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# Modeling arsenite oxidation by chemoautotrophic *Thiomonas arsenivorans* strain b6 in a packed-bed bioreactor

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ABSTRACT

Arsenic is a major toxic pollutant of concern for the human health. Biological treatment of arsenic contaminated water is an alternative strategy to the prevalent conventional treatments. The biological treatment involves a pre-oxidation step transforming the most toxic form of arsenic, As (III), to the least toxic form, As (V), respectively. This intermediate process improves the overall efficiency of total arsenic removal from the contaminated water. As (III) oxidation by the chemoautotrophic bacterium *Thiomonas arsenivorans* strain b6 was investigated in a fixed-film reactor under variable influent As (III) concentrations (500–4000 mg/L) and hydraulic residence times (HRTs) (0.2–1 day) for a duration of 137 days. During the entire operation, seven steady-state conditions were obtained with As (III) oxidation efficiency ranging from 48.2% to 99.3%. The strong resilience of the culture was exhibited by the recovery of the bioreactor from an As (III) overloading of $5300 \pm 400$ mg As (III)/L day operated at a HRT of 0.2 day. An arsenic mass balance revealed that As (III) was mainly oxidized to As (V) with unaccounted arsenic ($\leq 4\%$) well within the analytical error of measurement. A modified Monod flux expression was used to determine the biokinetic parameters by fitting the model against the observed steady-state flux data obtained from operating the bioreactor under a range of HRTs (0.2–1 day) and a constant influent As (III) concentration of 500 mg/L. Model parameters, $k = 0.71 \pm 0.1$ mg As (III)/mg cells h, and $K_s = 13.2 \pm 2.8$ mg As (III)/L were obtained using a non-linear estimation routine and employing the Marquardt–Levenberg algorithm. Sensitivity analysis revealed $k$ to be more sensitive to model simulations of As (III) oxidation under steady-state conditions than parameter $K_s$.



## 1. Introduction

Arsenic (As) is a toxic metalloid released to the environment as a result of both natural and anthropogenic activities (Parikh et al., 2010). One of the most important natural sources of As contamination in water resources is the dissolution of As bearing minerals such as arsenopyrite (FeAsS), realgar (AsS), and orpiment ($As_2S_3$) by weathering or erosion (Simeonova et al., 2005). The anthropogenic sources of As release include mining, smelting for copper and gold, manufacturing of glass and chemical weapons etc. (Simeonova et al., 2005). Luo et al. (2010) reported very high levels of As (>1 g/L) in the wastewater effluent from industries involved in smelting operations.

The toxicity of As arises from its carcinogenic effects both in humans and other forms of life (Lloyd and Oremland, 2006), as concentrations in the range of 20–100 μg/L have been shown to cause certain medical disorders and cancers in humans (Desesso et al., 1998). The environmental impact of As poisoning in drinking water has been widely reported in Bangladesh and India (Bagla and Kaiser, 1996; Nickson et al., 1998). To limit the toxic effect of As in drinking water, U.S. Environmental Protection Agency lowered the maximum contaminant level (MCL) of As from 50 to 10 μg/L (USEPA, 2001).

Several treatment technologies have been developed for the remediation of As contaminated water (Jiang, 2001; Bissen and Frimmel, 2003; Dambies, 2004; USEPA, 2000). Most of the conventional treatment methods such as coagulation/filtration, lime softening, ion exchange, adsorption on iron oxides and activated alumina etc. are not very efficient in removing As (III) due to its high mobility and toxicity (Rhine et al., 2006; Clifford, 1990; Johnston and Heijnen, 2001). Therefore, for effective As removal, a pre-oxidation step to transform As (III) to As (V) is needed. The pre-oxidation step may be accomplished using both chemical and biological oxidation methods. However, chemical oxidation methods may result in the formation of harmful byproducts that may be difficult to remove (Ghurye and Clifford, 2001). Biological oxidation using either autotrophic or heterotrophic bacterial strains presents as an alternative to the conventional chemical methods.

In biological oxidation of As (III), the heterotrophic strains may convert As (III) to As (V) for detoxification purposes (Cervantes et al., 1994), whereas, autotrophic strains can sustain growth by utilizing the energy released during the oxidation process (Santini et al., 2000; Battaglia-Brunet et al., 2002). During autotrophic As (III) oxidation, As

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(III) serves as the electron donor, whereas, oxygen is used as an electron acceptor, with $CO_2$/$NaHCO_3$ acting as the carbon source for cell synthesis. The energy for cell synthesis is provided by the exergonic nature of the oxidation reaction (Battaglia-Brunet et al., 2002, 2006; Santini et al., 2000). The autotrophic strains may be preferred over the heterotrophic ones for the oxidation process because of the lower nutritional requirements and lesser possibility of the formation of any organic harmful by-products during the oxidation reactions (Suttigarn and Wang, 2005; Dastidar and Wang, 2009).

The biological treatment of toxic species has been previously demonstrated in continuous flow bioreactor studies (Nkhalambayausi-Chirwa and Wang, 2001). As (III) oxidation in continuous flow bioreactors has been reported in very limited studies (Dastidar and Wang, 2010; Wan et al., 2010; Wang and Suttigarn, 2007). Wan et al. (2010) conducted a fixed-film reactor study for the total removal of arsenic using the cells of *Thiomonas arsenivorans* strain b6 and zero-valent iron-coated sand. The study involved the operation of two column reactors under 10 mg As/L and an HRT of 4 h and 1 h for a total duration of 33 days. In this study, the potential of a fixed-film reactor in transforming As (III) to As (V) was investigated using attached cells of *T. arsenivorans* strain b6. This study also represents the first ever modeling analysis of autotrophic As (III) oxidation study in a biofilm reactor. The steady-state data and a modified Monod biofilm model were used to estimate the biokinetic parameters. A validation study was also performed to evaluate the effectiveness of the parameters in predicting the overall steady-state performance of the bioreactor.

## 2. Materials and methods

### 2.1. Bacterial strain and feed composition

*T. arsenivorans* (LMG 22795T) strain b6 was obtained from the BCCM/LMG collection center (Belgium). Details regarding isolation, phylogenetic position in the *Thiomonas* genus, and growth aspects of the strain, have been earlier described (Battaglia-Brunet et al., 2006). The *T. arsenivorans* strain b6 grows on a modified CSM (MCSM) medium with inorganic $CO_2$ as the sole source of carbon and energy released from As (III) oxidation for cell synthesis (Bryan et al., 2009). The MCSM medium consisted of two solutions: solution A contained 0.5 g of $K_2HPO_4$, 0.5 g of $KH_2PO_4$, 0.5 g of NaCl, 0.5 g of yeast extract, 0.05 g of $(NH4)_2SO_4$, and 1 mL of trace elements solution in 500 mL of deionized distilled water. The pH of solution A was adjusted to 6 with $H_2SO_4$. Solution B contained 0.1 g of $CaCl_2$ and 0.1 g of $MgSO_4$ in 500 mL of deionized distilled (DD) water. Both solutions A and B were autoclaved at 121 °C for 15 min, cooled and then mixed. The trace elements solution was prepared by adding 6.5 mL of HCl (25%), 1.5 g of $FeCl_24H_2O$, 60 mg of $H_3BO_3$, 100 mg of $MnCl_2 \cdot 4H_2O$, 120 mg of $CoCl_2 \cdot 6H_2O$, 70 mg of $ZnCl_2$, 25 mg of $NiCl_2 \cdot 6H_2O$, 15 mg of $CuCl_2 \cdot 2H_2O$ and 25 mg of $Na_2MoO_4 \cdot 2H_2O$ to 1 L of DD water. The feed to the packed-bed reactor was a modified MCSM medium to which 5 g/L each of $K_2HPO_4$ and $KH_2PO_4$ was added as buffer, while the yeast extract was eliminated to ensure autotrophic growth conditions.

### 2.2. Reactor system

The biofilm reactor (Fig. 1) was constructed from an acrylic column with internal diameter and length measuring 2.30 ± 0.01 cm and 20.1 ± 0.04 cm, respectively. The reactor was packed with 2997 spherical pyrex glass beads (Fisher Scientific Co, Pittsburg, PA) of 3 mm diameter providing a total external surface area of 847.4 $cm^2$ for attachment of cells of strain b6. The reactor was set up in a walk-in 30 °C temperature room and operated in an upflow mode with an effluent recirculation ratio of 50:1 to ensure completely mixed flow pattern in the reactor. Precalibrated peristaltic pumps (Masterflex, Cole-Parmer Inst. Co., Niles, Illinois) were used both for the influent and recycle flows during the reactor operation. The pumps were calibrated to attain empty bed hydraulic retention times (HRTs) of 0.2, 0.48, and 1 day under feed flow rates of 83.7, 172.8, and 423.8 mL/day, respectively. The pump and the reactor tubings were autoclaved at 121 °C for 30 min, and the interior of the reactor rinsed with 95% ethanol before assembling under a germ free hood (Steril Gard Class II Model, The Baker Company, Stanford, ME). Any biological growth in the feed tubes was minimized by close monitoring and periodic replacement. Bolted flanges and rubber gaskets were used for connecting the central component of the reactor to the top and bottom parts to prevent any leakage during the reactor operation.

### 2.3. Reactor startup

All the components of the reactor were assembled under a laminar flow hood (Steril Gard, class II type A/B3, Baker Company, Sanford, ME), and packed with autoclaved oven-dried solid glass beads (Fisher scientific Co, Pittsburg, PA). The reactor was first operated under an influent As (III) concentration of 100 mg/L and an HRT of 1 day for at least 14 days without cells to serve as control. Sample analyses showed the effluent As concentration quickly attaining the influent level within 1 day of operation, indicating no abiotic As (III) oxidation in the reactor assembly. Inocula of the *T. arsenivorans* strain b6 were first grown overnight in the MCSM medium. Once the cells reached the exponential growth phase, they were harvested by centrifugation at 3580g for 20 min. The harvested cells were washed three times with 0.85% NaCl solution before use in the experiments. Finally, the reactor was inoculated with 30 mL of overnight grown pure cultures of *T. arsenivorans* strain b6, and then operated under an influent As (III) concentration of 500 mg/L and HRT of 1 day for at least 15 days until cell attachment was observed on the glass beads. After startup, the HRT was varied between 0.2 and 1 day under a constant influent As (III) concentration of 500 mg/L for the first fives phases (I–V) of operation. For the remaining three phases (VI–VIII), the reactor was operated under a constant HRT of 1 day, while the influent As (III) concentration was varied between 1000 and 4000 mg/L. The operating conditions of the reactor throughout the entire study were summarized in Table 1.

### 2.4. Analytical methods

The effluent samples from the reactor were collected using 1 mL sterile disposable pipets for the determination of As (III), As (V), total As, and total protein concentrations of the suspended and attached cells, respectively. The samples collected for As analyses were centrifuged at speed of 12,000g for 10 min using a microcentrifuge (Brinkmann Instruments Inc., West bury, NY). The supernatant was then acidified using 1% $HNO_3$ (pH$<$2), and preserved in 4 °C for no more than 7 days prior to analyses (APHA, 1995). As (III), As (V), and total As were analyzed using a silver diethyldithiocarbamate (SDDC) method (3500-As B, APHA, 1995). This method was slightly modified as described by Suttigarn and Wang (2005).

The biological samples were analyzed immediately to prevent any changes that may occur after collection. The viable suspended cell concentration was determined according to the spread plate technique outlined in section 9215C of the *Standard Methods for the Examination of Water and Wastewater* (APHA, 1995). The morphology of the bacterial colonies on the agar plates was also examined to ensure purity of the culture during the reactor operation. No contamination was observed throughout the experiment.

Once steady-state operating conditions were obtained, six glass beads were removed from the top and bottom of the reactor, respectively, under the laminar flow hood (Steril Gard Class II Model, The Baker Company, Stanford, ME). The removed beads were then immediately replaced by 12 sterile ones while maintaining a stable condition for the rest of the beads and the liquid content in the reactor. The collected beads were then placed each inside a 10 mL microreaction vessel (Supelco, Inc., Bellefonte, PA) containing 1 mL MCSM (without yeast extract) solution. The glass beads were then washed vigorously in the vessels for at least 5–10 min