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ORIGINAL PAPER

A new bacterial biosensor for trichloroethylene detection based on a three-dimensional carbon nanotubes bioarchitecture

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Abstract Trichloroethylene (TCE), a suspected human carcinogen, is one of the most common volatile groundwater contaminants. Many different methodologies have already been developed for the determination of TCE and its degradation products in water, but most of them are costly, time-consuming and require well-trained operators. In this work, a fast, sensitive and miniaturised whole cell conductometric biosensor was developed for the determination of trichloroethylene. The biosensor assembly was prepared by immobilising *Pseudomonas putida* F1 bacteria (PpF1) at the surface of gold interdigitated microelectrodes through a three-dimensional alkanethiol self-assembly monolayer/carbon nanotube architecture functionalised with *Pseudomonas* antibodies. The biosensor response was linear from 0.07 to 100 μM of TCE (9–13,100 $\mu g L^{-1}$). No significant loss of the enzymatic activity was observed after 5 weeks of storage at 4 °C in the M457 pH 7 defined medium (two or three measurements per week). Ninety-two per cent of the initial signal still remained after 7 weeks. The biosensor response to TCE was not significantly affected by *cis*-1,2-dichloroethylene and vinyl chloride and, in a limited way, by phenol. Toluene was the major interference found. The bacterial biosensor was successfully applied to the determination of TCE in spiked groundwater samples and in six water samples collected in an urban industrial site contaminated with TCE. Gas chromatography–mass spectrometric analysis of these samples confirmed the biosensor measurements.

Keywords Trichloroethylene · Whole cell biosensor · *Pseudomonas putida* F1 · Interdigitated microelectrodes · Single-wall carbon nanotubes · Self-assembly monolayer

Introduction

Trichloroethylene (TCE) has been extensively utilised over the last century as a dry cleaning agent and is still in use as solvent and metal degreaser. Owing to its widespread use, partial water solubility and volatility, as well as leakage and poor disposal practices, TCE has become one of the most common and persistent groundwater contaminants encountered at hazardous waste sites around the world [1–4]. TCE is known to be toxic to humans. Acute exposures to TCE at doses exceeding 250 ppm can impair the central nervous system, whilst long-term exposures are thought to induce hepatotoxicity, nephrotoxicity and neurotoxicity. TCE has been also classified in group 2A, "Probably carcinogenic in humans", by the International Agency for Research on Cancer [5]. Monitoring TCE in waters, understanding its mechanism of degradation and exploring effective remediation pathways are thus of great environmental significance.

Remediation technologies for TCE contamination mainly focus on biotic degradation. Microbial degradation of TCE can be achieved either by reductive dechlorination under anaerobic conditions or by co-metabolism under

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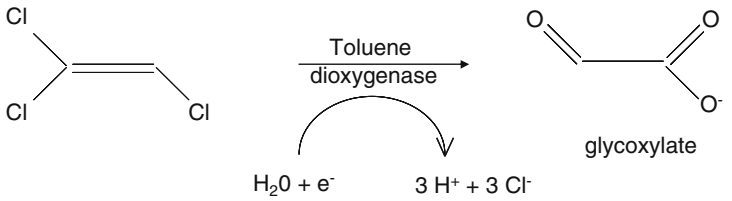
aerobic conditions. It has been shown that most of the naturally occurring anaerobic dechlorinating bacteria degrade TCE to the more toxic and persistent intermediates *cis*-DCE and vinylchloride (VC). Complete detoxification to the benign final product ethene can be achieved only by members of the genus *Dehalococcoides*. Under aerobic conditions, TCE, *cis*-DCE and VC are co-metabolised by bacteria containing oxygenase enzymes, such as methane monooxygenase, toluene monooxygenase or toluene dioxygenase [6]. To monitor the efficiency of natural attenuation or bioremediation processes, water samples have to be collected on the contaminated sites at regular intervals and analysed for their TCE, DCE and VC contents. For that, different analytical methods are available, mainly based on the chromatographic separation of the compounds followed by a mass spectrometric detection [7]. Among these techniques, headspace gas chromatography/mass spectrometry (GC/MS) is considered as one of the most promising. Very recently, a solid phase microextraction–gas chromatography/time-of-flight mass spectrometric technique with limits of detection lower than 0.8 nM has been proposed [8]. Nevertheless, samples have to be transported to the laboratory, with the risk of analyte volatilisation or degradation, and these performing methods are very time-consuming and require sophisticated instrumentation and well-trained operators. In this context, biosensors may constitute very attractive alternatives for the simple, rapid and in situ detection of pollutants. To our knowledge, only one bacterial biosensor (under three different versions), using *Pseudomonas aeruginosa* immobilised on a specific chloride electrode, has been already proposed for TCE detection, but its lifetime is very limited [9–11].

In this work, we propose an original whole cell conductometric biosensor based on the immobilisation of *Pseudomonas putida* F1 strain (PpF1) onto gold interdigitated microelectrodes functionalised with anti-*Pseudomonas* antibodies (Ab). PpF1 is a Gram-negative bacterium isolated from soil [12]. This bacterium has been extensively investigated as a model organism for the microbial degradation of aromatic compounds such as benzene, toluene or phenol but has also been found able to catalyse TCE degradation using the same enzyme called toluene dioxygenase (TOD) [12–16]. Several mutants defective in the TOD enzyme system have been isolated [17].

Immobilisation of the biosensing element onto the transducing surface is a key step of biosensor elaboration. Different strategies have been proposed for microbial cells, including covalent binding [18], cross-linking [19, 20], physical sorption [21] or entrapment into chemical/biological gels or polymeric matrices [22, 23]. The first two methods can affect the cell viability and/or the integrity of cell membrane biomolecules, reducing biosensor performances, whilst adsorption alone may lead to poor long-term stability due to cell desorption. For their part, organic or inorganic polymeric networks can efficiently protect the cells from external aggressions, but can form a diffusion barrier that restricts the accessibility of the cells to the substrate [24, 25]. These methods also suffer, in general, from low reproducibility and poor spatially controlled deposition [26]. In order to achieve a high and reproducible cell loading and favour both accessibility and long-term stability of the bioreceptor, we thus decided to immobilise PpF1 strain on the electrodes using a three-dimensional organised architecture constituted by a first alkanethiol self-assembly monolayer (SAM), covalently linked with a second layer of carbon nanotubes (CNTs) and a last layer of anti-*Pseudomonas* antibodies. Gold functionalisation with antibodies or enzymes using SAM has been extensively exploited in the last decade for biosensor construction [27] in view of the detection of small molecules [28, 29], proteins or bacteria [30–34]. Nanostructuration of electrodes either with randomly dispersed or with aligned CNT is also very promising in this field due to the unique chemical, structural, mechanical and electronic properties of CNT [35, 36]. Nevertheless, this is the first time, to our knowledge, that the resulting combination of SAM and CNT was conjugated to bacteria for the detection of a specific analyte.

In the biosensor proposed, PpF1 wild strain was immobilised in a mild way on the working electrode through antigen–antibody type interactions, whilst a mutant defective in todC1 gene (PpF4) [37] was immobilised following the same procedure on the reference electrode. As todC1 gene encodes the alpha subunit of TOD, PpF4 is not able to catalyse TCE degradation.

TOD enzyme, present in toluene-grown PpF1 and not in the genetically modified strain, catalyses the degradation of TCE into glyoxylate and formate by the following reactions [15]:



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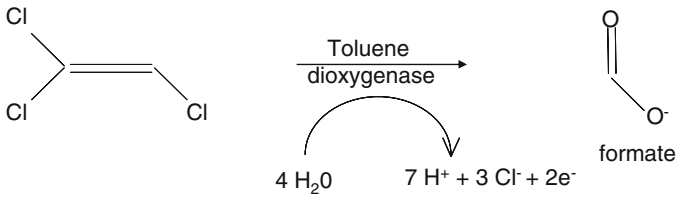

(2)

The ionic species produced by the reaction are detected by conductometry, which offers several advantages: (1) Thin-film electrodes are suitable for miniaturisation and large-scale production using inexpensive technology; (2) differential mode measurements allow cancellation of many interferences, (3) it is not light-sensitive; (4) the driving voltage can be sufficiently low to decrease significantly the power consumption; and (5) large spectrum of compounds of different nature can be determined on the basis of various reactions and mechanisms [38]. A differential mode of measurement between the PpF1-bearing electrode and the mutant-containing electrode is used to discriminate specific signals from differences in medium conductivity due to differences in its composition or temperature.

Experimental

Reagents

11-Amino-1-undecanethiol hydrochloride (99%), *N*-(3-dimethylaminopropyl)-*N*′-ethylcarbodiimide hydrochloride (protein seq grade), *N*-hydroxysuccinimide (NHS, 98%) and sodium dodecyl sulfate (SDS, >99%) were purchased from Sigma-Aldrich. Concentrated solutions of *cis*-1,2-dichloroethylene and vinyl chloride (2 mg/mL in methanol) were from Supelco and trichloroethylene (>99.5%) from Fluka. Toluene (>99.5%) and phenol (99%) were purchased from Laurylab. COOH-modified single-wall carbon nanotubes (SWNT-COOH, >80%) were obtained from Drop Sens. Guinea pig polyclonal anti-*Pseudomonas* antibodies (1 gL^{-1}) were purchased from GeneTex. Luria–Bertani broth (LBB) and Luria–Bertani agar (LBA) were obtained from Difco.

Phosphate buffer, pH 7.2, used for conductometric measurements was prepared from NaH_2PO_4 (>99%) and $Na_2HPO_4{\cdot}12H_2O$ (>99%) from Sigma-Aldrich, whilst buffer used during the electrode functionalisation process was phosphate-buffered saline (PBS) containing 140 mM NaCl, 2.7 mM KCl, 0.1 mM Na_2HPO_4 and 1.8 mM KH_2PO_4 and adjusted to pH 7 with NaOH. All aqueous solutions were prepared using 18 MΩcm ultrapure water obtained from an Elgastat UHQ II purification system (Elga Labwater, Le Plessis Robinson, France).

Microorganisms and culture conditions

P. putida F1 DSM99 (PpF1) was from the DSMZ collection and its todC1 gene defective mutant was a gift from Dr Rebecca Parales of the Section of Microbiology, University of California, Davis. PpF1 wild strain was first grown in LBB medium, and 0.1 mL of the microbial suspension, collected at early stationary phase, was used to inoculate a LBA plate. Then, a loopful of bacteria was aseptically transferred to chemically defined medium (M457) amended with toluene. M457 medium was prepared as follows: 2.44 g Na_2HPO_4, 1.52 g KH_2PO_4, 0.5 g $(NH_4)_2SO_4$, 0.2 g $MgSO_4{\cdot}7H_2O$ and 0.05 g $CaCl_2{\cdot}2H_2O$ were dissolved in 1 L of ultrapure water containing 10 mL of trace element SL-4 solution (0.5 gL^{-1} EDTA, 0.2 gL^{-1} $FeSO_4{\cdot}7H_2O$ and 1 mL of SL-6 solution). SL-6 solution is composed of 0.1 gL^{-1} $ZnSO_4{\cdot}7H_2O$, 0.03 gL^{-1} $MnCl_2{\cdot}4H_2O$, 0.3 gL^{-1} H_3BO_3, 0.2 gL^{-1} $C_OCl_2{\cdot}6H_2O$, 0.01 gL^{-1} $CuCl_2{\cdot}2H_2O$, 0.02 gL^{-1} $NiCl_2{\cdot}6H_2O$ and 0.03 gL^{-1} $Na_2MoO_4{\cdot}2H_2O$. pH was adjusted to 7 with 0.1 mM NaOH and the solution was sterilised by autoclaving at 121 °C for 15 min in a LaM-20-MCS-J (Adolf Wolf SANOclav, Bad Überkingen, Germany). Toluene, sterilised by filtration on a 0.2-μm cellulose acetate membrane, was subsequently added to the medium to get a final concentration of 10 $mmoll^{-1}$. PpF1 mutant pre-cultures and cultures were performed in LBB medium. Both bacterial strains were grown under aerobic conditions at 30 °C using a TH15 ping-pong type incubator from Edmund Bühler (Hechingen, Germany). After incubation, cells were harvested by centrifugation at 6,000 rpm for 10 min using a Sigma 2-16K model centrifuge. Bacterial pellets were collected, washed with PBS, pH 7, and resuspended in 5 mL of the same buffer. Cell counting was performed by plating 0.1 mL of successive dilutions of the PpF1 wild strain suspension on M457 agar plates and correlated to turbidity measurements (absorbance at 600 nm, UV-1601 CE, Shimadzu).

Sensor design

New conductometric transducers, consisting of two identical pairs of gold interdigitated thin film microelectrodes (Fig. 1), were fabricated on a silicon substrate at the Centro Nacional de Microelectronica (Barcelona) using a process

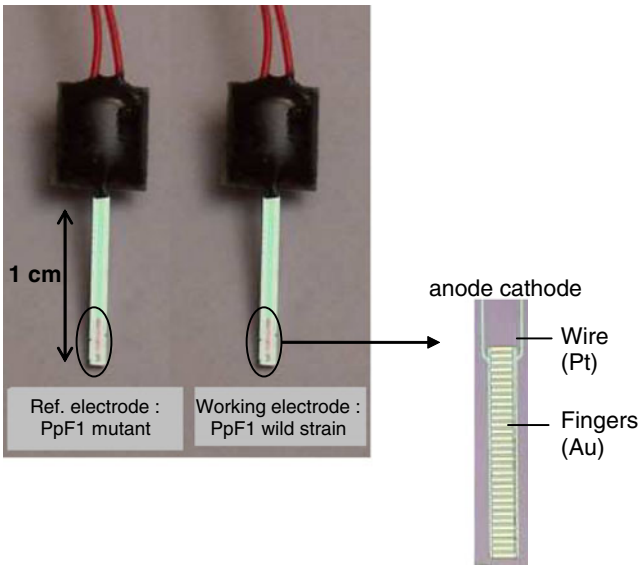

Fig. 1 General view of the interdigitated microelectrodes

with four photolithographic steps. The gold microelectrodes are located over silicon dioxide and fingers are interconnected by Pt conducting lines. The chip is covered by a silicon nitride layer (400 nm thick) except at the microelectrodes and contact pads. Both the digit width and the interdigital distance are 20 μm, and the active area is about 0.372 mm^2. One pair of microelectrodes was functionalised with PpF1 wild strain bacteria and the other with PpF1 mutant cells, according to the protocol described in the next paragraphs.

Construction of the microbial sensor

Gold pretreatment

Before use, gold electrodes were sonicated at room temperature for 10 min in acetone using a Branson 5210 ultrasonic bath (Branson Ultrasonics Corporation, Danbury, USA) rinsed with ultrapure water and then dried under nitrogen flow. After that, the gold surface was cleaned for 1 min with a freshly prepared "piranha" mixture (H_2O_2/H_2SO_4, 3:7, *v*/*v*) and rinsed carefully with large amounts of deionised water. Then, plates were washed with ethanol, rinsed with water and finally dried under nitrogen flow.

Functionalisation steps were further conducted in 500-μL Ependorf microtubes, and the reaction kinetics was followed by recording the evolution of the conductance of reactional media. A schematic view of the mechanisms implied in each step is presented in Fig. 2.

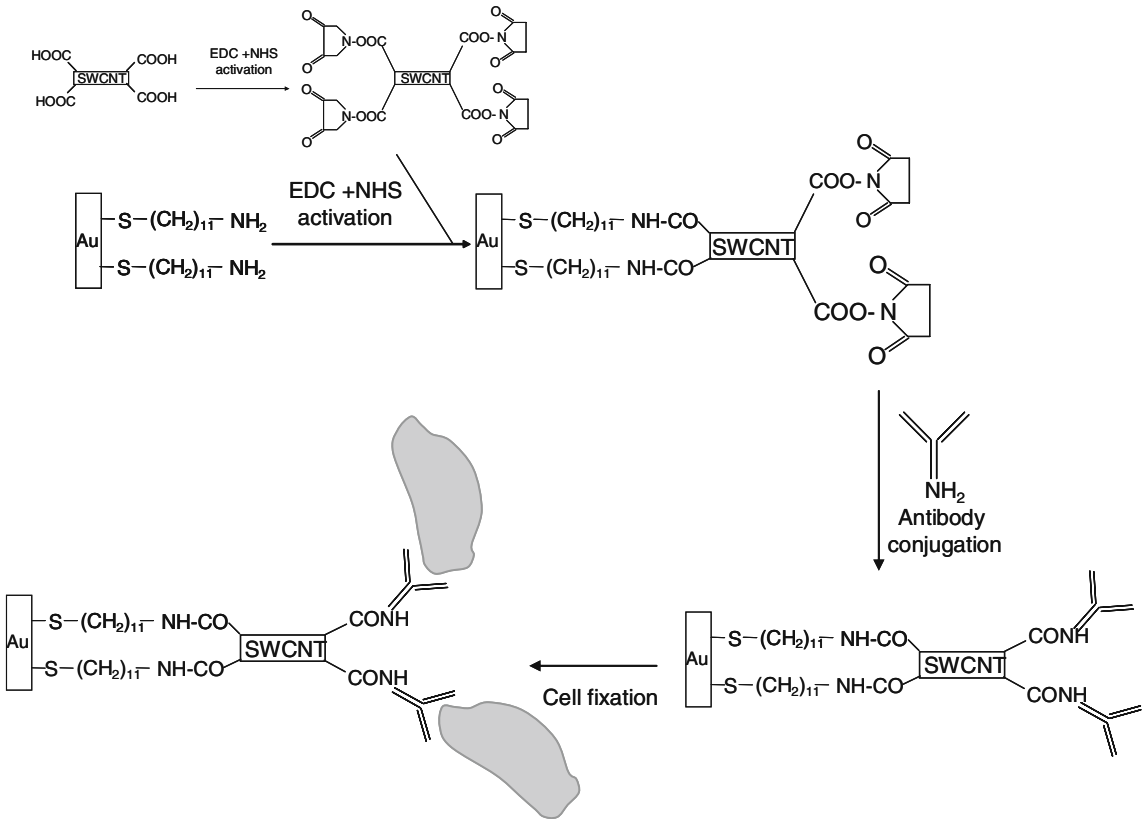

Fig. 2 Schematic illustration of the different steps of functionalisation used for the construction of the SAM/SWCNT/PpF1 biosensor. Not drawn to scale

SAM formation

The pretreated electrodes were immersed for 12 h at room temperature in 11-amino-1-undecanethiol (1 mM solution in ethanol) to give an alkanethiol SAM bearing terminal amino groups (SAM-NH_2). The modified electrodes were then rinsed with ethanol in order to remove unreacted thiols.

Activation of the carboxylic groups

Carboxyl groups of the carbon nanotubes were subsequently converted into active carbodiimide esters by reaction with an EDC/NHS solution in PBS for 1 h at room temperature. For that, a 0.17 gL^{-1} SWNT-COOH solution was prepared by dispersing the nanotubes in 0.1 M SDS, and 40 μL of this solution was treated with 40 μL of a 0.8 mM EDC/0.2 mM NHS.

Fixation of the carbon nanotubes

The electrodes were further modified by reacting the SAM-NH_2 electrodes with the activated SWNT-COOH solution for 2 h at room temperature and rinsed with ultrapure water to remove unreacted carbon nanotubes.

Antibody conjugation

The electrodes were then plunged into a 0.4 gL^{-1} anti-*Pseudomonas* solution for 1 h at room temperature. Antibodies in excess were removed by rinsing the electrodes with PBS, pH 7. Then, the antibody modified electrodes were treated with 0.1% caseine for 30 min to block the unreacted and non-specific sites, and then the substrate was rinsed with PBS, pH 7.

Bacteria capture

The PpF1 layer was finally constructed by plunging the electrodes in 100 μL PBS and proceeding to successive additions of 20 μL of a bacterial suspension (2,000 or 20,000 $UFC\,mL^{-1}$) until constant conductometric signal. The same amount of PpF1 mutant was immobilised on the reference electrode. Microelectrodes were finally rinsed with PBS.

Electrodes without nanotubes were fabricated following the same protocol, except that mercaptoundecanoic acid was used to create a first SAM layer bearing NH_2 terminal groups to which antibodies were directly bound.

Conductometric measurements

The experimental setup used in this work has already been described before [38]. Measurements were performed by applying to each pair of electrodes a small-amplitude alternating voltage (10-mV amplitude, 100-kHz frequency) generated by a low-frequency waveform generator (SR830 Lock-in amplifier from Stanford Research Systems). These conditions allowed reducing faradaic processes, double-layer charging and concentration polarisation at the microelectrode surface. The resistive contribution of the phase signals measured at both working and reference electrodes were compared and the difference was recorded as a function of time before and after substrate addition using the SR830 instrument.

Measurements were carried out at 23±2 °C in 500-μL Ependorf tubes filled with 100 μL of 5 mM phosphate buffer, pH 7.2. The two microelectrodes, taped together at their plastic upper end, were plunged in the solution and allowed to stand in this medium until the output signal was stable. Then, they were removed, the sample was added into the tube, the solution was rapidly homogenised by manual agitation, the electrodes were plunged again in the solution, and measurement was pursued. Injected volumes were between 5 and 50 μL for the standards prepared in 5 mM phosphate buffer, pH 7.2, and were limited to 20 μL for samples collected in the field.

GC/MS analyses

Headspace gas chromatography–mass spectrometry analyses (HS-GC/MS) were performed using a Trace DSQ II GC-MS instrument (Thermo Fisher Scientific, Waltham, MS, USA) equipped with a Combi PAL autosampler operating in headspace mode (CTC Analytics AG, Zwingen, Switzerland). A TR-V1 20 m×0.18 mm i.d. film GC column (0.18-μm thickness) was used for the separation of halogenated volatile organic compounds following the ISO 10301 protocol [34]. The samples were placed in 20-mL sealed vials equilibrated at 80 °C before injection.

Results and discussion

Electrodes functionalisation

As seen in Electronic supplementary material (ESM) Fig. S1, binding of the components in solution to the electrode induced a variation of the conductance of the medium, which allowed determining the optimal reaction time for the first four functionalisation steps. This time was about 1 h for carboxyl group activation and antibody binding, whilst it was close to 6 h for gold functionalisation with alkanethiol and 30 min for caseine fixation. In addition, saturation of antibody sites by bacteria was achieved after the addition of about 1,000 CFU onto the SAM/SWCNT modified electrodes (Fig. 3). This value was about ten times lower in the absence of CNT (data not shown).

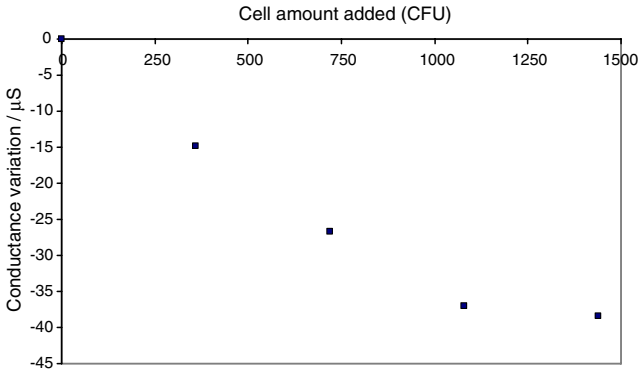

Fig. 3 Effect of cell loading on the antibody layer saturation during the elaboration of the SAM/SWCNT/PpF1 biosensor. Conductance variation measured in 5 mM phosphate buffer, pH 7.5. Temperature 23C±2 °C

Analytical characteristics of the microbial biosensor

The injection of TCE into the measurement cell caused an increase of the conductivity due to the enzymatic degradation of TCE. The steady-state response time, defined as the time required to reach 90% of the steady-state signal, was close to 8 min with the SAM/SWCNT/PpF1 biosensor, whilst it was 22 min with the SAM/PpF1 biosensor. Addition of the carbon nanotubes also increased the sensitivity by a factor of 80 (ESM Fig. S2). These results may be attributed to the presence of a higher amount of bacteria on the upper layer of the biosensor, to the improvement of substrate accessibility and/or to the electrocatalytic effect of the nanotubes [36] as electrons are also implied in the reaction process (Eqs. 1 and 2). Further performances were consequently evaluated on SAM/SWCNT/PpF1 biosensor only.

Linear range and limit of detection

The relationship between biosensor response and TCE concentration was examined in the 0- to 200-μM range. For that, eight standard solutions were used and five measurements were performed at each concentration level. Results are presented in Fig. 4.

The limit of detection (LOD), calculated as three times the signal-to-background ratio, was 0.07 μM, which corresponds to 0.4 μM in the sample if 20 μL is used for measurement. Although this value is much higher than the LOD obtained by classical GC/MS technique, it is suitable for the assessment of TCE degradation due to natural attenuation or induced remediation processes in slightly or highly polluted areas. This LOD value is close to the values reported for the TCE microbial biosensors reported before, which were 0.8 μM [9] and 0.2 μM [10, 11].

A lack of fit test at the 5% level proved that the biosensor response is linear in a wide range of concentration, from 0.07 to 100 μM TCE. The correlation coefficient and the sensitivity were 0.9952 and 0.4378±0.004 $\mu S\,\mu M^{-1}$, respectively, and the intercept was not significantly different from 0. Microbial biosensors based on *P. aeruginosa* exhibited a narrower linearity domain, 0.8–31 μM [9] and 0.2–15 μM [10, 11], respectively.

Short-term reproductibility and sensor stability

The short-term reproducibility of the biosensor response was tested at four concentration levels of TCE in the 10- to 100-μM range. The variation coefficient obtained from five measurements performed within 1 day with the same sensor was very good as it was between 2% and 4% in the concentration range studied.

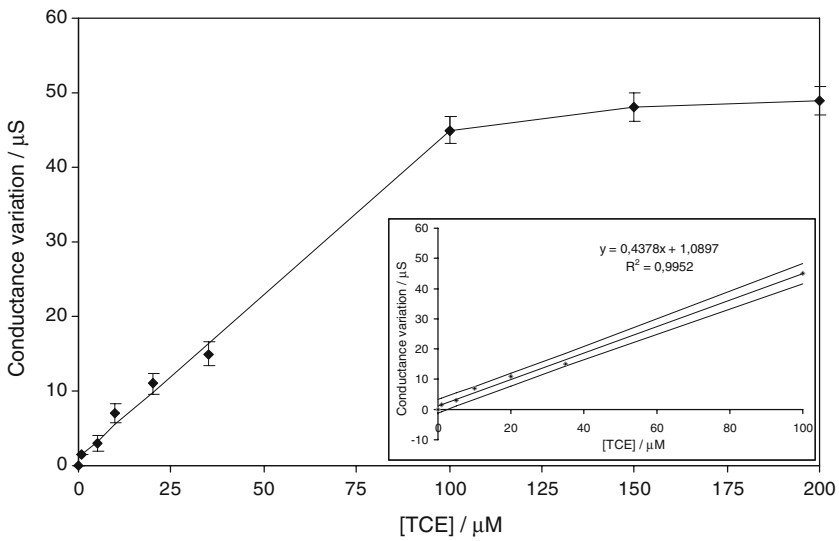

Fig. 4 Evolution of the SAM/SWCNT/PpF1 biosensor response with TCE concentration (n=5). The *inset* shows the linear part of the curve with the corresponding equation, correlation coefficient and 95% confidence bands. Measurement conditions as in Fig. 3

To test the SAM/SWCNT/PpF1 biosensor storage stability, one sensor was fabricated following the protocol described in "Experimental" and its response was measured three to four times a week over a 7-week period. Meanwhile, the biosensor was stored at +4 °C in M457 medium, pH 7. As seen in Fig. 5, the biosensor was stable over a very long period. The signal remained stable for 5 weeks. It is likely that a large amount of bacteria is no more metabolically active at this time, but it seems that the cells are able to protect TOD enzyme against degradation or leakage by keeping it safe inside the membrane. A slight decrease of the response was further observed, but only 8% of the initial value was lost after 7 weeks. This decrease may be attributed to a loss of activity and/or to enzyme leakage from the dead cells. *P. aeruginosa*-based biosensor reported in the literature exhibited a much shorter lifetime, with 40–50% of the initial activity lost over a 3- to 4-day period. This result was attributed to the release of NADH oxygenase cofactor. Oxygenase is the first enzyme involved in the TCE degradation by *P. aeruginosa*.

Interference study

The differential mode of measurement used allows eliminating many kinds of interference. TOD substrates are consequently expected to be the only possible interfering substances. *cis*-1,2-dichloroethylene (*cis*-1,2-DCE) and VC are the main TCE environmental degradation products. Hence, they may be found in waters together with TCE and are able to be dechlorinated by *P. putida* [14]. The toluene-induced cells of PpF1 can also oxidise phenol or induce the monohydroxylation of the aromatic ring of substituted phenols [13]. In addition, toluene is the major substrate of TOD enzyme. Interference study was consequently performed including these different compounds. The biosensor response towards toluene, phenol, DCE and VC was first evaluated at one single concentration (10 $\mu mol L^{-1}$) using individual substrates and compared to the TCE response obtained in the same conditions. As seen in Fig. 6a, relative responses for *cis*-1,2-DCE and VC are very low at this concentration level (6% and 4%, respectively). On the contrary, phenol interference is significant (35% response compared to that of TCE), and as expected, toluene constitutes a major interference in TCE determination. The biosensor signal was also recorded for toluene, *cis*-1,2-DCE and VC at different concentrations up to 200 μM (Fig. 6b). The biosensor response decreased in the order toluene>TCE>*cis*-1,2-DCE>VC in all the concentration domains. It was also linearly proportional to the substrate concentration up to 150 μM for toluene and VC and up to 100 μM for *cis*-1,2-DCE. Corresponding LODs were 0.04, 9 and 12 μM (5, 1,180 and 1,580 $\mu g L^{-1}$).

As competitive effects for enzyme sites may occur between TCE and toluene substrates, toluene/TCE mixtures were also investigated. Experiments were carried out at

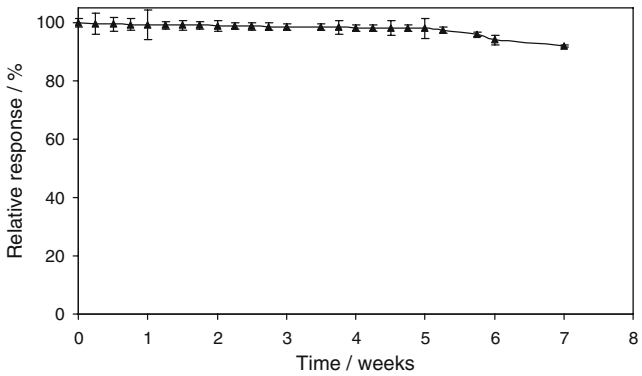

Fig. 5 Study of the SAM/SWCNT/PpF1 biosensor long-term storage stability. The electrodes were stored at 4 °C in M457 medium, pH 7, between two determinations. Three measurements were performed at 23±2 °C in 5 mM phosphate buffer, pH 7.2, for 10 μM TCE

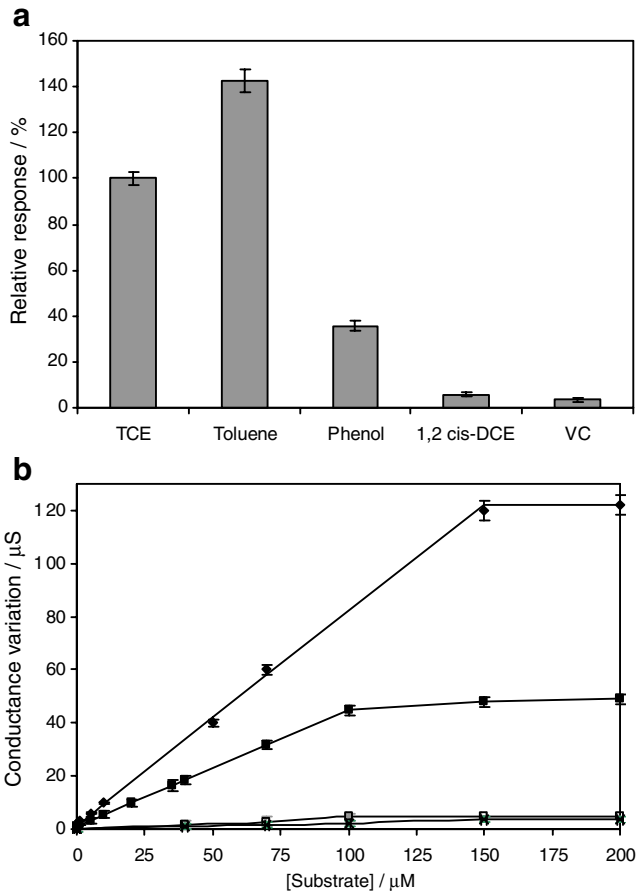

Fig. 6 Comparison of the biosensor responses recorded for TCE and possible interferents at a single concentration (10 μM) (**a**) and at different concentrations up to 200 μM (**b**): toluene (*filled diamond*), TCE (*filled square*), *cis*-1,2-DCE (*empty square*), VC (*cross*). Measurement conditions as in Fig. 3

Table 1 Influence of toluene on the SAM/SWCNT/PpF1 biosensor response

[Toluene] (μM)	[TCE] (μM)	[Toluene]/[TCE]	Biosensor relative response to TCE/%
5	5	1	108
10	5	2	116
30	5	6	156
10	10	1	109
20	10	2	118
50	10	5	190
7.5	15	0.5	110
15	15	1	119
30	15	2	140
30	20	0.5	112
15	20	1.5	137

Mean of three consecutive measurements

TCE concentrations of 5, 10, 15 and 20 μM and for toluene over TCE molar ratio varying from 0.5 to 6. In the same way, *cis*-1,2-DCE or VC can block enzyme binding sites and thus prevent the catalysis of TCE degradation. To check this, experiments were performed for a 100 μM TCE concentration and for 50, 100 and 150 μM *cis*-1,2-DCE or VC. In this latter case, no significant difference between biosensor responses was found, whatever the *cis*-1,2-DCE or VC concentrations. These two compounds do not significantly interfere with TCE determination. On the contrary, a competitive effect of TCE and toluene for TOD binding site was observed, confirming results reported by Hori et al. [16]. As shown in Table 1, toluene increases the TCE response by more than 10% for toluene/TCE molar ratio exceeding 0.5 at the highest concentrations tested (15 and 20 μM) and 1 at the lowest concentrations (5 and 10 μM). Ten per cent can be considered as a significant percentage taking into account measurement errors.

Application to real water samples

Six groundwater samples were collected in an urban industrial site located in northwestern France. A chlorinated solvent storage tank, located near well P2, was present on the site until 2004 and constitutes the source of TCE contamination. P1 well, located upstream of this area, remained uncontaminated. P3P, P3S, P4P and P4S locations follow the groundwater flow gradient. Toluene was not expected to interfere with TCE determination as its concentration, determined by GC/MS, was under the detection limit (5 $\mu g L^{-1}$) in both P3P and P3S samples and was very low in all other cases (13, 29 and 9 $\mu g L^{-1}$ in P2, P4P and P4S samples, respectively). To evaluate possible matrix effects, P1 sample was spiked with 5, 10, and 25 μM TCE, and 20 μL of each sample was analysed using the SAM/SWCNT/PpF1 biosensor. Three consecutive measurements of the samples were performed. TCE was not detected in P1 sample, whilst mean recoveries were respectively 102%, 98% and 99% for the 5-, 10- and 25-μM spiked samples. P2, P4P and P4S samples were also measured and biosensor signals were converted into concentration using the TCE calibration curve. Fivemicrolitres was injected in the 100-μL measurement cell for P2, whilst 20 μL was injected for the other samples. Results were then compared to TCE concentration obtained by HS-GC/MS. As seen in Table 2, biosensor results were in very good agreement with the HS-GC/MS method.

Conclusion

A rapid, sensitive and very stable conductometric biosensor based on *P. putida* bacteria has been developed for TCE determination. Inclusion of carbon nanotubes in the three-dimensional structure used for cell immobilisation, allowed improving bacterial loading, activity and accessibility, yielding faster and higher responses. Analytical performances, including limit of detection, stability and linearity domain, were much better than the characteristics of the bacterial biosensors already proposed for TCE analysis. A limit of detection of 0.07 μM (9 $\mu g L^{-1}$) was achieved, which is suitable for the assessment of environmental TCE degradation due to natural attenuation or induced remediation processes, as well as for water quality monitoring. The biosensor response to TCE was not significantly affected by *cis*-1,2-dichloroethylene and vinyl chloride and, in a limited way, by phenol. Toluene was the major

Table 2 TCE determination in groundwater samples by the SAM/SWNT/PpF1 biosensor and HS-GC/MS

Method	Concentrations (μM)	P2	P3P	P3S	P4P	P4S
Biosensor	TCE[a]	175.1±0.3	1.38±0.04	<0.42	<0.42	8.2±0.1
HS-GC/MS	TCE	175.6	1.45	0.023	0.030	8.40

Mean values and standard deviations of three consecutive measurements of the samples

[a] Concentration calculated from the biosensor response using TCE calibration curve

interference found. The biosensor, successfully applied to the determination of TCE in several groundwater samples, will be in the near future considered for direct on-site measurements.

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References

1. Moran MJ, Zogorski JS, Squillage PJ (2007) Chlorinated solvents in groundwater of the United States. Environ Sci Technol 41:74–81
2. Morioka Y (1996) Control of pollution of groundwater contaminated by toxic chemicals. J Jpn Soc Water Environ 19:529–533
3. Pooley K, Blessing M, Schmidt TC, Haderlein SB, Mac Quarrie KTB, Prommer H (2009) Aerobic biodegradation of chlorinated ethenes in a fractured bedrock aquifer: quantitative assessment by compound-specific isotope analysis (CSIA) and reactive transport modeling. Environ Sci Technol 43:7458–7464
4. Takeuchia M, Nanbab K, Iwamotoc H, Nireid H, Kusudae T, Kazaokae O, Owakid M, Furuyaa K (2005) In situ bioremediation of a *cis*-dichloroethylene-contaminated aquifer utilizing methane-rich groundwater from an uncontaminated aquifer. Water Res 39:2438–2444
5. Kim S, Kim D, Pollack GM, Collins LB, Rusyn I (2009) Pharmacokinetic analysis of trichloroethylene metabolism in male B6C3F1 mice: formation and disposition of trichloroacetic acid, dichloroacetic acid, S-(1,2-dichlorovinyl)glutathione and S-(1,2-dichlorovinyl)-L-cysteine. Toxicol Appl Pharmacol 238:90–99
6. Magnuson JK, Romine MF, Burris DR, Kingsley MT (2000) Trichloroethene reductive dehalogenase from *Dehalococcoides ethenogenes*: sequence of *tceA* and substrate range characterization. Appl Environ Microbiol 66:5141–5147
7. Delinsky AD, Bruckner JV, Bartlett MG (2005) A review of analytical methods for the determination of trichloroethylene and its major metabolites chloral hydrate, trichloroacetic acid and dichloroacetic acid. Biomed Chromatogr 19:617–639
8. Niri VH, Bragg L, Pawliszyn J (2008) Fast analysis of volatile organic compounds and disinfection by-products in drinking water using solid-phase microextraction–gas chromatography/time-of-flight mass spectrometry. J Chromatogr A 1201:222–227
9. Han TS, Kim YC, Sasaki S, Yano K, Ikebukuro K, Kitayama A, Nagamune T, Karube I (2001) Microbial sensor for trichloroethylene determination. Anal Chim Acta 431:225–230
10. Han TS, Sasaki S, Yano K, Ikebukuro K, Atsushi K, Nagamune T, Karube I (2002) Flow injection microbial trichloroethylene sensor. Talanta 57:271–276
11. Han TS, Sasaki S, Yano K, Ikebukuro K, Atsushi K, Nagamune T, Karube I (2003) Development of a reactor type bio-sensor for trichloroethylene. Anal Lett 36:539–547
12. Gibson DT, Koch JR, Kallio RE (1968) Oxidative degradation of aromatic hydrocarbons by microorganisms. I. enzymatic formation of catechol from benzene. Biochemistry 7:2653–2662
13. Spain JC, Gibson DT (1988) Oxidation of substituted phenols by *Pseudomonas putida* Fl and *Pseudomonas* sp. strain JS6. Appl Environ Microbiol 54:1399–1404
14. Wackett LP, Gibson DT (1988) Degradation of trichloroethylene by toluene dioxygenase in whole-cell studies with *Pseudomonas putida* Fl. Appl Environ Microbiol 54:1703–1708
15. Li S, Wackett LP (1992) Trichloroethylene oxidation by toluene dioxygenase. Biochem Biophys Res Commun 185:443–451
16. Hori K, Mii J, Morono Y, Tanji Y, Unno H (2005) Kinetic analyses of trichloroethylene cometabolism by toluene-degrading bacteria harboring a *tod* homologous gene. Biochem Eng J 26:59–64
17. Finette BA, Subramanian V, Gibson DT (1984) Isolation and characterization of *Pseudomonas putida* PpF1 mutants defective in the toluene dioxygenase enzyme system. J Bacteriol 160:1003–1009
18. Guedri H, Durrieu C (2008) A self-assembled monolayers based conductometric algal whole cell biosensor for water monitoring. Microchim Acta 163:179–184
19. Chouteau C, Dzyadevych S, Durrieu C, Chovelon JM (2005) A bi-enzymatic whole cell conductometric biosensor for heavy metal ions and pesticides detection in water samples. Biosens Bioelectron 21:273–281
20. Kumar S, Kundu S, Pakshirajan K, Dasu VV (2008) Cephalosporins determination with a novel microbial biosensor based on permeabilized *Pseudomonas aeruginosa* whole cells. Appl Biochem Biotechnol 151:653–664
21. Chu YF, Hsu CH, Soma PK, Lo YM (2009) Immobilization of bioluminescent *Escherichia coli* cells using natural and artificial fibers treated with polyethyleneimine. Bioresour Technol 100:3167–3174
22. Jha SK, Kanungo M, Nath A, D'Souza SF (2009) Entrapment of live microbial cells in electropolymerized polyaniline and their use as urea biosensor. Biosens Bioelectron 24:2637–2642
23. Yoo SK, Lee JH, Yun SS, Gu MB, Lee JH (2007) Fabrication of a bio-MEMS based cell-chip for toxicity monitoring. Biosens Bioelectron 22:1586–1592
24. D'Souza SF (2001) Microbial biosensors. Biosens Bioelectron 16:337–353
25. Lei Y, Chen W, Mulchandani A (2006) Microbial biosensors. Anal Chim Acta 568:200–210
26. Teles FRR, Fonseca LP (2008) Applications of polymers for biomolecule immobilization in electrochemical biosensors. Mat Sci Eng C 28:1530–1543
27. Chaki NK, Vijayamohanan K (2002) Self-assembled monolayers as a tunable platform for biosensor applications. Biosens Bioelectron 17:1–12
28. Mendes RK, Carvalhal RF, Kubota LT (2008) Effects of different self-assembled monolayers on enzyme immobilization procedures in peroxidase-based biosensor development. J Electroanal Chem 612:164–172
29. Jung SK, Namgung MO, Oh SY, Oh BK (2009) Fabrication of self-assembled oligophenylethynylenethiol monolayer for electrochemical glucose biosensor. Ultramicroscopy 109:911–915
30. Baldrich E, Laczka O, Del Campo FJ, Muñoz FX (2008) Gold immuno-functionalisation via self-assembled monolayers: study of critical parameters and comparative performance for protein and bacteria detection. J Immunol Methods 336:203–212
31. Chen H, Heng CK, Puiu PD, Zhou XD, Lee AC, Lim TM, Tan SN (2005) Detection of *Saccharomyces cerevisiae* immobilized on self-assembled monolayer (SAM) of alkanethiolate using electrochemical impedance spectroscopy. Anal Chim Acta 554:52–59
32. Hong SR, Choi SJ, Do Jeong H, Hong S (2009) Development of QCM biosensor to detect a marine derived pathogenic bacteria *Edwardsiella tarda* using a novel immobilisation method. Biosens Bioelectron 24:1635–1640

33. Laczka O, Baldrich E, del Campo FJ, Muñoz FX (2008) Immunofunctionalisation of gold transducers for bacterial detection by physisorption. Anal Bioanal Chem 391:2825–2835
34. Pyun JC, Kim SD, Chung JW (2005) New immobilization method for immunoaffinity biosensors by using thiolated proteins. Anal Biochem 347:227–233
35. Gooding JJ (2005) Nanostructuring electrodes with carbon nanotubes: a review on electrochemistry and applications for sensing. Electrochim Acta 50:3049–3060
36. Kim SN, Rusling JF, Papadimitrakopoulos F (2007) Carbon nanotubes for electronic and electrochemical detection of biomolecules. Adv Mater 19:3214–3228
37. Zylstra GJ, Mc Combie WR, Gibson DT, Finette BA (1988) Toluene degradation by *Pseudomonas putida* F1: genetic organization of the tod operon. Appl Environ Microbiol 54:1498–1503
38. Jaffrezic-Renault N, Dzyadevych SV (2008) Conductometric microbiosensors for environmental monitoring. Sensors 8:2569–2588