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Optimization and Characterization of a Flow Injection Electrochemical System for Pentachlorophenol Assay

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A flow injection (FI) electrochemical detection system has been developed and optimized for the determination of pentachlorophenol (PCP) in contaminated soil. PCP was oxidized to tetrachloro-1,4-benzoquinone (1,4-TCBQ) with a high yield using bis(trifluoroacetoxy)iodobenzene in 0.1 M tartaric acid, pH 2.0, at ambient temperature. Upon rapid reaction with immobilized glucose oxidase, the detection and amplification scheme was completed as the reduced form of 1,4-TCBQ or tetrachloro-1,4-hydroquinone was reoxidized to 1,4-TCBQ at the surface of the glassy carbon electrode (+ 0.40 V vs Ag/AgCl). Rapid electron exchange between the enzyme and its glucose substrate provided a non-rate-limiting current toward the electrode. The FI electrochemical system was linear up to 1 μM oxidized PCP with a detection limit of 10 nM and exhibited a reproducibility of ±0.6% over 165 repeated analyses during 14 h of continuous operation. When applied to PCP-contaminated soil samples, the results obtained from the FI electrochemical system compared well with those of the HPLC standard method.

Chlorophenols are an important class of organic pollutants in diverse applications. Chlorophenols have been formed as byproducts in the preparation of antioxidants, dyes, and drugs,[1,2] chlorination of drinking water, and chlorinated bleaching of paper.[3] They are also frequently present in the waste of the coal, gas, and petroleum industries.[4] Pentachlorophenol (PCP), commonly used as a wood preservative, is the most acutely toxic of all chlorophenol congeners. Recently, PCP regulations under the Resource Conservation and Recovery Act were created especially for wood treatment facilities in the United States. The large-scale use of PCP and other chlorinated phenols has led to the contamination of both aquatic and terrestrial ecosystems. With respect to their widespread and toxicological properties, several chlorinated phenols including PCP have been classified by the U.S. EPA as priority pollutants.[5] PCP is a widespread contaminant of groundwater and surface water worldwide, and the EPA maximum contaminant level is 3.7 nM PCP for potable water. Therefore, the monitoring and detection of PCP is of practical interest in environmental and food analysis for the investigation of animal and human exposure.

The inherent sensitivity, simplicity, and low cost of electrochemical detection make it very attractive and powerful for the determination of PCP in contaminated samples. Miniaturization of the electrode together with portable/compact instrumentation may also render the technique more applicable for on-line and field screening during site characterization and remediation activities. However, one major drawback of the method is that the surface of the electrode will be rapidly fouled, since the phenoxy radicals generated during PCP oxidations are subject to polymerization.[6] In addition, this method requires a high oxidation potential (700−900 mV), which leads to severe interferences, since contaminated samples will likely contain significant amounts of endogenous electroactive species. In contrast to PCP, tetrachloro-1,4-benzoquinone (1,4-TCBQ) can be easily detected at lower potentials and the amplification of the signal can be effected by coupling this oxidized form with an oxidoreductase such as glucose oxidase to increase the sensitivity.[7,8] A number of researchers have tried incorporating an oxidation step prior to electrochemical measurement with the aim of increasing analytical performance and aiding detection.[7−9] However, oxidation of phenolic compounds, especially PCP, in aqueous medium by enzymatic, photochemical, or chemical methods has not been very fruitful. Enzymatic reactions are generally not very effective with highly chlorinated phenols such as PCP, and a multitude of products including polymers are often formed.[10] Photochemical techniques involve various photosensitizers which often result in more complex reactions and the formation of a wide variety of product compounds.[11] Chemical reactions using oxidative re-

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agents such as hydrogen peroxide in conjunction with catalysts, unfortunately, result in low yields and/or multiple byproducts.[12]

This article describes a sensitive electrochemical method for the detection and determination of PCP in contaminated soil. PCP was oxidized to 1,4-TCBQ by bis(trifluoroacetoxy)iodobenzene (BTFAIB) since this high-yield reaction was recently reported for the oxidation of various chlorinated phenols including PCP under mild conditions.[13,14] A flow injection (FI) electrochemical system was constructed by immobilization of glucose oxidase on a glassy carbon electrode. In addition to characterization of the electrode and the oxidation step, the 1,4-TCBQ was then demonstrated as an efficient mediator to shuttle electrons between the enzyme and an electrode when coupled with immobilized glucose oxidase in the presence of excess glucose. Additional relevant data were also presented to demonstrate the applicability of the novel electrochemical scheme for the determination of PCP. To our knowledge, this is the first demonstration of a hybridized electrochemical detection system being used in the assay of PCP in real contaminated soil samples.

EXPERIMENTAL SECTION

Reagents. Glucose oxidase (EC 1.1.3.4, type X-S, 182 units/mg), L-tartaric acid, and glutaraldehyde (25% w/w) were obtained from Sigma (St. Louis, MO). PCP, BTFAIB, tetrachloro-1,2-benzoquinone (1,2-TCBQ), 1,4-TCBQ, and Nafion (5% w/w solution) were purchased from Aldrich (Milwaukee, WI). D-Glucose stock solutions (2 M) were allowed to mutarotate for at least 24 h before use. All chemicals were of the highest grade commercially available and used without further purification. Certified PCP-contaminated soil samples were obtained from Resource Technology Corp. (Laramie, WY). PCP-contaminated samples were obtained from a wood-preserving plant in the Montreal region.

Enzyme-Based Electrode Preparation. A dual glassy carbon electrode (BioAnalytical Systems (BAS), West Lafayette, IN) was polished with 1-μm diamond paste followed by 0.05-μm alumina slurry (Buehler) and washed with deionized water. The electrode surface was then cleaned for enzyme immobilization by sonication for 10 min in a Branson sonicating bath. Glutaraldehyde was added to glucose oxidase to initiate cross-linking, and 10 μL of this mixture was dropped onto one of the glassy carbon surfaces. The mixture was dried completely (10−15 min) under vacuum using water aspiration to form a thin film. A drop (10 μL) of Nafion solution was then dropped onto this film and dried under vacuum (10−15 min) to form a protective film over the enzyme layer. The effects of glutaraldehyde, enzyme, and Nafion concentration on the 1,4-TCBQ response were examined.

FI-Electrochemical Detection Apparatus. The FI-electrochemical system consisted of a peristaltic pump (FIA Pump 1000, Eppendorf North America, Madison, WI) which delivered the sample and buffer (100 mM L-tartrate (pH 3.5), 40 mM glucose) at a preset flow rate. Oxygen was removed from the buffer and sample by constant nitrogen bubbling. A 100-μL sample was injected into this stream by a motorized injection valve (EVA injector, Eppendorf). The loading and injection times were controlled from the EVA injector. The detector was a LC-44 cross-flow thin-layer electrochemical cell (BAS) which was placed downstream from the injector. The electrochemical cell consisted of an Ag/AgCl (3 M NaCl) reference electrode, a stainless steel auxiliary electrode, and the dual glassy carbon working electrode containing the glucose oxidase film. The target analyte (1,4-TCBQ) was reduced to tetrachloro-1,4-hydroquinone (1,4-TCHQ) in the presence of reduced glucose oxidase and then electrochemical detection of the oxidation of 1,4-TCHQ at the electrode surface was performed at +0.40 V using a CV1B voltammograph (BAS). Digitization and data acquisition were accomplished using a DAS-8 A/D card (MetraByte, Taunton, MA) connected to an IBM-AT computer with custom software such that peak height or peak area could be obtained. Note that all lines in contact with the sample had to be stainless steel to avoid severe peak tailing due to adsorption of the analyte within the system.

Optimization of FI-Mediated Electrochemical System. The 1,4-TCBQ response with respect to glucose concentration, flow rate, response time, and buffer pH was tested. The sensitivity and reproducibility of the 1,4-TCBQ signal were investigated as well as the stability of the enzyme-based electrode. The optimized system was then applied to samples of PCP which had been oxidized by BTFAIB (see below). The substrates and products of this reaction were tested for possible interferences.

PCP Oxidation Using Bis(trifluoroacetoxy)iodobenzene. Reactions were performed in 0.1 M tartrate buffer (20 mL) using PCP (10 mM in ethanol) and freshly prepared BTFAIB (250 mM in ethanol) stock solutions. The reactions were carried out at room temperature, with light protection for 1 h. At the end of the reaction, hydrogen peroxide (500 μM) was added to neutralize any unreacted BTFAIB and concentrated NaOH (8 M, 250 μL) was added to increase the pH to 3.5. The effect of buffer type, pH, and the BTFAIB to PCP concentration ratio were optimized. PCP-spiked tap water samples as well as extracts from soil samples were oxidized by BTFAIB under the optimized conditions and tested using the electrochemical detection system.

Preparation of Soil Extracts. Soil samples (2 g) were extracted with 100 mL of 10 mM NaOH for 4−8 h under light protection. After centrifugation, the supernatants were collected and stored in the dark at 4 °C. PCP from soil extracts could be partially purified and concentrated if necessary by using Sep-Pak tC18 cartridges (Waters Corp., Milford, MA). Cartridges were wetted with methanol and then washed with 50 mM phosphate (pH 8.0). The samples (20−40 mL) were neutralized and passed through the cartridges. Contaminants were released from the cartridges using a 50% methanol solution (2 mL). The PCP was only eluted from the cartridges when the methanol concentration was increased to 100%. The sample was then diluted back to the original volume for analysis using HPLC or the electrochemical system. For samples containing low levels of PCP (<50 ppm), the concentrated methanol extract could be used directly without dilution for HPLC analysis since the limit of detection was much higher than that of the electrochemical system. The sample preparation technique using the Sep-Pak cartridges resulted in recoveries of PCP of greater than 95%. It should be noted that less than 2.5% PCP was recovered in the 50% methanol fraction when standard PCP at 1 μM was analyzed.

PCP Analysis by HPLC. The HPLC system consisted of a WISP 710B injector (Waters), a Waters model 590 pump controlled

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by a Waters automated gradient controller, and a Waters Lambda-Max model 481 LC spectrophotometer set at 214 nm for PCP detection and a 4.6 mm i.d. × 15 cm Supelcosil LC-PAH column (Supelco, Bellefonte, PA). The mobile phase (1 L) was a mixture of 50% water, 50% acetonitrile containing 59 μL of 85% phosphoric acid. The flow rate was set at 1.0 mL/min with an injection volume of 20 μL. The calibration was established between 2 and 50 μM PCP. The detection limit was ~1 μM, and the PCP retention time was ~20 min. Reproducibility for the peak height expressed as the coefficient of variation (standard deviation/mean) was ~5% for all measurements.

RESULTS AND DISCUSSION

Enzyme-Based Electrode Preparation. Glucose oxidase (1 mg/mL) was mixed with various concentrations of glutaraldehyde to form different cross-linked enzyme films. A Nafion (0.7 mg/mL) layer was formed over these enzyme films and the response to 1 μM 1,4-TCBQ was determined at a flow rate of 44 mL/min. Initial investigation of the effect of glutaraldehyde on the enzymatic activity showed that a resilient film was formed at 0.35−0.4% glutaraldehyde with good mechanical strength and stability (figure not shown). At lower levels (<0.1%), the layer was soft and insufficient protein cross-linking led to the washing away of the enzyme, whereas at higher concentrations of glutaraldehyde (>0.5%), extensive cross-linking can cause diffusion resistance and destruction of the enzyme-active sites (about 86 and 79% of the activity at 0.5 and 0.8% glutaraldehyde, respectively). As a result, 0.35% glutaraldehyde was used in all subsequent film preparations.

Further studies showed that the level of glucose oxidase used in immobilization was crucial to the sensitivity of the enzyme-based electrode. The maximum response was obtained at about 1.5−2 mg/mL, while at higher concentrations (>6 mg/mL), the sensitivity decreased due to diffusion resistance as observed by the presence of a thicker film (~90% of the result obtained at 2 mg/mL glucose oxidase). The signal without glucose oxidase was about 2−3% of the maximum signal, and this signal likely resulted from the oxidation of 1,4-TCHQ which could be present as a contamination in the 1,4-TCBQ sample. In the absence of glucose oxidase, 1,4-TCBQ should not provide any signal at +0.4 V applied potential. The recycling of the mediator effected an amplification of the signal of ~10-fold, since the signal of 1,4-TCBQ reduced by zinc powder without the glucose oxidase present was ~10-fold lower than the signal for 1,4-TCBQ with the enzyme-based electrode. In the case of the enzyme-based electrode, the signal for 1,4-TCBQ and 1,4-TCHQ were the same, indicating that the oxidation of 1,4-TCHQ at the electrode surface was not the rate-determining step. In all subsequent enzyme film preparations, the level of glucose oxidase was 4 mg/mL.

The film covering the glucose oxidase layer was formed by the cation exchanger Nafion. The optimal concentration of Nafion was 0.7 mg/mL, whereas at higher concentrations, the response to 1,4-TCBQ decreased drastically with the signal at 2.0 mg/mL only 20% that of the maximum. It was reasoned that the presence of the Nafion layer would help to protect the enzyme film from the harsh flow conditions as well as plausibly eliminate the passage of some interfering anionic components. Very good reproducibility (±4% at 95% confidence interval) was obtained for the formation of the enzyme electrode as reflected by the response to 1 μM 1,4-TCBQ for 20 different electrode preparations (9.88 ± 0.44 μA·s).

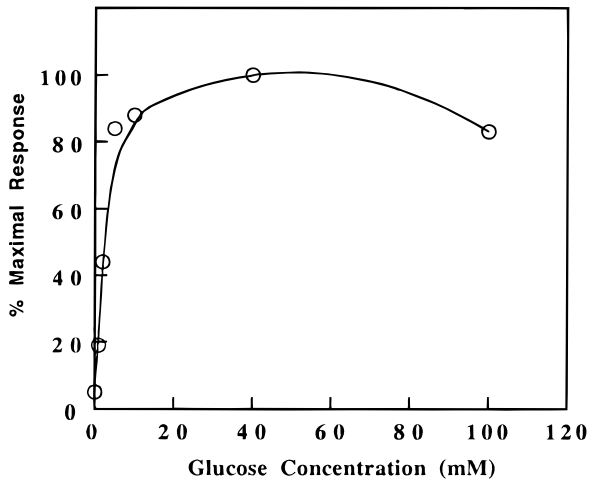
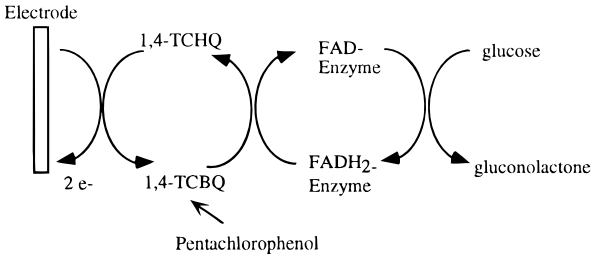


Figure 1. Effect of glucose concentration on the FI response to 1 μM 1,4-TCBQ using immobilized glucose oxidase (4 mg/mL) cross-linked by glutaraldehyde (0.35%). The enzyme-based electrode was covered by a Nafion (0.7 mg/mL) film, and the flow rate was set at 44 mL/h.

Optimization of FI-Electrochemical Detection for 1,4-TCBQ. Enzyme-based electrodes were prepared as described above and used to optimize some of the running conditions of the FI system. The amplification of the current is obtained due to the cycling of the mediator between the enzyme and the electrode surface. To achieve this amplification, the glucose must be in excess so that the electrode response will be dependent only on the 1,4-TCBQ concentration and the kinetics of the reaction between the enzyme and the mediator.



As shown in Figure 1, the optimal concentration for glucose was ~40 mM, and this concentration was used throughout the study. It should be noted that if glucose was omitted from the sample, the response was still ~90% compared to that of the same sample containing 40 mM glucose. Alternatively, if glucose was omitted from the buffer line but present in the sample, the response was ~80% compared to that of the same sample with 40 mM glucose in the buffer stream. The response to 1,4-TCBQ increased with a decrease in the flow rate as expected since at lower flow rates more analyte would react with the glucose oxidase. The response time also increased with decreasing flow rate. As a compromise between sensitivity and sample throughput (assays per hour), a flow rate of 44 mL/h resulting in a response time of about 3−4 min was selected for all subsequent studies.

The response to 1,4-TCBQ was independent of the pH of the L-tartrate buffer between 2.5 and 3.5 as similar responses were

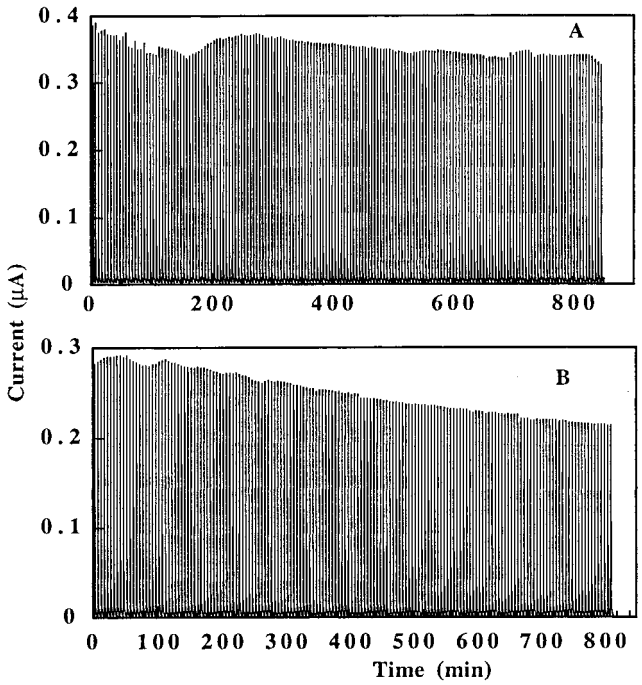


Figure 2. (A) Stability of the glucose oxidase-based electrode as determined by repeated injections of 1 μM 1,4-TCBQ. The flow rate was 44 mL/h, and the FI system was operated for 14 h with an injection every 5 min. (B) Stability of the glucose oxidase-based electrode as determined by repeated injections of 1 μM PCP oxidized for 1 h in the presence of 500 μM BTFAIB, tartaric acid (pH 2.0). The flow rate was 44 mL/h, and the FI system was operated for 14 h with an injection every 5 min.

obtained. However, the long-term stability of the enzyme electrode was dependent upon the pH, so as a result, a pH of 3.5 was always used in subsequent experiments. The signal for 1,4-TCBQ in the presence of nitrogen bubbling was only slightly higher (10%) in comparison to the signal without nitrogen bubbling. However, nitrogen bubbling was considered necessary since it eliminated the oxygen competition with the mediator which could adversely affect the sensitivity of the glucose oxidase based electrode.

An excellent linear relationship between the response of the electrochemical system and 1,4-TCBQ up to 4 μM was obtained ($R^2 = 0.999$). The sensitivity of the electrochemical system (95% confidence interval, $n = 12$) was determined to be 11.0 ± 0.10 μA·s/μM. Good reproducibility ($\pm$0.5% at 95% confidence interval) was also obtained as reflected in Figure 2A by the average response for 170 repeated analyses of 1 μM 1,4-TCBQ (11.18 ± 0.05 μA·s/μM). The detection limit (signal/noise = 3) was determined to be ~5 nM, and it should be noted that there was no signal in the absence of 1,4-TCBQ. The enzyme-based electrode was stable for ~14 h if run continuously with an injection every 5 min. After this time the signal began to decrease rapidly (~30%), and such behavior could be due to a long-term inhibitory effect of product formation on the enzyme activity. It should be noted that the enzyme electrode can be stored at 4 °C for over 30 days with no loss of activity.

PCP Detection Using the FI-Electrochemical System. PCP was oxidized by reaction with BTFAIB. In some previous studies,[13,14] the reaction was carried out at pH 1.0 with 100 mM trichloroacetic acid (TCA) for 30 min. Unfortunately TCA was found to decrease both the long-term stability of the enzyme and the sensitivity of the 1,4-TCBQ signal, likely due to the inhibition of the glucose oxidase. Among a variety of buffers tested, the reaction in acetate buffer (pH 3) was not complete until 2 h and the conversion yield from PCP to 1,4-TCBQ was below 70%. Further studies showed that lowering the pH of the acetate buffer to 2.0 caused inhibition of the enzyme. The reaction yield was very low when carried out in phosphate buffer, so attempts were made using tartrate buffer. At 100 mM tartaric acid (pH 2.0), the reaction yield from PCP to 1,4-TCBQ after 1 h was always greater than 70%. As the pH was increased, the yield dropped so as a result tartaric acid at pH 2.0 was used for all subsequent experiments.

Although the yield was not 100%, there was no PCP remaining after the reaction as indicated by HPLC. It is possible that some of the PCP could be oxidized to 1,2-TCBQ. The reaction yield would be lower, since it was observed that 1,2-TCBQ gave a response of <10% that of the same concentration of 1,4-TCBQ at the applied potential of +0.4 V. Alternatively, the lower yield could be due to an instability of the 1,4-TCBQ product. It was verified that the two byproducts of the PCP/BTFAIB reaction, trifluoroacetic acid and iodobenzene, exhibited no interference on the signal of 1,4-TCBQ. Hydrogen peroxide (500 μM) was added at the end of the reaction to destroy unreacted BTFAIB since it was observed that BTFAIB decreased the sensitivity of the 1,4-TCBQ response, possibly due to inhibition of the glucose oxidase. However, hydrogen peroxide per se did not interfere with the 1,4-TCBQ response.

The concentration ratio of BTFAIB to PCP was critical for a good reaction yield. In the previous study, the optimal ratio was 50:1 when 10 μM PCP was oxidized.[13,14] Initially, in this study, 1 μM PCP was oxidized with 50 μM BTFAIB and the yield was observed to be only 10−20%. Increasing the BTFAIB concentration to 500 μM increased the yield to over 70%, while higher concentrations (>1 mM) of BTFAIB did not improve the yield and actually effected precipitation. As a result, 500 μM BTFAIB was used for all PCP oxidation reactions. An excellent linear relationship between the response of the electrochemical system and oxidized PCP up to 1 μM was obtained ($R^2 = 0.999$). The sensitivity of the electrochemical system (95% confidence interval, $n = 8$) was determined to be 9.1 ± 0.2 μA·s/μM. Good reproducibility (0.6% at 95% confidence interval) was obtained as reflected in Figure 2B by the average response for 165 repeated analyses of 1 μM oxidized PCP (8.84 ± 0.06 μA·s/μM). It should be noted that although the peak height was decreasing slowly with time the peak area was not altered significantly (<10%). In the case of Figure 2A with standard 1,4-TCBQ, the peak height remained relatively unchanged with time. Such a result could imply that the surface of the film was altered with time by the components of the oxidation reaction resulting in a change in the diffusion characteristic of the film. The detection limit (signal/noise = 3) was determined to be 10 nM. The enzyme-based electrode was stable for ~14 h if run continuously with an injection every 5 min. As was the case with 1,4-TCBQ, the signal began to drop rapidly after this time. However, the response was more stable (16−18 h) when the electrochemical system was tested for a sample containing only 0.4 μM oxidized PCP. Although electrode fouling is a possibility, such behavior could also be due to the long-term inhibitory effect of product formation on the enzyme activity.

A blank response (i.e., no PCP) occurred when the BTFAIB reaction was carried out with tartaric acid, indicating that an electroactive species was being generated from the buffer in the presence of BTFAIB. The blank signal was equivalent to about 10−20 nM 1,4-TCBQ, which would cause a significant problem in determining low levels of PCP. It was also observed that the blank signal was dependent on the applied potential to the electrode surface. In the previous study,[12] the applied potential was +0.45 V, and it was observed in this study that lowering the applied potential to +0.40 V decreased the blank signal by 50% while dropping the signal due to 1,4-TCBQ only 10−15%. Further decreases in the applied potential (+0.30 V) almost completely eliminated the blank; however, the signal for 1,4-TCBQ was also drastically reduced (>50%) so as a compromise between sensitivity and the blank signal +0.40 V was used in all studies. The blank signal could be reduced another 50% by the addition of 1 mM ethylenediaminetetraacetic acid (EDTA) to the reaction buffer. This would seem to imply that metal ions may have some role to play in the blank signal. EDTA itself did not interfere with the 1,4-TCBQ signal, although there was a slight decrease in the stability of the enzyme electrode with time. EDTA at a concentration of 1 mM was added to the 100 mM tartaric acid (pH 2.0), 40 mM glucose reaction buffer to monitor low levels of PCP, especially in water samples. As a result of these improvements to the PCP oxidation reaction conditions, the blank was now equivalent to less than 5 nM 1,4-TCBQ, making it possible to detect lower levels of PCP accurately. It should be noted that the highest grade of L-tartaric acid (Sigma T-8277) was used in all studies as it was observed that lower grades resulted in higher blanks.

PCP Determination in Spiked Tap Water and Contaminated Soils. PCP was added to tap water at seven concentrations varying from 10 nM to 1 μM. The PCP oxidation reaction with BTFAIB was carried out, and the results were compared to the same PCP oxidized standards in double-distilled water. The sensitivity of the electrochemical system (95% confidence interval) for oxidized PCP was 8.5 ± 0.4 μ and 7.7 ± 0.2 μA·s/μM, respectively, for the standards in double-distilled water and tap water. The slight drop in sensitivity (10%) for oxidized PCP in the tap water likely indicated a small matrix effect.

The concentration of PCP in contaminated soils was determined by HPLC and compared to the values obtained by the electrochemical system for oxidized PCP. The PCP contamination level for the 22 samples tested ranged from 1 to 3000 ppm (μg of PCP/g of soil). Table 1 indicates excellent agreement between the two techniques with an overall discrepancy of only 5 ± 1.5% at the 95% confidence interval. For example, the certified soil sample from Resource Technology Corp. (sample 21, reference value 1425 ppm, confidence interval 1109−1742 ppm) gave a value of 1490 ppm with HPLC and 1470 ppm with the electrochemical system. When the electrochemical system values were plotted against those of HPLC, a straight line resulted with a slope of 1.02 ± 0.01 and a correlation coefficient of 0.999 (95% confidence interval, n = 22). Such good agreement thus validated the applicability of the mediated electrochemical system for measuring PCP in contaminated samples.

The stability of the electrochemical system was tested using a PCP-contaminated soil sample (~100 ppm) which was diluted 20-fold before analysis. The average response for 160 repeated analyses (13 h) was 2.62 ± 0.03 μA·s (95% confidence interval) and the peak area signal decreased ~10% during this time (figure not shown). As was the case with standard PCP (Figure 2B), periodic recalibration of the system at a regular time interval was required to account for the decrease in signal response. The same sample analyzed without the pretreatment procedure using the Sep-Pak cartridges resulted in a 50% reduction in the peak area signal over the same 13-h period. This certainly illustrates the importance of the pretreatment procedure in alleviating the soil matrix effect on the electrochemical detection system. Another contaminated soil sample (~2 ppm) was also analyzed, and since no dilution was necessary, this would represent the worst possible matrix effect. The peak area signal after 1 and 4 h dropped 5 and 30%, respectively. Without pretreatment the same sample resulted in a drop of 50 and 70% of the peak area signal after 1 and 4 h, respectively. As expected, the more concentrated sample matrix resulted in a more detrimental effect on the sensitivity of the electrochemical system.

Table 1. Comparison of Determinations of Concentrations of Pentachlorophenol in Contaminated Soil

sample no.	PCP conc (ppm) HPLC method	PCP conc (ppm) electrochem method[a]	diff[b] (%)
1	0.68	0.72	6
2	0.98	0.94	−4
3	1.8	2.0	11
4	2.2	2.4	9
5	2.3	2.2	−4
6	3.9	4.0	3
7	5.9	5.7	−3
8	10.2	9.9	−3
9	16.4	15.8	−4
10	18.4	17.7	−4
11	27.4	23.4	−15
12	100	96	−4
13	160	147	−8
14	160	156	−3
15	190	200	5
16	200	190	−5
17	610	650	7
18	700	710	1
19	1330	1370	−3
20	1470	1430	3
21	1490	1470	−1
22	2960	3080	4

[a] The value for the electrochemical method was determined from the average of triplicates. The reproducibility expressed as the coefficient of variation (standard deviation/mean) varied from 0.6 to 2.8%, and the overall coefficient of variation was determined to be 1.4 ± 0.5% (at 95% confidence interval). [b] Difference = 100(HPLC$_{(value)}$ − electrochemical system$_{(value)}$)/HPLC$_{(value)}$.

CONCLUSIONS

An automated, hybridized enzyme-based electrode has been developed and optimized for PCP assay employing immobilized glucose oxidase and a chemical reaction using bis(trifluoroacetoxy)iodobenzene for the oxidation of PCP to tetrachloro-1,4-benzoquinone, which shuttles electrons between the enzyme and the electrode. The electrochemical detection system exhibited a low detection limit for oxidized PCP and rapid response to PCP

concentration. For the first time, the enzyme-based electrode demonstrated the ability to assay contaminated soil samples, providing results equivalent to those from the HPLC standard method. Undoubtedly, the method could be extended for analysis of other PCP-contaminated samples such as groundwater, foodstuffs, and biological samples. Good detection limit and reproducibility together with inexpensive analytical instrumentation make the technique attractive for field analysis during site characterization and remediation activities. The remarkable sensitivity of the flow injection enzyme-based electrode permits significant dilution of contaminated soil samples, allowing for a substantial minimization of matrix effects.

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