Optical fiber whole cell bioluminescent sensor

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INTRODUCTION

The whole-cell optical fiber sensor is proposed as a real in-situ detector for on-line monitoring of the environmental pollution in remote localities (Close 2009). Intensity of bioluminescence produced with bioluminescent bioreporters (Genetically engineered cells producing bioluminescence in presence of certain compounds.) in contact with pollutants is low. This limits biosensor sensitivity especially in case of tiny optical fiber sensors.

The performance of whole-cell optical fiber sensor system was examined as a function of gel probe matrix volume, bacterial cell density and numerical aperture of the fiber core. Alginate gel with living bioluminescent bioreporters was applied on the fiber tip in a length of 1 cm. An optimal response to a model genotoxicant was achieved with six alginate/bacterial layers on a 1 cm exposed fiber-optic core. Polyak et al. (2001) showed that if the core diameter was etched down, photon detection efficiency increases although to a lesser extent than that expected from theoretical calculations. Another approach to fixation of bioluminescent reporter cells on the fiber end is conjugation of biotinylated alginate microspheres with encapsulated cells to the surface of a streptavidin-coated optical fiber (Polyak 2004).

Silica based polymers possess some of the most desirable properties for immobilization of biosensors; biocompatibility, transparency and chemical, thermal and dimensional stability. Previous study (Trögl 2005) demonstrated that bioluminescent bioreporter *Ps. fluorescens* HK44 entrapped in silica gel was kept alive and repeatedly producing bioluminescence for three month. The fiber taper makes possible to increase the photon detection efficiency by increasing of a number of the light sources, which are on the wider fiber end (Vrbova 2009).

In this study we present an optical fiber biosensor with bacteria *Pseudomonas putida* TVA8 (Applegate 1998), producing bioluminescence selectively in contact with benzene and its derivatives (BTEX), entrapped into silica gel on the tapered optical fiber element.

MATERIALS AND METHODS

Microorganism cultivation
P. putida TVA8 was kindly delivered from the CEB, University of Tennessee, Knoxville, USA. The cells were cultivated overnight in Luria–Bertani medium plus kanamycin at 50 mg/L at 28 ◦C to an optical density OD_{600}= 0.35. Cultures were washed once and then resuspended in an equal volume of minimal salts medium (Kuncova 2011).

Entrapment of cells into silica gel
Tetramethoxysilane (TMOS) (4.1 g) was stirred with distilled water (2 mL) and cooled 5 min at 4°C and 0.1M HCl (0.5 mL) was slowly added. After disappearance of two phases a clear solution was left to pre-polymerize for 24 h at 4°C. Pre-polymerized TMOS (50 µL) was mixed with 0.05M NaOH (50 µL) and cells suspension (50 µL). This mixture was dipped on the polished end face of the tapered fiber (d_{min}= 0.8 mm, d_{max}= 8.8 mm, length 19 cm), which was a rest of quartz preform from drawing of polymer cladded silica fibers.

Bioluminescence measurement
The tapered optical fiber element was connected by SMA optical fiber bare connector to a detector (photon-counter, Perkin–Elmer 3954-P-087) as is depicted on Fig. 1. Bioluminescence of *Pseudomonas putida* TVA8 was daily induced by immersion into toluene solution (26.5 mg/L) in phosphate saline buffer (pH 7.2).

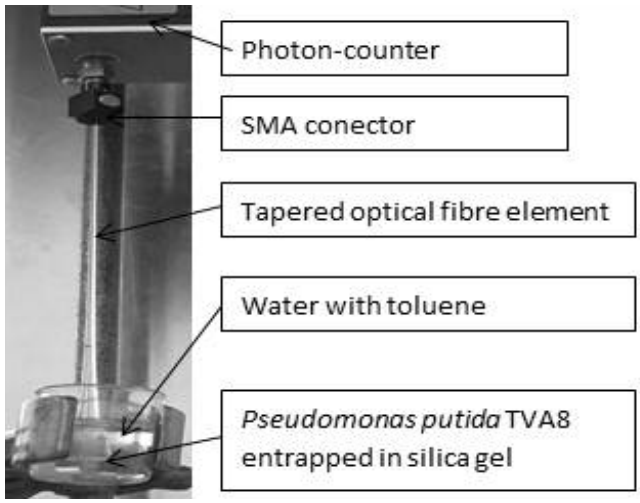

Figure 1: The tapered optical fiber element connected with photon counter

RESULTS AND DISCUSSION

The silica gel lens with entrapped cells was slowly dissolved during 32 days (Fig. 2). Within this period bioluminescence was daily induced with toluene (Fig. 3). First week the lens covered completely the fiber

end. With freshly entrapped cells the maximum bioluminescence appeared after 12 hours. In the following days these response times became shorter due to biofilm formation on the surface of silica gel lens. Minimum response times, 1 - 2 hours, were observed between 7th and 18th day. Later the response times were again prolonged and bioluminescence

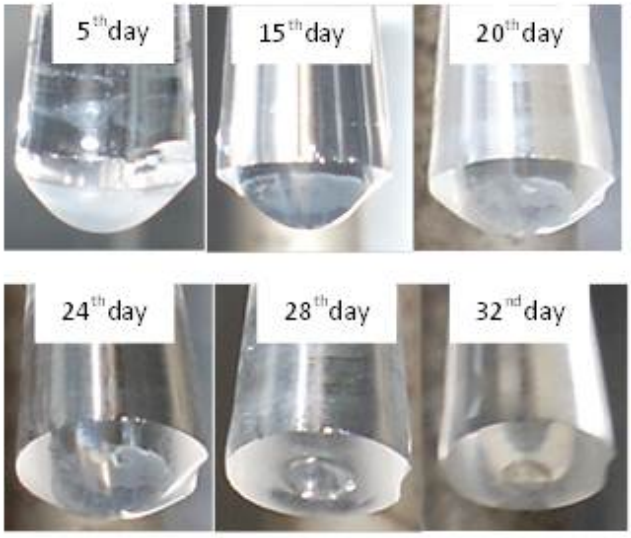

Figure 2: The silica gel lens with entrapped *Pseudomonas putida* TVA8

dropped down as the lens waned. The intensity of bioluminescence maximum was highest on 18th day.

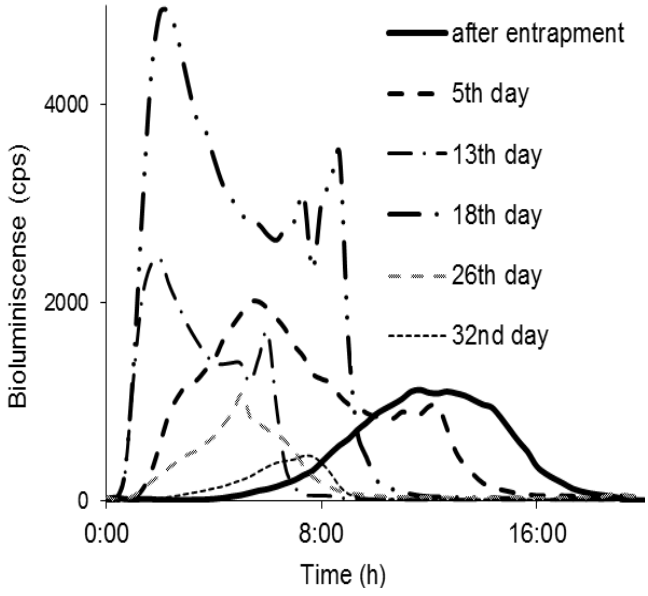

Figure 3: Bioluminescence of the silica gel lens with entrapped *P. putida* TVA8 induced with toluene. (Selected curves)

Immobilized cells were released and also probably consumed toluene and grew, which might be deduced from an appearance of the second and third bioluminescence maximum after 6–10 hours immersion into buffer with toluene.

CONCLUSIONS

The preparation and application of the optical fiber biosensor of BTEX with bioluminescent bioreporter *Ps. Putida* TVA8 entrapped into silica gel on tapered fiber was demonstrated. The sensor bioluminescence was repeatedly induced with toluene (26.5 mg/L) for 32 days. The recorded response times of bioluminescence maxima were from 1 to 12 hours. Between 7th and 18th day the response times were 1-2 hours and bioluminescence maxima from 2000-5000 cps. At least within this period the sensor worked as reliable detector of toluene.

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