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Assessment of chromium-contaminated groundwater using a thiosulfate-oxidizing bacteria (TOB) biosensor

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HIGHLIGHTS

- A biosensor is developed using thiosulfate oxidizing bacteria (TOB) for groundwater.
- Primary detection of TOB activity is with increase in electrical conductivity (EC).
- Increasing thiosulfate concentration (100–600 mg L^{-1}) induced a lag period in EC rise.
- SOB was inhibited by 16.7–100% in the presence of Cr^{6+} (50–1000 μg L^{-1}).
- Real groundwater (Cr^{6+} contaminated) with SOB has given EC_{50} of Cr^{6+} 78.96 μg L^{-1}.

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ABSTRACT

The effect of Cr^{6+}-contaminated groundwater was assessed using thiosulfate-oxidizing bacteria (TOB). Electrical conductivity (EC), pH, and sulfate production were determined based on thiosulfate oxidation. Final pH values in the different test treatments of Cr^{6+}-contaminated groundwater (50–1000 μg Cr^{6+} L^{-1}) ranged from 2.02 ± 0.09 to 7.76 ± 0.07 and EC ranged from 5.95 ± 0.03 to 3.63 ± 0.03 mS cm^{-1}. Inhibition of TOB due to Cr^{6+} was between 16.7% and 100%, with higher levels of inhibition occurring at higher Cr^{6+} concentrations. The median effective concentration (EC_{50}) was 78.96 μg Cr^{6+} L^{-1}. These data demonstrate that TOB can detect less than 100 μg L^{-1} of Cr^{6+} in the groundwater and can be used as an effective bioassay for toxicity assessment.

1. Introduction

Seepage of heavy metals from disposal sites to groundwater poses serious environmental and human health concerns at locations in several countries (Sharma and Al-Busaidi, 2001). Anthropogenic sources of heavy metal contamination include landfill leachate as well as several industries, such as plating, ceramics, glass, mining, and battery manufacturing (Aziz et al., 2004). Although there are natural sources of heavy metals, such as ore deposit leachate and volcanic extruded products (Marcovecchio et al., 2007), significant risks to human populations are typically associated with metals from artificial sources.

Chromium is an essential micronutrient for plant and animal metabolism; however, at high levels of exposure, it can cause nausea, skin ulcerations, and lung cancer (Ajmal et al., 1984). Usually, uncontaminated groundwater contains less than 10 μg L^{-1} chromium (Peterson et al., 1996), but concentrations at a contaminated site may reach several hundred parts per million (Loyaux-Lawniczak et al., 2001). Groundwater systems may contain two types of chromium species: trivalent (Cr^{3+}) and hexavalent (Cr^{6+}), based on redox conditions. Of these two, hexavalent Cr is substantially more soluble, toxic, and mobile (Nikolaidis et al., 1999). If Cr^{6+} is orally ingested, some is likely to be converted to Cr^{3+} in the stomach because of the low pH, but toxicity will be little affected, and it will move across cell membranes, potentially damaging the DNA (Sedman et al., 2006).

Several methods have been developed for indirectly and directly measuring toxicity, including analytical chemistry techniques e.g., chromatographic systems High Performance Liquid Chromatography (HPLC), Gas Chromatography (GC), and Atomic Absorption Spectrometry (AAS) and bioassays (van Wezel et al., 2010). Chromatographic methods are very sensitive and quantitative, but are laborious, expensive, and can only detect specific chemicals at any one time (Woutersen et al., 2011). In addition,

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quantitative chemistry measurements do not take into account factors that may ameliorate or exacerbate the environmental toxicity of some contaminants. Different bioassays have been developed that utilize endpoints such as acute and long-term mortality, growth rate, photosynthetic activity, respiration, amperometry (Hassan et al., 2012), and bioluminescence (Hassan and Oh, 2010). Numerous organisms have been well-studied for their use in toxicity bioassays, including vertebrates (e.g, *Pimephales*), invertebrates (e.g., *Daphnia*), algae, and bacteria, such as *Vibrio fischeri*, iron-oxidizing bacteria, and sulfur-oxidizing bacteria (Oh et al., 2011). Bioassays are direct measures of toxicity, and their results are applicable to a wide range of toxic chemicals.

The biological oxidation of reduced sulfur compounds (S°, $S_2O_3^{2-}$, H_2S^{2-}, and SO_3^{2-}) is a key reaction in the sulfur cycle. These reactions are carried out by Archaea, bacteria, and phototrophic organisms (Friedrich et al., 2001), which can be found in various ambient and extreme conditions, including hypersalinity, extreme acidity to alkalinity, and a wide range of temperatures. Acidophiles have historically received more attention from researchers due to their importance in the bioleaching process (Sorokin et al., 2006). The complete oxidation of thiosulfate may result in sulfate or tetrathionate, according to the following equations (Robertson and Kuenen, 1999):

$$Na_2S_2O_3 + 2O_2 + H_2O \rightarrow Na_2SO_4 + H_2SO_4 \quad (1)$$

$$4Na_2S_2O_3 + O_2 + 2H_2O \rightarrow 2Na_2S_4O_6 + 4NaOH \quad (2)$$

$$2Na_2S_4O_6 + 7O_2 + 6H_2O \rightarrow 2Na_2SO_4 + 6H_2SO_4 \quad (3)$$

Thiosulfate-oxidizing bacteria (TOB) utilize thiosulfate as an electron donor and produce sulfate by decreasing pH and increasing electrical conductivity (EC). In this study, TOB activity was exploited for detecting toxicity in chromium-contaminated groundwater.

2. Materials and methods

2.1. Thiosulfate master culture reactor

A thiosulfate master culture reactor (TMCR) was designed to receive a constant supply of TOB culture. Soil (10 g) from an agricultural field near Kangwon National University was used as an inoculum to the TMCR. A 1-L media bottle (Pyrex, Germany) filled with 500 mL of Thiosulfate containing nutrient mineral buffer (NMB) solution was used for the TMCR. The concentration of thiosulfate was 1 g L^{-1}. The synthetic stream water was prepared by diluting the NMB solution 100 times. The NMB solution consisted of: $NaHCO_3$ (3.13 g L^{-1}), NH_4Cl (0.31 g L^{-1}), $NaH_2PO_4{\cdot}H_2O$ (0.75 g L^{-1}), KCl (0.13 g L^{-1}), NaH_2PO_4 (4.22 g L^{-1}), Na_2HPO_4 (2.75 g L^{-1}), and trace metal and vitamin solutions, as previously described by (Lovley and Phillips, 1988).

Air was supplied into the TMCR through a stone diffuser (flow rate: 150–250 mL min^{-1}). The TMCR incubation temperature was 30 °C. The TMCR was operated in fed-batch mode by removing 250 mL of waste and refilling with 250 mL of Thiosulfate NMB solution at 3–5-d intervals. The initial pH, EC, and alkalinity of the TMCR were 6.76 ± 0.01, 5.07 ± 0.03 mS cm^{-1}, and 19.33 ± 1.15 mg L^{-1} as $CaCO_3$, respectively.

2.2. Batch test strategy

A 100-mL media bottle (Pyrex) was used as the test reactor; each bottle received a mixture of 40 mL of sodium thiosulfate (1 g L^{-1})-containing groundwater (Table 1). A 10 mL culture from the TMCR was added to the reactor as an inoculum. The pH of the inocula was raised to 6.0 with NaOH to prevent thiosulfate precipitation. To assess the impact of varying levels of thiosulfate, concentrations from 100 to 600 mg L^{-1} were selected and tested in a 100-mL reactor containing 40 mL of thiosulfate-containing groundwater, with 10 mL of inoculum. Hexavalent chromium was selected as the chemical for testing, using nominal Cr^{6+} concentrations of 0 (control), 50, 100, 250, 500, 750, and 1000 μg L^{-1}. The test bottles were covered with aluminum foil to minimize water evaporation and provide adequate gas exchange. All test reactors were incubated at 30 °C with agitation at 150 rpm for 48 h. Toxicity tests were performed in three replicates, and results were found to be within ±5%.

Table 1
Groundwater characteristics (mean and standard deviation (SD) calculated from five replicates).

Parameters	Mean ± SD*
pH	7.53 ± 0.06
Conductivity, μS cm^{-1}	153.8 ± 0.56
Cl^- (mg L^{-1})	8.09 ± 0.10
NO_2^--N (mg L^{-1})	ND**
NO_3^--N (mg L^{-1})	3.76 ± 0.05
PO_4^{2-} (mg L^{-1})	ND**
SO_4^{2-} (mg L^{-1})	1.31 ± 0.06
Alkalinity mg L^{-1} as $CaCO_3$	35.00 ± 1.79
Cr^{6+}	ND**
Cd^{2+}	ND**
Cu^{2+}	ND**
As^{2+}	ND**
Zn^{2+}	ND**

* % RSD was not more than 5%.
** Not detected.

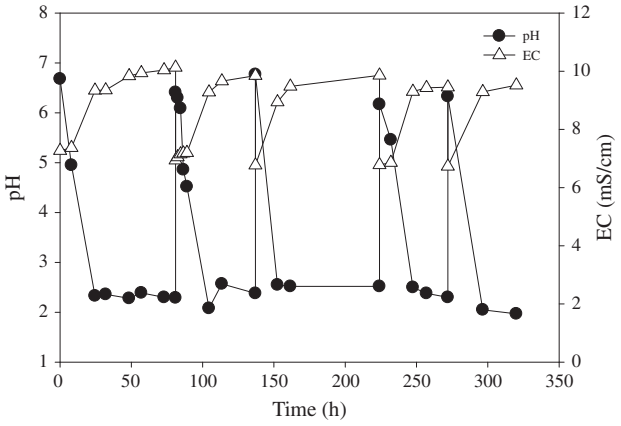

Fig. 1. Thiosulfate MCR pH and EC profiles in fed-batch mode.

For the detection of TOB activity EC was selected as in the presence of ions the value of EC increases. TOB produce sulfate which is a strong anion and has the ability to increase EC. As more sulfates are produced EC keeps on increasing and helps us to monitor TOB activity easily.

2.3. Chemicals and analysis

All chemicals used in this study were purchased from Sigma Aldrich (St. Louis, MO, USA) and were of analytical grade. An Orion 410A pH meter was used to measure pH, and EC was determined using an Inlab 737 meter (Mettler Toledo, Columbus, OH, USA). Alkalinity was measured by titration according to Standard Methods 2320 B (Clesceri et al., 1996). For determination of chloride,

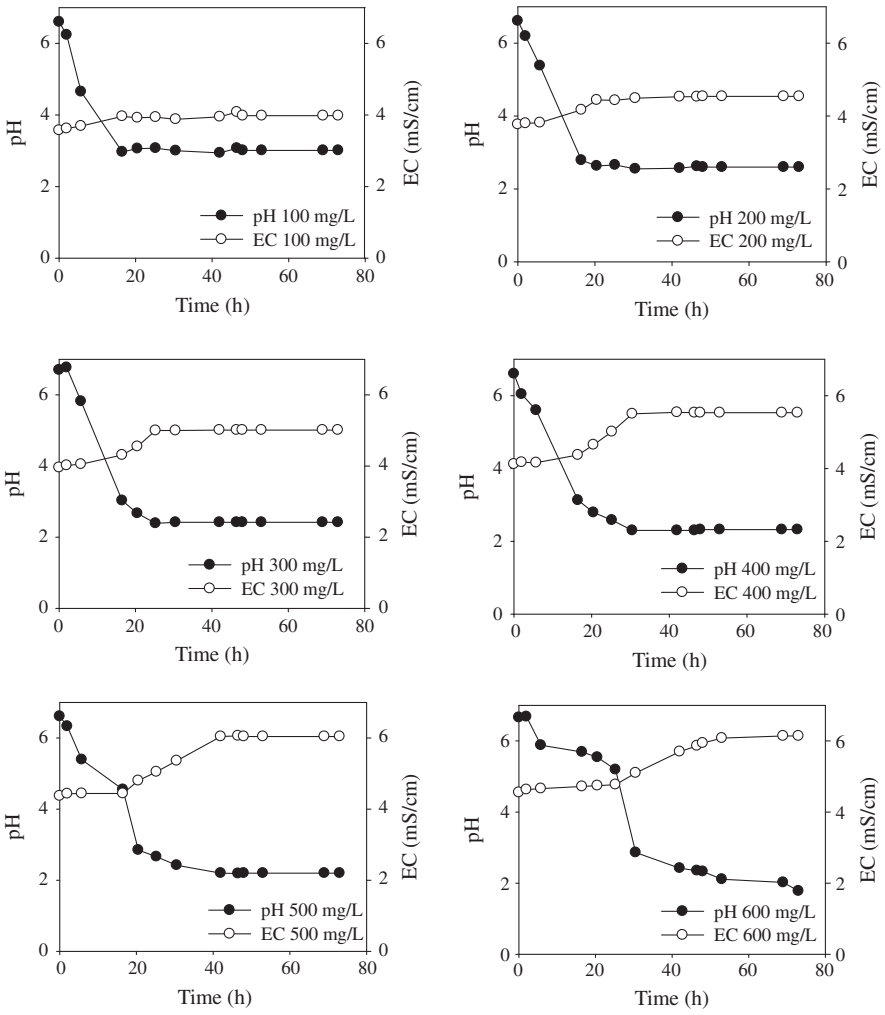

Fig. 2. pH and EC profiles for the utilization of different thiosulfate concentrations.

nitrite, nitrate, phosphate, sulfate (SO_4^{2-}), and thiosulfate ($S_2O_3^{2-}$), samples were first filtered through a 0.45-μm syringe filter, followed by analysis by ion chromatography (ICS-900, Dionex Corp with AS40 Column) with a suppressed conductivity detector. The total concentrations of Cr^{6+}, Cd^{2+}, Cu^{2+}, As^{2+}, and Zn^{2+} were determined using an inductively coupled plasma (ICP-OES) spectrometer (Optima DV, Perkin-Elmer, Waltham, MA, USA).

The inhibitory effect of Cr^{6+} on TOB growth was calculated using the following equation:

$$\text{Inhibition of SOB}(\%) = \text{EC}_{(\text{Control})} - \text{EC}_{(\text{Sample})}/\text{EC}_{(\text{Control})} * 100 \quad (4)$$

where $EC_{(Control)}$ is the EC without inhibition, and $EC_{(Sample)}$ is the EC of the media with inhibition. The median effective concentration (EC_{50}) responsible for 50% growth inhibition was calculated by using TOXCALC (v5.0.32, Tidepool Scientific Software, McKinleyville, CA, USA). These values were calculated by the Maximum Likelihood-Probit analysis test. Similarly, the toxicity unit (TU) was calculated using the EC_{50} (as TU = 100/EC_{50}).

3. Results and discussions

The groundwater used in this study was obtained from a village near Chuncheon, South Korea. The pH was neutral and EC was very low. Alkalinity of the groundwater was low (35 mg L^{-1} as $CaCO_3$), and there was only a small amount of sulfate (1.31 mg L^{-1}). The selected heavy metals (Cr^{6+}, Cd^{2+}, Cu^{2+}, As^{2+}, and Zn^{2+}) were not detected in the groundwater.

The TMCR pH was adjusted to less than 6.0 so that thiosulfate would not precipitate upon the initial addition of thiosulfate. The pH, EC, and alkalinity of thiosulfate containing the NMB medium were 6.76 ± 0.01, 5.07 ± 0.03 mS cm^{-1}, and 19.33 ± 1.15 mg L^{-1} as $CaCO_3$, respectively. After 13 h, thiosulfate was consumed from the TMCR, and the pH dropped to 4.0, as depicted in Fig. 1. When the pH exceeded 3.0, thiosulfate oxidation was maintained. The pH of the TMCR varied from 2.3 to 6.68, while the EC ranged from 7.27 to 10.04 mS cm^{-1}. The TMCR was monitored for 2 months before conducting the toxicity experiments. Changes in pH and EC were measured during this process.

3.1. Effect of thiosulfate concentration

Batch tests with various concentrations of thiosulfate (100–600 mg L^{-1}) were conducted using inoculum from the TMCR (Fig. 2). The pH and EC of the reactors were checked at the appropriate times for evaluation purposes. The reactors containing 100–400 mg L^{-1} of thiosulfate quickly demonstrated an increase in EC and a decline in pH. When the thiosulfate was higher, however, there was a slow increase in EC followed by a more rapid rise. Similar behavior was seen in the solution pH, which was between 7.0 and 5.0, but experienced a rapid decline until pH reached 2.0. This response in the solutions containing higher thiosulfate levels

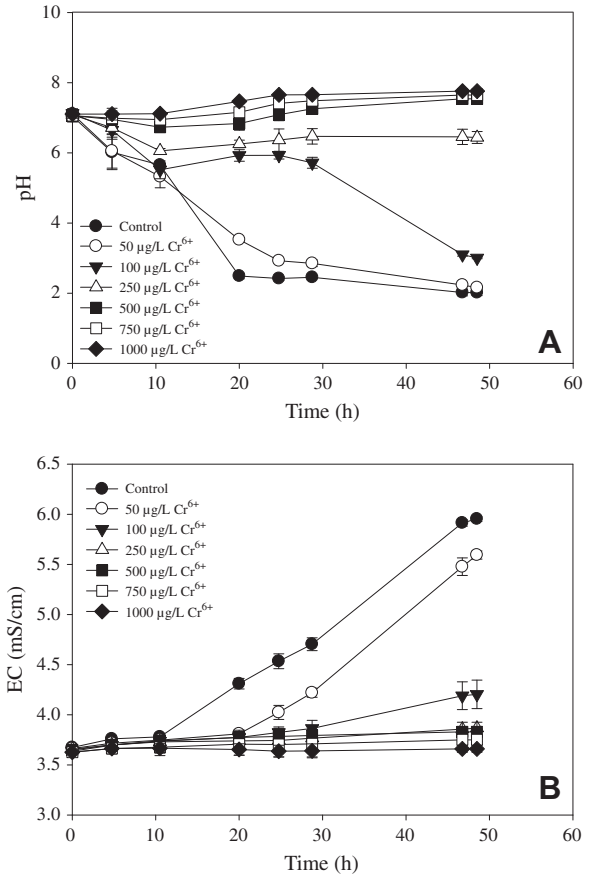

Fig. 3. Effect of different Cr^{6+} concentrations on TOB activity.

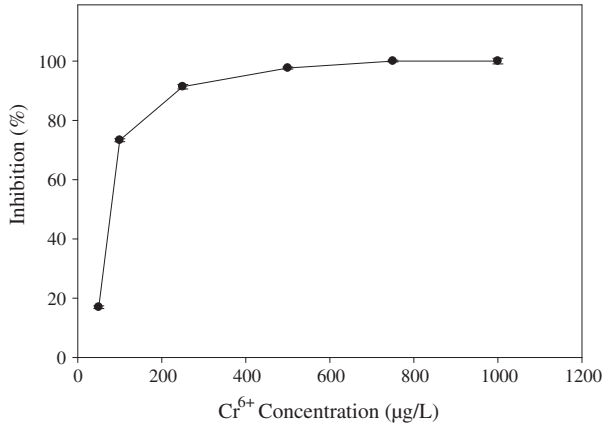

Fig. 4. Inhibition of TOB activity at different concentrations.

suggests the formation of an intermediate molecule (e.g. tetrathionate), which may have slowed the initial EC increase. Wentzien et al. also observed same behavior of *Thiobacillus intermedius* culture. The pH and redox potential remained low until the exhaustion of thiosulfate then pH dropped and redox potential increased. Presence of different intermediates was also observed (Wentzien et al., 1994).

3.2. Toxicity of Cr^{6+} on TOB

The initial pH of all reactors was 7.08 ± 0.03, which is comparatively stable for thiosulfate (Fig. 3(A)). In the control reactor, a small lag phase was observed from initiation until approximately 10 h. After that lag phase, the pH declined sharply until 40 h, followed by a final drop to less than 2.5 in 48 h. There was a slight difference in the pH drop between control and reactor with 50 μg L^{-1} Cr^{6+}. In the reactor containing 100 μg L^{-1} Cr^{6+}, there was a relatively longer lag phase. In the 250 μg L^{-1} Cr^{6+} treatment, only a slight decrease in pH was observed initially and later on pH started increasing and finally stabilized. At the higher Cr^{6+} concentrations, the pH initially increased above 7.5 for up to 30 h, followed by stabilization. The final pH values were 2.02 ± 0.09, 2.16 ± 0.02, 3.0 ± 0.02, 6.44 ± 0.17, 7.54 ± 0.06, 7.65 ± 0.08, and 7.76 ± 0.07 with the control, 50, 100, 250, 500, 750, and 1000 μg L^{-1} Cr^{6+} treatments, respectively. These data suggest that the solution chemistry was similar in the control and first two concentrations, but changed dramatically in the 250 μg L^{-1} and higher treatments.

The initial ECs were 3.67 ± 0.01, 3.66 ± 0.01, 3.64 ± 0.01, 3.66 ± 0.01, 3.65 ± 0.01, 3.63 ± 0.01, and 3.63 ± 0.01 mS cm^{-1} with the control, 50, 100, 250, 500, 750, and 1000 μg L^{-1} Cr^{6+} treatments, respectively. EC was generally stable for approximately 20 h. EC then climbed sharply until approximately 48 h post-initiation. The presence of Cr^{2+} caused a decrease in TOB activity (Fig. 3(B)), which resulted in a more substantial, though gradual, decline in EC at the end of the test. Final ECs were 5.95 ± 0.03, 5.59 ± 0.04, 4.20 ± 0.14, 3.87 ± 0.02, 3.83 ± 0.10, 3.65 ± 0.11, and 3.63 ± 0.03 mS cm^{-1} with the control, 50, 100, 250, 500, 750, and 1000 μg L^{-1} Cr^{6+} treatments, respectively.

Sulfate is produced as the end product of thiosulfate oxidation. As the sulfate concentration increases, the pH decreases and EC rises with time. In this study, sulfate was measured in the reactors to evaluate the effect of Cr^{6+} on TOB activity. The initial sulfate concentration was 355.53 ± 5.03 mg L^{-1} for all reactors. In the control reactor, there was an immediate increase in the sulfate concentration; the maximum concentration was reached at the end of the study. Finally, the amount of sulfate produced after 48 h was 947.17, 786.45, 252.82, 81.76, and 22.19 mg L^{-1} with the control, 50, 100, 200, and 500 μg L^{-1} Cr^{6+} treatments, respectively, whereas no sulfate was produced in the reactors containing 750 and 1000 μg L^{-1} Cr^{6+}. The percent inhibition was calculated in comparison with the EC changes in control reactors. Using the control response as a base value, the percent EC inhibition was 16.7 ± 0.45, 73.31 ± 0.50, 91.37 ± 0.77, 97.66 ± 0.34, 100.0 ± 0.30, and 100.0 ± 0.07 in the 50, 100, 250, 500, 750, and 1000 μg L^{-1} Cr^{6+} reactors, respectively (Fig. 4). An EC_{50} of 78.96 μg L^{-1} was measured for the TOB sensor.

The toxicity microbial systems in current practice have demonstrated less sensitivity to Cr^{6+} (Table 2). Cho et al. measured 30 min IC_{50} of 17 500 μg Cr^{6+} L^{-1} and Hsieh et al. reported 15 min EC_{50} of 18 000 μg Cr^{6+} L^{-1} for *Vibrio fischeri* (Cho et al., 2004; Hsieh et al., 2004). In the current study, the EC_{50} was estimated to be

Table 2
Comparison of relative toxicity of Cr^{6+} of other systems with TOB.

Toxicity assessment systems	Concentration of Cr^{6+} (μg L^{-1}) (EC_{50})	Reference
Thiosulfate oxidizing bacteria (TOB) (48-h) EC_{50}	78.96	This study
Selenastrum capricomatum (72-h) EC_{50}	207.23	Radetski et al. (1995)
Daphnids (24–48 h) EC_{50}	260–1790	Hsieh et al. (2004)
Hyalella azteca (7-d) LC_{50}	3139.50	Borgmann et al. (2005)
Fish (96-h) LC_{50}	11 200–133 000	Hsieh et al. (2004)
V. fischeri (0.5-h) IC_{50}	17 500	Cho et al. (2004)

78.96 μg L^{-1}, which is notably lower than these earlier reported values. This study has shown that toxic chemicals in groundwater can be detected using TOB. A distinct response at low levels of Cr^{6+} suggests that TOB may be an effective tool for assessing the presence of Cr^{6+} and its effects in groundwater.

4. Conclusions

An initial study of Cr^{6+} assessment by TOB was conducted. Thiosulfate was maintained in stable form at higher pH (>4). A decrease in TOB activity was verified in terms of pH, EC, and sulfate production in the presence of Cr^{6+}. Inhibition concentrations were very low, and an EC_{50} of 78.96 μg L^{-1} was measured. The inhibition of bacterial activity ranged from 16.7% to 100% in the presence of Cr^{6+}. The TOB studied here can be cultured through the careful maintenance of pH, and then applied, when needed, for groundwater toxicity monitoring.

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