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Short communication

Semi-continuous detection of toxic hexavalent chromium using a sulfur-oxidizing bacteria biosensor

Anup Gurung [a], Sang-Eun Oh [a,*], Ki Duck Kim [b], Beom-Soo Shin [b,**]

[a] Kangwon National University, Gangwon-do, Chuncheon 200-701, South Korea
[b] Department of Biosystems Engineering, Kangwon National University, Gangwon-do, South Korea

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ABSTRACT

Toxicity testing is becoming a useful tool for environmental risk assessment. A biosensor based on the metabolic properties of sulfur-oxidizing bacteria (SOB) has been applied for the detection of toxic chemicals in water. The methodology exploits the ability of SOB to oxidize elemental sulfur to sulfuric acid under aerobic conditions. The reaction results in an increase in electrical conductivity (EC) and a decrease in pH. Five hours after Cr^{6+} was added to the SOB biosensor operated in semi-continuous mode (1 min rapid feeding and 29 min batch reaction), a decrease in effluent EC and an increase in pH (from 2–3 to 6) were detected due to Cr^{6+} toxicity to SOB. The SOB biosensor is simple; it can detect toxic levels of Cr^{6+} on the order of minutes to hours, a useful time scale for early warning detection systems designed to protect the environment from further degradation.

1. Introduction

Due to the complex toxic nature of industrial effluents, the biological effects of industrial effluents and wastewaters discharged into aquatic ecosystems is a matter of increasing concern (Farre and Barcelo, 2003; Gómez et al., 2001; Sponza, 2003; Tonkes et al., 1999). In recent years, bioassays and biosensors, using species representing different trophic levels, have been developed and applied to identify the relevant toxic substances present in wastewaters and to evaluate the impact of their release into the environment (Farre and Barcelo, 2003; Liu et al., 2002; Sponza, 2002). Unlike conventional physiochemical methods, toxicity testing (or biomonitoring) can reveal synergistic effects of complex compounds (including unknown and undetermined substances) that are present in wastewaters (Farre and Barcelo, 2001; Sahu et al., 2008; Van Ginkel et al., 2011).

In particular, sulfur-oxidizing bacteria (SOB) biosensors, used in combination with acute toxicity tests, could be valuable screening tools that may enable researcher to better understand the toxicity of an environmental toxicant (Hassan et al., 2010; Oh et al., 2011; Van Ginkel et al., 2010). SOB can be used to assess whether wastewaters pose a risk to aquatic life. SOB are chemoautotrophic bacteria that can use reduced sulfur compounds as an energy source to produce sulfuric acid (H_2SO_4) under aerobic conditions. Some SOB, such as those of the Genus *Acidithiobacillus*, can use elemental sulfur (S°) particles as the electron donor and O_2 as an electron acceptor, as illustrated in Eq. (1) (Madigan et al., 2009):

$$S^\circ + H_2O + 1.5O_2 \rightarrow SO_4^{2-} + 2H^+, \Delta G^{\circ\prime} = -587\ \text{kJ/reaction} \quad (1)$$

In this reaction, SOB oxidize S° to sulfate (SO_4^{2-}) and protons (H^+), ultimately reducing the pH of the medium to ~2. In addition, the formation of sulfate ions increases the electrical conductivity (EC) of the medium (Madigan et al., 2009). EC is a measurement of the ability of the medium to carry an electric current and is proportional to the concentration and type of ions in solution (Oh et al., 2011). The presence of toxic chemicals inhibits the activity of SOB that stops the oxidation of sulfur to sulfate and pH changes from acidic to neutral environment (Gurung et al., 2011; Oh et al., 2011). SOB can be used for monitoring toxicity by observing changes in pH and EC, since the SOB biosensor is validated using EC and pH data (Oh et al., 2011). In this study, a semi-continuous SOB biosensor was applied for the detection of hexavalent chromium, Cr^{6+}, in an aqueous environment and the measured values of EC and pH of the influent and effluent were compared.

* Corresponding author. Tel.: +82 33 250 6449; fax: +82 33 241 6640.
** Corresponding author. Tel.: +82 33 250 6493; fax: +82 33 255 6406.
E-mail addresses: ohsangeun@kangwon.ac.kr (S.-E. Oh), bshin@kangwon.ac.kr (B.-S. Shin).

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2. Materials and methods

2.1. Culture and medium

Aerobic return activated sludge (1 mL) was used as the inoculum (source of SOB) to SOB biosensors packed with S° (50 mL; 2–4 mm diameter). The sludge was taken from the Chuncheon Wastewater Treatment plant in Chuncheon City, Kangwon-do, Republic of Korea. Synthetic stream water was prepared by diluting the following nutrient mineral buffer (NMB) solution 100 times: $NaHCO_3$ (3.13 g L^{-1}), NH_4Cl (0.31 g L^{-1}), NaH_2PO_4 (4.87 g L^{-1}), KCl (0.13 g L^{-1}) and Na_2HPO_4 (2.75 g L^{-1}). Trace metal and vitamin solutions previously described by Oh et al. (2004) were diluted 100 times and added to the synthetic stream water. The pH, EC, and alkalinity of the synthetic stream water were 7.2 ± 0.2, 0.17 mS cm^{-1}, and 45 mg L^{-1} as $CaCO_3$, respectively, similar to actual stream water (Gongi stream in Chuncheon), which, according to our measurements, had a pH of 6.9–7.1, EC = 0.18–0.20 mS cm^{-1}, and alkalinity of 75 mg L^{-1} as $CaCO_3$ at the time of these experiments.

2.2. SOB biosensor construction and operation

Three identical cylindrical reactors were constructed as described previously (Oh et al., 2011) (Fig. 1). Each biosensor was packed with 50 mL (82 g) of S° particles (2–4 mm diameter). Air was introduced from the bottom at a flow rate of 150–250 mL min^{-1}. The biosensors were inoculated with sludge and incubated at 30 °C for ~3 d in batch mode. The biosensors were then fed synthetic stream water semi-continuously (50 mL/min; 1 min rapid feeding and 29 min batch reaction) in up-flow mode using adjustable peristaltic pumps. The influent synthetic stream water is distributed throughout the surface of the sulfur particles during the rapid feeding (1 min) and then the reaction initiates (i.e. 29 min batch oxidation of sulfur particles) as the microorganisms come in contact with sulfur (substrate). Upon reaching steady-state conditions (i.e. stable EC and pH values), chromium hexavalent of 1 mg/L (in the form of $K_2Cr_2O_7$, Sigma–Aldrich, St. Louis, MO) was applied to the biosensor influents, and the resultant effluent EC and pH values were monitored. Reaction times were varied (30, 20, 15, and 10 min) to ascertain the shortest reaction time possible for toxicity monitoring.

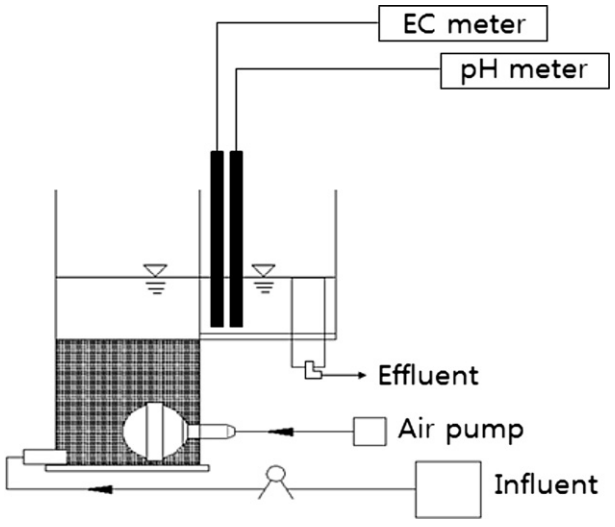

Fig. 1. Schematic of the SOB biosensor including sulfur particles, air supply, EC and pH meters.

2.3. Chemical and analyses

EC and pH were measured using meters from LUTRON (models #-YK 22 CT and #-pH 208c, respectively). The EC and pH meters were connected to a personal computer and all data were automatically recorded every 2 min. For SO_4^{2-} analysis, samples were collected from the effluent, filtered through a 0.2-μm syringe filter (Fisher Scientific), and stored at 4 °C prior to ion chromatography analysis (DX-100, Dionex Corp, AS14 column).

3. Results and discussion

3.1. Steady-state conditions

The SOB biosensor was inoculated and operated in batch mode for 3 d without added toxic compounds, fed with synthetic stream water to achieve the baseline steady-state EC and pH. Fig. 2 illustrates the influent and effluent EC and pH values during the steady-state conditions. The influent EC and pH values of the synthetic stream water were 197 μS/cm and pH 7.0, respectively. Using actual stream water from the Gongi stream in Chuncheon, South Korea with an EC of 197 μS/cm and a pH of 7, the effluent EC stabilized in the range of 422–778 μS/cm and pH stabilized in the range of 2–3, depending on the reactor used (see next paragraph below). These values were maintained over a period of 60 d for a reactor fed the synthetic stream water. The observed increase in EC and decrease in pH indicate that SOB attached to S° particles and produce H_2SO_4 under aerobic condition.

The inter-reactor variability in EC and pH that was observed was likely due to different sulfur surface areas resulting in different masses of acclimated bacteria becoming attached to the S° particles. Although the same volume (50 mL) of sulfur was added to each biosensor, the total mass and surface area of sulfur can be different between biosensors (Van Ginkel et al., 2010), leading to different production rates of H_2SO_4. However, this phenomenon is not a significant concern with respect to SOB biosensor use; changes in in-reactor EC and pH are more relevant for the purpose of monitoring toxicity (Oh et al., 2011; Van Ginkel et al., 2010).

3.2. Effect of reaction time

Four different reaction times (30, 20, 15, and 10 min) were tested to determine the minimum time effective for monitoring toxicity. Fig. 3 illustrates how EC values changed as a function of reaction time. Samples were taken for each reaction time period and the SO_4^{2-} concentration was measured. The influent EC and SO_4^{2-}

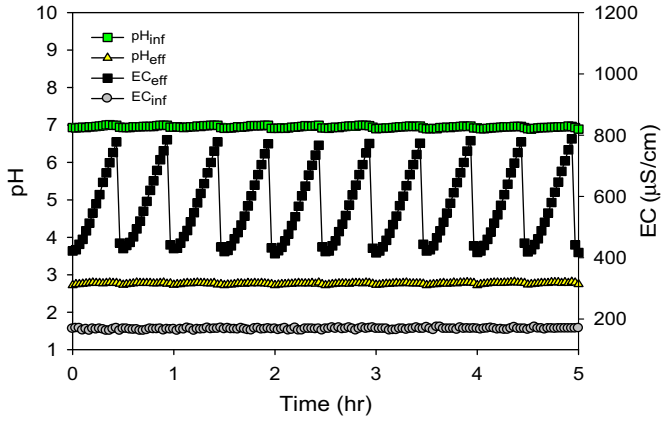

Fig. 2. Influent and effluent EC and pH values at stable conditions during 30 min reaction time (1 min feeding, 29 min batch).

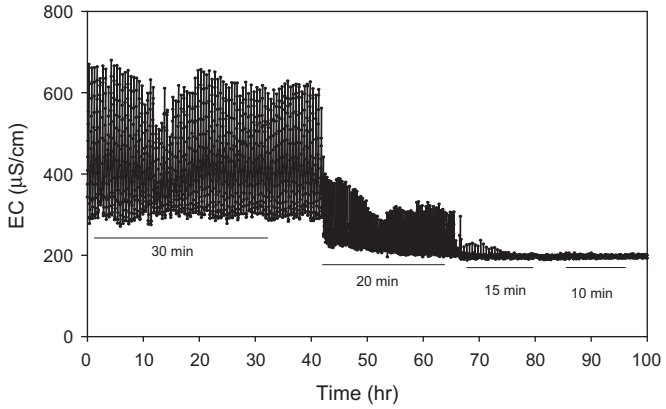

Fig. 3. Change of EC value under different reaction time i.e. at 10, 15, 20 and 30 min reaction time.

concentration were 197 μS/cm and 4.2 ± 0.7 mg/L, respectively. At 30 min reaction time (1 min rapid feeding and 29 min batch), the SO_4^{2-} concentration of 95 ± 0.5 mg/L was obtained. The effluent's EC averaged at 650 ± 0.6 μS/cm while the pH values were in the range of 2–3.

Decreasing the reaction time from 30 to 20 min decreased the effluent EC from ~650 to 412 μS/cm and decreased the SO_4^{2-} concentration from 95 to 43 mg/L. Higher pH values (~3.5) were observed after a 20-min reaction relative to a 30-min reaction (~2.5), presumably due to the short reaction time of SOB. At a reaction time of 15 min, the effluent EC was approximately 227 μS/cm and the SO_4^{2-} concentration was also decreased significantly. The SO_4^{2-} concentration at the 15-min reaction period was 25 mg/L, which is less than one-third of that observed after a 30-min reaction period. The 10-min reaction time period proved to be too short to observe changes in in-reactor EC and pH compared to the influent water and produced a minimal amount of SO_4^{2-} (14 mg/L), the production of which is limited by the duration that water stays in the reactor. Although reaction times of 15 and 20 min allowed detectable changes in EC and pH values, the reaction time of 30 min was chosen for further toxicity testing in this analysis because it produced more stable and significant changes.

3.3. Effects of Cr^{6+}

Fig. 4 illustrates Cr^{6+}-dependent EC changes. Effluent EC decreased immediately upon administration of 1 mg/L Cr^{6+} to the system. After 5 h, the effluent EC returned to the influent EC (197 μS/cm) level and SOB activity was inhibited almost completely. The SOB biosensor can resume its original function after addition of synthetic stream water to the reactors (Fig. 4). After the addition of Cr^{6+} to the SOB biosensors, the in-reactor EC decreased to the level of the influent EC. However, after 3 cycles of feeding synthetic stream water without Cr^{6+} into the system, batch operation in-reactor EC increased over time showing a 1 h lag phase and a linear increase of EC. Thereafter, when the system operated in semi-continuous mode, stable pH and EC values were maintained as shown in the Fig. 1. In addition, batch test was performed using new sulfur and Cr^{6+} without SOB sludge. There was no change in influent and effluent Cr^{6+} concentration during the batch test.

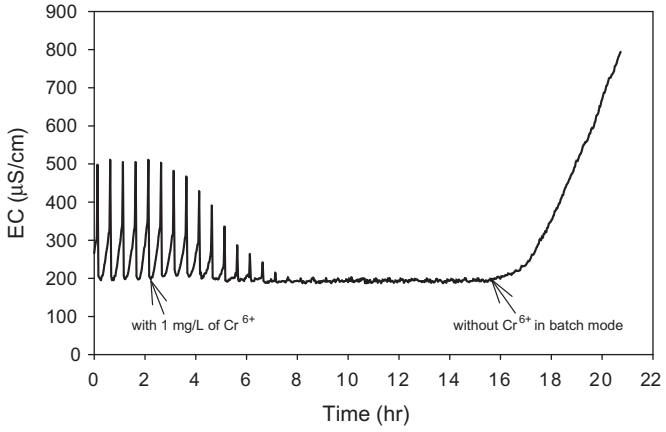

Fig. 4. EC changes after addition of 1 mg/L Cr^{6+} to the SOB biosensor operated in 30 min reaction time (1 min rapid feeding and 29 min batch).

4. Conclusions

In this study, a biosensor using SOB was operated in a semi-continuous mode for the detection of the toxic chemical in water. When Cr^{6+} was administered into the biosensor, the effluent EC decreased and the pH increased due to the inhibition of SOB activity. It was shown that the monitoring of toxicity in water was possible by using the SOB biosensor in semi-continuous mode. The results demonstrate that the SOB biosensor can detect the toxicity on the orders of minutes to hours which can serve as an early warning signal in the environment to protect ecosystem.

Acknowledgments

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