8 weeks. It is unclear why no Mn(II) removal occurred in the column.

As(III) in Mn(II) pre-loaded columns

Although the start-up of Mn(II) pre-loaded columns was unsuccessful, these columns received 20 µg/L As(III) nonetheless.

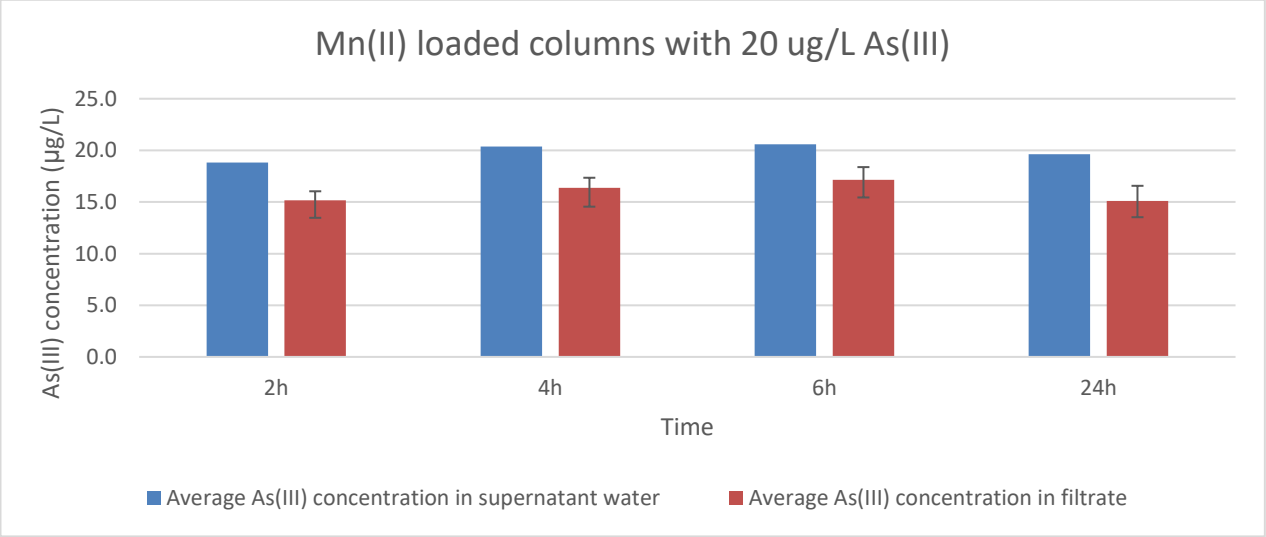

Figure 13: As(III) measured before and after column filtration, of the Mn(II) pre-loaded columns

From figure 13 can be observed that these columns have a minor As(III) oxidation capacity. Comparing the oxidation capacity of Mn(II) pre-loaded columns with the tap water pre-loaded columns, the Mn(II) pre-loaded columns have a slightly higher oxidation capacity. This suggests that something has changed in the columns, although the results are minimal.

3.2 Exploring As(III) oxidation mechanisms, continued on most promising results

Based on the results described in section 3.1, the tap water only and the NH_4^+ pre-loaded column experiments were repeated until full nitrification was reached, where after 100 µg/L As(III) was dosed in both columns for 21 days to observe their As oxidizing capacity.

3.2.1 Loading of columns with NH_4^+ and tap water

The start-up of the NH_4^+ pre-loaded columns took 36 days, until effluent of NH_4^+ and NO_2^- was below 0.01 mg/L NH_4-N and NO_2-N. Dosing of 100 µg/L of As(III) started 3 days later. NH_4^+ and NO_2^- removal was faster compared to the first start-up (described in section 3.1), there NO_2^- was found in the effluent after 42 days. A possible explanation for this difference was that the first time NH_4^+ removal start-up was from cleaned and disinfected columns. Prior to the second start-up, the columns have not been cleaned. This might have resulted in traces of NH_4^+- and NO_2^-- related bacteria left in the column from the previous experiment with NH_4^+ pre-loaded columns, making it easier for quick accumulation of the right bacteria.

3.2.2 Dosing 100 µg/L As(III) in NH_4^+ and tap water pre-loaded columns

Based on the results of the sudden oxidation of half of the 20 µg As(III)/L after 24h with NH_4^+ dosing observed in the previous section, NH_4^+ removing columns are now fed with 100 µg/L As(III) to observe their oxidation capacity and the results were compared with (randomly grown biomass in the) tap water columns. At the end of this section a comparison was made with the results from fresh sand from section 3.1.

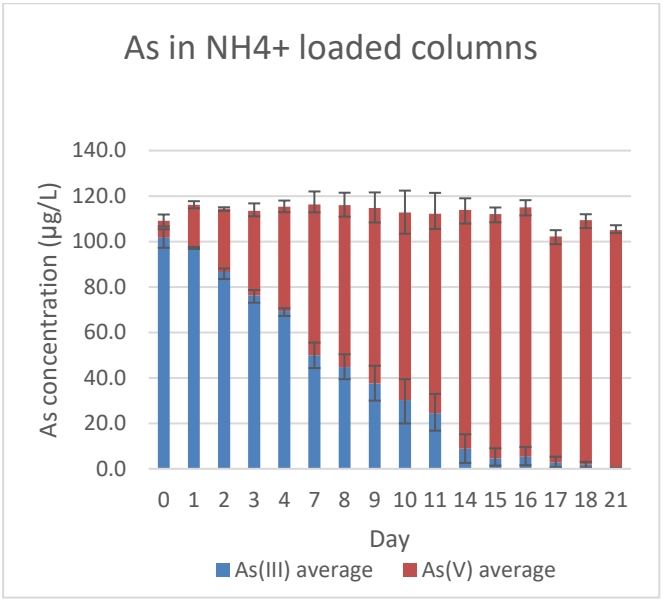

Figure 14: As (III and V) measured in the effluent of NH_4^+ pre-loaded columns

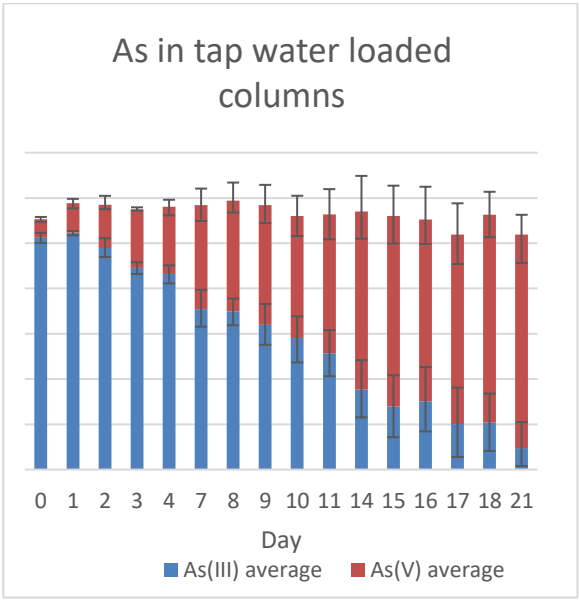

Figure 15: As (III and V) measured in the tap water pre-loaded columns

Figures 14 and 15 show the oxidation of As(III) and corresponding As(V) production with error bars, during the 21 days of accumulation. The 50% oxidation of As(III) observed in section 3.1.2 after one day, was not observed here. In figure 14 the gradual oxidation of As(III) by the biomass created by NH_4^+ dosing was observed. In 14 days these bacteria have oxidized 100 ug As(III)/L below 10 µg As(III)/L.

In the tap water pre-loaded columns (figure 15) a similar decrease of As(III) and increase of As(V) can be observed. The rate however is slower (seven days) compared to the NH_4^+ pre-loaded columns, a value under 10 µg/L As(III) was reached. From the data can be learned that even biomass loaded with tap water as influent was able to adapt to an environment with As(III) by oxidizing As(III). Looking back at the ATP results (section 3.1.5), it was observed that there was on average 15 times more bacterial activity in NH_4^+ pre-loaded columns compared to the tap water pre-loaded columns. This might explain the faster rate of oxidation in NH_4^+ columns, compared to the tap water columns.

The results of the tap water columns can be explained by the fact that these columns had a biomass before the experiment, from here bacteria were selected on their capability to oxidize As(III). In Lami et al. (2013) no As(III) oxidation was observed during the 22 days of the experiment with sterilized columns and As(III) spiked tap water. Two possible explanation can be given for the different results obtained in this study and theirs. In their study sterilized sand was used, our sand was taken straight out of a sand bag, without pre-treatment. Secondly, the concentration of As in our study was much lower compared to Lami et al. (11-19 mg As(III)/L).

3.2.3 As(III) influencing NH_4^+ and NO_2^- removal

During the 100 µg As(III)/L dosing, NH_4^+ was continuously dosed too. Only in the first week of introducing As(III), NH_4^+ and NO_2^- removal decreased slightly in the effluent, where after it was stable until the end of the experiment (see table 3).

Table 3: N concentrations during As(III) dosing

Concentrations (mg N /L)	Max values day before As(III) dosing	Max values first week of As(III) dosing	Max values of remaining weeks of As(III)
NH_4-N	0.016	0.018	< 0.01
NO_2-N	0.0007	0.044	0.005

Looking at the table, it can be safely said that introducing 100 ug/L As(III) in the system did not have any noticeable influence on the removal of NH_4^+ and NO_2^-. In literature was found that microbial communities might shift based on the capability of microbes to detoxify As (Macur et al., 2004). With the results of table 3 was speculated that the bacteria have either already shifted or the large amount of bacteria were not (yet) poisoned by the incoming As since no interference with NH_4^+ removal was observed.

3.2.4 Comparing pre-loaded columns with fresh sand

The decreasing As(III) concentrations in the NH_4^+ and tap water pre-loaded columns can now be compared with the As(III) concentrations of the As(III) pre-loaded columns from section 3.1. In figure 16 the results of all three experiments are combined.

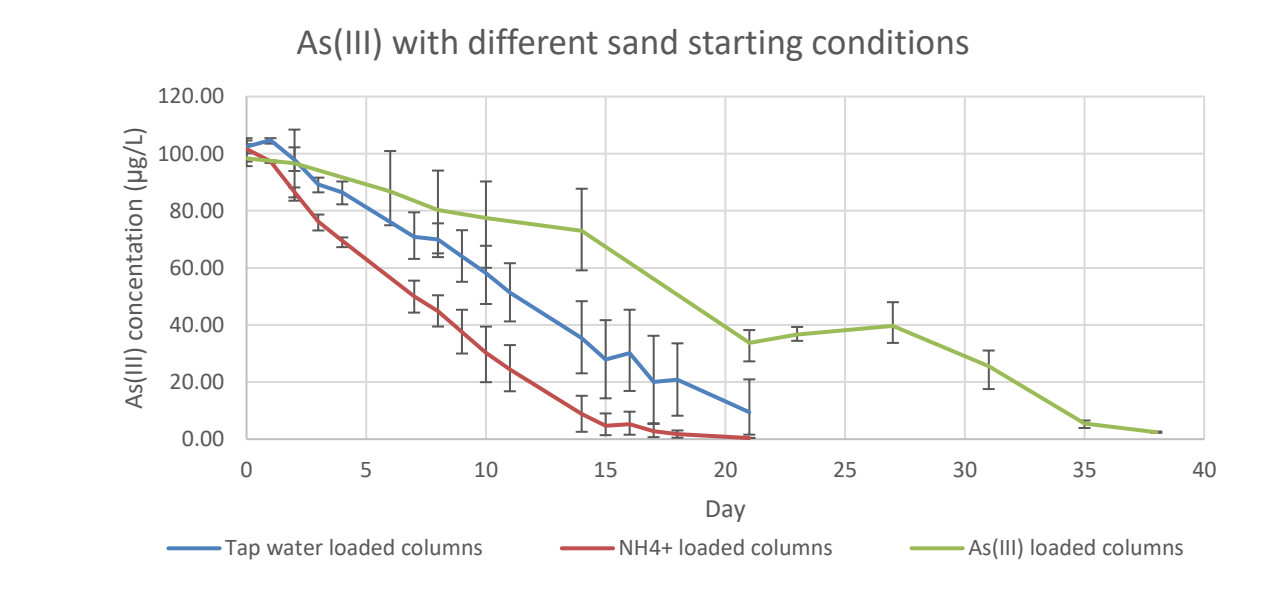

Figure 16: Reduction of As(III) oxidation with NH_4^+, tap water and As(III) pre-loaded columns (tap and NH_4^+ from 3.2.2 and fresh sand from section 3.1)

Looking at figure 16 the As(III) pre-loaded biomass fed with only As(III) (from section 3.1) were almost two times slower in reaching the same As(III) effluent values compared to the NH_4^+ pre-loaded column and were

slower than randomly accumulated bacteria as well. Columns pre-loaded with NH_4^+ appear to have the shortest ripening time, excluding the pre-loading time of total NH_4^+ and NO_2^- removal.

The results suggest that each environment with biomass tends to create a less toxic environment by oxidizing As(III). Whether this is just a detoxification process or for metabolic purposes was not determined in this research. Oxidation of As(III) seems to be independent of the starting conditions in tap waters, just a matter of time and continuous As(III) dosing. This is supported by the idea of Costerton et al., (1987), who found that 99% of all microorganisms on earth can be found in a biofilm. More discussion about figure 16, combined with the results obtained from the ATP analysis, will be done in section 4.1.

3.3 As(III) oxidation mechanisms in raw water loaded columns

The results mentioned so far have all been gathered using tap water to explore As(III) oxidation mechanisms. So far it is clear that bacteria play a great role in the oxidation of As. In the next section one set of columns will be pre-loaded with 100 µg/L As(III) to create a biomass capable of oxidizing the influent, while the other set of columns will start with fresh filter sand. Both types of columns will receive raw groundwater. In table 4 an overview of water quality parameters is shown, which characterizes the raw water influent. All values are an average of 26 measurements.

Table 4: Water quality parameters measured to determine influent quality

	As(T)	As(III)	As(V)	Fe	Mn	NH_4^+	NH_4-N	PO_4^{3-}
	µg/L	µg/L	µg/L	mg/L	mg/L	mg/L	mg/L	mg/L
Average	12.68	10.56	2.12	1.46	0.04	0.63	0.49	0.40
Max-average	0.91	1.45	1.59	0.09	0.00	0.15	0.12	0.10
Average-min	0.97	1.48	1.07	0.09	0.00	0.15	0.11	0.40

3.3.1 As oxidation in pre-loaded and fresh columns

Start-up of As oxidation in pre-loaded columns

The pre-loaded As(III) columns received 100 µg/L As(III) for 28 days until effluent concentrations were lower than 10 µg/L As(III) for all three columns. For two days the columns received only tap water, giving the column time to release adsorbed As(V). After this influent changed to natural raw water. Compared to the ripening time of the columns in section 3.1.1 (36 days before concentrations <10 µg/L As(III) were found), columns in this experiment required less days to reach similar As(III) effluent concentrations. Similarly as observed in NH_4^+ pre-loaded column, the reason for faster As(III) oxidation was found in possible remaining bacteria on the inner wall surface of the column after previous experiments with As(III) pre-loading (column used in this experiment are the same columns as used for experiment in 3.1.1 and were not disinfected).

Raw water and As

In the following two graphs the As(III) and As(V) concentrations are shown, during the 50 days of start-up.

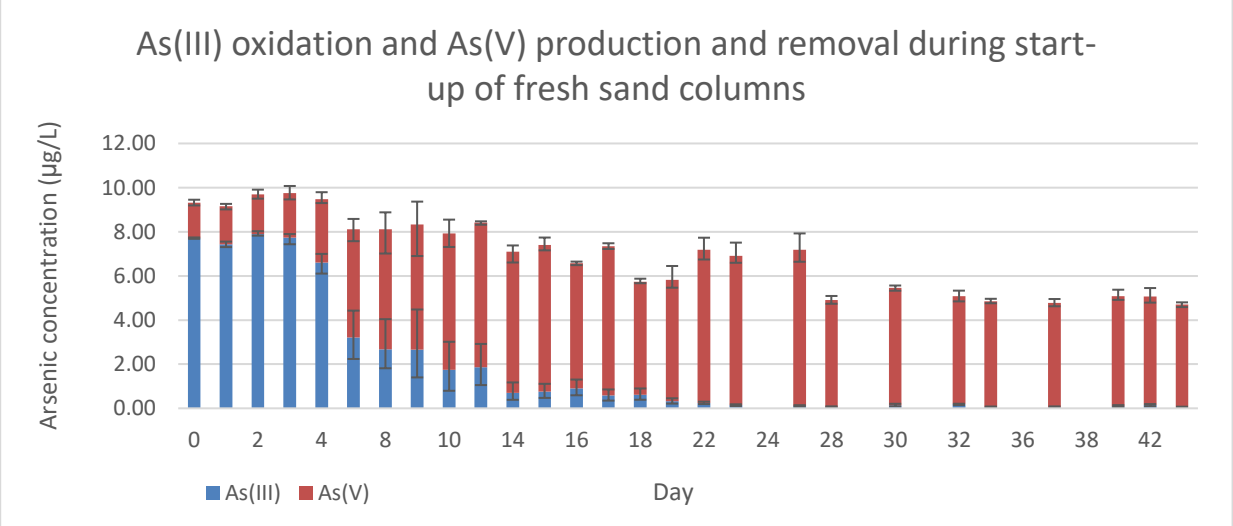

Figure 17: As(III and V) concentrations measured in columns filled with fresh sand

From figure 17 it can be seen that it took 14 days for As(III) effluent concentrations to reach values lower than 1 µg/L in the columns with fresh sand as filter medium. The first days concentrations below 2 µg As(V)/L are measured in the effluent, indicating several days required for As oxidation to start. After day 4 the concentration As(V) starts to increase rapidly, indication oxidation of As(III). Between day 14 and 25 As(V) concentrations fluctuate from 6-7 ug/L As(V). After day 21 the As(III) concentration was below 0.5 µg/L As(III), while after day 28 almost no As(III) is found in the effluent.

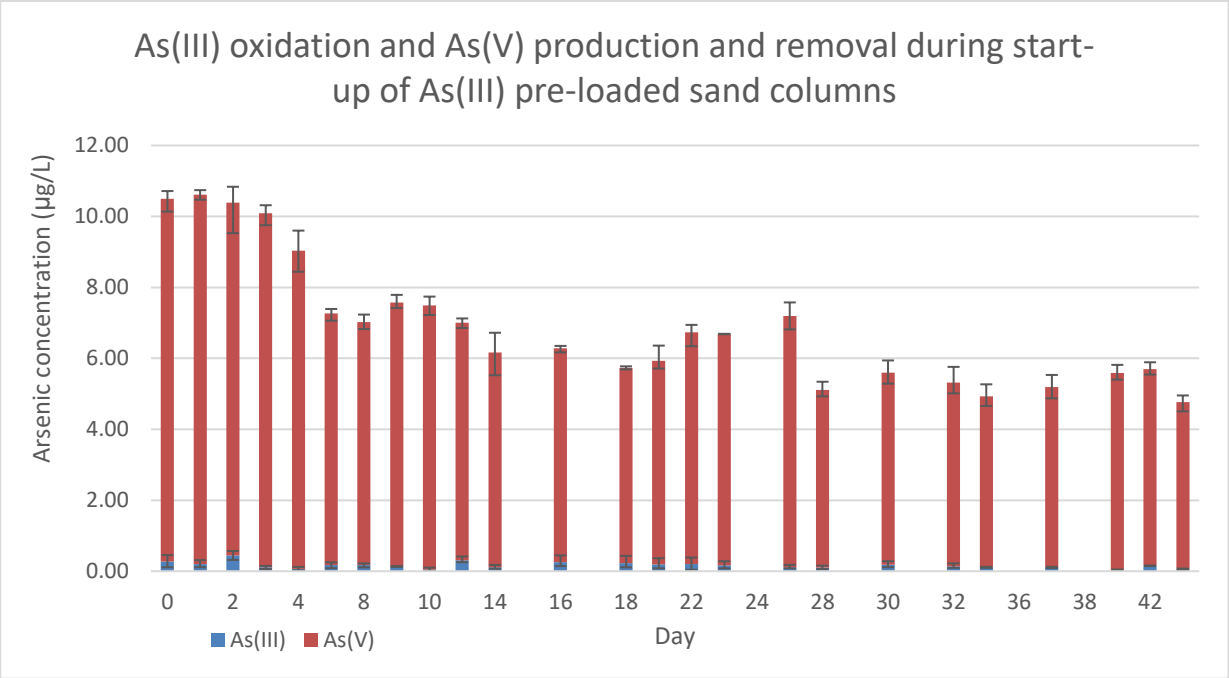

Figure 18: As(III and V) concentrations measured in pre-loaded As(II) columns

The columns with pre-loaded As(III) (figure 18) had a constant outflow of low As(III) concentration, indicating that the accumulated biomass was able to survive in this raw water environment while oxidizing As(III) the entire time. In the pre-loaded As(III) columns a delay of 4 days can be observed at the beginning of the experiment. This might be the result of some time required before adsorptive Fe oxide are formed on the filter sand, which seems to take a couple of days. After this period the removal starts to increase and the As(V) concentrations follow a similar pattern described in the fresh columns.

From the right side of both figures can be observed that the final As(V) concentrations are around 5 µg/L and seem to be stabilizing around that value. Pre-loading of As(III) seems to have no additional effect on the As(V) removal, it only improves the As(IIII) effluent concentration from the first moment of raw water dosing. However, after 21 days the fresh sand columns have reached the same As(III) outflow concentration, resulting in the conclusion that pre-loading of As(III) does not increase the amount of As(V) removed with respect to the fresh sand columns. Removal of As(V) in this study was approximately 60%, which is in the same order of magnitude as in a study done by Katsoyiannis et al. (2008). In their full scale treatment plant removal efficiency of As (21 µg/L initially, 6 µg/L in effluent) was approximately 70%.

In literature no studies were found with columns pre-loaded with As(III) to create an As(III) oxidizing biomass, therefore studies with AsOB inoculated columns were used to evaluate the results obtained. In a study done by Macur et al. (2004) was observed that with AsOB inoculated sand columns (length 100 mm, diameter = 35 mm) complete As(III) oxidation was reached almost directly, during the course of the experiment (total length 14 days). Initially the columns in Macur et al. (2004) could not process the high As(III) loads (5.6 mg/L As influent), but after two days almost no As(III) was found in the effluent. The delay observed in the latter study is probably due to the high influent concentration, which is evidently much higher than the concentration in this study. However, the results of constant As(III) oxidation are similar.

Removal efficiency of fresh columns

In figure 19 the removal efficiency of As(III), Fe, NH_4-N and P are shown, NO_2-N is included in figures 22 and 23. The graph is made to compare selected relevant quality parameters in groundwater with As(III) removal. The As(III) pre-loaded columns are discussed in the other figures of this section, except for $PO_4^{3-}$and Fe. Due to the similarity of the removal efficiencies of the latter two compared to the efficiencies in the fresh column, they can be found in annex 4.

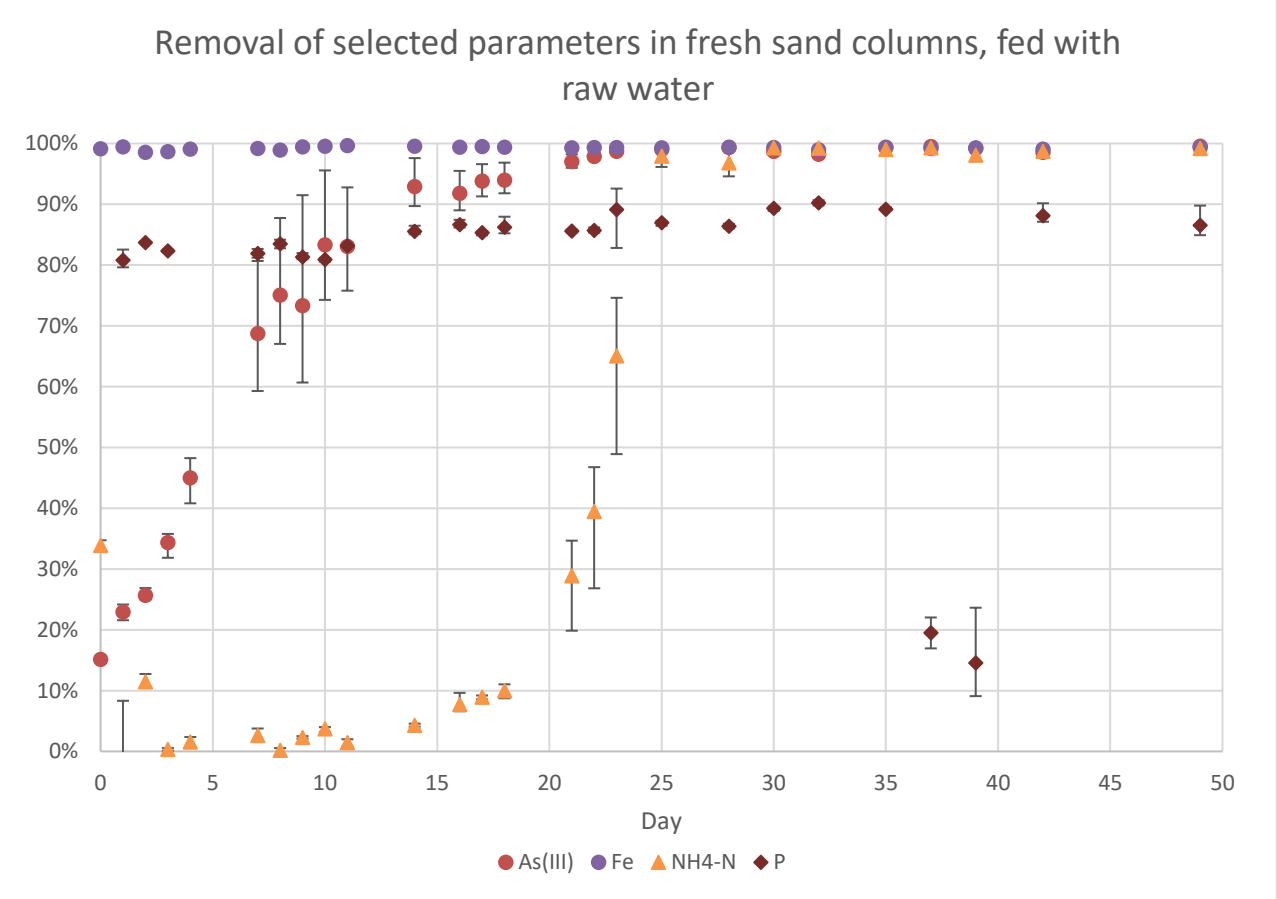

Figure 19: Average removal efficiencies of As(III), NH_4^+-N, Fe and P (as PO_4^{3-}) in fresh sand columns

In the graph was observed that Fe was removed constantly over time (99-100%), effluent values did not exceed 15 µg/L. Also PO_4^{3-} was removed from the start of the experiment and removal efficiency fluctuated between 80 % in the beginning of the experiment, slowly rising to around 90%. The two points around day 37-39 are seen as outliers. As(III) removal started from the beginning of the experiment and reached 90% in 14 days. NH_4^+ measurements during the first three days were oddly elevated, resulting in unclear removal efficiencies and were also seen as outliers. From day 4 to 14 removal stayed constant and below 5%. From day 15 till 25 NH_4^+ was completely removed, to an efficiency above 95% and constant from then on.

Instant and almost complete removal of Fe was observed by Bai et al. (2016) as well. In that study with Mn bacteria inoculated columns (50 cm height, 3 cm diameter, residence time 0.17h, backwash every 48-72h) were compared to columns without inoculum. And although the concentration of Fe (6 mg/L) was higher in the latter study, the removal efficiency of the bio augmented columns was >90% instantly and continuous over time (120 days). Non bio-augmented columns had a removal efficiency of 60%, reaching >90% removal after 10 days and stable total Fe removal for the rest of the experiment. The columns in figure 19 were not bio-augmented, but removed Fe almost instantaneously. The non-bio augmented columns in Bai et al. (2016) needed some adjustment time before complete removal of Fe, probably due to the 4 time higher Fe concentration. From the graph can be seen that As(III) was almost (>90%) oxidized before NH_4^+ removal is started.

ATP in raw water columns

Similar to previous experiments, ATP concentrations were measured using ultrasonic waves to vibrate bacteria from the sand grains. At three depths ATP was measured to get an indication of the location of the bacterial activity and the magnitude. The ATP tests were taken in duplicate, resulting in two sets of ATP data per column type. The results are shown in figure 20 and 21.

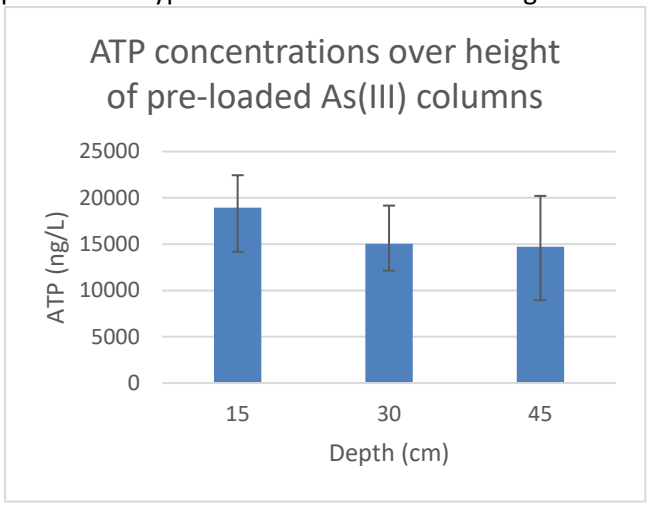

Figure 20: ATP measured over the height in the As(III) pre-loaded columns

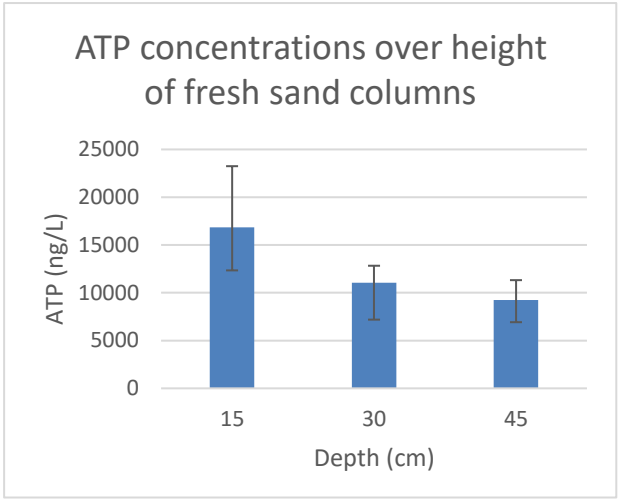

Figure 21: ATP measured over the height in the fresh sand columns

On average most ATP was found in the top layer, where 30 and 45 cm depth show on average almost identical values (figure 20). The error bar of the 45 cm depth is large, which complicates the interpretation of the results. The shape of ATP distribution in the fresh columns is similar to what was observed so far, with the highest ATP found in the top layer, decreasing over the height (figure 21). ATP in the fresh sand was on average lower compared to the As(III) pre-loaded columns.

The spread of bacteria, especially in the As(III) pre-loaded columns, shown by the error bars was not surprising and probably a result of the backwash procedure three times per week. Redistribution of sand grains was observed in literature (Jessen et al., 2005). The ATP results are in range of what was found in top layers of rapid sand filters in 9 Dutch WTP plants: 16.000-2.592.000 ng/L (Magic-Knezev and Kooij, 2004). Results obtained in this study met the lower boundary of ATP concentrations found in the latter study, this might be the result of differences between full scale and pilot scale columns and the age of the filter sand (2-20 years in Magic-Knevez and Kooij, vs 50-83 days in this study).

The concentration found in the top layer was similar to the concentration found in sand fed with tap water and NH_4^+ (section 3.1.2). In the tap water feed however ATP concentrations of 30 and 45 cm depth were significantly lower, probably since no backwash was performed in those columns and no redistribution of bacteria could take place. Unfortunately the exact type of bacteria could not be identified within the time limitation of this thesis. Results of DNA extraction will be published elsewhere.

3.3.2 N and Mn removal

In the sections so far NH_4^+, NO_2^-, P, Fe and As(III and V) have been shown together or only focussed on speciation of As. In this section NH_4^+ and NO_2^- from As-ripened and fresh sand will be evaluated with As plotted in the same graph. Finally Mn removal will be discussed.

Influence of N removal on As

In figures 22 and 23 the As(III and V), NH_4^+ and NO_2^- concentrations are shown in both columns types for further comparison.

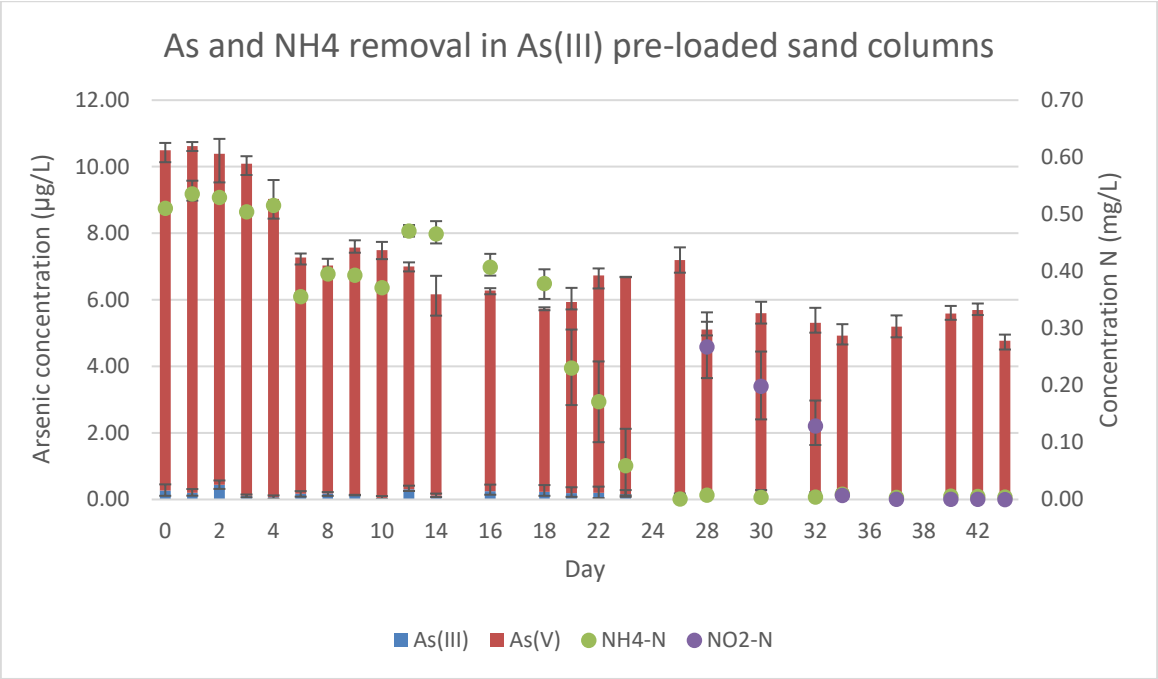

Figure 22: As(III and V) and NH_4-N and NO_2-N concentrations in As(III) pre-loaded columns

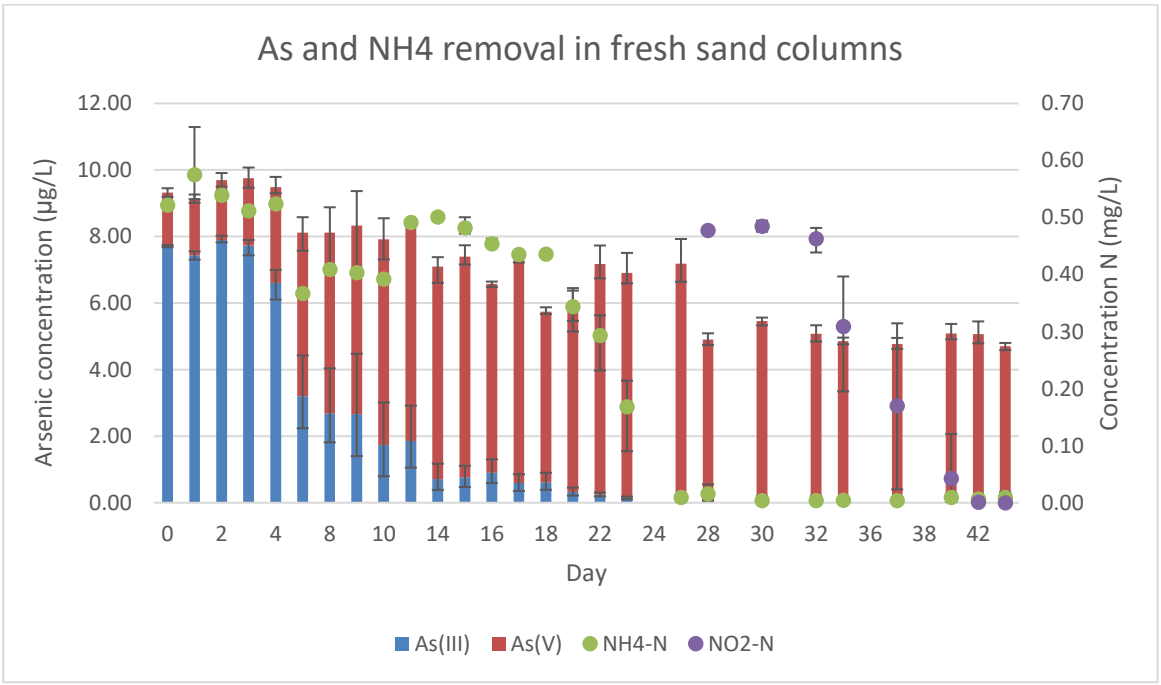

Figure 23: As(III and V) and NH_4-N and NO_2-N concentrations in fresh sand columns

From graph 22 can be seen that neither NH_4^+ removal by bacteria nor NO_2^- removal had a clearly visible influence on As(III) oxidation, as As(III) effluent stayed stable below 0.25 µg As(III)/L. Also in the fresh sand columns (figure 23) influence of NH_4^+ and NO_2^- on As(III) oxidation was not visible. Before NH_4^+ removal started As(III) was already oxidized below 1 µg/L. Once NH_4^+ and NO_2^- started to be removed, no increase of As(III) was observed in either of the column types. As(III) oxidation before NH_4^+ removal is consistent with previous observations (Lytle et al., 2007).

In both graphs can be seen that NH_4^+ removal in both types of columns followed the same path, and even though the As(III) pre-loaded columns are expected to have a larger biomass after its pre-loading stage with As(III), this does not influence the conversion of NH_4^+. The decrease in NH_4^+ effluent observed from day 5 till 10 was the result of a decrease of NH_4^+ in the raw water. Because raw groundwater was used, the water quality per well varied slightly. As a result the NH_4^+ concentration was lower in this period. Before and after days 5 till 10, NH_4-N concentrations of the influent fluctuated less. NO_2^- removal of the two column types diverged more than NH_4^+ removal, although the difference is small: seven days delay of fresh sand until full NO_2^- removal.

This difference might be the result of the differences in biomass, the As(III) pre-loaded columns had a biomass already present resulting from As(III) pre-loading in their columns. Because of this, it might be possible that within this biomass some bacteria with NO_2^- preferences have been growing. The difference was however seven days, and the effluent concentration after 50 days is almost equal. An effort to shorten the start-up time of a rapid sand filter by pre-loading of As(III) to achieve faster NO_2^- removal seems redundant. It seems that NH_4^+ oxidizing biomass does not influence oxidation of As(III) vigorously, and inversely a biomass pre-loaded with As(III) does not decrease the time required for complete NH_4^+ oxidation, although some reduction of ripening was observed for NO_2^- oxidation.

Another observation can be made around day 19 till 25, where the total As influent actually increased slightly, at the same time NH_4^+ removal was completed and NO_2^- started to occur in the effluent. As in the influent was not elevated enough to have caused this increase of As in the effluent. The exact reason was not found.

Mn

The influence of Mn-oxides seems to be negligibly small, As(III) was already completely oxidized before Mn removal began. Although after 29 days Mn removal seemed to show progress, after 50 days the concentrations were almost equal, as can be seen in annex 5. All removal processes showed that Mn in this concentration during these experiments had no influence on As(III) oxidation.

The small contribution of Mn was also seen in Katsoyiannis et al. (2004), where As(III) in small concentrations (25-50 µg/L) showed minor differences in removal, even though Mn concentration were doubled (from 0.4 mg/L to 0.9 mg/L). Concentrations of Mn in this research were even ten times smaller, which adds to the hypothesis that Mn played an insignificant role in the current water quality matrix and experiments. Lee et al. (2014) found with similar influent quality (0.032 mg/L Mn) a start-up time of 4 months to be sufficient for complete Mn removal.

4. Discussion, conclusions and recommendations

4.1 Overall discussion

4.1.1 Alternative oxidation scenarios

An alternative explanation to biotic As(III) oxidation would be homogeneous oxidation of adsorbed As(III) and the subsequent substitution of fresh As(III) by already adsorbed and oxidized As(V), which would explain the increasing As(V) concentrations in the filtrate over time. However, calculations show that maximum adsorbed As in the columns would be 24 mg, assuming all incoming As(III) would exchange for As(V) (which is already unlikely). In that case the average residence time of As(III) in the columns would be increased to 40 h. From own experiments done with homogeneous oxidation, 14% oxidation in 24 h was observed, linear extrapolation to 50h would result in a maximum of 23% As(III) oxidation. This is by far not the complete oxidation of As(III) we observed during the 38 days of the experiment. Therefore, this alternative scenario would never be a substitute of our suggested biotic As(III) oxidation process, merely adding a small percentage to the overall As(III) oxidation.

Another alternative explanation for As(III) oxidation would be accumulation of a substance from the tap water, which leads to the oxidation of As(III) over time (f.e. traces of $NaMnO_4$). Therefore the tap water column can be used as an example and directly as disproof, since tap water columns received tap water for 83 days and after 24 hours of As(III) dosing no sign of As(III) oxidation occurred. The subsequent experiment with pre-loading of tap water for 39 days and loading of As(III) for 21 days showed slow As(III) oxidation.

4.1.2 Type of biomass/bacteria

Biotic As oxidation was not possible in our experiments without pre-loading of As(III) substrate or groundwater containing As(III). Pre-loading of columns with groundwater inert elements (NH_4^+, Mn(II)) did not result in As(III) oxidation, only when As(III) was dosed for a longer period of time.

Looking at the water quality from all experiments with As(III) was observed that As(III) concentrations reduced, while As(V) concentrations increased. Also was observed that ATP concentrations of sand in tap water pre-loaded column (83 days) was lower than As(III) pre-loaded columns (34 days). Biomass grown in pre-loaded tap water columns did not oxidize As(III) in 24 h (ripening time: 83 days), and was only able to oxidize As(III) slowly over a time span of 21 days (with a ripening time 39 days). Finally, in the experiments with pre-loaded As(III) Proteobacteria were found, which are often related to As(III) oxidizing abilities (Cavalca et al., 2013). These observations combined lead to the hypothesis that As(III) oxidation is a result of As(III) oxidizing bacteria, growing specifically on As(III) as substrate.

Furthermore, in the tap water experiments spiked with As(III) 45% more ATP was found compared to columns with tap water only. This indicates that biomass gained energy from the oxidation of As(III) (redox reaction) and were able to grow. Another source of energy would be sunlight, this is however unlikely because the columns were covered to block sunlight. Generally it is seen that in tap water and groundwater nutrition levels are low, in tap water because of the strict regulation and in groundwater because of the long residence time in the ground. Therefore inorganic compounds are expected to be electron donor and carbon source for the microbes living in our columns. Combining all these characterisations, the type of bacteria found in our columns can be hypothesized to be chemolithoautotrophic bacteria.

4.1.3 Lag, growth and steady state phases

In figure 16 the reduction of As(III) concentrations with different filter media were shown. Based on the ATP results of figure 4 can be said that the amount of microbial activity in the NH_4^+ pre-loaded columns was a factor 15 times higher compared to the activity in the tap water pre-loaded columns. We can only hypothetically explain this with figure 16. It seems that the bacteria in the NH_4^+ pre-loaded columns had the shortest lag phase, since after day 3 till 15 there was an almost linear decrease of As(III) measured in the effluent. The bacteria in the tap water pre-loaded columns seem to have had a longer lag time, while after

8 days there was a very similar linear decrease of As(III) visible. It was speculated that between the large amount of bacteria in the NH_4^+ pre-loaded columns, more bacteria were able to adapt to the environment with As as a form of selection, as bacteria react to their environment and adapt in order to survive (Jefferson, 2004). This results in a relatively short lag phase, followed by a linear reduction of substrate during the growth phase. In the tap water columns much less bacteria were present at the start of the experiment, therefore this follows as the main reason why the lag phase in the tap water pre-loaded columns is longer.

This seems to exclude the option that NH_4^+ oxidizing bacteria were co-responsible for oxidation of As(III). It is expected that between the larger biomass formed during NH_4^+ removal the chance that As(III) oxidizing genes or bacteria are able to be activated or grown increases. This coincides with the absence of literature (to the best knowledge of the author) linking NH_4^+ removal (oxidation) to As(III) oxidation. Sun et al., (2010) found that under anaerobic conditions an alternative electron acceptor for oxidation of As(III) could be found in NO_3^-, replacing oxygen as electron acceptor. However, with the aerobic influent water it was assumed that the influence of NO_3^- as alternative electron acceptor would be limited and therefore left out of the scope of this research.

4.1.4 As(III) oxidation mechanism(s)

Based on the discussion mentioned above, and the fact that in raw groundwater NH_4^+ removal is achieved after full As(III) oxidation (see results 3.3.1), it is strongly suggested that As(III) oxidation is not coupled to nitrification. In this report was already described that all experiments including Mn(II) were unsuccessful, which leads to an even stronger suspicion that Mn has a minor role in As(III) oxidation in low As(III) and low Mn(II) concentrations. In other research Mn was believed to be of significant importance in the oxidation of As(III) (Yang et al., 2015). Yang et al. concluded that the microbial community responsible for the oxidation of Mn(II) (2-5 mg/L) was also responsible for the oxidation of As(III) as well (300-2500 µg/L). However, there the concentration of As were much higher and also the Mn(II) concentrations were (much) higher than concentrations encountered in this study.

4.2 Conclusions

The aim of this study was to investigate whether microorganisms commonly found in rapid sand filters (nitrifying and Mn oxidizing bacteria) are able to oxidize As(III). With this knowledge As(III) oxidation in rapid sand filters would no longer be a black box.

A biomass was capable of growing on fresh sand fed with tap water spiked with 100 µg As(III)/L, oxidizing 97% of the As(III) to As(V) in 38 days. Columns loaded with tap water only and tap water spiked with Mn(II) did not show As(III) oxidation after 24 hours of As(III) loading, whereas NH_4^+ loaded columns oxidized 50% of As(III) after 24 hours. Biomass growing within tap water pre-loaded and NH_4^+ pre-loaded columns, showed to oxidize As(III) after continuous (21 days) dosing of As(III) in the influent. NH_4^+ and NO_2^- removal was not inhibited by addition of As(III) (resulting from NH_4^+ pre-loaded columns).

In raw water columns with fresh sand was observed that As(III) oxidation was completed before NH_4^+, NO_2^- and Mn removal, where Fe and PO_4^{3-} were removed from the start of the experiment. After complete oxidation of As(III) (in 14 days above 90%), the effluent concentration stabilized around 4.5-5 µg/L As(V) (60% removal of As). As(III) pre-loaded raw water columns had a continuous outflow of As(V), showing the robustness of the bacteria and their capability of surviving in a complex water matrix. Removal efficiencies of Fe, PO_4^{3-}, NH_4^+ and As were similar for both types of raw water columns, whereas NO_2^- removal was completed seven days earlier in As(III) pre-loaded columns.

From results with tap water and the raw groundwater columns, in combination with the absence of literature found about co-oxidation of As(III) in NH_4^+ or NO_2^- oxidation, can be concluded that As(III) is not co-oxidized during the nitrifying process. Since Mn(II) removal was unsuccessful, the added contribution is unknown. However, in the raw water matrix can be observed that As(III) was oxidized within two weeks, before Mn(II)

oxidation, showing that Mn oxides and Mn oxidizing bacteria had no role in oxidation of As(III) in our experiments.

The overall conclusion was that microorganisms play a crucial role in rapid and continuous oxidation of As(III). Oxidation of As(III) is a matter of time, but was surprisingly easily achieved in tap water and raw groundwater experiments.

4.3 Future research and practical applications

What now remains is the practical application and implementation of the results found in this research, and a preview of future research directions. This research is mainly done to discover the mechanisms of As(III) oxidation, or to at least rule out mechanisms which are unlikely to have any additional effect on the oxidation of As. Looking back at the research done, measurements over the height were missed the most. In order to reduce As concentrations, the focus should be on the exact location of As(III) oxidation (hypothesized to be in the top layer) and subsequently removal of As(V) deeper in the filter bed. One option for follow-up research includes experiments measuring As(III) oxidation over the height, to confirm the hypothesis of As(III) oxidation in the top layer of the filter bed. In this way changes in design and operational parameters are systematically monitored.

The main idea is that after As(III) oxidation, As(V) needs to be removed by adsorptive material deeper in the filter bed, separating As(III) oxidation and As(V) adsorption currently happening in one column. One possibility is a two layered filter, with an upper layer solely capable of oxidizing As(III) by offering sites for biomass to grow on porous material, but letting the Fe(II) pass. In this first layer As(III) could be oxidized, while Fe(II) is still present in the water and no flocs are formed yet. The second layer would consist of regular filter sand, where Fe flocs can be formed for As(V) adsorption. Another possibility would be a pre- and after-filtration step, with two filter stages in separated columns. In the first filter stage As(III) is oxidized and other parameters are removed as well (Fe, NH_4^+, Mn), while in the second stage Fe (II or III) is added to form Fe oxides for As(V) removal and polishing the water with trace elements. A third option would be addition of Fe(II or III) half way the filter, by injecting Fe, for additional adsorption capacity to remove As(V). A final option would be by changing operational parameters, such as supernatant water level, to stimulate more Fe(II) entering deeper layers of the filter bed. In such a way Fe(II) can enter the filter bed without floc formation on the surface, but deeper in the bed, where it can adsorb As(V).

Which of the proposed applications is most suitable or is most promising, should be researched in future experiments with measurement points over the height to observe which processes occurs in the layers beyond As(III) oxidation.

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Annex 1: Salt concentration test

In figure 24 concentrations in supernatant (blue line) and effluent (orange line) can be seen. Salt was put in the reservoir, which is being pumped into the columns with 1 ml/min.

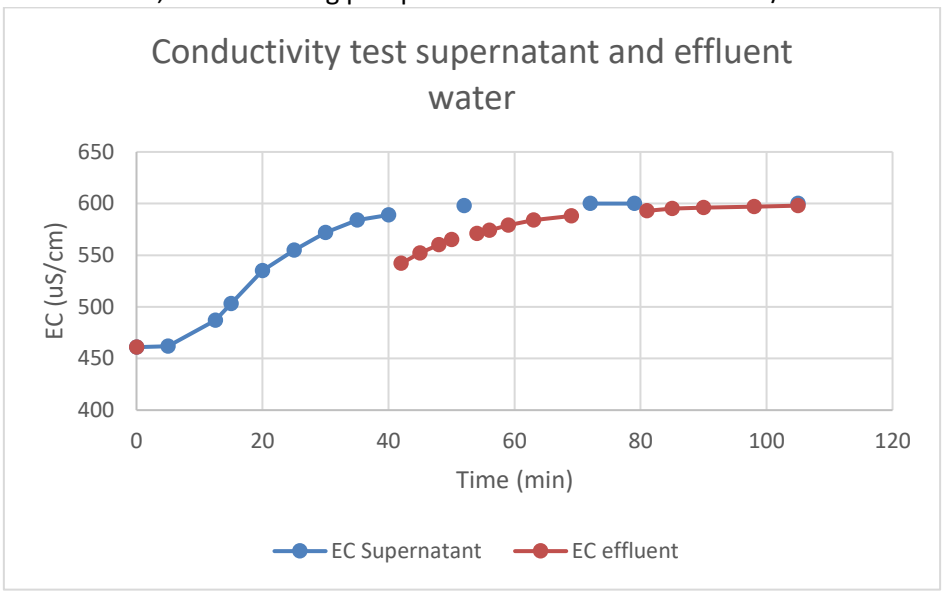

Figure 24: Electrical conductivity (EC) test to check the adaptability of the sand column to changes (salt concentration changed) in the system

Annex 2: Start-up of NH_4^+

In table 5 the concentrations of NH_4^+, NO_2^- and NO_3^- are shown during start-up of NH_4 removal. It can be seen that after 35 days (3-5-2016 till 7-6-2016) NH_4^+ was removed, but NO_2^- was still present in the effluent. The experiment was continued until 25-7-2016 without changing any of the variable. This resulted in complete NH_4^+ and NO_2^- removal after 85 days.

date	spr 4 [NH4] mg/L	spr 5 [NH4] mg/L	spr 6 [NH4] mg/L	fl 4 [NH4] mg/L	fl 5 [NH4] mg/L	fl 6 [NH4] mg/L	fl 4 [NO2] mg/L	fl 5 [NO2] mg/L	fl 6 [NO2] mg/L	fl 4 [NO3] mg/L	fl 5 [NO3] mg/L	fl 6 [NO3] mg/L
3-5-2016				1.42								
4-5-2016												
11-5-2016												
17-5-2016	1	1	1.07	0.99								
23-5-2016	0.95			0.89								
31-5-2016	0.95	0.94	0.97	0.01	0.01	0.01						
1-6-2016												
6-6-2016	0.87	0.87	0.9	0.01	0.01	0.01	2.22	2.15	2.37	1.87	1.77	1.74
7-6-2016	0.91			0.01			2.25			1.45		
7-6-2016	0.95			0.01			2.29			1.41		
25-7-2016	0.7879			0.012			0.003			5.047		

Table 5: Results of supernatant (spr #) and effluent (fl #) during start up of NH_4 and NO_2 removal.

Annex 3: NH_4^+ and NO_2^- removal during As(III) dosing.

In table 6 the concentrations of NH_4^+ and NO_2^- are shown in the supernatant and filtrate water. The small concentration of NO_2^- found in the supernatant water (27-7-2016: 1600) is due to oxidation of NH_4^+ during transport to the water lab. However, since no NO_2^- was measured in the other three measurements and NO_2^- concentrations in the effluent remained consistently low, it is assumed that this delay in transport did not affect the outcome of the test.

	Supernatant		Filtrate	
2h	0.253269	mg/L NH4-N	0.004	mg/L NH4-N
			0.000	mg NO2-N/L
4h	0.370869	mg/L NH4-N	0.005	mg/L NH4-N
	0.089906	mg NO2-N/L	0.000	mg NO2-N/L
6h	0.40215	mg/L NH4-N	0.001	mg/L NH4-N
			0.000	mg NO2-N/L
24h	0.362556	mg/L NH4-N	0.005	mg/L NH4-N
			0.000	mg NO2-N/L

Table 6: Concentrations of NH_4^+ and NO_2^- in supernatant and effluent water during 20 ug/L As(III) dosing

Annex 4: Removal efficiencies columns As(III) pre-loaded with.

In figure 25 the removal efficiency of the columns pre-loaded with As(III) is shown. Due to its similarity to the removal efficiency to the fresh columns, and As(III) and NH_4-N of As(III) pre-loaded columns are shown here in the annex.

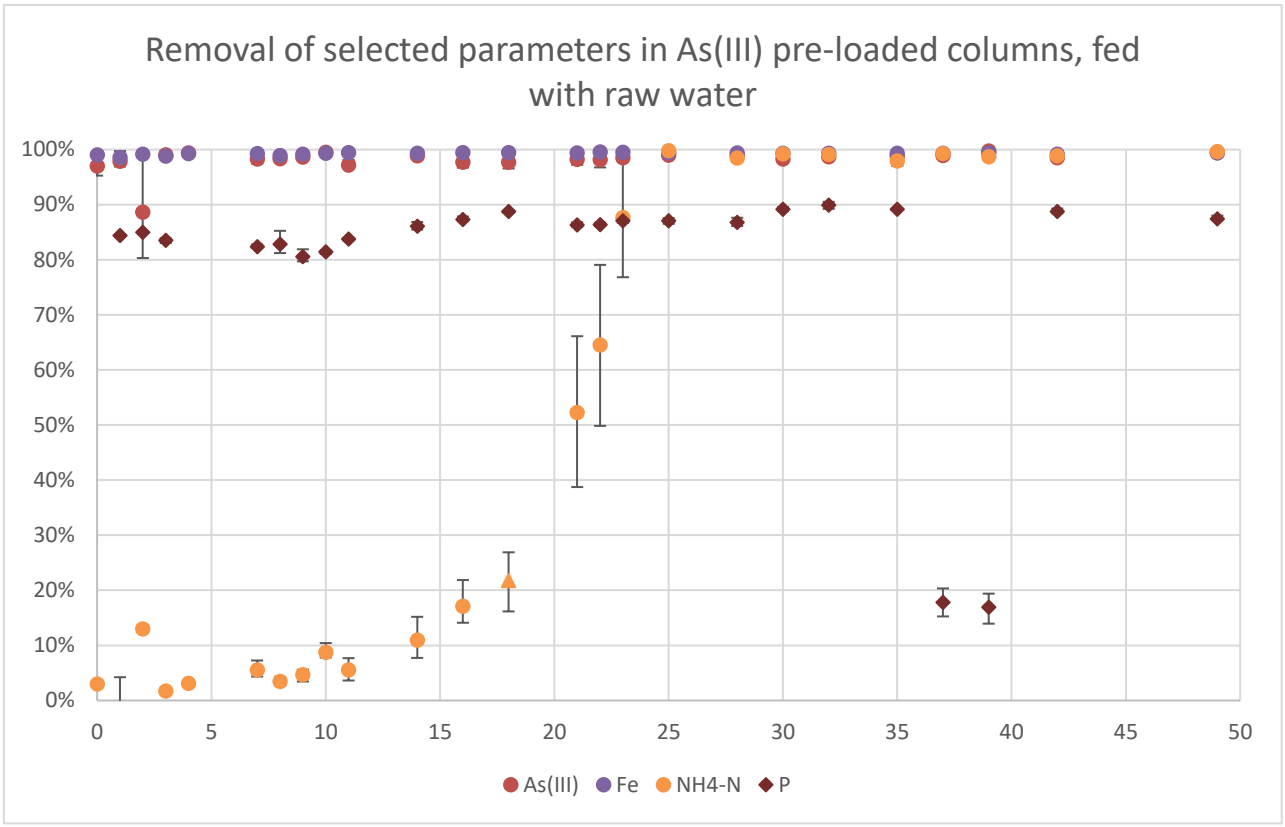

Figure 25: Removal efficiencies of selected parameters of the As pre-loaded columns

Annex 5: Removal of Mn in raw water fed columns

In the figure 26 the attempt to create a Mn(II) oxidizing column are shown. As can be observed, no Mn oxidation occurred during the 50 days experiment. Due to the higher Mn effluent concentrations compared to the influent (until day 24) this data was difficult to understand. Since no Mn(II) removal was reached within the time of the experiment, no further investigation was done to explain the higher Mn(II) effluent concentrations.

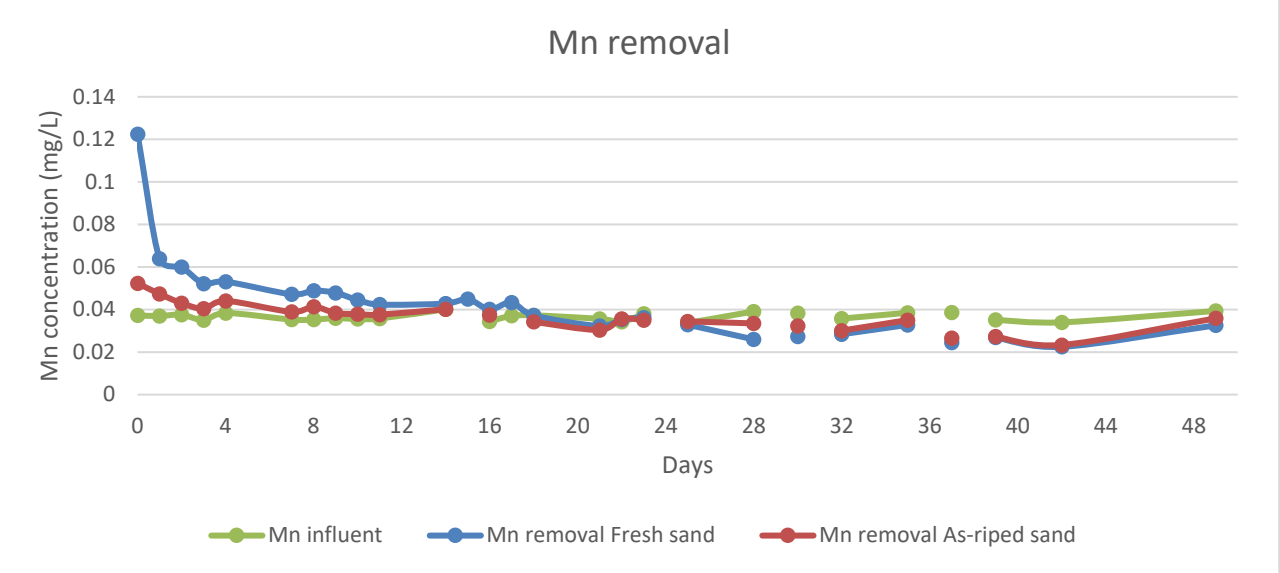

Figure 26: Mn concentration in influent and effluent of both types of columns (fresh sand and As(III) pre-loaded)