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Rapid detection of heavy metal-induced toxicity in water using a fed-batch sulfur-oxidizing bacteria (SOB) bioreactor

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ABSTRACT

A fed-batch bioreactor based on sulfur-oxidizing bacteria (SOB) was tested for rapid detection of heavy metal-induced toxicity in water. For this evaluation, SOB were exposed to water contaminated by selenium, mercury, hexavalent chromium, arsenic, cyanide, cadmium, and lead for 2 h and their inhibition rates were analyzed based on changes in electrical conductivity (EC). The results demonstrate that SOB were highly inhibited by selenium, mercury, hexavalent chromium, and arsenic but not by cyanide, cadmium, and lead. The 2 h half maximum effective concentrations (EC_{50}) of SOB for selenium, mercury, hexavalent chromium, and arsenic were estimated to be 0.33, 0.89, 1.18, and 0.24 mg/L, respectively, which are comparable or lower than earlier reports in the literature. However, the EC_{50} or EC_{20} values of SOB for cyanide, cadmium, and lead were notably higher compared to findings from previous toxicity tests that employed other microorganisms. The findings from the current study suggest that the fed-batch SOB bioreactor is suitable for rapid detection of toxicity induced by selenium, mercury, hexavalent chromium, and arsenic in water.

1. Introduction

There has been increasing concern over threats to ecological and public health associated with heavy metal-induced environmental contamination (Nagajyoti et al., 2010; Tchounwou et al., 2012; Jaishankar et al., 2014). In general, heavy metals are referred to as metals having relatively high densities and atomic weights (Fergusson, 1990). In trace amounts, they are vital to biochemical and physiological functions in plants and animals (Kabata-Pendias, 2010; Tchounwou et al., 2012). However, excess amounts of heavy metals lead to cellular and tissue damage and also cause various adverse effects on the ecology and human disease (Chang et al., 1996; Tchounwou et al., 2012). Therefore, monitoring and detecting toxicity induced by heavy metals in aquatic environments is of great significance to ensure the safety of water resources and human health.

Monitoring toxic chemicals in water based on analytic chemistry techniques including chromatographic and spectrometric methods shows high accuracy and sensitivity, whereas these analyses are expensive and laborious and require lengthy processing times (van Wezel et al., 2010; Woutersen et al., 2011; Hassan et al., 2013). Moreover, they detect only the quantity of specific contaminants without indicating impacts of contaminants on environments or living organisms (Hernando et al., 2005; Hassan et al., 2013). Hence, there is great demand for cost-effective, simple, and rapid analyses for monitoring contaminants in aquatic environments (Farré and Barceló, 2003).

Microbial bioassays have been considered alternative methods for evaluating toxicity in water (Sponza, 2002; Farré and Barceló, 2003; Oh et al., 2011;). Microbial bioassays detect toxicity of contaminants *via* monitoring changes in microbial activities such as growth rate, respiration, photosynthesis, motility, and bioluminescence (McFeters et al., 1983; Tothill and Turner, 1996; Kim, 2001; Eilersen et al., 2004; Ahmed and Häder, 2010; Hassan and Oh, 2010; Kong, 2011). A number of microorganisms including bacteria in activated sludge and microbial fuel cells, biolumiscent bacteria, nitrifying bacteria, oligotrophic bacteria, iron-oxidizing bacteria, and *Escherichia coli* have been studied for their use in microbial bioassays to evaluate toxicity of herbicides, organics, and heavy metals in water (Tada et al., 2001; Farré and Barceló, 2003; Kim et al., 2003; Cho et al., 2004; Eilersen et al., 2004; Tencaliec

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et al., 2006; Zlatev et al., 2006; Tront et al., 2008).

Our recent studies have shown successful toxicity monitoring in water using a microbial bioassay based on sulfur-oxidizing bacteria (SOB) (Oh et al., 2011; Gurung et al., 2012; Hassan et al., 2013; Ahmed and Oh, 2018). SOB are chemoautotrophic bacteria that use the energy obtained from oxidation of reduced sulfur compounds under aerobic conditions (Madigan et al., 2003). Particularly, some SOB including *Acidithiobacillus*, *Thiospirillopsis*, and *Thiovulum* can utilize elemental sulfur particles (S^0) as electron donors and grow attached to their surface (Madigan et al., 2003; Oh et al., 2011). Oxidation of S^0 by SOB results in a formation of sulfate (SO_4^{2-}) (Eq. (1)), which leads to an increase in electrical conductivity (EC) (Madigan et al., 2003).

$$S^0 + H_2O + 1.5O_2 \rightarrow SO_4^{2-} + 2H^+,\ \Delta G^{\circ\prime} = -587\ \text{kJ/reaction} \quad (1)$$

This is because that EC is a measurement of the ability to carry a current and generally proportional to the concentration of ions (Oh et al., 2011). However, in the presence of toxic substances, microbial activity of SOB is inhibited, resulting in less generation of sulfate (SO_4^{2-}) and lower EC values. Therefore, the SOB-based bioassay is able to detect toxicity in water by comparing EC values, which are easily measured by an EC meter. This simplicity is an advantage of the SOB-based bioassay over conventional potentiometric bioassays requiring sophisticated measurement techniques with a reference electrode in addition to a working electrode for toxicity monitoring (Farré et al., 2009).

Our earlier SOB studies have tested employment of SOB in various toxicity tests, which are mostly 6–24 h detection time-based (Van Ginkel et al., 2011; Oh et al., 2011; Gurung et al., 2012; Hassan et al., 2012; Ahmed et al., 2016). However, given that changes in EC rapidly respond to toxic substances in a SOB-based bioassay, SOB have potential for being used in toxicity tests with shorter detection times. The objective of the current study is evaluating suitability of the SOB-based bioassay for rapid detection of heavy metal-induced toxicity in water. For this evaluation, a fed-batch SOB bioreactor was developed and its effluent EC during 2 h exposure to seven selected heavy metals - selenium, mercury, hexavalent chromium, arsenic, cyanide, cadmium, and lead - was monitored. The half maximum effective concentration (EC_{50}) of SOB for each heavy metal was analyzed. Based on the comparisons of these EC_{50} values with those in earlier reports that employed other microorganisms, sensitivity of the fed-batch SOB bioreactor for detecting heavy metal-induced toxicity in water is discussed.

2. Materials and methods

2.1. Sulfur master culture reactor

Fig. 1 presents the sulfur master culture reactor (SMCR). The SMCR, constructed of polycarbonate, has a rectangular shape; its dimensions are 10 cm of a length, 10 cm of a width, and 15 cm of a height with 1.3 L of working volume. Approximately 1 L of the SMCR was filled with S^0 (0.8–2.8 mm diameter). As inoculum, 50 g of sulfur particles from another SMCR already in operation was added to the current SMCR. Dechlorinated tap water was used as medium. Dechlorination of tap water was performed by adding granular activated carbon (200 mg/L) and sparging with air over 24 h prior to use. The SMCR was operated in a fed-batch mode by feeding 1 L of medium for 10 min every 2 h under aerobic conditions. The SMCR was operated inside an incubator maintained at 45 °C but the actual operating temperature of the SMCR was 38 °C (The door of the incubator was incompletely closed due to the influent and effluent tubing of SMCR, causing a decrease in temperature inside the incubator). Aeration was provided using a rubber diffuser from the bottom of the SMCR at a flow rate of 1.5 L/min. Every 1 min, effluent EC values were automatically monitored by an EC meter and all data were recorded to a computer connected with an EC meter. The SMCR was maintained for more than 1 month prior to using the inorganic sulfur particles in subsequent toxicity tests.

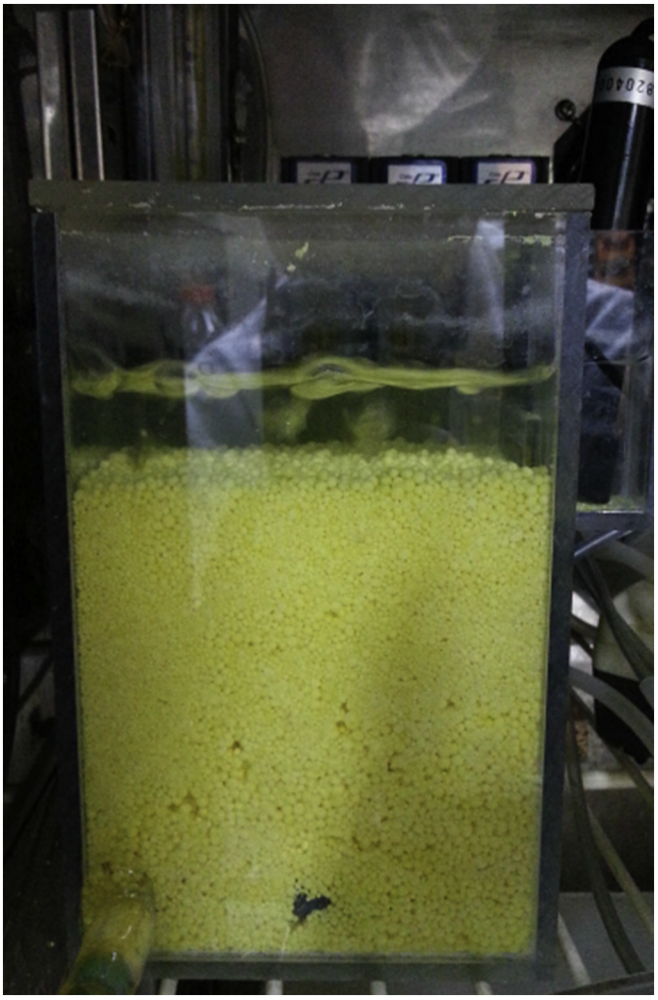

Fig. 1. Sulfur master culture reactor (SMCR).

2.2. fed-batch SOB bioreactor

A rectangular SOB bioreactor was constructed using polycarbonate (Fig. 2). The SOB bioreactor has 3.5 cm of a length, 3.5 cm of a width, and 9.2 cm of a height; its working volume was approximately 75 mL. For toxicity tests, the SOB bioreactor was packed with 50 g of sulfur particles from the SMCR and operated in fed-batch mode by feeding 70 mL of 100 times diluted nutrient mineral buffer solution (NMB) for 1 min every 10 min. The NMB was prepared as described by Oh et al. (2011). The fed-batch SOB bioreactor was operated inside the incubator and the working temperature of the SOB bioreactor was fairly constant at 38 °C. Aeration was introduced from the bottom of the bioreactor by a stone diffuser at a flow rate of 100 mL/min. A total of seven heavy metals - selenium, mercury, hexavalent chromium, arsenic, cyanide, cadmium, and lead - was tested with various concentration ranges, which is summarized in Table 1. Heavy metals were introduced to the fed-batch SOB bioreactor 2–3 reaction cycles later after beginning operation of the SOB bioreactor. During entire operations of the fed-batch SOB bioreactor (3 h), effluent EC values were automatically monitored by an EC meter every 1 min and recorded to a computer. All toxicity tests were performed in duplicate and the average values were reported.

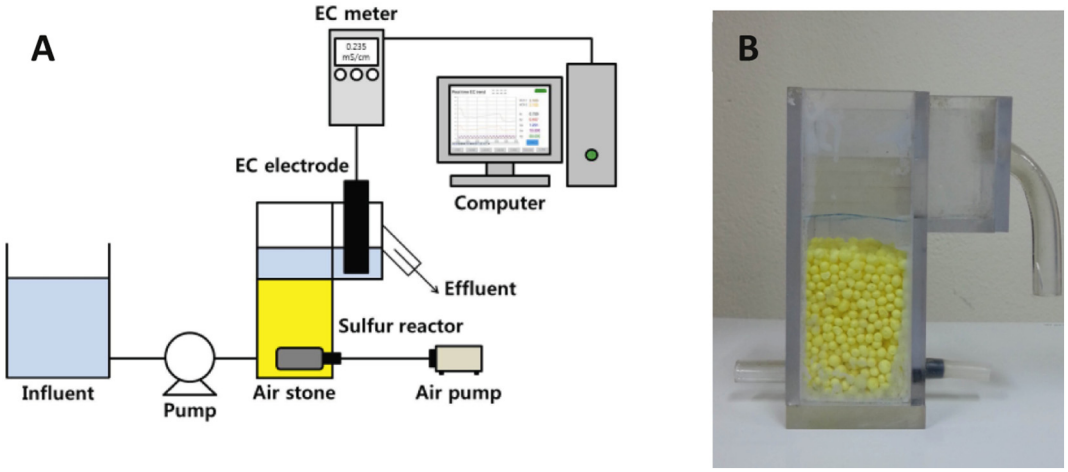

Fig. 2. Configuration of toxicity monitoring system (A) and fed-batch SOB bioreactor (B).

Table 1
Concentration ranges of heavy metals for toxicity tests.

Target heavy metal	Tested concentration range (mg/L)
Selenium	0.1, 0.3, 0.5, 0.7, 1
Mercury	0.1, 0.5, 1, 1.5, 5
Hexavalent Chromium	0.1, 0.5, 1, 2, 3, 5, 7, 10
Arsenic	0.1, 0.3, 0.5, 0.7, 1, 10
Cyanide	1, 10, 25, 50, 100
Cadmium	10, 30, 50, 70, 100
Lead	10, 30, 50, 70, 100, 200

2.3. Chemicals and analytical techniques

Chemicals used in the current study were analytical grade and were used without further purification. For the toxicity tests of selenium, mercury, hexavalent chromium, arsenic, cyanide, cadmium, and lead, sodium selenite, mercury nitrate, potassium dichromate, sodium arsenate, potassium cyanide, cadmium chloride, and lead nitrate were used, respectively (Sigma-Aldrich, St. Louis, MO, US). Measurement of EC values was conducted using an Inlab 737 EC meter (Mettler Toledo, Columbus, OH, US). Inhibition rates of SOB by heavy metals were calculated using the following Eq. (2).

$$Inhibition\ rate\ (\%) = \left(1 - \frac{rate\ of\ EC\ change\ in\ test}{rate\ of\ EC\ change\ in\ control}\right) \times 100 \quad (2)$$

The half effective concentration (EC_{50}) responsible for 50% growth inhibition (or EC_{20} responsible for 20% growth inhibition) for each heavy metal was estimated using Sigma Plot 10 (Systat Software Inc., San Jose, CA, US) based on the Hillslope EC_{50} method.

3. Results and discussions

3.1. Operation of SMCR

The SMCR was operated to cultivate SOB attached to S^0 and supply them for toxicity tests of heavy metals. Influent EC was fairly constant with an average value of 99 μS/cm. Fig. 3 illustrates changes in effluent EC in the early stages of SMCR operation. Once the SMCR was started, variations in EC values during one reaction cycle rapidly increased and then reached a stable range approximately from 3 mS/cm to 5 mS/cm in 90 h. This increase in effluent EC suggests formation of sulfate (SO_4^{2-}) by oxidation of S^0 as a result of the microbial activity of SOB.

Despite the absence of sequencing analysis of the microbial community in the SMCR during the current study, it is highly expected that microorganisms in the SMCR are mostly comprised of *Acidithiobacillus* sp. The inoculum of the current SMCR was sulfur particles from another SMCR already in operation. Our earlier study (Hassan and Oh, 2010) and regular analyses of the microbial community in that SMCR similarly report that approximately 99.8% of microorganisms were chemolithoautotrophic bacteria *Acidithiobacillus* sp.; the remaining 0.2% were heterotrophic bacteria. As S^0 was used as the only electron donor and no organic carbon was provided to the SMCR, it is reasonable that chemolithoautrophic SOB is highly dominant in the SMCR.

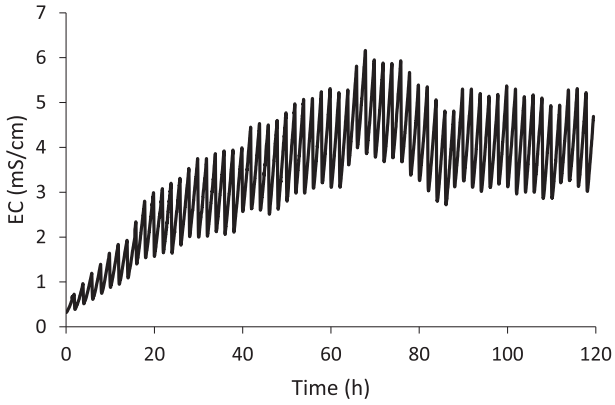

Fig. 3. Changes in effluent EC from SMCR.

3.2. Detecting toxicity of heavy metals to SOB

Fig. 4 illustrates changes in effluent EC during operations of the fed-batch SOB bioreactor with introduction of various concentrations of selenium. Before selenium was introduced, effluent EC during one reaction cycle varied approximately from 0.12 mS/cm to 0.35 mS/cm, showing 0.24 mS/cm of the effluent EC increase in one reaction cycle. In the presences of 0.1, 0.3, 0.5, 0.7 and 1 mg/L of selenium for 2 h, the effluent EC increases in one reaction cycle accounted for approximately 0.20, 0.14, 0.06, 0.003, and 0.002 mS/cm, respectively. The 2 h inhibition rates of SOB by selenium depending on its concentrations are depicted in Fig. 5. As this figure shows, microbial activity of SOB was highly inhibited by even minute amounts of selenium. Introduction of 0.5 mg/L of selenium for 2 h resulted in 96.8% of SOB inhibition rate. The 2 h half maximum effect concentration (EC_{50}) of SOB for selenium was estimated to be 0.32 mg/L, which is comparable or lower than those in previous studies. For example, Johnston (1987) used *Daphnia magna* in toxicity tests for selenium and reported 0.44–1.65 mg/L of EC_{50} values depending on the chemical forms of selenium (selenite or selenate) and exposure times (1, 2, or 4 d). A study by Ibrahim and

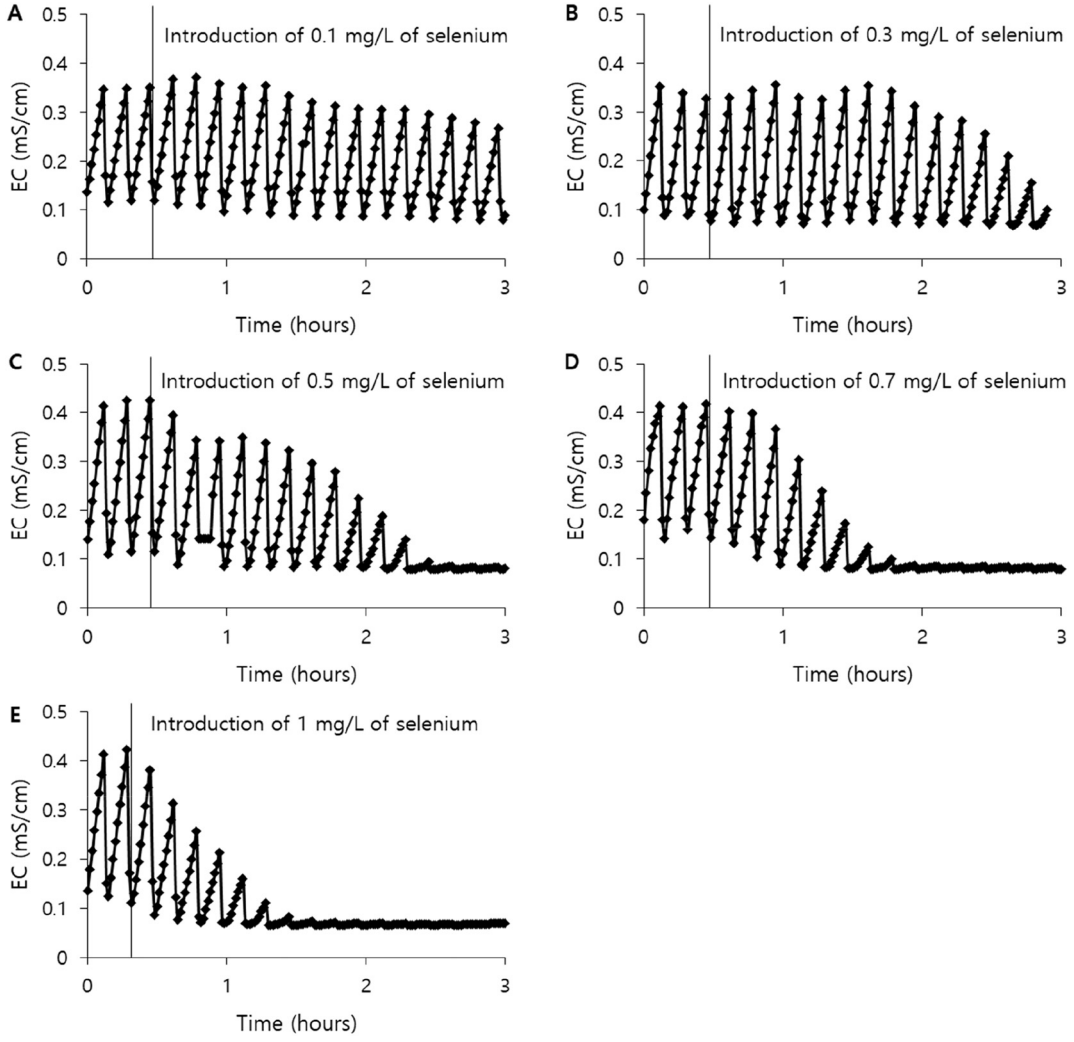

Fig. 4. Changes in effluent EC during operations of the fed-batch SOB bioreactor with introduction of various concentrations of selenium. (A) 0.1 mg/L; (B) 0.3 mg/L; (C) 0.5 mg/L; (D) 0.7 mg/L; (E) 1 mg/L.

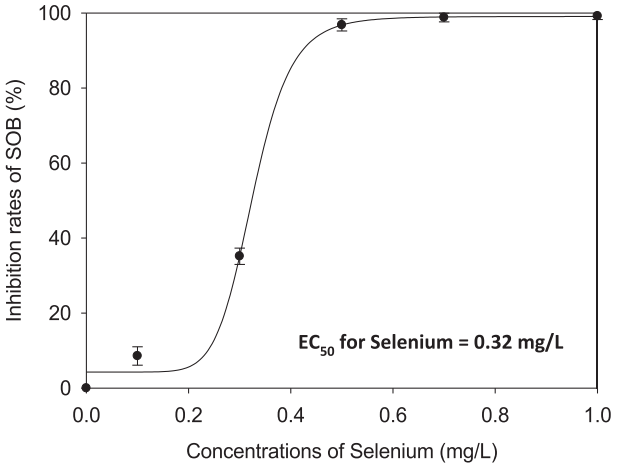

Fig. 5. Inhibition rates of SOB by selenium. Error bars represent the range of the results from the duplicate tests.

Spacie (1990) that investigated inhibition rates of *Selenastrum capricornutum* by selenium found 61.5–143 mg/L of EC_{50} values at various exposure times (1, 2, 4, or 6 d). Although Hsieh et al. (2004) observed a slightly lower EC_{50} value (0.114 mg/L) with *Vibrio fisheri* than the finding in the current study, our significantly shorter detection time (2 h) compared to that (22*h*) by Hsieh et al. (2004) is noteworthy. A low EC_{50} value indicates that the microorganism is more readily inhibited by a contaminant, suggesting higher sensitivity for detecting toxicity of that contaminant. Hence, one may conclude that our fed-batch SOB bioreactor is sensitive for evaluating toxicity induced by selenium in water with a rapid detection time.

The 2 h inhibition rates of SOB by various concentrations of mercury, hexavalent chromium, arsenic, cyanide, cadmium, and lead are graphed in Fig. 6. Similar to selenium, small amounts of mercury, hexavalent chromium, and arsenic significantly inhibited microbial activity of SOB. Introduction of 1–2 mg/L of mercury, hexavalent chromium, and arsenic for 2 h resulted in approximately 75–90% of SOB inhibition rates. The 2 h EC_{50} values of SOB for mercury, hexavalent chromium, and arsenic were analyzed to be 0.92, 1.17, and 0.20 mg/L, respectively. Table 2 compares our EC_{50} values to those in earlier toxicity tests based on nitrifying bacteria, activated sludge, algae, water fleas, bioluminescent bacteria, and *Escherichia coli*. Although some earlier reports in Table 2 show lower EC_{50} values than our findings, our EC_{50} values relatively fall within the lows. Moreover, given that our tests were conducted with a very short detection time (2 h), Table 2 suggests that our fed-batch SOB bioreactor performs favorably for detecting toxicity induced by mercury, hexavalent chromium, and arsenic in water.

However, SOB appears to be not suitable for monitoring toxicity

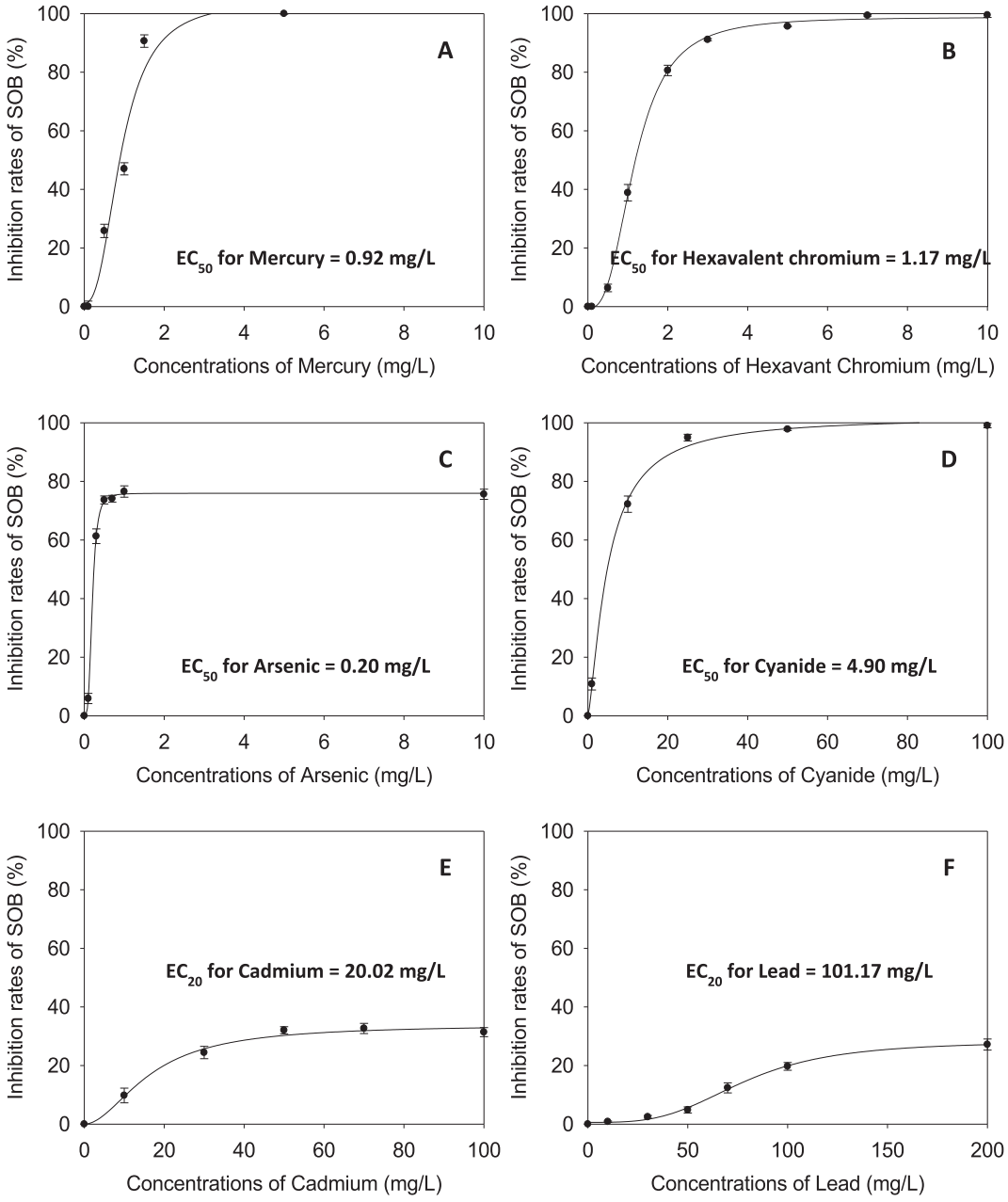

Fig. 6. Inhibition rates of SOB by mercury (A), hexavalent chromium (B), arsenic (C), cyanide (D), cadmium (E), and lead (F). Error bars represent the range of the results from the duplicate tests.

caused by cyanide, cadmium, and lead in water. Microbial activity of SOB was minimally affected by introduction of low amounts of cyanide. Exposure of 1 mg/L of cyanide for 2 h led to only 10.8% of SOB inhibition rate. The 2 h EC_{50} value of SOB for cyanide in the current study was estimated to be 4.90 mg/L, which is substantially higher than previously reported. For example, Liu et al. (2007) reported 38.38 and 26.37 μg/L for 10-min EC_{50} values for cyanide with employment of luminescent bacteria and *Escherichia coli*, respectively. Jaafarzadeh et al. (2013) also presented significantly lower EC_{50} values than ours for cyanide, ranging from 0.019 mg/L to 0.171 mg/L depending on the detection times (1, 2, 3, or 4 d). These earlier studies demonstrate that SOB are relatively insensitive for evaluating toxicity induced by cyanide in water.

Impacts of cadmium and lead on microbial activity of SOB were also insignificant. In the presence of even 100 mg/L of cadmium and 200 mg/L of lead, the 2 h SOB inhibition rates accounted for only 31.4 and 27.2%, respectively. With our testing ranges, the EC_{50} values of SOB for cadmium and lead cannot be estimated. Instead, the EC_{20} values for cadmium and lead were analyzed to be 20.02 and 101.17 mg/L, respectively. Earlier studies have reported that there is a large variation in EC_{50} values for cadmium and lead, ranging from 0.024 mg/L to 22.2 mg/L and from 0.122 mg/L to 20.4 mg/L, respectively, depending on the microorganisms, end-point measurements, and detection times (Mount and Borgerg 1984; Cho et al., 2004; Guéguen et al., 2004; Catterall et al., 2010; Magdaleno et al., 2014; Mansour et al., 2015). However, as shown in Table 2, numerous previous studies observed lower EC_{50} values for cadmium and lead than that found in the current study, indicating that the fed-batch SOB bioreactor is unsuitable for detecting toxicity caused by cadmium and lead in water.

In summary, the results from the current study found that a fed-batch SOB bioreactor is sensitive for detecting toxicity induced by selenium, mercury, hexavalent chromium, and arsenic, but not by

Table 2
Comparison of the EC_{50} values of heavy metals found in the current and earlier studies.

Microorganism	End-point measurement	Detection time	EC_{50} (LC_{50}) values (mg/L)							Reference
			Selenium	Mercury	Hexavalent Chromium	Arsenic	Cyanide	Cadmium	Lead	
SOB	Electrical conductivity	2 h	0.32	0.92	1.17	0.2	4.9	20.02 (EC_{20})	101.17 (EC_{20})	Current study
Daphnia magna	Mortality	2 d	–	–	0.022	3.8	–	0.118	4.4	Mount and Norberg (1984)
Daphnia pulex	Mortality	2 d	–	–	0.048	1.9	–	0.068	5.1	Mount and Norberg (1984)
Ceridaphnia reticulata	Mortality	2 d	–	–	0.045	1.8	–	0.066	0.53	Mount and Norberg (1984)
Simocephalus vetulus	Mortality	2 d	–	–	0.05	1.7	–	0.024	4.5	Mount and Norberg (1984)
Daphnia magna	Mortality	1, 2, 4 d	0.44–1.65	–	–	–	–	–	–	Johnston (1987)
Selenastrum capricornutum	Microbial growth	1, 2, 4, 6 d	61.5–143	–	–	–	–	–	–	Ibrahim and Spacie (1990)
Selenastrum capricornutum	Microbial growth	3 d	–	–	0.0657–0.209	–	–	0.0206–0.0427	–	Radetski et al. (1995)
Escherichia coli PMP 101	Electrical current	0.5 h	–	1.6	–	–	–	–	–	Bentley et al. (2001)
Escherichia coli PMP 101	Luminescence	0.5 h	–	1.5	–	–	–	–	–	Bentley et al. (2001)
Nitrifying bacteria	Nitrification	2 h	–	–	35.6	–	–	–	–	Dalzell et al. (2002)
Vibrio fischeri	Luminescence	0.5 h	–	–	22.5	–	–	–	–	Dalzell et al. (2002)
Activated sludge	Respiration	3 h	–	–	36.2	–	–	–	–	Dalzell et al. (2002)
Activated sludge	ATP luminescence	0.5 h	–	–	37.5	–	–	–	–	Dalzell et al. (2002)
Activated sludge	L-alanine-aminopeptidase	0.25 h	–	–	228.5	–	–	–	–	Dalzell et al. (2002)
Janthinobacterium lividum YH9-RC	Luminescence	5, 15, 30 min	–	0.2–0.3	5.9–7.5	4.3–28.4	–	1.1–4.6	–	Cho et al. (2004)
Vibrio fischeri	Luminescence	5, 15, 30 min	–	0.8–1.6	17.2–18.9	16.5–25.2	–	21.7–22.2	–	Cho et al. (2004)
Selenastrum capricornutum	Microbial growth	30 min	–	–	0.04–0.9	–	–	0.065–0.074	–	Guéguen et al. (2004)
Vibrio fischeri	Luminescence	30 min	–	–	13.756	–	–	7.965	0.122	Guéguen et al. (2004)
Vibrio fischeri	Luminescence	22 h	0.114	0.0338	0.0124	0.821	–	0.0504	0.669	Hsieh et al. (2004)
Vibrio-qinghaiensis	Luminescence	10 min	–	–	–	–	0.03838	–	–	Liu et al. (2007)
Escherichia coli	Electrical current	10 min	–	–	–	–	0.02637	–	–	Liu et al. (2007)
Escherichia coli	Respiration	1 h	1.41	2.03	–	–	–	7.8	20.4	Catterall et al. (2010)
Scenedesmus obliquus	Cell counting	4 d	–	–	–	–	–	0.058	–	Monteiro et al. (2011)
Desmodesmus pleiomorphus	Cell counting	4 d	–	–	–	18.3	–	1.92	–	Monteiro et al. (2011)
Daphnia magna	Mortality	1, 2, 3, 4 d	–	–	–	–	0.019–0.171	–	–	Jaafarzadeh et al. (2013)
Psychrobacter sp.	Electrical current	30 min	–	0.8	14	–	–	47.3	110.1	Wang et al. (2013)
Ankistrodesmus fusiformis	Cell density	4 d	–	–	–	–	–	0.141	–	Magdaleno et al. (2014)
Chlorella ellipsoidea	Cell density	4 d	–	–	–	–	–	0.429	–	Magdaleno et al. (2014)
Monoraphidium contortum	Cell density	4 d	–	–	–	–	–	0.191	–	Magdaleno et al. (2014)
Scenedesmus acuminatus	Cell density	4 d	–	–	–	–	–	0.397	–	Magdaleno et al. (2014)
Chaetoceros gracilis	Cell counting	4 d	–	–	–	–	–	2.37	–	Suratno (2015)
Isochrysis sp.	Cell counting	4 d	–	–	–	–	–	0.49	–	Suratno (2015)
Daphnia magna	Mortality	30 min, 1 d	–	–	–	–	–	6.48, 0.254	14.76, 0.413	Mansour et al. (2015)
Vibrio fischeri	Luminescence	5, 15 min	–	–	–	–	–	4.53, 4.47	6.60, 5.83	Mansour et al. (2015)

cyanide, cadmium, and lead in water. Based on the findings in the current study, it is hard to present a clear explanation for this dissimilarity in toxicity assessment ability of SOB for different heavy metals. One possible explanation is that this difference might be associated with characteristics of microorganisms and toxicological mechanisms of the actions of heavy metals. In general, toxicity of heavy metals is known to be caused by interaction between heavy metals and cellular components such as nuclear protein and DNA (Chang et al., 1996; Flora et al., 2008). This interaction leads to conformational changes causing cell cycle modulation and damage to DNA (Chang et al., 1996; Wang and Shi, 2001; Beyersmann and Hartwig, 2008). However, heavy metal-induced toxicity involves many unknown mechanisms, and each heavy metal has its unique physical-chemical properties that confer its specific toxicological mechanisms of action (Tchounwou et al., 2012). The impact of toxicity of heavy metals on living organisms also varies depending on the microbial characteristics including genetics and metabolisms (Chang et al., 1996; Tchounwou et al., 2012). Thus, it is speculated that some microorganisms can be inhibited by some heavy metals more readily than by others, possibly showing species specificity in response to the toxicity of heavy metals.

Advantages of the SOB bioassay for toxicity tests include its high sensitivity and ease of operation for evaluating toxicity along with rapid detection times and cheap operating cost (Hassan et al., 2012; Ahmed and Oh, 2018). Moreover, according to our earlier studies, the SOB bioassay can be operated under severe conditions such as low organic supply and pH, due to the SOB used in toxicity tests being mainly acidophilic chemolithoautotrophic bacteria, with no production of by-products (Hassan et al., 2013; Gurung et al., 2014). These advantages make the SOB bioassay a promising tool for on-site acute toxicity tests for various contaminants. Our future research will focus on evaluating feasibility of the SOB bioassay for detecting toxicity of various contaminants including volatile organic compounds and emerging contaminants such as BPA and pharmaceuticals like phenytoin and atenolol. Furthermore, our future research will attempt to improve the sensitivity of the fed-batch SOB bioreactor by optimizing its operational conditions. Table 2 demonstrates that some studies employed identical microorganisms and heavy metals but found different EC_{50} values. This can be attributed mainly to differences in operational conditions of the bioassay including amounts of microorganisms, operating temperature, and characteristics of the medium such as nutrients, pH, and alkalinity. It is expected that these future studies will facilitate in extending application of the SOB bioassay for various on-situ acute toxicity tests.

4. Conclusion

A fed-batch SOB bioreactor was tested for rapid detection of toxicity caused by heavy metals in water. For this analysis, changes in EC values during 2 h exposures of heavy metals were monitored and SOB inhibition rates were analyzed. The results show that SOB are readily inhibited by toxicity induced by selenium, mercury, hexavalent chromium, and arsenic but not by cyanide, cadmium, and lead. Estimated EC_{50} values for selenium, mercury, hexavalent chromium, and arsenic were fairly low; however, those for cyanide, cadmium, and lead were significantly higher, compared to reports in the literature. These results suggest that the fed-batch SOB bioreactor is sensitive and suitable for rapid detection of toxicity induced by selenium, mercury, hexavalent chromium, and arsenic in water.

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