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# Biological As(III) oxidation in biofilters by using native groundwater microorganisms

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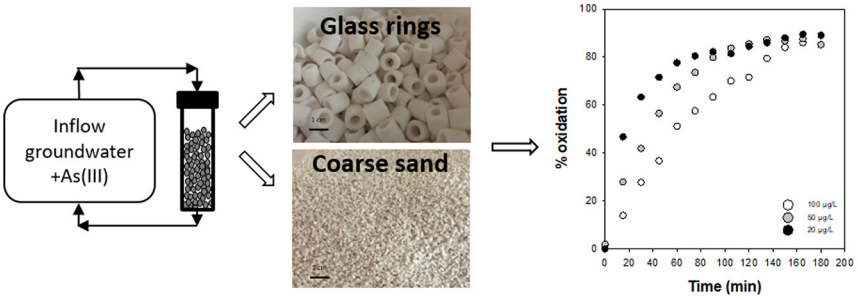
## HIGHLIGHTS

- Evaluation of the process efficiency in biofilters under varying operation conditions
- Development of highly performing As (III) oxidizing biofilms in biofilter reactors
- Enrichment of *aioA* genes in biofilters treating contaminated groundwater

## GRAPHICAL ABSTRACT

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## ABSTRACT

Arsenic (As) contamination in drinking water represents a worldwide threat to human health. During last decades, the exploitation of microbial As-transformations has been proposed for bioremediation applications. Among biological methods for As-contaminated water treatment, microbial As(III)-oxidation is one of the most promising approaches since it can be coupled to commonly used adsorption removal technologies, without requiring the addition of chemicals and producing toxic by-products. Despite the As(III) oxidation capability has been described in several bacterial pure or enrichment cultures, very little is known about the real potentialities of this process when mixed microbial communities, naturally occurring in As contaminated waters, are used. This study highlighted the contribution of native groundwater bacteria to As(III)-oxidation in biofilters, under conditions suitable for a household-scale treatment system. This work elucidated the influence of a variety of experimental conditions (i.e., various filling materials, flow rates, As(III) inflow concentration, As(III):As(V) ratio, filter volumes) on the microbially-mediated As(III)-oxidation process in terms of oxidation efficiency and rate. The highest oxidation efficiencies (up to 90% in 3 h) were found on coarse sand biofilters treating total initial As concentration of 100 μg $L^{-1}$. The detailed microbial characterization of the As(III) oxidizing biofilms revealed the occurrence of several OTUs affiliated with families known to oxidize As(III) (e.g., *Burkholderiaceae*, *Comamonadaceae*, *Rhodobacteraceae*, *Xanthomonadaceae*). Furthermore, As-related functional genes increased in biofilter systems in line with the observed oxidative performances.



## 1. Introduction

Arsenic (As) is a well-known carcinogenic element widely distributed in natural aquatic environments representing a serious threat to

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human health worldwide (Nordstrom, 2002). Several physical-chemical methods are used for arsenic removal, such as coagulation/filtration, ion exchange, enhanced lime softening, adsorption and reverse osmosis (Ng et al., 2004; Nicomel et al., 2016).

Nevertheless, in recent years the research interest is moving towards the adoption of biotechnological solutions to be used in combination with traditional chemical As treatment processes to enhance the sustainability and cost-effectiveness of the process (Plewniak et al., 2018). Indeed, despite the high toxicity, some microorganisms are able to withstand high As levels and use it for energetic metabolism (Huang, 2014). Among the possible microbial As-transformations, the redox reactions involving As(III) oxidation and As(V) reduction are the most investigated for bioremediation purposes (Kumari and Jagadevan, 2016). In particular, microbiological As(III)-oxidation is one of the most promising application as a precursor step in As removal from contaminated groundwater, since conventional iron-based treatments are more effective in removing As(V) rather than As(III) (Fazi et al., 2016a). Pre-oxidation process is commonly performed through the addition of chemical reagents such as potassium permanganate, chlorine, ozone, hydrogen peroxide or manganese oxide that can cause secondary problems arisen by the presence of residuals or from by-products formation, inducing a significant increase in operational costs (Driehaus et al., 1995; Katsoyiannis and Zouboulis, 2004; Kim and Nriagu, 2000).

Microorganisms involved in As(III)-oxidation were retrieved in a variety of As-rich environments including mine, arsenical pesticides or smelter-impacted sites, geothermal sites, geyser, soil and sediments (Engel et al., 2013; Heinrich-Salmeron et al., 2011; Lami et al., 2013; Quéméneur et al., 2010, 2008; Sultana et al., 2012). Microbial As(III) oxidation represents a detoxification process in heterotrophic microorganisms as *Herminiimonas arsenicoxydans*, *Hydrogenophaga* NT-14, *Stenotrophomonas* sp. MM-7 (Bahar et al., 2012; Muller et al., 2003; Vanden Hoven and Santini, 2004), or an energetic metabolism in chemolithoauthotrophic microbes, such as *Rhizobium* NT-26 and *Thiomonas arsenivorans* (Battaglia-Brunet et al., 2006; Garcia-Dominguez et al., 2008; Hoeft et al., 2007; Santini et al., 2000). Both oxidation mechanisms are carried out by the enzyme arsenite oxidase, firstly purified in *Alcaligenes faecalis* (Anderson et al., 1992). This enzyme is composed of a small subunit containing a Rieske [2Fe-2S] cluster and a large subunit harboring molybdopterin guanosine dinucleotide at the active site and a [3Fe-4S] cluster (Ellis et al., 2001). The two genes encoding for large and small subunits of the arsenite oxidase were named as *aioA* and *aioB*, respectively (Lett et al., 2012). Sometimes, the combination of As(III) oxidation with nitrate or chlorate reduction has been observed in microorganisms such as *Acidovorax* NO1 and *Azoarcus* DAO1 (Huang et al., 2012; Zargar et al., 2012).

During last decades, the potentialities of microbial As(III) oxidation were investigated in lab-scale experiments by using planktonic cells (Battaglia-Brunet et al., 2002), biofilms (Michel et al., 2007) and immobilized bacteria (Dastidar and Wang, 2012; Ito et al., 2012; Michon et al., 2010). Battaglia-Brunet et al. (2002) reported an oxidation rate of 166 mg $L^{-1}$ $h^{-1}$ in a fixed bed column inoculated with a autotrophic As(III)-oxidizing population selected from an As-rich mine site. Furthermore, As(III)-oxidizing microorganisms, such as *T. arsenicoxydans*, were also used to operate biofilters coupled with arsenic removal treatment based on activated alumina and metallic Fe adsorbents (Ike et al., 2008; Wan et al., 2010). Other studies proposed the application of biotic As(III) and Fe(II)/Mn(II) oxidation in a fixed-bed upflow filtration unit for the oxidation and simultaneous removal of arsenic and dissolved Fe and Mn (Hassan et al., 2009; Katsoyiannis and Zouboulis, 2004; Katsoyiannis et al., 2004; Tani et al., 2004). Recently, the potentialities of biological As(III) oxidation were investigated by using mixed microbial communities in bioreactors filled with sand or perlites (Gude et al., 2018; Li et al., 2016). The laboratory-scale biofilter, inoculated with an enriched population of As(III)-oxidizing microorganisms from realgar mine sediments, showed the capability to oxidize 1100 μg As(III) $L^{-1}$ within 10 min (Li et al., 2016). This process was also evaluated in sand filters by using a mixed microbial community from raw groundwater. About 98% of As(III) at the initial concentration of 116 μg $L^{-1}$was oxidized in 38 days without acclimation to As(III) contaminated water and within three weeks when the biofilter was previously exposed to As-rich groundwater (Gude et al., 2018). Other investigations showed the ability of microorganisms grown on quartz sand to simultaneously remove arsenic (100–150 mg $L^{-1}$), iron (0.8–1.5 mg $L^{-1}$) and manganese (1–1.2 mg $L^{-1}$) from groundwater, with As removal up to 98.2% within 180 days (Yang et al., 2014). Although the high potentialities of the microbially-mediated As(III) oxidation, this process has received only scant attention (Crognale et al., 2017a). The majority of studies were performed by using As(III)-oxidizing microorganisms isolated from extreme environments (such as for example acid mine drainage, mine sediments and geothermal environments) and very little information is available on the process performances for the treatment of contaminated groundwater. The frequently reported long oxidation times (days or weeks) are not satisfactory to practically and efficiently couple this preliminary biological treatment to the fast As removal by adsorption. Overall, specific information about As-related functional genes and microorganisms involved in biological As(III)-oxidation was largely disregarded and only few studies reported the employment of mixed microbial communities (Crognale et al., 2017a).

This study aimed to evaluate the potentialities of biological As(III)-oxidation in laboratory scale biofilters treating As-contaminated groundwater through the selection and the establishment of biofilms composed by native water microbial communities. The oxidative performance of the biofilters was evaluated by using a variety of experimental conditions (e.g., various filling materials, flow rates, As(III) inflow concentration, As(III):As(V) ratio, filter volumes) in order to elucidate the best conditions to efficiently couple the proposed biological pre-oxidation to household-scale treatment units. The As(III)-oxidizing biofilms in the bioreactors were explored by applying an advanced microbial community characterization approach through flow cytometry, qPCR and high-throughput 16S rRNA gene sequencing.

## 2. Materials and methods

### 2.1. Biofilter set-up

Four polycarbonate columns (Ø 30 mm, height 135 mm) were used for the construction of biofilters with a bed volume (BV) of 0.1 L. Two biofilters with BV of 0.7 L (Ø 65 mm, height 200 mm) were also used in order to test As(III)-oxidation performance in systems with larger volumes (see Fig. S1). Sintered glass rings (porosity 56.7%) and coarse sand (porosity 26.4%), chosen based on their easy availability and low cost, were separately used as filling materials for the construction of biofilters (herein named “glass” and “sand” respectively). Groundwater with As concentration ranging from 2.5 to 4.5 μg $L^{-1}$ was used for biofilm growth. The water was let to circulate for 20 days throughout the biofilters (Fig. S1), afterwards inflowing water was continuously spiked with 100 μg As(III) $L^{-1}$ and the water circulated in a closed system throughout the columns for a different amount of time. The biofilters exposed to short-term As(III) acclimation period (~15 days) were hereinafter named “STA biofilters”. The biofilters operated with a long-term acclimation period (around 40 days) were named “LTA biofilters”. Oxidation efficiency was periodically checked until the biofilm was able to oxidize >60% of As(III) in 2 h under the selected conditions.

Once this performance was achieved, biofilters with 0.1 L and 0.7 L BV were used in kinetic experiments, respectively, under different operation conditions (see Section 2.2). Water tanks and biofilters were kept in the dark at 25 °C temperature for the entire duration of the experiments to prevent As(III) photo-oxidation. Possible As(III) oxidation within the tanks was absent within a period of 6 h. Kinetic experiments were carried out by recirculating the same water from the inflow tank