A Fiber-Optic Biosensor Based on Monooxygenases and Sol–Gel Entrapped Fluoresceinamine for Trichloroethene and Tetrachloroethene

D. Ryoo*, Y. Kim*, K. Jeun*, T. Wood** and Y. Choi*
*Jeonju University, Dept. of Env. Sci. & Tech. , Jeonju, Jeonbuk, Korea, dry@jj.ac.kr
**University of Connecticut, Dept. of Chem. Eng., CT, USA, twood@eng.uconn.edu

ABSTRACT

Directed evolution was performed for toluene-o-monooxygenase (TOM) and toluene/xylene-o-monooxygenase (ToMO) to obtain differential and improved reaction rates with respect to each chlorinated ethenes. Peroxide shunt was applied to remove the requirement for NADH, thus eliminating the need for complex NADH regeneration schemes for live cells on the biosensor tip and long-term application of enzyme biosensor would be possible. A three-layer sandwich structured sensing tip was constructed. The monooxygenases were immobilized on the outer layer, consisting of hydrophilic modified polyvinylidenefluoride membrane. The membrane was in contact with an intermediate sol–gel that incorporated fluoresceinamine (FLA), layered on an inner glass disk. The sensor operated in a static mode at room temperature and the intensity variation caused by hydrogen ion served as an analytical signal. Calibration curves were obtained for TCE and PCE, with concentration ranges 0.2–100 mg/l. The detection limits were 10μg/1 for TCE and PCE. The method reproducibility was tested. The method was successfully applied to the detection and determination of these chlorinated ethenes in water samples, without sample preparation steps

Keywords: TCE, PCE, optical fiber, biosensor

1 INTRODUCTION

Tetrachloroethene (a.k.a. perchloroethene, PCE) and trichloroethene (TCE) are the most frequently detected groundwater contaminants which are suspected to cause cancer in human[1]. Monitoring of these carcinogens suffers from high costs and inaccuracy caused by cumbersome sampling procedure altering the analyte concentration through volume averaging. A couple of whole cell biosensors were tested for TCE [2], but no sensor has been developed for PCE. Especially, it was previously thought that PCE was completely resistant to oxygenase attack; however, it was showed that the fully chlorinated PCE may be degraded by aerobic systems using toluene/xylene-o-monooxygenase (ToMO) [3].

Directed evolution has been successfully used to improve enzyme activity for many purposes. In this study, shuffled toluene-o-monooxygenase (TOM) and toluene/xylene-o-monooxygenase(ToMO) were applied for the detection of TCE and PCE[4].

Usually oxygenases in live cells use NADH and oxygen as electron donor and acceptor, respectively. Importantly, it was known that hydrogen peroxide can be used instead of NADH to donate both electrons and atomic oxygen in the monooxygenase system.

A biosensor system based on a fiber-optic enzyme sensor was developed to detect TCE and PCE. Shuffled TOM and ToMO were used to obtain improved reaction rate. A three-layer sandwich structured sensing tip was constructed. Peroxide shunt was applied to remove the requirement for NADH, thus eliminating the need for complex NADH regeneration schemes for live cells on the biosensor tip and long-term application of enzyme biosensor would be possible.

2 MATERIALS AND METHODS

2.1 Bacterial strains and growth conditions.

Escherichia coli strain TG1 with shuffled genes of TOM and ToMO [7] was routinely cultivated at 37°C in Luria-Bertani (LB) medium, and 100 μg/ml kanamycin was added to maintain plasmids. Stationary-phase cultures were obtained to achieve maximum enzyme activity. To remove metabolic by-products, the grown cells were washed three times with 1M phosphate buffer, pH 7.0

2.2 Instrumentation

The experimental set up for the optical measurements consisted of a portable fiber optic spectrometer (Ocean optics INC, USA) that incorporated a blue LED pulsed light source(LS-450), a CCD (USB 2000) and a back scattering sensor(R400-7-uv/vis). The optical fiber has a core and surrounding hexagonal fibers. Light was emitted through the hexagonal fibers and detected by CCD through the core fiber (Fig. 1).

2.3 Sensing unit

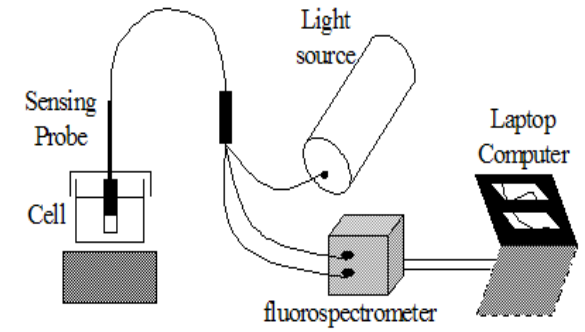
Bioactive disk sandwich was consist of sol-gel/indicator part and immobilized enzyme part. The hybrid silica sol contained tetraethoxysilane (Sigma-Aldrich, USA), phenytrimethoxysilane (Sigma-Aldrich, USA), water, ethanol and HCl were casted on a cleaned glass disk. The solvent was left to spread and evaporate. Fluorescent dyes

and color Hydrophilic membrane (Durapore, USA) was gently placed on the sticky surface of the sol-gel/indicator modified glass surface after 1 min of evaporation [5].

Purified oxygenase was immobilized by closslinking with glutaraldehyde. 80µl of enzyme solution was prepared in phosphate buffer (5mM phosphate, pH 7.0) and contained 5 µl of purified enzyme and 2.5 mg bovin serum albumin. The glutaraldehyde solution was prepared by mixing 40 µl fresh glutaraldehyde solution (8% aqueous solution, Grade I) with 100 µl of above phosphate buffer. 20 µl of the glutaraldehyde solution was rapidly mixed with 5 µl of the enzyme solution and 10 µl of mixture was deposited on the moist outer surface of the membrane.

2.4 Measurement procedure

Deionized water was sealed in a 300-ml round bottom flask without headspace, and 100 µM TCE of PCE was added to the flask with a syringe. The sensing unit was installed and the sample was stirred with a magnetic stirrer. Intensity of visible spectrophotometer or fluorospectrometer was measured through OOIBase32 spectrometer operating software.



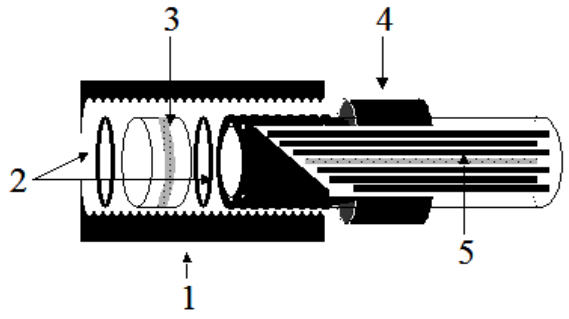


Fig. 1. Schematic of experimental equipment and detail structure; 1. screwable terminal holding ring, 2. Sealing 'O' rings, 3. biosensing sandwich(left to right : Durapore membrane with immobilized enzyme, middle sol-gel/indicator layer, supporting glass disk), 4. Probe tip, 5. optical fiber.

3 RESULTS AND DISCUSSION

Toluene monooxygenases, such as TOM and ToMO, release the acidic anion and result in a decrease of the pH. This pH decrease was transduced to an optical measurable signal by the immobilized indicators such as bromocresol purple(BCP) and fluoresceinamine(FLA). FLA was most sensitively indicated the variation of pH as a result of chlorinated ethenes variation. FLA was excited at 490nm and emitted at 520nm.

Figure 2 shows typical rate curves obtained for 100 µM of TCE. Stable response was reached after 2.5 min of contacting time. Response of analytical signal for TCE in TOM enzyme sensor was shown in Fig. 3. The response showed typical Michelis-Menton type response for chlorinated ethenes. TOM and ToMO were reactive on TCE and PCE. Former research showed that PCE is degraded only by ToMO not by TOM in live cell. TOM and ToMO using hydrogen peroxide shunting could be successfully applied for the detection of TCE and PCE.

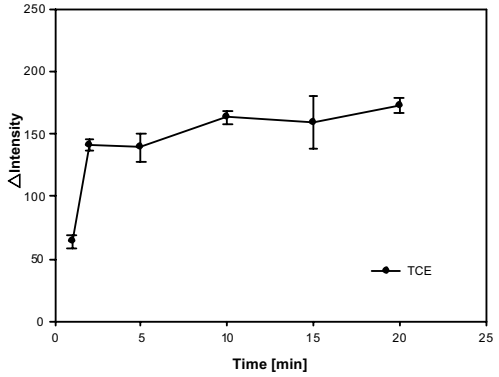


Fig. 2. Typical response curve of biosensing system for 100 µM of TCE.

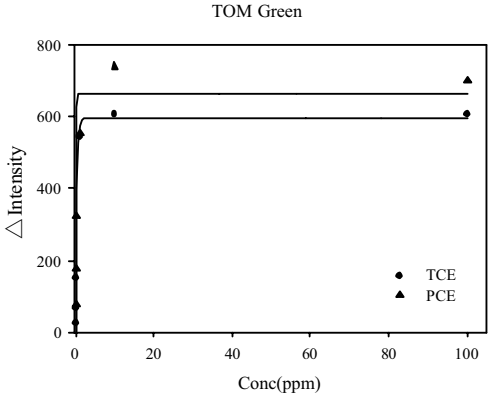


Fig. 3. Response of analytical signal for TCE in TOM enzyme sensor

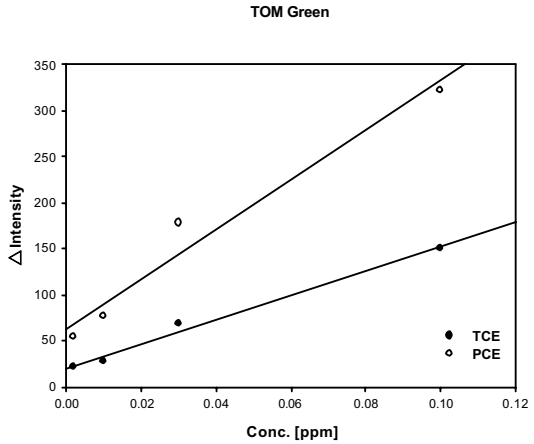


Fig. 4. Comparison △intensity of low concentration contaminants in TOM green (TCE correlated equation : $y = 1326x + 20.145$, $R^2 = 0.9892$, PCE correlated equation : $y = 2697x + 62.582$, $R^2 = 0.9638$)

Figure 4 shows the calibration curve obtained for the low concentration range of TCE and PCE for TOM. It can be seen that ? Intensity can be linearly related to the chlorinated ethenes concentration in bulk solution. A similar calibration curve was obtained for ToMO (data not shown). Calibration curves were obtained for TCE and PCE, with concentration ranges 0.2–100 mg/l. The detection limits were 10μg/1 for TCE and PCE.

ACKNOWLEDGEMENTS

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