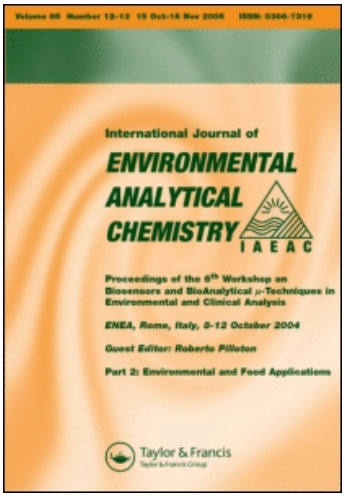

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Simultaneous Determination of Chromate and Aromatic Hydrocarbons in Environmental Samples by Capillary Electrophoresis

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SIMULTANEOUS DETERMINATION OF CHROMATE AND AROMATIC HYDROCARBONS IN ENVIRONMENTAL SAMPLES BY CAPILLARY ELECTROPHORESIS

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An analytical method was developed to determine simultaneously, the inorganic anion CrO_4^{2-}, and organic aromatic compounds including benzoate, 2-Cl-benzoate, phenol, m-cresol and o-/p-cresol by capillary electrophoresis (CE). Chromate and the aromatics were separated in a relatively short time with the use of a tetradecyltrimethylammonium bromide (TTAB) modified buffer solution. The detection limits of all the analytes were in the sub mg/L level with the consumption of a very small volume of sample (<0.06 μL). Calibration curves with high regression coefficient (r^2) values (0.999–1.000) were obtained within two orders of magnitude of concentrations. Factors affecting the separation and the determination of chromate and the organic aromatics, such as the buffer electrolyte concentration, the voltage applied and the TTAB concentration, were investigated. The effect of TTAB on the direction and the magnitude of the electroosmotic flow (EOF) was also discussed. A study of the microbial catalyzed chromate reduction coupled with benzoate oxidation demonstrated the application potential of the proposed method.

Keywords: Capillary electrophoresis; reversed EOF; chromate reduction

INTRODUCTION

The industrial discharge of chromium and aromatic compounds has posed a serious hazard to the environment. It is well known that chromate and aromatics are toxic substances to human health and are listed as priority pollutants by the U.S. Environmental Protection Agency.[1] To date, biological methods have been

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explored as remedial technologies for aromatic and chromate contaminated soils and groundwater.[2] Microorganisms have been shown to use the highly toxic and soluble chromate ion as a terminal electron acceptor, and reduce it to a less toxic and less soluble trivalent form. The reduction of chromate can be coupled with the oxidation of an electron donors, such as H_2 or aromatic compounds.[2–3] The *in situ* microbial reduction of chromate utilizing aromatic compounds as an electron donor has the ultimate environmental significance. It represents the potential for co-detoxification of both the inorganic and the organic contaminants, since they are often concurrently detected at many contaminated sites.[4] To evaluate the microbial reduction of chromate during the anaerobic degradation of aromatic compounds, frequent determinations of the concentrations of chromate and the aromatic compounds are necessary. Repetitive sampling and long term monitoring of the experimental systems limit the size of the subsamples available for process evaluation. An analytical technique with high sensitivity and a small sample size requirement is desirable. It would also be more efficient if inorganic chromate and the organic aromatics can be analyzed simultaneously.

Capillary electrophoresis (CE) appears to meet the analytical criteria by providing high efficiency, fast analysis, minute sample volume requirements and operational simplicity.[5,6] CE has been used for a variety of environmental analysis in the past decade.[7] Since then, it has encompassed applications from the analysis of aromatic sulfonic acids in leachate samples from a hazardous waste site,[8] to the determination of organic and inorganic anions in rain water,[9] and the detection of fatty acids in the ambient air.[10] Furthermore, separation of phenolic compounds has been shown by Cartoni et al.[11] and Masselter et al.[12] Recently, chromate determination by CE has also been reported.[13] Although much attention has been paid to the separation of organic or inorganic anions,[9,14,15] simultaneous determination of chromate and aromatic compounds has not been demonstrated. However, due to the nature of the biodegradation study, only a limited volume of sample can be taken each time. It is of extreme importance that the concentration of both chromate and aromatic compounds are determined simultaneously. Moreover, simultaneous determination minimizes the system errors between different analytical methods and thus provides data that better meet the requirements for mass balance and stoichiometry analysis.

CE separates ions according to their mobility in the electrolyte within an applied electric field. Detection of the ions can be achieved with a variety of available detectors. In a "standard" CE module, anions with high mobility escape or take very long time to reach the detector which located at the cathode end of the capillary, due to the fact that they travel towards the opposite direction of the electroosmotic flow (EOF). To solve this problem, surfactants such as sodium dodecyl sulfate (SDS) or tetradecyltrimethylammonium bromide

(TTAB)[13,16,17] have been introduced into buffer solutions at a concentration level at which hemimicelles formed. The hemimicelles reverse the bulk liquid flow direction, hence, the detector can capture those ions that would otherwise escape or prolong the run time needed for detection.

The purpose of this paper is to evaluate the potential of utilizing CE for the simultaneous separation/quantification of the inorganic and organic anions. Parameters governing the separation of chromate and aromatics, such as the buffer electrolyte concentration and the applied voltage, were examined. The effect of the TTAB concentration on the direction and the magnitude of the EOF, the analytes' migration time and sensitivity, as well as the separation resolution was investigated. Analytical reproducibility, detection limits and linear dynamic ranges were also determined.

EXPERIMENTAL

Instrumental

The CE instrument was a Spectra PHORESIS 500 (Thermal Separation Product, San Jose, CA, USA) equipped with a build-in wavelength variable detector, a polarity reversible power supply (0–30 kV) and an autosampler which was capable of housing a maximum of 80 samples. A deuterium lamp and a tungsten lamp were used as light sources for UV and visible region detection. The UV/Vis absorbance was measured in-line directly. An open-tubular fused silica capillary column of 70.0 cm × 75 μm I.D. (Thermal Separation Products, San Joes, CA) was used for separation. The effective length of the column was 62.6 cm. Samples were injected into the capillary using a hydrodynamic mode by applying a vacuum to the buffer chamber for 9.9 sec. The oven temperature was maintained at 30°C for the analysis. Data acquisition and processing were achieved using a Dynamax MacIntegrator (Rainin, Woburn, MA, USA) software and a Macintosh IIx computer.

Reagents

All solutions were prepared with analytical grade reagents and deionized water (18 MΩ). Standards of chromate and aromatic compounds were prepared by a series dilution of the 1000.00 mg/L stock solutions of chromium (as K_2CrO_4, Aldrich, Milwaukee, WI, USA), benzoate (as sodium benzoate), 2-Cl-benzoate (as sodium 2-Cl-benzoate), phenol, m-cresol, o-cresol and p-cresol. Buffer solu-

tion was prepared from Na_2SO_4 daily. The HPLC grade TTAB was added to the buffer for the reversal of the EOF. The 1 M NaOH washing solution was prepared from NaOH pellet (JT. Baker Chemical Co., Phillipsburg, NJ, USA). Phenol was obtained from Mallinckrodt (St. Louis, MO, USA), while sodium sulfate, TTAB and other aromatic compounds were obtained from Sigma Chemical Company (St. Louis, MO, USA). Environmental microcosms were prepared from subsurface soils, mineral salts medium and microbial enrichments in an anaerobic chamber.[3] The pH of microcosms was buffered at 7 with ammonium phosphate.

Procedure

The buffer solution and washing solutions were filtered via a 0.22 μm microfilter (Nalge, Co., Rochester, NY, USA) before use. Samples were centrifuged at 10,000 rpm for 10 min. Prior to any sample analysis each day, the capillary was conditioned by washing with 1 M NaOH for 5 min, followed by a 3 min wash with deionized water and finally, a 10 min wash with the carrier electrolytes. No voltage was applied during the capillary conditioning. Supernatant of 0.2 mL from the pre-centrifuged sample was introduced into each glass sample vial (2 mL) and placed on the autosampler. For separation, a negative voltage was applied. During the analysis, the capillary was washed for 3 min with the buffer electrolytes before sample injection, and 1 min with the deionized water following each sample analysis. A vacuum was applied to the buffer chamber during each washing cycle to empty it and then aspirate the wash buffer into the capillary. The washing cycle was critical for obtaining reproducible results.

RESULTS AND DISCUSSION

The UV absorption spectra showed an absorption maximum at 273 nm for chromate and at 220 nm for benzoate and other aromatics of interest. In order to determine chromate and benzoate simultaneously, 254 nm was chosen as a representative detection wavelength where both analytes exhibited reasonable absorption. In a normal CE module, samples are injected from the anode end of the capillary. Upon applying a high voltage across the two electrodes, the EOF, a bulk fluid flow of buffer and analytes, travels towards the cathode and through the detector. The detector is located near the cathode end of the capillary. Anions with high mobilities often miss the detection because the negative charges they carry drive them the opposite direction of the EOF. This effect has been ob-

served in our experiments, in which neither chromate nor benzoate had been detected within a 60 min time frame under the "standard" CE module. However, reversing the polarity of the electrodes alone could not solve the problem either, because anions still traveled in a direction against the EOF, thus lengthening the run time excessively. Previous studies by Huang et al[17] and Martinez[13] et al have shown that adding a TTAB surfactant to the buffer solution could cause the reversal of the EOF, which made it possible for them to separate 6 carboxylic acids or detect chromate. In our experiment, a 0.50 mM TTAB was also added to the Na_2SO_4 buffer, and the electropherogram of chromate and benzoate was obtained (Figure 1). As is seen in Figure 1, both chromate and benzoate had sharp, symmetric and well resolved peaks. The migration time of chromate and benzoate (2.2 and 3.8 min respectively) were short and analytically applicable.

The EOF reversal behavior is related to the inner surface of the capillary which is composed of silanol groups (Si-OH). These negatively charged groups attract the cations in the buffer electrolyte and result in the intrinsic EOF direction. TTAB is a cationic surfactant, which can interact with the negative charges on the inner wall of the capillary. The EOF was suppressed or reversed when the negative charges on the inner surface of the capillary were reduced or totally shielded by the TTAB surfactant. Table I lists the change of electroosmotic mobility (μ_{eo}) in magnitude and direction, when different concentrations of

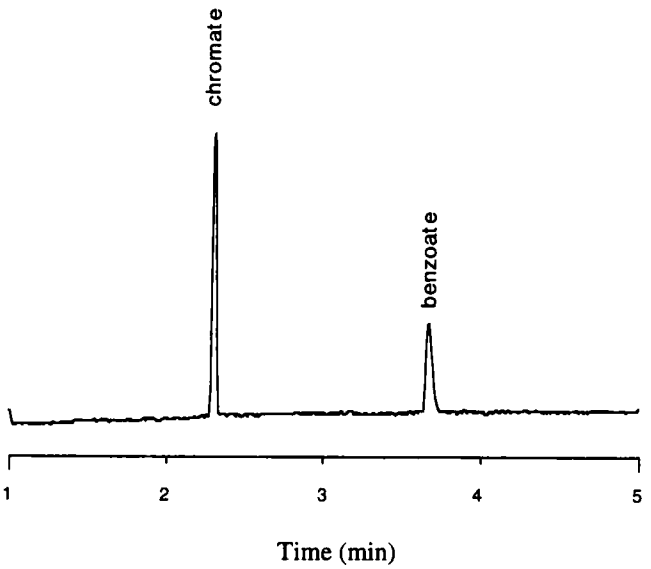

FIGURE 1 Electropherogram of 2.50 mg/L chromate and benzoate. Experimental conditions: buffer solution, 5.00 mM Na_2SO_4 and 0.50 mM TTAB; temperature, 30°C; hydrodynamic injection time, 9.9 sec; separation voltage, 25 kV; detection wavelength, 254 nm.

TABLE I Electroosmotic mobility at various TTAB concentrations

Concentration of TTAB (mM)	*Electroosmotic mobility (10^{-4} cm^2 s^{-1} V^{-1})*
0.00	2.0
0.01	1.7
0.05	1.6
0.08	−1.2
0.10	−1.3
0.50	−4.2
1.00	−4.7
2.50	−5.1
5.00	−4.4
10.00	−4.9

TTAB were added to the buffer solution. It is noted that at the lower TTAB levels, negative charges on the inner capillary surface were reduced, resulting in a suppressed EOF. As the TTAB concentration increased, more negative charges were shielded and a reversed EOF was indicated by a negative sign of μ_{eo} at TTAB concentrations of 0.08 mM and above. The μ_{eo} of the reversed EOF increased with a further increase in the TTAB concentrations up to 2.50 mM, then leveled off after the capillary inner surface was saturated with TTAB. The optimum TTAB concentration was determined by examining the migration time of chromate and benzoate (Figure 2). It is seen in Figure 2 that a longer migra-

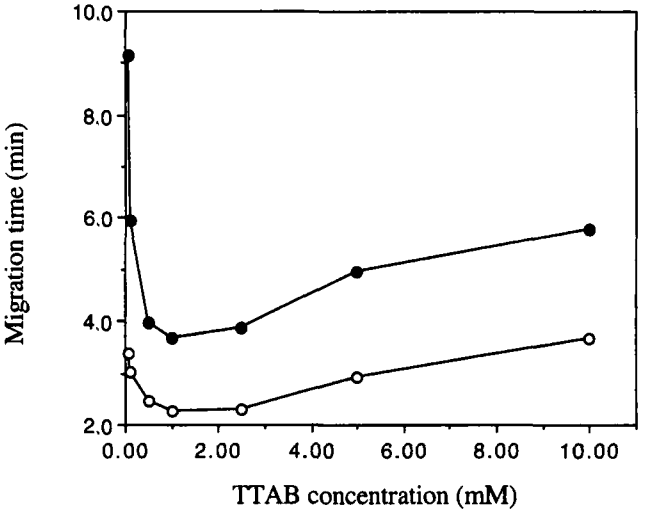

FIGURE 2 Migration times of chromate (○) and benzoate (●) using 5.00 mM Na_2SO_4 buffer solution supplemented with different concentrations of TTAB.

tion time was obtained for both chromate and benzoate at a lower TTAB concentration. The migration time of both analytes decreased drastically with an increase in the TTAB concentration from 0.08 to 0.50 mM. The shortest migration times for both analytes were obtained when the TTAB concentration laid between 0.5 and 2.5 mM. Further increasing the TTAB concentration above 2.5 mM, on the contrary, caused an increase in the migration time of both analytes. The reason for this behavior was the formation of micelles (the critical micelle concentration of TTAB is 3.4 mM[13]). When micelles were formed in the solution, anions were electrostatically adsorbed on the micelle surface, reducing their migration velocities. Therefore, TTAB concentrations between 0.50 and 2.50 mM were the most suitable for the determination of chromate and benzoate.

The best CE separation is achieved when the mobilities of analytes match that of the electrolyte. Na_2SO_4 was chosen as the buffer electrolyte for our experiments rather than phosphate because the microcosm samples were usually amended with phosphate. It is known that the buffer electrolyte concentration affects the separation in many ways.[18] Increasing the buffer concentration provides a better peak shape for high concentration analytes. Even for a moderate analyte concentration, the influence of the buffer concentration on the analytes' migration time and sensitivities can still be observed. This effect can be seen in Figure 3, which shows the sensitivities and the migration times of chromate and benzoate in different concentrations of Na_2SO_4. The sensitivity is defined as the peak area per unit concentration. It is noted in Figure 3 that both chromate and benzoate had a slightly shorter migration time at a low concentration of Na_2SO_4 (1.00 mM), but with poor sensitivities. Benzoate had good sensitivity at a higher Na_2SO_4 concentration (20.00 mM), but not chromate. Moreover, migration times for both analytes were also slightly longer at higher Na_2SO_4 concentrations. A moderate Na_2SO_4 concentration (3.00–5.00 mM) resulted in an improved sensitivity and a relatively short migration time for both chromate and benzoate, thus was used for the experiment.

pH is another factor that influences the CE separation. Usually, anions have better separation under alkaline conditions.[10,19,20] Unfortunately, the mineral salts medium in our experiment was buffered at pH 7 for optimal biological activities. Adjusting the sample pH means to introduce a relatively large amount of base into a small volume of sample. This could significantly alter the sample ionic strength, hence, the migration time and the analytical sensitivity, and potentially interfere with the analysis. To minimize the interference, no pH adjustment was performed in our experiments. The pH of samples and standards were approximately at 7.

To obtain better sensitivity for the determination of chromate and the aromatic compounds (benzoate, 2-Cl-benzoate, phenol, m-cresol, o-cresol and p-cresol),

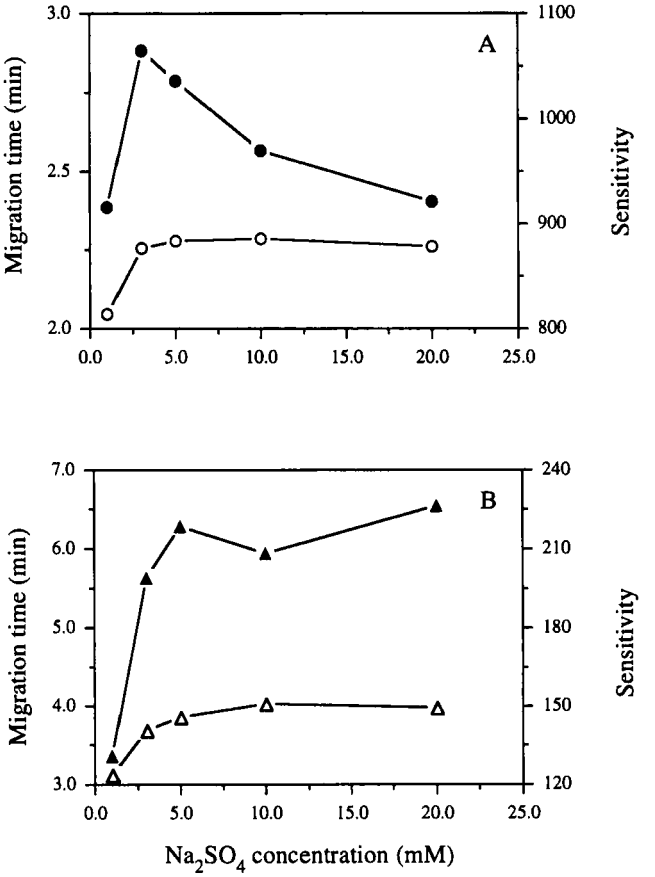

FIGURE 3 Migration times (○ and △) and sensitivity (● and ▲) of (A) chromate and (B) benzoate in different concentrations of Na_2SO_4 with the addition of 0.50 mM TTAB.

the 273 nm wavelength was used for the chromate detection and the 220 nm for the aromatic compounds detection. The wavelength change can be performed at a time between the elution of chromate and benzoate through the detector. For the 500 model Spectra PHORESIS CE, wavelength switch during one run can be done manually. On a later CE model such as Spectra PHORESIS 1000, wavelength change within one run can be programmed and conducted automatically. The electropherogram of chromate and the 6 aromatic compounds is showing in Figure 4. All of the anions, except o- and p-cresol, separated relatively well in a short time (6 min). Taking into account the 3 min pre-wash and the 1 min post-wash time, a total of only 10 min was required for each run. The

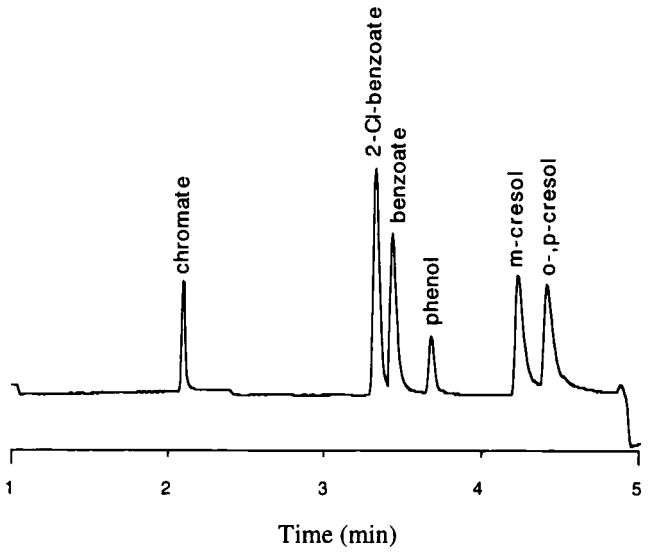

FIGURE 4 Separation of chromate, 2-Cl-benzoate, benzoate, phenol, m-cresol and o-/p-cresol. Experimental conditions: buffer solution, 5.00 mM Na_2SO_4 and 2.50 mM TTAB; temperature, 30°C; hydrodynamic injection time, 9.9 sec; separation voltage, 25 kV; detection wavelength: 273 nm for chromate and 220 nm for the aromatics.

o- and p-cresols co-eluted under the experimental conditions. Further study on the separation of these two compounds is still in progress, however, separation of the isomers was not critical to the bioremediation study.

Voltage applied across the capillary plays an important role in the separation resolution. Figure 5 shows the effect of voltage on the migration times of chromate and the 6 aromatic compounds. It was noticed that low voltages resulted in an improved separation, but at the expense of a longer analysis time. Although high voltages had significantly shortened the migration time of all analytes, the resolution between 2-Cl-benzoate and benzoate, and that of m-cresol and o-/p-cresol was diminished. Moreover, a higher voltage led to a rise of Joule's heat which caused peak broadening and a decrease of efficiency.[12] A voltage of 25 kV was selected in our experiment to maximize the resolution and a short analysis time.

The TTAB concentration had a direct effect on the separation of the anions, especially the aromatic compounds. Electropherograms of the 6 aromatic compounds were obtained at different TTAB concentrations (Figure 6). As shown in Figure 6, a high concentration of TTAB (4.00 mM) was incapable of separating m-cresol from o-/p-cresol. At a TTAB concentration of 3.00 mM, all the compounds were separated (except o- and p-cresol), but with broadened peaks. A

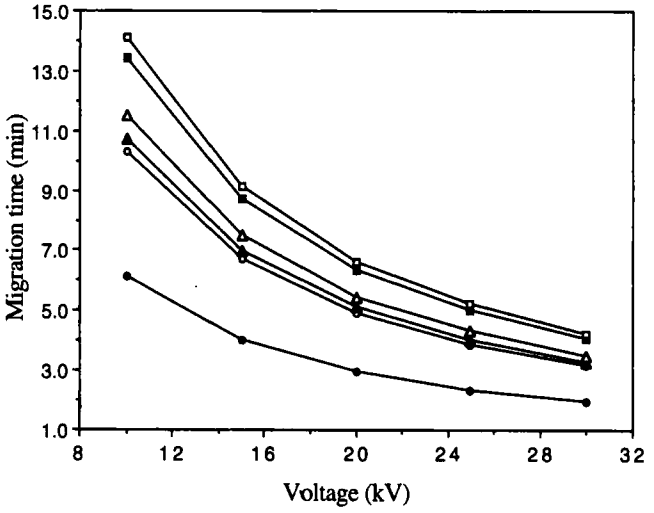

FIGURE 5 The effect of the applied voltage on the migration time of chromate (●), 2-Cl-benzoate (○), benzoate (▲), phenol (△), m-cresol (■) and o-/p-cresol (□), using a buffer solution of 5.00 mM Na_2SO_4 and 2.50 mM TTAB.

TTAB concentration of 2.50 mM appeared to be the best choice because (i) all the aromatics were better separated than at other TTAB concentrations tested; (ii) peaks were sharper and more symmetric than that at the TTAB concentration of 3.00 mM; (iii) the migration times of all the aromatics were shorter than that at higher TTAB concentrations. However, further reducing the TTAB concentration to 2.00 mM diminished the resolution between 2-Cl-benzoate and benzoate. It was obvious that TTAB at a concentration of 2.50 mM best facilitated the separation of the aromatic compounds.

Calibration curves for all analytes from 0.50 to 25.00 mg/L concentrations were obtained. Peak area was used for the calibration curve construction. The regression coefficients (r^2) of the calibration curves for chromate and the 6 aromatic compounds are listed in Table II. The r^2 values, ranging from 0.999 to 1.000, were obtained for all the tested anions over two orders of magnitude of concentrations. To improve the sensitivity for the analysis, a large volume of sample could be injected, i.e., a longer sample injection time could be selected as long as a symmetric and sharp peak shape is maintained. A 9.9 sec injection time was employed in our experiment. The relative standard deviations (R.S.D.s, %) for 5 consecutive analysis of each analyte were below 5.0% (Table II). The detection limits of each analyte were calculated using two times the standard deviations generated by the baseline noise. These detection limit values (Table II) were sufficiently low for the study of microbial reduction of chromate during

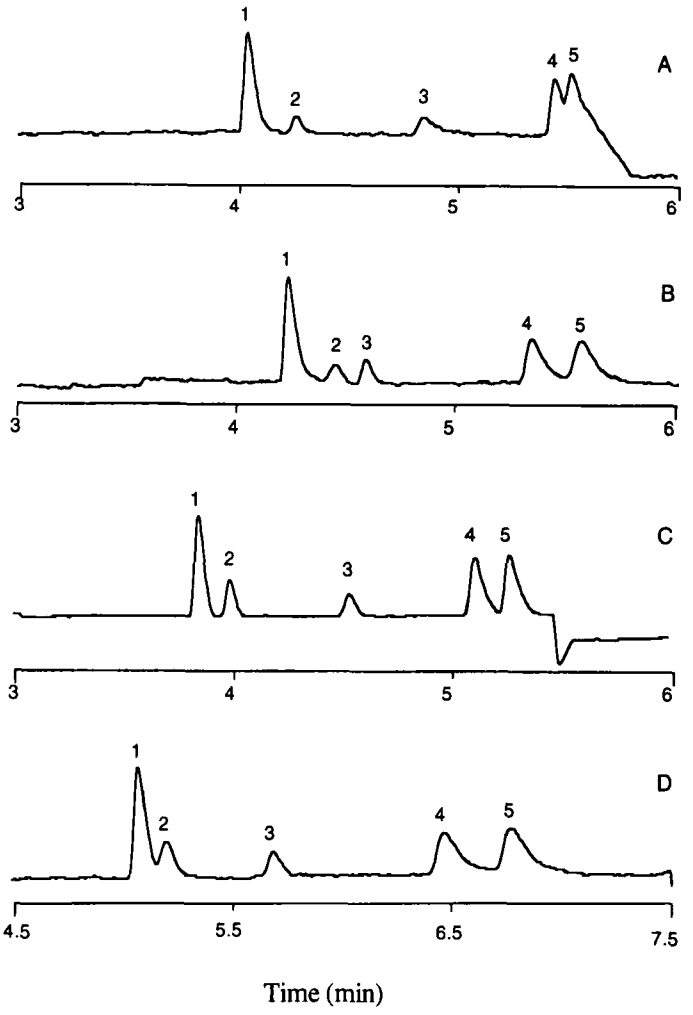

FIGURE 6 Separation of anions in a 5.00 mM Na_2SO_4 buffer solution supplemented with a TTAB concentration of (A) 4.00 mM; (B) 3.00 mM; (C) 2.50 mM and (D) 2.0 mM. Peak identification: 1 = 2-Cl-benzoate, 2 = benzoate, 3 = phenol, 4 = m-cresol and 5 = o-/p-cresol.

the anaerobic degradation of the aromatic compounds. The detection limits could be lowered if the electrokinetic injection mode was utilized,[21] but the linear dynamic range might be poor. This type of injection is very useful when qualification or semi-quantification of the analytes are of interest.

Subsequently, this method of simultaneous determination of chromate and benzoate by CE was used to study the biological linkage between chromate reduction and the benzoate oxidative catabolism by anaerobic microorganisms.[3]

TABLE II Regression coefficients (r^2) for calibration curves, relative standard deviations and detection limits

Compound	r^2	*R. S. D. (%) (n = 5)*	*Detection limit (mg/L)*
chromate	1.000	2.4	0.06
2-Cl-benzoate	0.999	3.0	0.03
benzoate	1.000	2.0	0.09
phenol	1.000	1.8	0.15
m-cresol	0.999	2.8	0.08
o-cresol	1.000	2.3	0.11
p-cresol	0.999	4.3	0.05

Figure 7 shows a typical electropherogram of chromate and benzoate from an environmental microcosm sample. The concentrations of chromate and benzoate were plotted individually versus the incubation time (Figure 8). As can be seen from Figure 8 that neither chromate nor benzoate concentration varied much during the first 4 days of incubation. This is the adaption phase of the microorganisms. Then starting from day 5 both chromate and benzoate concentrations decreased drastically. Chromate and benzoate were added to the microcosms repetitively upon their complete consumption. Arrows in Figure 8 showed the times when chromate or benzoate were re-amended. It is also noted from Figure 8 that benzoate degradation occurred concurrently with chromate reduction.

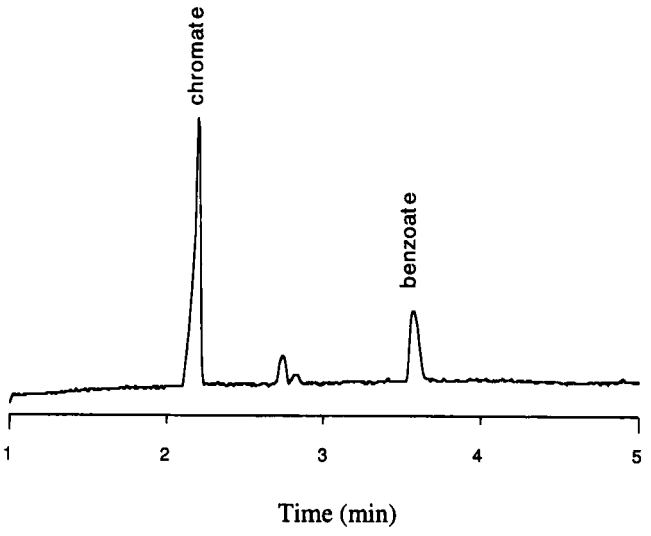

FIGURE 7 Electropherogram of chromate and benzoate from a typical microcosm sample for the study of microbial chromate reduction and benzoate oxidation under anaerobic conditions. Other experimental conditions were the same as Figure 1.

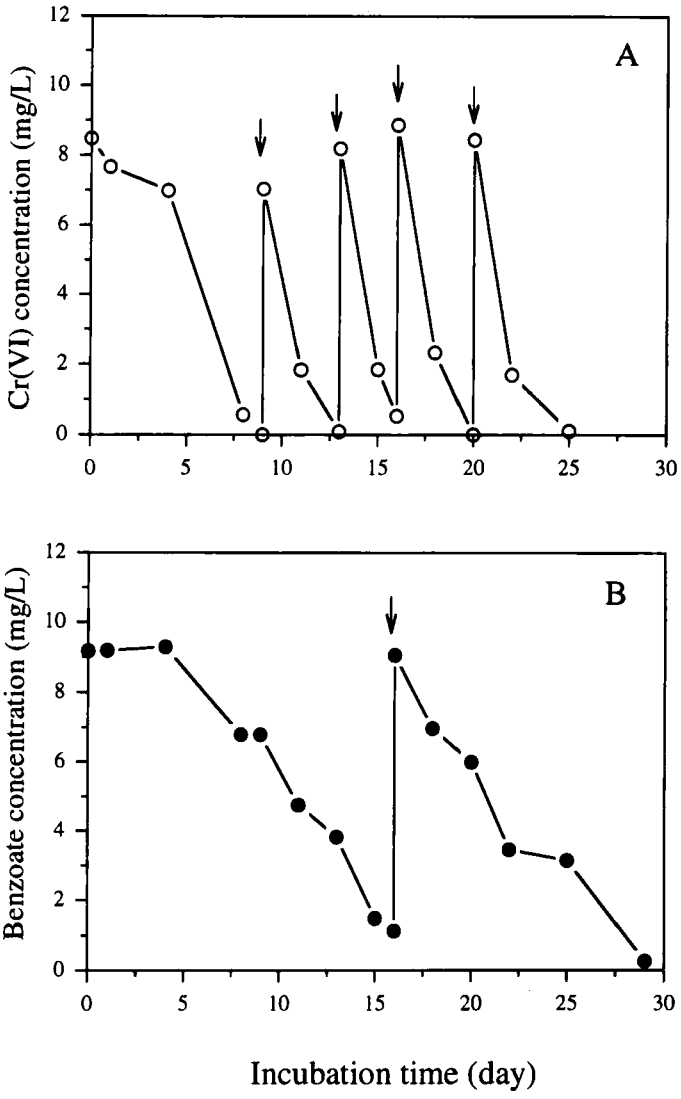

FIGURE 8 Relationship between the anaerobic chromate reduction (A) and the benzoate consumption (B) in the enriched microcosms. The results were averaged from triplicate incubations.

Quantitative analysis of the concentration change during a total 28 days incubation period suggested that benzoate consumption was linked to chromate removal. A statistical study indicated that the consumption of benzoate by chromate was in a 1 : 10 molar ratio, which was consistent with the stoichiometric calculation.[3] The results suggested that the chromate reduction was coupled with

the anaerobic oxidation of benzoate, and that the microorganisms catalyzed the electron transport from benzoate to chromate. The study has represented a potential of using aromatic compound to detoxify chromate. It has also offered an opportunity for the *in situ* microbial co-detoxification of metal and aromatic contaminants.

CONCLUSIONS

The results presented above have demonstrated the potential of utilizing CE for the determination of chromate and aromatic compounds simultaneously to evaluate a microbial treatment system. Inorganic chromate and organic aromatic compounds were separated in a relatively short time with the use of a TTAB modified buffer. The total run time for each sample was 10 min. Quantification capability of the method was proved by the calibration curves with high r^2 values (0.999–1.000) within two orders of magnitude concentrations (0.50 to 25.00 mg/L) for all analytes. The detection limits for each analyte were sufficiently low for monitoring significant biotransformation activities. Another advantage of the method was the minute sample volume (<0.06 μL) required for CE analysis, which made it suitable for monitoring the biological system in a long term experiment.

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