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Effect of organics and alkalinity on the sulfur oxidizing bacteria (SOB) biosensor

Sedky H.A. Hassan [a], Steven W. Van Ginkel [b], Sang-Eun Oh [a,*]

[a] Department of Biological Environment, Kangwon National University, Kangwon-do, 200-701 Chuncheon, Republic of Korea
[b] School of Civil and Environmental Engineering, Georgia Institute of Technology, Mason Building, 790 Atlantic Drive, Atlanta, GA 30332-0355, United States

HIGHLIGHTS

- A biosensor based on sulfur-oxidizing bacteria (SOB) for detection of toxic chemicals in water was developed.
- The bioassay based on SOB inhibition in the presence of toxic chemicals by measuring changes in EC and pH.
- The presence of organic matters increases the SOB activity.
- The sensitivity of SOB to toxic chemicals increased at low alkalinities.
- SOB biosensor can operate in low nutrient environments.

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ABSTRACT

The environmental risk assessment of toxic chemicals in stream water requires the use of a low cost standardized toxicity bioassay. Here, a biosensor for detection of toxic chemicals in stream water was studied using sulfur oxidizing bacteria (SOB) in continuous mode. The biosensor depends on the ability of SOB to oxidize sulfur particles under aerobic conditions to produce sulfuric acid. The reaction results in an increase in electrical conductivity (EC) and a decrease in pH. The biosensor is based on the inhibition of SOB in the presence of toxic chemicals by measuring changes in EC and pH. We found that the SOB biosensor can detect Cr^{6+}at a low concentration (50 ppb) which is lower than many whole-cell biosensors. The effect of organic material in real stream water on SOB activity was studied. Due to the presence of mixotrophic SOB, we found that the presence of organic matter increases SOB activity which decreases the biosensor start up period. Low alkalinity (22 mg L^{-1} $CaCO_3$) increased effluent EC and decreased effluent pH which is optimal for biosensor operation. While at high alkalinity (820 mg L^{-1} $CaCO_3$, the activity of SOB little decreased. We found that system can detect 50 ppb of Cr^{6+} at low alkalinity (22 mg L^{-1} $CaCO_3$) in few hours while, complete inhibition was observed after 35 h of operation at high alkalinity (820 mg L^{-1} $CaCO_3$).

1. Introduction

Even though water covers more than 70% of the total area of the Earth, drinking water is an increasingly precious natural resource threatened by pollution. Recently, the USEPA reported that more than 45% of stream miles, 47% of lake acres, and 32% of bay and estuarine acres were classified as contaminated (EPA, 2007). Moreover, in 42 US states, public water supplies were found to be contaminated with 260 toxic chemicals which have no established safety standards (Heilprin, 2005).

The seriousness of these environmental problems demonstrates a growing number of initiatives and legislative actions for water pollution control and increasing scientific activity in this area: e.g., the European Union Water Frame Work Directive ensured the establishment of adequate monitoring programs for the identification and monitoring of a wide range of different chemical groups in aquatic environments.

In spite of the high accuracy and sensitivity in measuring toxic chemicals in water samples based on chemical or physical analyses, there are many disadvantages. These methods enable the detection of a single compound or a group of structurally related compounds without any indication of their effect on living organisms. In addition, most chemical and physical methods require skilled trainers, are expensive, and it may take a long time to obtain the results of the test. Thus, there is a huge demand for simple, fast, and cost-effective methods for the screening and monitoring of a wide range of toxic contaminants in aquatic environments (Farr and Barcel, 2003).

* Corresponding author. Address: Department of Biological Environment, Kangwon National University, 192-1, Hyoja 2-dong, Kangwon-do, Chuncheon 200-701, Republic of Korea. Tel.: +82 33 250 6449; fax: +82 33 241 6640.
E-mail address: ohsangeun@kangwon.ac.kr (S.-E. Oh).

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Bioassays are one of the recent technologies, in which living organisms, tissues, or enzymes can be used for the detection of toxic chemicals in water. Some examples of living organisms include algae, *Daphnia*, shrimp, and various types of fish (Marty et al., 1991; Crane et al., 1995; Farr and Barcel, 2003; Pandey et al., 2005).

Microbial bioassays have some advantages over other bioassays as they can functionally represent higher organisms. Also, they are inexpensive and less time consuming compared to bioassays involving higher organisms. Microbial bioassays can be based on changes in growth rate (Eilersen et al., 2004), enzymatic activity (Bierkens et al., 1998; Brohon et al., 2001), photosynthetic activity (McFeters et al., 1983; Ahmed and Hader, 2010), motility (Ahmed and Hader, 2010), oxygen consumption using respirometry or electrodes (Tothill and Turner, 1996; Tzoris et al., 2005; Del Campo et al., 2007), bioluminescence output (Hassan and Oh, 2010), or amperometry (Farr and Barcel, 2003). The most common bacterial tool for monitoring toxicity is Microtox in which the light intensity of bioluminescent bacteria in water samples potentially containing toxic chemicals is monitored (Tizzard et al., 2004).

Recently, sulfur-oxidizing bacteria (SOB) can be used for the continuous monitoring of toxicity and their use circumvents many of the obstacles associated with conventional assays (Hassan et al., 2010; Van Ginkel et al., 2010; Oh et al., 2011). SOB, first identified by Sergey Winogradsky in 1885, are chemoautotrophic bacteria that oxidize reduced sulfur compounds to sulfuric acid in the presence of oxygen (O_2). Some SOB, such as genus *Acidithiobacillus* use elemental sulfur (S°) particles as the electron donor, as shown by the following equation (Hassan et al., 2010):

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

Oxidation of S° results in the formation of sulfate (SO_4^{2-}) and two protons (H^+), which lowers the medium pH to ~2. The production of SO_4^{2-} also increases the electrical conductivity (EC) of the medium. Thus, the decrease in pH and the increase in EC reflect the formation of sulfuric acid as the result of sulfur oxidation by SOB and can easily be detected using simple EC and pH meters (Oh et al., 2011). In the presence of toxic chemicals, the activity of SOB will be inhibited, which will cause an increase in pH and a decrease in EC.

S° particles can be oxidized aerobically by autotrophic SOB and the rate of S° oxidation can be increased when SOB grow mixotrophically in the presence of dissolved organic matter (DOM) or when industrial effluents contain a high chemical oxygen demand (COD) (Yang et al., 2010). For example, *Acidithiobacillus caldus* can oxidize S° to sulfate ions and simultaneously metabolize glucose (Hassan et al., 2010). Natural stream water usually contains DOM which can enhance the growth of heterotrophic SOB (Yang et al., 2006; Oh et al., 2011). Thus, it is important to determine if high organic loading to the SOB biosensor can reduce sensitivity by maintaining high EC values in the presence of toxic chemicals. DOM may also significantly decrease the toxicity by reducing the bio-availability toxic chemicals (Yang et al., 2006; Abskharon et al., 2008).

Industrial effluents may also contain high alkalinity which decreases the activity of SOB by buffering the pH at a higher level than the optimal pH for SOB (pH ~ 2) (Cotman et al., 2008). Furthermore, high pH reduces the sensitivity of the SOB biosensor by putting oxidized contaminants into their ionized forms (NO_3^-, ClO_4^-, etc.) which is less able to penetrate the cell membrane than their un-dissociated forms (Van Ginkel et al., 2011). Heavy metals also exude a lower toxic effect at high pH (Hassan et al., 2010). Thus, it is crucial to operate the SOB biosensor at low pH values where SOB activity is high and contaminants are bioavailable. Here, we investigated the presence of organic matters and alkalinity on the activity of SOB using continuous systems.

2. Materials and methods

2.1. Reactor construction and operation

The SOB biosensor construction was described elsewhere (Hassan et al., 2010; Van Ginkel et al., 2010; Oh et al., 2011). Briefly, the biosensors contained 50 mL of S° particles (2–4 mm), were inoculated with swine waste water, and incubated at 30 °C for 3 d in batch mode. Air was introduced from the bottom (150–250 mL min^{-1}) to supply O_2 as an electron acceptor. The biosensors were then fed synthetic stream water continuously in up-flow mode using adjustable peristaltic pumps. The biosensors were operated at a hydraulic retention time (HRT) of 30 min for approximately 2–3 d each to reach steady-state conditions (i.e., stable EC and pH values). A control reactor without inoculation with SOB was operated in the same time in order to be sure that the changes are come only from SOB.

2.2. Medium

Synthetic stream water was made 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}), $NaH_2PO_4{\cdot}H_2O$ (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}). Trace metal and vitamin solutions described in (Lovley and Phillips, 1988) were also diluted 100 times and added to the water. The pH, EC, and alkalinity of the synthetic stream water were 6.9 ± 0.2, 0.12 mS cm^{-1}, and 45 mg L^{-1} as $CaCO_3$, respectively.

2.3. Effect of organic material on SOB activity

To study the effect of organic material on the activity of SOB, different concentrations of glucose (0.2, 0.5, 1.0, and 2.0 g L^{-1}) were introduced to the influent of the reactors and the effluent pH and EC were monitored. To investigate the effect of real stream water on SOB activity, real stream water from the Gongi-ji stream in Chuncheon, South Korea, was used in this study. The real stream water was introduced to the influent of biosensor instead of synthetic stream water, and the system was operated for more than 100 h. In order to check the ability and activity of SOB in the presence of low nutrient concentrations, the synthetic stream water was replaced by tap and distilled water. The physicochemical properties of the real stream, tap, and distilled waters are shown in Table 1.

2.4. Effect of alkalinity on SOB activity

Alkalinity in water results from the presence of carbonate (CO_3^{2-}), bicarbonate (HCO_3^-), and hydroxides (OH^-) of calcium, so-

Table 1
The physicochemical properties of the Gonji stream water, tap and distilled water.

	Tap water	Distilled water	Stream water
NO_3–N (mg L^{-1})	4.8	2.49	8 ± 0.1
Cl^- (mg L^{-1})	6.957	1.92	41 ± 0.9
SO_4^{2-} (mg L^{-1})	5.29	1.49	11 ± 0.2
PO_4^{3-} (mg L^{-1})	0.055	0.005	0.3
Na^+ (mg L^{-1})	4.575	1.497	ND
K^+ (mg L^{-1})	1	0.395	ND
NH_4–N (mg L^{-1})	0.045	0.021	29 ± 2
Mg^{2+} (mg L^{-1})	0.97	0.294	ND
Ca^{2+} (mg L^{-1})	6.72	2.502	ND
pH	6.9	7.05	6.9 ± 0.23
EC (mS/cm)	0.062	0.012	0.18 ± 0.04
Alkalinity[a]	18.75	ND	85 ± 0.7

ND: not detected.
[a] (mg as $CaCO_3$/L).

dium, potassium, magnesium and ammonia. Alkalinity resists changes in pH and may maintain a fairly stable pH even in the presence of S° oxidation by SOB. Stream waters with low alkalinity are very susceptible to changes in pH as the buffering capacity of the water is consumed and will be suitable for the growth of SOB. Thus, in order to study the effect of alkalinity on the activity of SOB, the NMB medium was diluted 100, 50, 25, 10, 5, and 2 times (22–820 mg L^{-1} $CaCO_3$) and the effluent pH and EC were monitored. The toxicity of Cr^{6+} was studied using two different influent alkalinities (100 and 10 times diluted NMB medium; 22 and 195 mg L^{-1} as $CaCO_3$, respectively).

2.5. Chemicals and analyses

All reagents were of analytical grade and were used without further purification. Cr^{6+}($K_2Cr_2O_7$) was used as the toxic chemical (50 ppb) in the experiments (Sigma Aldrich, St. Louis, MO). Cr^{6+} was spiked to the synthetic stream water and the toxicity was monitored by measuring changes in the electrical conductivity (LUTRON meter model # – YK 22 CT) and the pH (LUTRON meter model # – pH 208c). The EC and pH meters were connected to a computer and all data were automatically recorded every10 min. Alkalinity and COD were measured according to standard methods (APHA, 1998.). The concentration of NH_4^+–N was measured by an ammonia-gas sensing electrode (Orion, 9512, Thermo, USA) connected to a meter (Orion 5 Star Bechtop). The concentrations of Cl^-, NO_3^-–N, NO_2^-–N, SO_4^{2-}, and PO_4^-–P were measured using an ion chromatograph (ICS-900, Dionex, USA) equipped with an IonPack® AG14 guard column (4 × 50 mm), an IonPack®AS14 analytical column (4 × 250 mm), a Dionex ASRS-II suppressor, a CDM-3 conductivity detector, and an AS40 automated sampler. Liquid samples were filtered through sterile, non-pyrogenic hydrophilic membrane filters (Sartorius, Germany) with 0.20 μm pore size prior to chromatographic analysis.

3. Results and discussion

3.1. Effect of glucose on the SOB biosensor

The effect of different concentrations of organic matter (glucose) on SOB activity is shown in Fig. 1a. When glucose was added to the influent at0.2 g L^{-1}, the effluent EC increased from 0.5 to 0.56 mS cm^{-1}. At 2 g L^{-1} glucose, the effluent EC increased by 45% compared to when no glucose was added. The influent EC increased as well due to the added glucose from 0.1 to 0.2 mS cm^{-1} while the effluent EC increased from 0.5 to nearly 0.8 mS cm^{-1} due to the enhanced S° oxidation. Hassan et al. (2010) isolated *A. caldus* from an SOB biosensor and also showed that SOB can grow mixtotrophically in the presence of glucose. (Yang et al., 2010) also reported that the heterotrophic growth of SOB can increase the rate of S° oxidation. Since natural stream waters naturally contain organic matter, mixotrophic growth may increase SOB activity and maintain a high EC even in the presence of toxic chemicals.

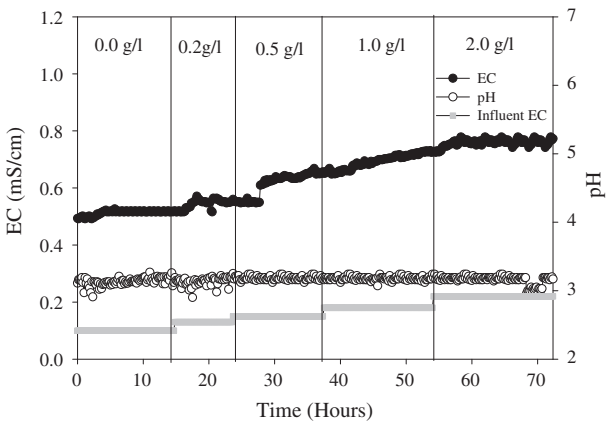

Fig. 1a. Effect of glucose concentration on the SOB biosensor.

3.2. Real stream water effect on SOB biosensor

Real stream water from Gongi-ji stream in Chuncheon, South Korea was used as influent to an SOB biosensor (Fig. 1b). The physicochemical properties of the stream water are presented in Table 1. The pH, EC, and alkalinity were 6.9 ± 0.23, 0.178 ± 0.036 mS cm^{-1}, and 85 ± 0.73 mg L^{-1} as $CaCO_3$, respectively. The pH, EC, and alkalinity are similar to the synthetic stream water reported in (Oh et al., 2011). The COD was 29 ± 2 mg L^{-1} which is less than the standard COD discharge limit set by the Korean government (40 mg L^{-1}). When synthetic stream water was replaced by the real stream water, there was a slight increase in EC as shown in Fig. 1b. The effluent EC increased from 1.84 and began to stabilize at∼2.04 mS cm^{-1}. This increase in EC may be due to the mixotrophic growth of the SOB on the organic matters in the real stream water (Hassan et al., 2010). The high effluent EC and low effluent pH suggests SOB can use the real stream water as a medium for bacterial growth.

3.3. Effect of alkalinity on the activity of SOB biosensor

When low alkalinity (NMB diluted 100 times) water was fed to the SOB biosensor, the effluent pH was ∼2.8 and the effluent EC was higher than the influent EC which shows that the SOB were active (Fig. 2). Similarly, when we decreased the dilution from 100 times to 10 times, changes in EC grew which suggests increased SOB activity. However, when we decreased the dilution to 5× and 2× diluted NMB, the effluent pH increased to ∼4.5 and ∼5.5, respectively, which is out of the optimum pH range for SOB. These higher pH values dampened the difference between influent and effluent EC as less SO_4^{2-} was produced. Thus, high alkalinity streams reduce the growth of SOB and should be avoided as the SOB favor acidic conditions. However, when the medium was changed back to 100× NMB, the SOB activity increased which decreased the pH back to ∼2.7.

3.4. Effect of alkalinity on detection of Cr^{6+} by the SOB biosensor

The effect of two different alkalinities on the detection of 50 ppb Cr^{6+} is shown in Fig. 3A and B. At low alkalinity, before

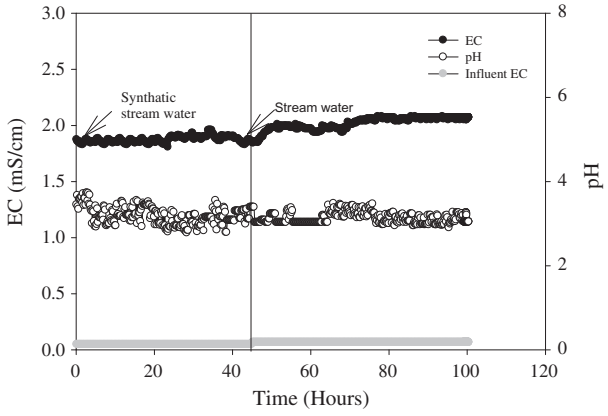

Fig. 1b. Effect of real stream water on the SOB biosensor. The influent was switched to real stream water on day 43.

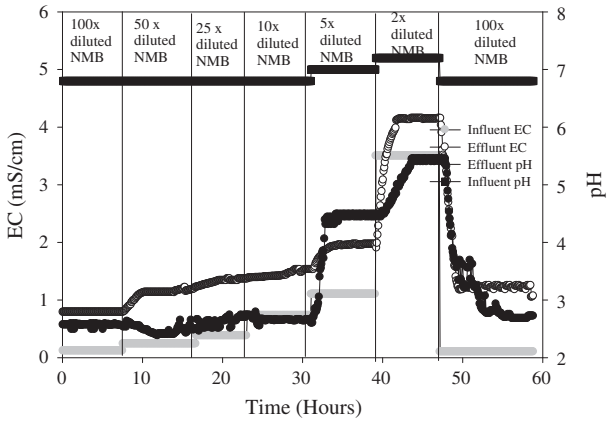

Fig. 2. Effect of different alkalinities on SOB biosensor.

Cr^{6+} was added, the influent and effluent ECs and pH were 0.1–0.93 mS cm^{-1}, and 6.9 and 2.3, respectively. While at high alkalinity, the influent EC was much higher at 0.8 mS cm^{-1} and the effluent EC was approximately 1.30 mS cm^{-1}. The influent and effluent pH values were 7.3 and 2.9.

When toxic Cr^{6+} (50 ppb) was added to the low alkalinity medium, the effluent EC decreased rapidly and complete inhibition of SOB was observed after 20 h of operation. When using the high alkalinity medium,, the effluent EC slowly decreased after 5 h of Cr^{6+} injection and SOB activity was completely inhibited after 30 h. This can be explained by higher Cr^{6+} toxicity at the lower pH values observed when using the low alkalinity medium. (Van Ginkel et al., 2011) reported that the toxicity of oxidized contaminants (NO_2^-, NO_3^-, $Cr_2O_7^{2-}$, ClO_4^-) to the SOB biosensor was increased

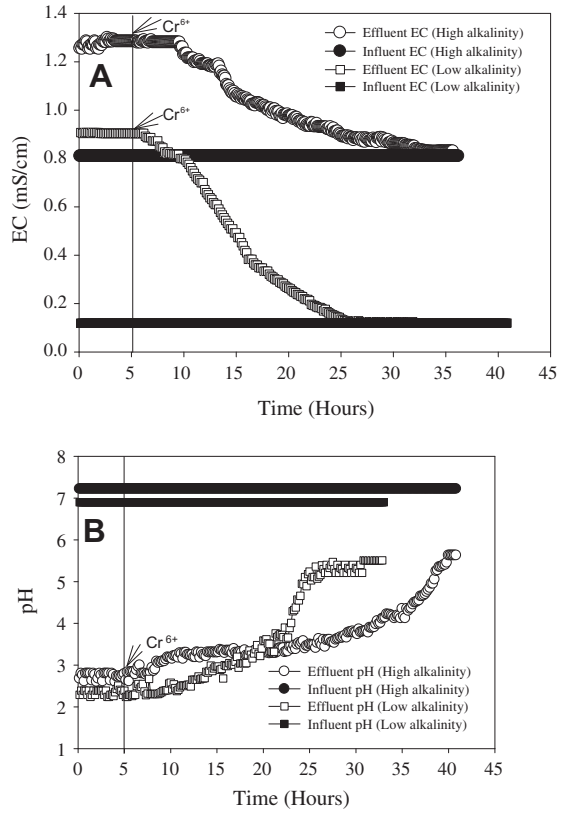

Fig. 3. Effect of alkalinity (22 and 195 mg L^{-1} as $CaCO_3$) on the detection of 50 ppb $Cr6^+$ by the SOB biosensor.

at low alkalinity since under acidic conditions the oxidized contaminants, which also are acids, are in their free and protonated form which may act as an un-coupler and reduce the activity of SOB. When using higher alkalinity media, the reactor pH tends to be higher which decreases the concentration of the protonated form and reduces the sensitivity of SOB biosensor.

3.5. Effect of influent EC on SOB activity

To study the effect of different influent EC on the oxidation rate of S° particles by SOB, the influent EC of 0.1 mS cm^{-1} (NMB diluted 100 times) was adjusted to 0.5, 1, 2 mS cm^{-1} using a 1 $MMgSO_4$ and KCl solution. The effect of different influent ECs on the SOB biosensor is shown in Fig. 4. When the influent EC was changed from 0.5 to 1.0 mS cm^{-1}, the effluent EC gradually increased and stabilized around 3.07 mS cm^{-1}. Similarly, the effluent EC increased by100% when the influent EC was changed to 1 or 2 mS cm^{-1}. The increase in effluent EC is due to the presence of ions in the influent. The fluctuation in effluent EC indicates the sensitivity and response of the SOB biosensor to the influent. Under the low alkalinity of the 100× diluted NMB media, Figs. 1b and 4 suggest that the effluent EC should be a near mirror image of the influent EC as the pH is favorable to S° oxidation. However, as stream water and industrial effluents are affected by the presence or absence of ions, organic matter, and alkalinity which will lead to an increase or decrease of influent EC and the resulting EC changes, these changes need to be taken into account when using the SOB biosensor.

3.6. Effect of tap and distilled water on SOB activity

In order to test the nutrient level needed to sustain SOB activity, we then used tap and distilled water as the influents to the SOB biosensor. After the inoculation and operation of SOB biosensors using S° particles (2.0–4.0 mm) in continuous mode at an HRT of 30 min using the 100× diluted NMB, the effluent EC and pH values began to stabilize at 1.0 ± 0.1 mS cm^{-1} and 2.2 ± 0.2, respectively. After reaching steady state after 10 h, we switched to using tap water. Fig. 5 represents influent and effluent EC values of the synthetic stream water and tap water. The effluent EC slightly decreased in 1 h and remained constant thereafter. The slight decrease in EC is likely due to the low influent EC and the low ion concentrations in the tap water compared to the synthetic stream water. The physicochemical properties of tap water are presented in Table 1. The pH, EC, and alkalinity of tap water are 6.9 ± 0.23, 0.062 ± 0.002, 19 ± 0.7 mg as $CaCO_3$/L which are similar to the synthetic stream water. This experiment shows that SOB

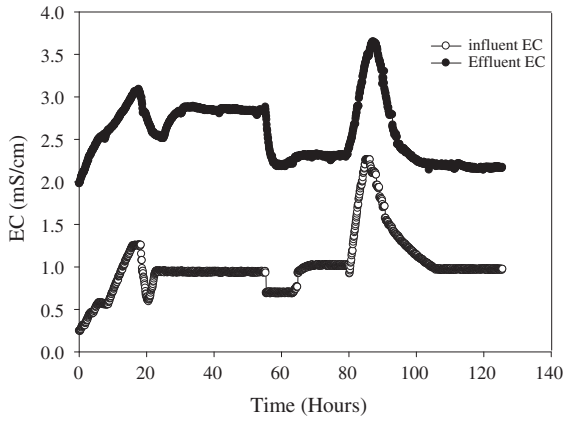

Fig. 4. Effect of different influent ECs on the activity of SOB biosensor.

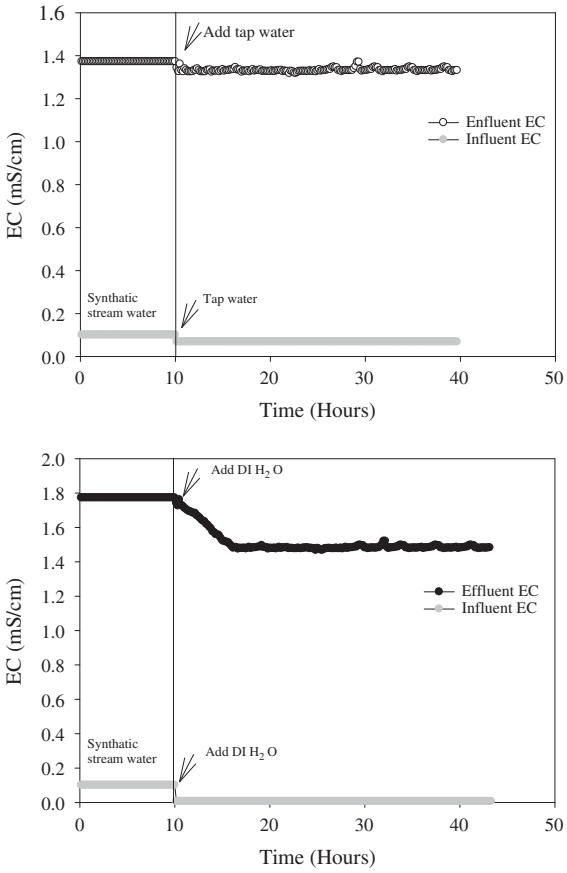

Fig. 5. Effect of tap and distilled water on SOB activity.

activity can be sustained by the nutrients and ions in the tap water. Similarly, when synthetic stream water was replaced by distilled water, the effluent EC decreased from 1.87 to 1.43 mS cm^{-1} and become stable from more than 1 month (Fig. 5). To check the presence of ions and nutrients in the distilled water, ion chromatography (IC) was used to analyze the anions and cations. The data indicated the presence of ions in low concentration compared to tap water as shown in Table 1. X-ray fluorescence spectra (XRF) was used in order to identify the elements and composition of S° particles. From XRF data (Table 2) it was found that S° particles contain 99.8% as elemental S°, and low percentages of other elements which serve as macro- and micro-nutrients for the growth of SOB. This indicates that SOB can grow and oxidize S° particles in tap or distilled water as they and S° particles already contain some nutrients to support SOB activity.

Table 2
Sulfur particle composition from XRF data.

Elements	%
Na	0.034
Mg	0.014
Al	0.011
Si	0.03
P	0.007
S	99.8
Cl	0.035
K	0.0246
Fe	0.007
Ca	0.0187
Ba	0.037

4. Conclusions

The SOB biosensor was developed for the detection of toxic chemicals in water in continuous mode based on the oxidation of S° particles and by measuring the EC and pH in the effluent. Elemental S° oxidation in the SOB biosensor is governed alkalinity, organic matter, and nutrients which change the rate of S° oxidation and the ability to detect toxic chemicals. The detection of Cr^{6+} by the SOB biosensor is more sensitive when the reactor pH is low due to a low influent alkalinity. The activity of SOB was increased in the presence of organic matter (glucose) which supports mixotrophic growth of SOB. At low alkalinities, the effluent EC should be a mirror image of the influent EC. However, the difference between effluent and influent EC can be changed when organic matter or higher alkalinity is present. Tap and distilled water supported the operation of the SOB biosensor which shows that the biosensor can operate under very low nutrient concentrations. The advantages of the SOB biosensor include its simplicity, easy operation since measuring the pH and EC is sufficient for detecting toxicity, the ability to monitor toxicity in real-time, and high sensitivity due to the increased bioavailability of contaminants at low pH. In addition, S° particles are very cheap, no toxic by-products are produced and the SOB biosensor can operate in low nutrient environments.

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