Bioluminescent biosensor of toluene

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INTRODUCTION

Monitoring of environmental pollution and early detection of harmful agents requires analysis of many samples. Typically, water would be pumped up from wells and sent to an off-site laboratory for contaminant analysis using gas chromatography/mass spectrometry (GC/MS) techniques. GC/MS analysis is extremely sensitive and accurate, and is by far the best method available for detecting chemical contaminants in environmental sources. However, it also requires expensive and bulky instrumentation, a trained technician, the use of hazardous chemicals, and a significant allotment of time. As an alternative, bioreporters were proposed as sensors for the groundwater contaminants.

Bioluminescent bioreporters were originally constructed as whole-cell, bacterial biosensors that responded to specific chemicals or physical agents in their environment via the production of visible light (Sayler et al., 1998). Due to the widespread use of petroleum products the number of localities contaminated with benzene, toluene, ethylbenzene (BTEX) is increasing. Bioluminescent bioreporter *Pseudomonas fluorescens* TVA8 was constructed by introducing *tod–luxCDABE* fusion into the chromosome of *Pseudomonas putida* F1, bacterium capable biodegradation of toluene (Applegate et al. 1998).

A construction of analytical instruments for *in situ* real time monitoring needs biorecognition elements containing bioluminescent bioreporters encapsulated in a matrix that is strong enough to endure the rigour of the environment yet resilient enough to viably maintain the fragile cells.

In this study we present an applicapability of bioluminescence bioreporter - *Pseudomonas putida* TVA8 as a sensor of environmental pollution. We attempted to induce bioluminescence in this strain with 23 compounds to better discriminate response selectivity. The repeatable sensors were prepared by encapsulation of cells into silica based matrices. The cells were encapsulated both in thick layers and on the tips of optical fibres to prepare biosensors in remote localities. The biosensor applicability was demonstrated by comparison of its bioluminescence response towards exposition to influent and effluent of the waste water treatment of the chemical factory in the Czech Republic.

MATERIAL AND METHODS

Microorganisms cultaivation : *Pseudomonas putida* TVA8 cells were cultivated in Lauria-Bertani (LB) medium (28°C, 80 rpm) with kanamycine (50 mg/l) overnight to OD_{600}~ 0.3 ±0.15, centrifuged (7.74 G, 25°C, 10 min), resuspended in mineral medium (MSM), centrifuged again and diluted by MSM to concentration 10^9 cells/ml.

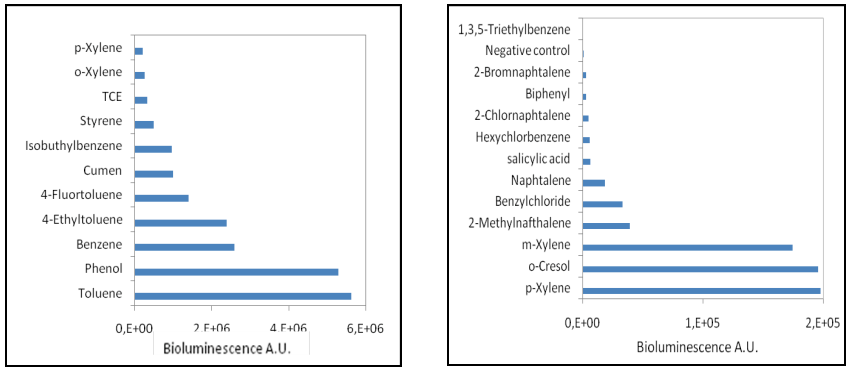
Selectivity of bioluminescence response with free cells : The cells were left starving overnight (28°C, 80 rpm). Bioluminescence was induced in MSM medium (10 mL) with cells concentration 10^8 cell/mL at 25°C after addition of tested compound (10 µL). An aliquots of 0.5 mL were pipetted to measure bioluminescence (Berthold FB-12 luminometer) in regular interval 30 min.

Pre-polymerization of tetramethoxysilane (TMOS) : TMOS was mixed with distilled water and HCl in TMOS:H_2O:HCl mol ratio = 1:5:10^{-2}, to form a clear solution and left to pre-polymerize for 24 h at 4°C (Trogl J. et al. (2005).

Cell encapsulation : Pre-polymerized TMOS (0.1 ml) was mixed with 0.05 M NaOH (0.1 ml) and with TVA8 cell suspension in LB medium (0.4 ml, concentration of 10^8 cells ml^{-1}). The mixture was poured into Petri dishes Ø 3.5 cm to form bio-silica film thickness of ~ 2 mm. The cells in silica sol were also drop on the tip of quartz optical fibre ($Ø_{core}$= 600 µm) and the tapered fibre ($Ø_{core}$= 10 mm). Bioluminescence of TVA8 encapsulated in films was monitored using Berthold FB-12 luminometer and on the tip of optical fibre or tapered fibre with luminometer SAFIBRA.

RESULTS AND DISCUSSION

The results presented in Figure 1 demonstrate TVA8 responds to a broad spectrum of compounds. For biosensing of environmental pollutants this can be advantageous since it provides a wide range of chemically similar target. On the other hand this strain cannot be used for selective detection of toluene. Comparison of inducing compounds reveals that highest bioluminescence produced with BTEX. In addition of that, BTEX bioluminescence maxima were reached in shorter time ~1 hour in contrast to ~ 6 hours for tetrachlorethylene, TCE, (bioluminescence time scan data are not shown).



(a) **(b)**

Figure 1: Bioluminescence maxima of free *Pseudomonas putida* TVA8 (10^8cell/mL) induced with the contaminants (10 µL). Compounds with high bioluminescence (a), compounds with low bioluminescence (b).

The cell encapsulation into silica biofilm was reproducible. These silica biofilms performed repeatable and reproducible induction with the main inducer toluene (Figure 2). The silica biofilm

was applied on the tip of optical fibre. With aim to increase durability and sensitivity of biorecognition element of optical fibre sensor the sensitive biofilm was coated on the tappered tip (Figure 3). This arrangement of sensor biorecognition element prolonged lifespan from days, to at least one month and the response time decreased from 12 to 3 hours. Fixation of cells in silica matrix on the tip of optical fibre makes possible in–line and long time monitoring in remote localities.

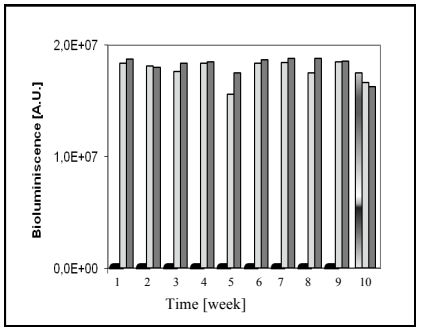


Figure 2: Bioluminescence maxima of silica films with encapsulated cells TVA8. Three identical bio-silica films were induced each week. The first film ■ was negative control (except the 10th week ▨), the second ▭ and the third ▬ were induced with toluene 26.5 mg/L.

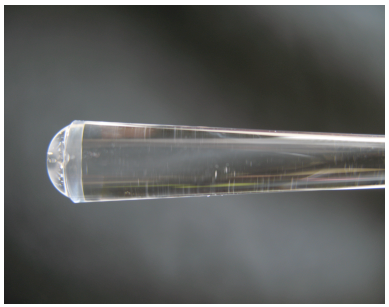


Figure 3: The tip of tappered quartz optical fibre (Ø=10 mm) coated with *Pseudomonas putida* TVA8 encapsulated in silica gel.

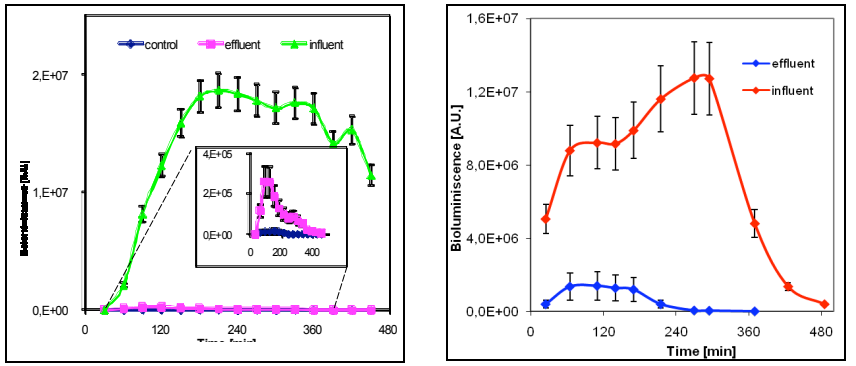
Contaminant	Composition µg/L (±20%)	
	Influent	Effluent
benzene	28900	0.299
toluene	18900	1.45
ethylbenzene	4720	0.156
(m+p) xylene	2240	260
styrene	422	< 0.1
o-xylene	1320	207

Table 1: Composition of water from waste water treatment plant determined by GLC.

The practical applicability of biosensing with whole cell biosensor *Psedomonas putida* TVA 8 was demonstrated by bioluminescence induction with water from influent and effluent of the waste water treatment plant (GLC compositionof water see Table 1). The bioluminescence of free and silica immobilized cells increased after beeing in contact with highly contaminated water from influent to the waste water treatment plant. A slight bioluminescence was observed also after contact with effluent, which contained 10^3 times less highly bioluminescent inducing toluene. Bioluminescence induced with free cells reached maxima after ~ 3 hours and gradually decreased after ~ 5 hours. Encapsulated cells reached bioluminescence maxima after 5 hours.

The different time course, slow and with temporal maximum, of free and encapsulated cells can be attributed to slow diffusion of contaminants and metabolites in microporous silica matrix .

Commonly, this contaminated water induced higher and faster bioluminescence response than was observed in corresponding water spiked with the same amount of BTEX (data not shown).



(a) **(b)**

Figure 4: The bioluminescence response of TVA8 to water in the influent and the effluent to the waste water treatment plant. Measured with free cells (a) and with cells encapsulated in silica biofilm (b).

CONCLUSIONS

The biofilms prepared by encapsulation of bioluminescent bioreporter *Pseudomonas putida* TVA8 into prepolymerized tetramethoxasilane have been reproducible and repeatable biorecognition elements of optical sensors. They have been able to sense contamination in real waters.

REFERENCES

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