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Short Communication

Detecting endocrine disrupting compounds in water using sulfur-oxidizing bacteria

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ABSTRACT

For the rapid and reliable detection of endocrine disrupting compounds in water, a novel toxicity detection methodology based on sulfur-oxidizing bacteria (SOB) has been developed. The methodology exploits the ability of SOB to oxidize elemental sulfur to sulfuric acid in the presence of oxygen. The reaction results in an increase in electrical conductivity (EC) and a decrease in pH. When endocrine disrupting compounds were added to the system, the effluent EC decreased and the pH increased due to the inhibition of the SOB. We found that the system can detect these chemicals in the 50–200 ppb range, which is lower than many whole-cell biosensors to date. The SOB biosensor can detect toxicity on the order of min to h which can serve as an early warning so as to not pollute the environment and affect public health.

1. Introduction

In recent years, more than 100 000 new chemicals of unknown toxicity and effects to humans and wildlife are released into our waterways (Rodriguez-Mozaz et al., 2004; Lean, 2008). The European Commission has stated that 99% of these are not adequately regulated and there is no proper safety information for 95% of them (Lean, 2008). Furthermore, since there are >600 known chemicals listed in the Toxics Release Inventory which could be released, it is difficult to quickly analyse the potential toxicity of each of these chemicals using conventional analytical methods. Hence, the rapid and continuous monitoring of toxicity, in general, is of great importance.

The so-called emerging contaminants include compounds used in everyday life, such as surfactants, pharmaceuticals, plastics, paints, and hormones. These chemicals are of particular concern both because of the volume of these substances used and because of their activity as endocrine disrupting compounds (EDCs) (Rodriguez-Mozaz et al., 2004). When these chemicals enter a wastewater treatment system, their degradation leads to unknown products, such as nonylphenols, which are also produced during the synthesis of polyethoxylate detergents (Farre and Barcelo, 2003). Furthermore, the degradation products of EDCs can be more toxic than the original compounds (Warhurst, 1995).

A wide variety of chemicals can be considered EDCs since they are defined by their biological effect. EDCs have been attributed to cancer, birth defects, declining sperm counts, feminisation of male vertebrates, and other developmental problems in both humans and wildlife (Sadik et al., 2000; Rodriguez-Mozaz et al., 2004; Lean, 2008). With the reuse of water supplies, the concentrations of EDCs in drinking water will likely increase. Even though EDCs in drinking water are not yet regulated in the United States, the US Food and Drug Administration requires environmental risk assessments for new pharmaceutical EDCs with predicted environmental concentrations >1 $\mu g\ L^{-1}$ (Snyder et al., 2000). Bisphenol A, nonylphenol, and diethylstilbestrol are among the chemicals currently being studied as potential EDCs which are partly the focus of this study (Sadik et al., 2000).

According to Hernando et al. (2005), the assessment of contamination of water emerging from contaminated sites or as effluents based on the conventional, analytical detection of specific pollutants is not sufficient to assess environmental risk since toxicity is a biological response (Hernando et al., 2005). Furthermore, the toxicity of pollutants is synergistic meaning the toxicity of multiple pollutants is greater than the sum of toxicity of the individual pollutants (Eaton, 1973; Fernandez-Alba et al., 2002; Hsieh et al., 2006). Therefore, means to evaluate toxicity on living organisms is needed.

Sulfur-oxidizing bacteria (SOB) can be used for monitoring toxicity and their use circumvents many of the obstacles associated with biosensors. SOB, first identified by Sergey Winogradsky in

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1885, are chemolithotrophic bacteria that oxidize reduced sulfur compounds to sulfuric acid in the presence of oxygen. Some SOB, such as Genus *Thiobacillus*, use elemental sulfur particles as the electron donor, as shown by Eq. (1) (Madigan et al., 2003):

$$S^o + H_2O + 1.5\ O_2 \rightarrow SO_4^{2-} + 2H^+ \quad \Delta G^{o'} = -587\ \text{kJ/reaction} \tag{1}$$

Since elemental sulfur is extremely insoluble, SOB must grow attached to sulfur particles. It is known that extracellular, outer-membrane proteins are considered to play key roles in sulfur mobilization and transport (Rohwerder and Sand, 2003; Zhang et al., 2008). Oxidation of elemental sulfur results in the formation of sulfuric acid (sulfate and hydrogen ion), which lowers the medium pH to ~2.5. The production of sulfate ions also increases the electrical conductivity (EC) of the medium. Generally, EC is proportional to the concentration of ions in the medium and is a measurement of the ability of a medium to carry an electric current and varies both with the number and type of ions the medium contains. Thus, the decrease in pH and the increase in EC reflect the formation of sulfuric acid as the result of sulfur oxidation and can easily be detected using simple EC and pH meters (Madigan et al., 2003). 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 (Oh, 2009). Traditional potentiometric microbial biosensors measure the difference between a working electrode and a reference electrode and the signal is correlated to the concentration of the analyte. This method requires a very stable reference electrode and may be a limitation of these transducers (Farre et al., 2009). However, the SOB biosensor does not have a reference electrode and recalibration is not needed since only changes in pH are needed to detect the inhibition of SOB.

Rogers and Williams note that simplicity, ruggedness (insensitivity to external conditions), and cost-effectiveness are key components of biosensors (Rogers and Williams, 1995). Biosensors should be extremely versatile both in the range of compounds and matrices for which they can be adapted. Biosensors able to detect a variety of chemicals will be most competitive since the market for detecting any single pollutant is small. This work expands on the work of Oh et al. (2010) which used the SOB-based biosensor to detect a variety of heavy metals in water.

2. Materials and methods

2.1. Culture and medium

Aerobic return activated sludge was used as the inoculum to the SOB biosensors packed with elemental sulfur. The sludge was collected from the Chuncheon Wastewater Treatment plant in Chuncheon City, Kangwondo, Korea. 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 previously described were also diluted 100 times and added to the water (Lovley and Phillips, 1988). 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, which is similar to the actual stream water which had a pH 6.8–7.0, EC = 0.18 mS cm^{-1}, and alkalinity of 75 mg L^{-1} as $CaCO_3$.

2.2. Reactor construction and operation

The SOB biosensors tested were two identical cylindrical reactors (100 mL) constructed from acrylic (Fig. 1). Each biosensor was packed with 50 mL of sulfur particles (2–4 mm diameter) and 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 three d in a batch mode. The biosensors were then fed synthetic stream water continuously in up-flow mode using adjustable peristaltic pumps. The biosensors were operated at a HRT of 30 min for approximately 2–3 d each to reach steady-state conditions (i.e. stable EC and pH values) and then several EDCs were spiked to the influents of the biosensors and the effluent EC and pH values were monitored.

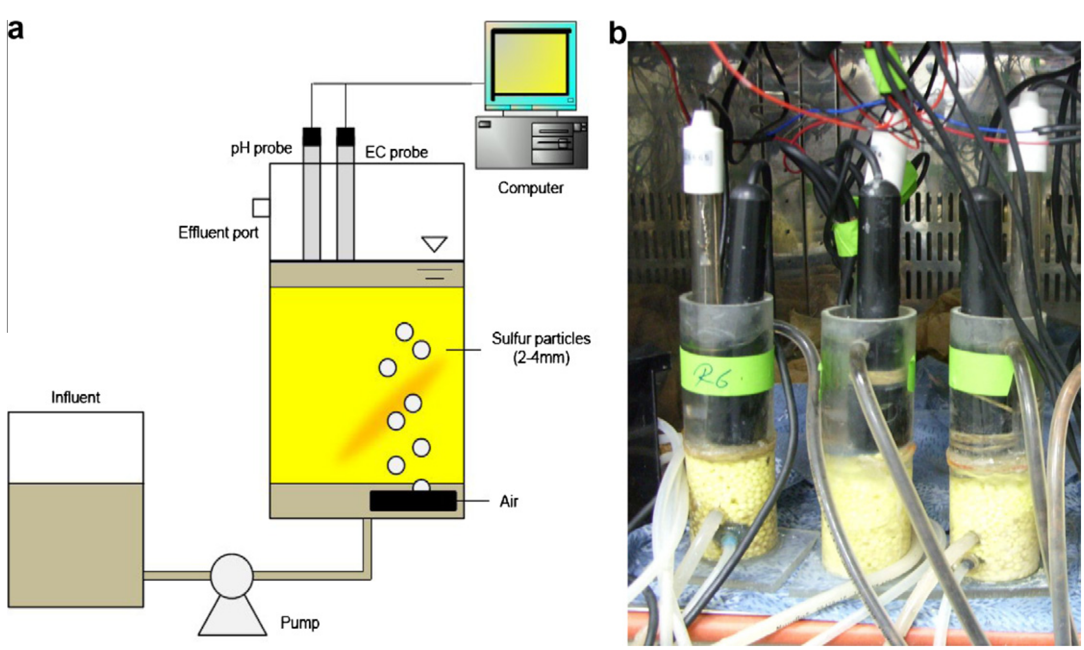

Fig. 1. The SOB biosensor: (a) schematic of the biosensor including sulfur particles, air supply, EC and pH meters. (b) Picture of SOB biosensors used in this study.