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Use of ion-exclusion chromatography for monitoring fatty acids produced by bacterial anaerobic degradation of tetrachloroethene in ground water

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ABSTRACT

    A rapid method for the simultaneous quantification of non-volatile and volatile fatty acids in aqueous sample by ion-exclusion chromatography is described. The sample is directly injected into the column and detected by a chemically suppressed conductivity detector, connected in tandem with an UV detector at 210 nm. The method allows detection of fatty acid as low as 1 ppm with a linear dynamic range up to 1000 ppm. At least 13 fatty acids can be determined within 50 min. This technique has been used to monitor the common fermentation products (lactate, acetate, propionate and butyrate) of the reductive dehalogenation of tetrachloroethene by anaerobic bacteria.

INTRODUCTION

    Gas chromatography (GC) has been used to determine non-volatile and volatile carboxylic acids in aqueous solution. [1]. Volatile fatty acids are extracted with ether prior to the analysis by GC. Then non-volatile fatty acids are derivatized to form dimethyl esters and are extracted into an organic solvent before GC analysis. Later, this time-consuming procedure was simplified by direct injection of the aqueous sample into the column [2]. Polar stationary phase, such as free fatty acid phase (FFAP) is used with either packed or capillary column. With this column, one can analyze both volatile and non-volatile fatty acids by using different conditions; therefore, separate analyses are required.
    The simultaneous analysis of both type of fatty acids by high-performance liquid chromatography (HPLC) has been reported [3]. At least 20 *p*-nitrobenzyl (PNB) esters of fatty acids in coffee were determined with 20 min by gradient reversed-phase HPLC. This technique requires extensive sample cleanup to remove the excess deriv-

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atizing agent. It is also unsuitable for samples containing chloride ions, because the formation of PNB chloride will interfere with the propionic acid PNB ester.

Ion-exclusion chromatography on a cation-exchange resin is widley used for the determination of fatty acids [4]. Strong-acid anions are excluded from the resin according to the Donnan principle and are eluted at the void volume of the column. Weaker-acid anions, existing largely in the molecular form, are retained on the stationary phase by a combination of ion exclusion, size exclusion and hydrophobic interactions. Typically, a mineral acid solution is used as the eluent to ensure that the fatty acids are predominantly in non-ionized form. However, water has also been used as eluent [5]. Detection of the eluted weak acids has been carried out by UV detection at 195–220 nm. A non-suppressed conductivity detector was also used with benzoic acid as the eluent to determine the C_1–C_4 aliphatic fatty acids [6]. Although this method can achieve detection limits from 60 ppb[a] to 1.4 ppm, the linearity is only one order of magnitude. Nadkarni and Brewer [2] reported the use of a chemically suppressed conductivity detector with ion-exclusion chromatography to determine volatile and non-volatile fatty acids in aqueous samples to obtain detection limits below 1 ppm with linearity over 2–3 orders of magnitude. However, resolution was not sufficient to determine more than 6 components simultaneously.

Contamination of aquifers with recalcitrant chlorinated compounds, including chloroethenes, creates a situation difficult to remedy. Although reductive dechlorination process can be effective in removing highly chlorinated compounds, as yet little is known about the factors controlling these reactions. In this study, the effects of the addition of common fermentation products (acetate, lactate, propionate and butyrate) on the reductive dehalogenation of tetrachloroethene (PCE) by anaerobic bacteria were examined. It was important that all four acids could be analyzed and quantified over the life of the experiment to identify changes in their concentrations and to compare those changes to the appearancce of chlorinated daughter products from the reduction of PCE.

EXPERIMENTAL

Reagents and chemicals

Pyruvic, malonic, methylmalonic, succinic, lactic, fumaric, acetic, propionic, butyric, isobutyric, valeric and isovaleric acids were purchased from Sigma (St. Louis, MO, U.S.A.). PCE, trichloroethenes (TCEs) and dichloroethenes (DCEs) were obtained from Aldrich (Milwaukee, WI, U.S.A.). Tetrabutyl ammonium hydroxide (TBAOH) and hexanesulfonic acid were high-purity grade and obtained from Dionex (Sunnyvale, CA, U.S.A.). Analytical reagent-grade sulfuric acid (H_2SO_4) (Fisher Scientific, Springfield, NJ, U.S.A.) was also used.

Reagent solutions

Sulfuric acid (0.05 M) was prepared by diluting concentrated sulfuric acid with deionized water (18 mΩ). This solution was used to prepare mobile phase at a concentration range from 1 to 7.5 mM. Hexanesulfonic acid 1 mM was prepared by diluting the stock solution (0.1 M) with deionized water. TBAOH (5 mM) was prepared by

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[a] Throughout this article, the American billion (10^9) is meant.

pipetting 50 ml of the stock solution (100 mM) into a 1-liter volumetric flask and diluting to volume with deionized water. These solutions were filtered through a 0.45-μm Millipore filter before use. Fatty acids were dissolved in water, aqueous methanol or methanol in the concentration range 1–1000 ppm.

Liquid chromatography

The apparatus consisted of the following components: a Model M6000 pump (Waters Associates, Milford, MA, U.S.A.), a Waters Autosampler Model 710B, a chemically suppressed conductivity detector, equipped with an anion micromembrane suppressor, Model AMMS-ICE (Dionex) and a Waters UV detector Model 490 at 210 nm. A Series A pump (Eldex Labs., San Carlos, CA, U.S.A.) delivered a regenerant TBAOH 5 mM) into the suppressor at a flow-rate of 1 ml/min. A 100-μl sample loop was used for loading the sample on the Ionguard GC-801 guard column (Interaction Chemicals, Mountainview, CA, U.S.A.), attached to the Interaction Ion-300 fatty column (300 × 7.8 mm). The columns were kept at 70°C in a Waters electric column heater. For routine analysis of the fatty acids, the mobile phase was 1 mM hexane sulfonic acid at a flow-rate of 0.5 ml/min. The chromatograms from the two detector were recorded simultaneously by a Model 2600 integrator (PE Nelson, Cupertino, CA, U.S.A.).

Methods

Anaerobic slurries were made with aquifer solids and sterile sulfide-reduced spring water supplemented with nitrogen and phosphorus. Aquifer solids were collected using the aseptic/anaerobic coring procedure [7]. Sealed, collected cores were placed in an anaerobic chamber where 50 g each of saturated core material was added aseptically to sterile 160 ml serum bottles. They were then completely filled with sterilized spring water amended with ammonium phosphate (10 mM ammonium and 5 mM phosphate). Reducing conditions were maintained by the addition of sodium sulfide (1 mM final concentration) and resazurin was added as a redox indicator. These slurries were spiked with 30 μM PCE or were unamended. Four concentrations (0, 0.1, 1 and 10 mM) of mixed organic acids (lactate, acetate, propionate and butyrate) were tested. The slurries were incubated anaerobically at room temperature, protected from light. They were sampled periodically and analyzed for PCE and its degradation products (TCE and DCE) by a headspace analysis technique, using the HP5890A gas chromatograph, equipped with HP 19395A headspace sampler. The aqueous portions were also centrifuged at 14 000 g for 5 min and the supernatant (100 μl) was injected into the HPLC column.

RESULTS AND DISCUSSION

The retention of the organic acid on the ion-exclusion column was mainly controlled by the column temperature and the mobile phase concentration. The column temperature was varied between 40 to 60°C and the concentration of sulfuric acid was varied between 1 to 7.5 mM. The effect of column temperature on selectivity is shown in Fig. 1. At 40°C, fumaric acid is coeluted with acetic acid and lactic acid is not separated from succinic acid. On the other hand, fumaric acid and formic acid are unresolved at 60°C. When 3 mM sulfuric acid is used as the mobile phase, the opti-
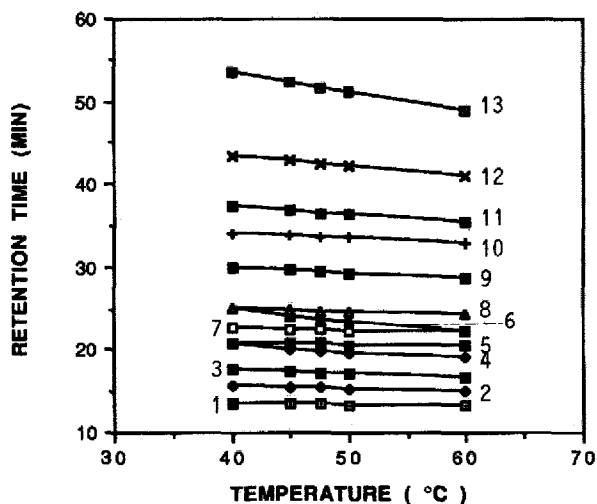
Fig. 1. Effect of column temperature on the retention times of fatty acids using 3 mM of sulfuric acid as the mobile phase. For compounds numbers, see Table I.

mum temperature lies between 45 and 50°C. Subsequently, the concentration of sulfuric acid was varied from 2.5 to 3.5 mM and the carboxylic acids were analyzed at 47.5°C (Fig. 2). To achieve good resolution of formic acid, fumaric acid and acetic acid at a column temperature of 47.5°C, the mobile phase concentration must be 3 mM (Fig. 3). Serial dilutions of fatty acid standard, having concentrations from 1 to 1000 ppm, were prepared and analyzed to construct the calibration curve. Table I shows the limit of detection and the linear range of 13 carboxylic acids when the UV detector is set at 210 nm.
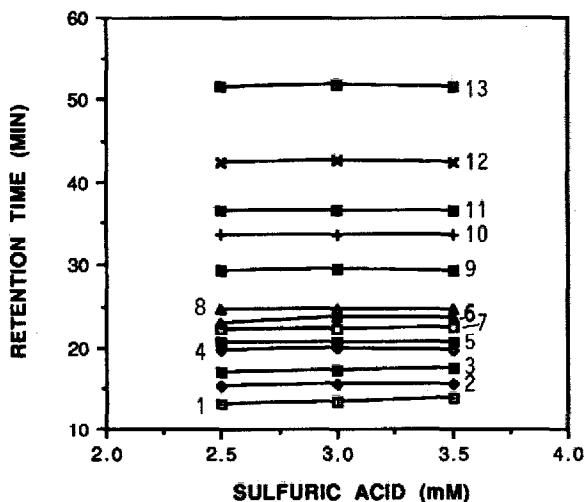

Fig. 2. Effect of sulfuric acid concentration used as the mobile phase on the retention time of fatty acids at 47.5°C.
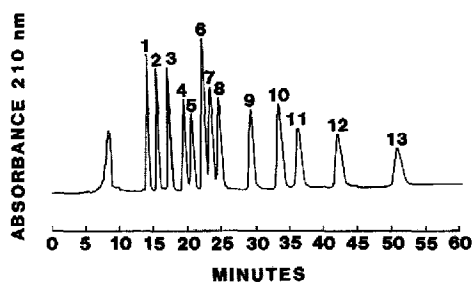
Fig. 3. Chromatogram of fatty acids on the I-300 fatty acid column. Mobile phase 3 mM H$_2$SO$_4$; flow-rate 1 ml/min; UV detector at 210 nm. Acids: 1 = pyruvic; 2 = malonic; 3 = methylmalonic; 4 = succinic; 5 = lactic; 6 = formic; 7 = fumaric; 8 = acetic; 9 = propionic; 10 = isobutyric; 11 = butyric; 12 = isovaleric; 13 = valeric.

Acidified ground water (200 ml of water + 2 drops of concentrated sulfuric acid), collected from Traverse city, MI U.S.A., was spiked with carboxylic acid mixtures at two concentration levels (Table II). Deionized water, spiked with the same amount of acid, was used as the control sample. Each sample was analyzed for carboxylic acids, along with blank sample. The recovery of pyruvic acid was low at both concentration levels. It may react with compounds in ground water and then be degraded to other by-products. The recoveries of other fatty acids were above 90% with a coefficient of variation of < 5% at the low concentration level and < 1% at the high concentration level. This ion-exclusion method works well for a determination of fatty acids in ground water at a detection limit above 10 ppm.

To improve the sensitivity of this technique, a chemically suppressed conductivity detector was connected to the UV detector in tandem. However, the sulfuric acid solution (3 mM) used as the mobile phase has such a high background conductivity

TABLE I

ANALYTICAL PERFORMANCE OF ION-EXCLUSION CHROMATOGRAPHY FOR THE DETERMINATION OF FATTY ACIDS WITH THE UV DETECTOR AT 210 nm

No.	Acid	Detection limit (ppm)	Upper dynamic range (ppm)
1	Pyruvic acid	0.3	100
2	Malonic acid	4	1000
3	Methylmalonic acid	3	1000
4	Succinic acid	7	1000
5	Lactic acid	5	1000
6	Fumaric acid	0.01	50
7	Formic acid	5	1000
8	Acetic acid	7	1000
9	Propionic acid	8	1000
10	Isobutyric acid	8	1000
11	Butyric acid	12	1000
12	Isovaleric acid	12	1000
13	Valeric acid	15	1000

TABLE II

RECOVERIES OF FATTY ACIDS FROM GROUND WATER

Acid	Spike level 1				Spike level 2			
	ppm	Average recovery $(n = 3)$	S.D.	C.V.	ppm	Average recovery $(n = 3)$	S.D.	C.V.
Pyruvic acid	4.0	27.5	10.4	27.7	40	14.3	3.6	25.3
Malonic acid	40.0	94.0	0.3	0.4	400	95.8	0.9	0.9
Methylmalonic acid	34.0	95.0	1.0	1.1	340	97.7	0.3	0.3
Succinic acid	73.0	97.0	0.4	0.4	730	99.5	0.4	0.4
Lactic acid	41.0	96.1	0.8	0.8	410	96.9	0.1	0.1
Fumaric acid	0.4	96.7	1.0	1.0	4	99.1	0.7	0.7
Formic acid	80.0	98.1	0.5	0.5	800	98.8	0.2	0.2
Acetic acid	80.0	103.5	1.1	1.0	800	100.6	0.2	0.2
Propionic acid	80.0	114.1	2.2	1.9	800	98.2	0.2	0.3
Isobutyric acid	80.0	102.5	1.9	1.8	800	96.8	0.2	0.2
Butyric acid	80.0	103.8	2.1	2.0	800	99.5	0.4	0.4
Isovaleric acid	80.0	106.9	5.9	5.5	800	109.9	0.3	0.3
Valeric acid	76.0	99.2	5.3	5.3	760	107.3	0.3	0.2

that it cannot be suppressed to an acceptable level. Hexane sulfonic acid (1 mM) was used as the mobile phase with TBAOH as the regenerant to suppress the background conductivity. Because the concentration of the hexane sulfonic acid was much less than the concentration of sulfuric acid previously used, the retention times of the carboxylic acids decreased. Since only four acids were investigated in the experiment, the column temperature was increased to 70°C to shorten the analysis time.

Applications

The method developed has been applied to the study of the reductive dechlorination processes of PCE in the presence of carboxylic acids, including lactate, acetate, propionate and butyrate. It is important that all four acids can be determined over the life of the experiment. The changes of their concentrations are compared with the changes in the chlorinated daughter products from the reduction of PCE. Fig. 4 shows the typical chromatograms of standard fatty acids detected by the conductivity detector and the UV detector. The benefit of having two detectors connected in tandem is that sensitivity and selectivity increase. The conductivity detector has an average detection limit of 1 ppm for fatty acids with a linear dynamic range up to 100 ppm. The UV detector has the average detection limit of 10 ppm with the linear dynamic range up to 1000 ppm. Therefore, the analyst can determine fatty acids over a much wider linear dynamic range with high sensitivity. For the sample that contains high concentrations of carbonate, it is difficult to quantify butyrate with the conductivity detector because carbonate peak overlaps the butyrate peak (Fig. 5). On the other hand, the UV detector can detect butyrate quite nicely.

Fig. 6A shows the change of the PCE concentration and the concentrations of its daughter products in the slurry containing 10 mM of carboxylic acids. The change of carboxylic acids concentrations, is shown in Fig. 6B for comparison. As the buty-
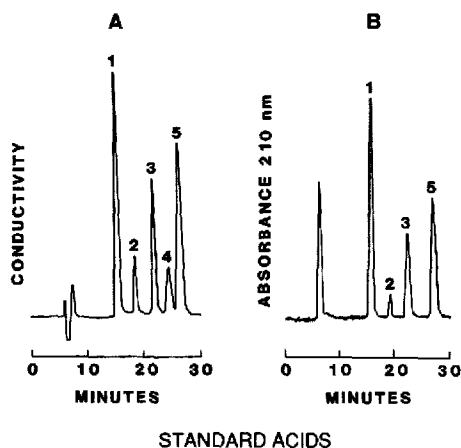
STANDARD ACIDS

Fig. 4. Chromatograms of a mixture of standard fatty acids: 1 = lactic (82 ppm), 2 = acetic (13 ppm), 3 = propionic (47 ppm), 4 = carbonate (trace) and 5 = butyric (92 ppm) detected by (A) the chemically suppressed conductivitity detector and (B) the UV detector at 210 nm.

rate was oxidized to acetate, the PCE was reduced to TCE and DCE after 51 days of incubation. Lactate was used up after the first 20 days. There was no change in propionate concentration. The incubation times before reductive dechlorination of PCE was observed were 51 days (10 mM acids), 65 days (1 mM acids), 86 days (0.1 mM acids), and over 175 days (no acids added). After 175 days incubation, no PCE was detectable in any of the fatty acid-supplemented slurry, but 27 to 29 μM of combined tri- and dichloroethenes were detected. These results indicate that the avail-
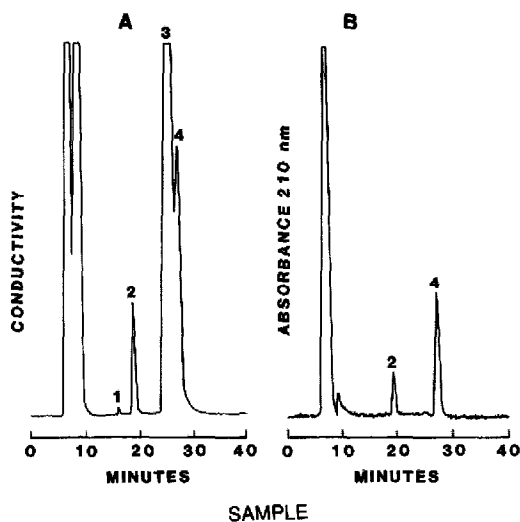

SAMPLE

Fig. 5. Chromatograms of the slurry containing high concentration of carbonate detected by (A) the chemically suppressed conductivity detector and (B) the UV detector at 210 nm. Peaks: 1 = lactic acid; 2 = acetic acid; 3 = carbonate; 4 = butyric acid.
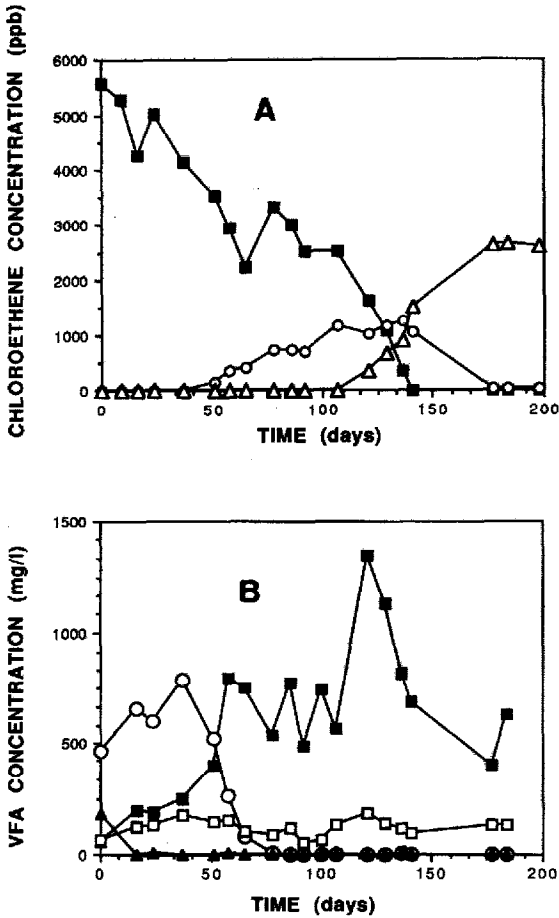
Fig. 6. (A) Relationship between the incubation period and the concentration of PCE with its daughter products in the slurry containing 10 m*M* of fatty acids: ■ = PCE; ○ = TCE; △ = DCE; (B) Relationship between the incubation period and the fatty acids concentrations in the slurry. VFA = Volatile fatty acids. ■ = Acetate; ○ = butyrate; ▲ = lactate; □ = propionate.

ability of organic electron donors are stimulatory to reductive dechlorination processes.

CONCLUSION

Ion-exclusion chromatography is suitable for the analysis of fatty acids in an aqueous sample. It requires neither sample cleanup nor precolumn derivatization. Two parameters, column temperature and mobile phase concentrations, play an important role in a successful separation. To increase the sensitivity and selectivity of the analysis, connecting a chemically suppressed conductivity detector and a UV detector in tandem is recommended.

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