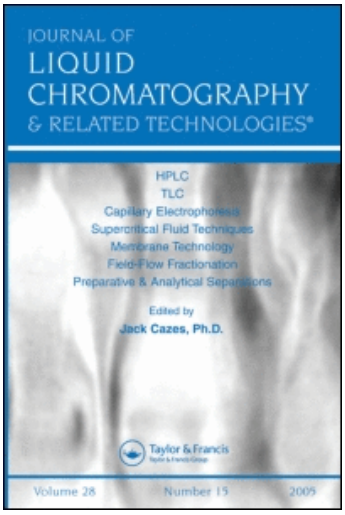

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DEVELOPMENT OF HPLC METHOD FOR THE ANALYSIS OF CHLOROPHENOLS IN SAMPLES FROM ANAEROBIC REACTORS FOR WASTEWATER TREATMENT

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ABSTRACT

This article describes an HPLC method for routine samples analysis of laboratory scale anaerobic immobilized sludge reactors used for degradation of pentachlorophenol (PCP) in synthetic wastewater. The method was developed to quantify pentachlorophenol and six chlorophenol isomers produced during the degradation of pentachlorophenol by anaerobic bacteria. The validated method was used for anaerobic reactor samples and was able to quantify the chlorophenol intermediates produced.

INTRODUCTION

The contamination of aquatic environments by organic toxic chemicals represents a worldwide threat to the maintenance of the quality of the water for

human consumption. Nowadays among the thousands of organic compounds used for many purposes, some represent environmental pollutants and their introduction in ecosystems is due to improper disposal of industrial wastes, domestic sewage, accidental spills, and use of considerable amounts of pesticides in crops.[1]

In the class of organochlorine contaminants, chlorophenols play an important part since they are present in pulp mill effluents and have been used as pesticides, wood preservatives, and they are also toxic to a variety of living organisms.[2] The toxicity exhibited by chlorophenols, has put them in the list of the eleven priority phenols of USA-EPA, which cannot be present in drinking water at levels above 0,01 mg/L for total phenols.[3]

In recent years, environmental research has concentrated efforts in the development of new technologies related to the restoration of contaminated aquatic ecosystems by the use of bioremediation techniques. Organochlorines are biologically degraded in anaerobic processes called reductive dechlorination, where the number of chlorine atoms in the molecule are reduced and also its toxicity.[4,5]

Analytical chemistry is an important tool in environmental research since it provides mechanisms to detect environmental problems and supports the improvement of remediation techniques.[6,7,8] In this article the development of an HPLC method is described, for monitoring of the performance of laboratory scale anaerobic digesters used for the degradation of pentachlorophenol.

In the reductive dechlorination of pentachlorophenol, less chlorinated intermediates are formed according to metabolic pathways of the microorganisms. Six chlorophenol isomers and also pentachlorophenol were selected to be included in the group of analyzed compounds.

EXPERIMENTAL

Chemicals

The following chlorophenol standards obtained from Supelco were used: *meta*-chlorophenol, *para*-chlorophenol, 3,4-dichlorophenol, 3,5-dichlorophenol, 2,3,5-trichlorophenol, 2,4,5-trichlorophenol, and pentachlorophenol. Methanol (HPLC grade) was obtained from Malinckrodt and Carlo Erba. Water was obtained from a Milli-Q system (Millipore, Bedford, MA, USA). Potassium hydrogenophosphate (Merck) and phosphoric acid (Merck) were used for the preparation of buffer solutions used as part of the mobile phase. Supelclean LC-18 (500 mg Supelco) cartridges were used for the clean up of

the samples. The analytical column (15 x 0,46 cm I.D.) used was packed with silica ODS Hypersil (5 μm, 120 Å) from Shandon (Cheshire, UK).

Equipment

The HPLC system consisted of a Waters Model 510 pump, an injection valve (Rheodyne, Model 7125) and an UV detector (Shimadzu, Model SPD 6AV) operated at 280 nm. The data acquisition was carried out using the Class LC10 software (Shimadzu) in a personal computer. A Shandon packer was used to pack the analytical column.

Method

The mobile phase consisted of methanol and a 0.01 $mol.L^{-1}$ potassium hydrogenophosphate buffer adjusted to pH 4 with phosphoric acid which were used in a step gradient with a flow rate of 2 $mL.min^{-1}$. Methanol:buffer (30:70 v/v) was used from 0 to 15 min and from 15 to 45 min. Methanol:buffer (60:40 v/v).

Individual 10^3 mg/L stock solutions of each chlorophenol were prepared and stored in the dark at 4°C. These stock solutions were used to prepare the standard solutions of mixed chlorophenols at the following nominal concentrations: 1, 3, 5, and 8 $mg.L^{-1}$.

Extraction Procedure

Supelclean LC-18 Cartridges (500 mg, Sulpelco) were conditioned with methanol (5 mL) and water acidified to pH 2 with phosphoric acid (5 mL) prior to use. An aliquot of the sample (3 mL) was passed through the cartridge and then washed with the water at pH 2 (4 mL). The cartridges were dried before the chlorophenols were eluted with methanol (3 mL). The methanol extracted (20 μL) was then analyzed by HPLC.

RESULTS AND DISCUSSION

To achieve good resolution for the seven chlorophenols a wide range of mobile-phases was investigated in order to see the effects on the capacity factors of these congeners. The wide variety of capacity factors obtained for the series made an isocratic run impossible. For the elution of *para* and *meta* - chlorophenol a weak solvent was necessary if complete separation was the goal, while for the other chlorophenols a much stronger solvent was necessary. The larger capacity factor obtained for the pentachlorophenol in a weak solvent

would make the run not practical while affecting the sensibility of the analysis. A good compromise was obtained by the use of the step gradient as described in Experimental.

Figure 1 shows a chromatogram of the separation of the seven chlorophenols obtained in these conditions and also the elution order, which was identified by co-injection of the individual compounds.

Method Validation

Linearity

Calibration curves were made by plotting the peak area against the concentration of each compound, injected in triplicate, of standard solutions. Regression analysis of the least-square line for the data showed an excellent straight line fit over the concentration examined (1 to 8 $mg.L^{-1}$) with r over 0.998 for the seven chlorophenols. Figure 2 shows the calibration curves obtained.

Taking a signal-to-noise ratio of 2 as criteria to the limit of detection (LOD) and as ten times the LOD for the limit of quantification (LOQ), the LOD of this method was found to be 0.08 $mg.L^{-1}$ and the LOQ of 0.8 $mg.L^{-1}$.

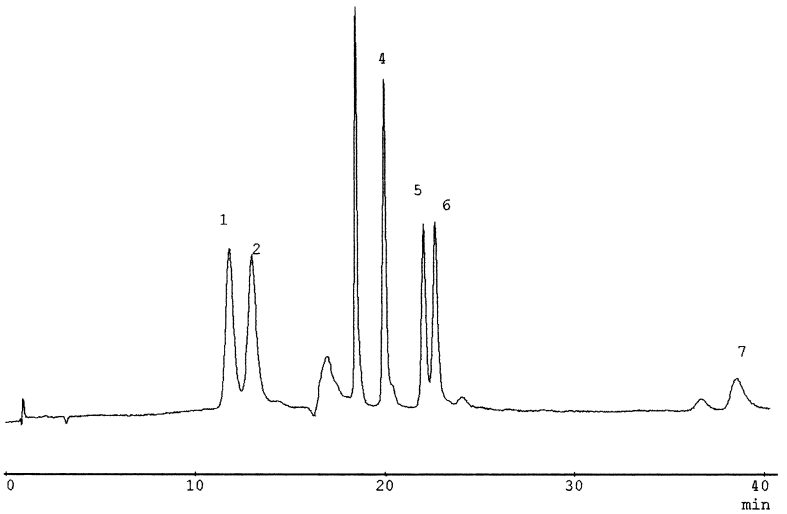

Figure 1. Chromatogram of the separation of seven chlorophenols in an ODS - Hypersil column (15 x 0,46 cm I.D., 5 μm, 120 Å). 1) *para*-chlorophenol; 2) *meta*- chlorophenol; 3) 3,4 -dichlorophenol; 4) 3,5-dichlorophenol; 5) 2,3,4 -trichlorophenol; 6) 2,3,5-trichlorophenol; 7) pentachlorophenol. For chromatographic conditions see text.

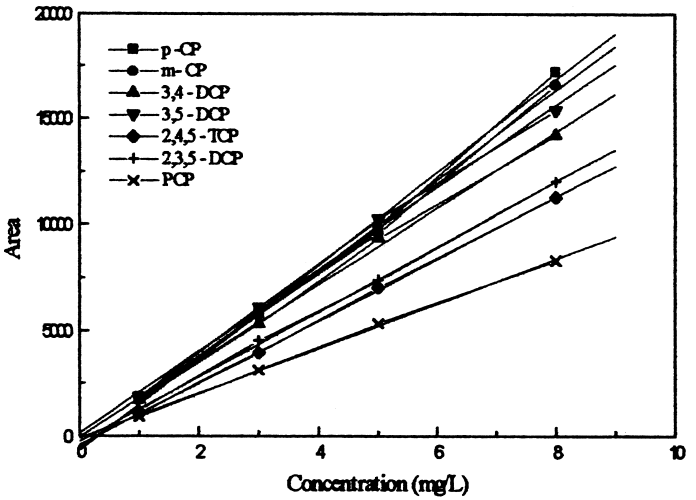

Figure 2. Calibration curves for the seven chlorophenols studied.

Precision

The result of the intra-day variability was calculated for multiple injections (n = 7) at two concentrations 1 and 3 mg.L^{-1} levels. The Coefficient of Variation (CV %) was in the range of 1.6 to 11.6%. The inter-day precision was examined for a 3 mg.L^{-1} solution analyzed in quintuplicate in three different days (n = 15). In this case, the CV (%) obtained was between 3.2 and 11.0 %.

Analytical Recovery

The anaerobic reactor is usually operated by the introduction of an aqueous solution of inorganic salts representing macro and micronutrients for the population of anaerobic bacteria. This matrix was used to prepare three samples spiked with chlorophenols at 3, 5, and 8 mg.L^{-1} levels. Each solution was extracted in triplicate and each extraction was analyzed also in triplicate. The recovery of each chlorophenol was calculated by comparison of the areas of the extracted and non extracted compounds in each concentration. Table 1 shows the recoveries obtained for each chlorophenol in each concentration and their CV %. The specificity of the method was also examined by running an extracted blank matrix where no interfering peaks were noticed.

Table 1

Recoveries of the Seven Chlorophenols Analyzed

Compound	Recovery (%)	CV (%)
para-Chlorophenol	98 - 111	3.0 - 4.8
meta-Chlorophenol	96 - 103	3.3 - 8.0
3,4-Chlorophenol	102 - 112	1.7 - 15.0
3,5-Chlorophenol	79 - 104	4.6 - 6.3
2,4,5-Chlorophenol	106 - 116	6.1 - 10.2
2,3,5-Chlorophenol	103 - 109	6.0 -10.2
Pentachlorophenol	81 - 95	8.5 - 15.0

Blinded Unknowns

Two samples were prepared by spiking the blank matrix with concentrations of chlorophenols unknown to the analyst. The samples were extracted as described above and analyzed in duplicate.

Since the calibration curve was not an extracted one but was made with standard solutions, a correction factor which represented the percent of recovery exhibited by each compound analyzed was used. Fifteen percent was the limit adopted as the accuracy acceptance criteria. The results of the blinded analysis can be seen in Table 2.

Table 2

Results for the Determination of Blinded Unknowns

Compound	Added ($mg.L^{-1}$)	Sample Determined ($mg.L^{-1}$)	Recovery (%)	Added ($mg.L^{-1}$)	Sample Determined ($mg.L^{-1}$)	Recovery (%)
para-CP	<LOQ	<LOQ	---	<LOQ	<LOQ	---
meta-CP	1	<LOQ	---	1.5	1.1 ± 0.0	74
3,4-DCP	<LOQ	<LOQ	---	2.0	2.2 ± 0.1	110
3,5-DCP	1	1.0 ± 0.0	100	3.0	3.1 ± 0.4	103
2,4,5-TCP	1.5	1.1 ± 0.6	74	2.0	1.8 ± 0.3	90
2,3,5-TCP	3.5	3.2 ± 1.3	91	2.5	2.1 ± 0.4	84
PCP	2.0	2.0 ± 0.2	100	2.5	2.6 ± 0.1	104

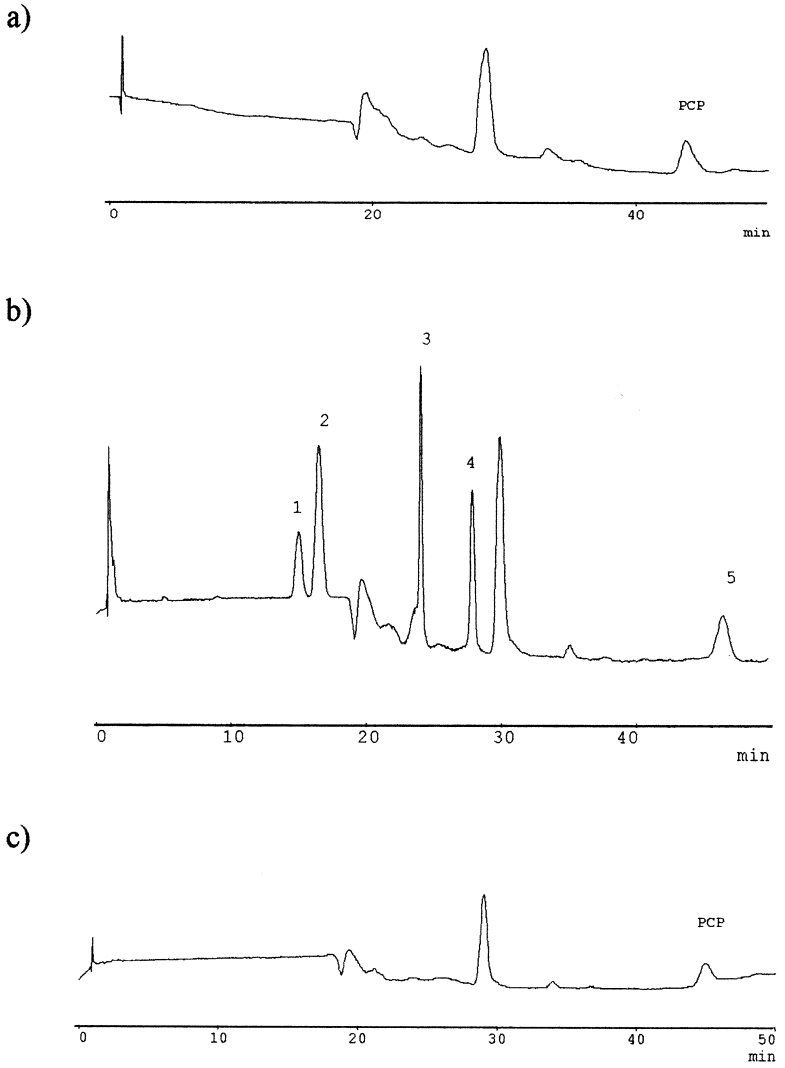

Figure 3. a) Chromatogram of the reactor effluent containing pentachlorophenol; b) Chromatogram of the analysis of the second anaerobic reactor sample: 1) *para*-chlorophenol; 2) *meta*- chlorophenol; 3) 3,5-dichlorophenol; 4) 2,3,5 -trichlorophenol; 5) pentachlorophenol. c) Chromatogram of the effluent of the reactor containing pentachlorophenol not degraded. For chromatographic conditions see text.

Table 3

Identified Compounds with Their Concentrations of the Anaerobic Reactor's Samples Analyzed

Compound	Concentrations ($mg.L^{-1}$) Inlet	Middle Point	Outlet
para-CP	---	2.2 ± 0.4	---
meta-CP	---	5.8 ± 1.4	---
3,5-DCP	---	3.8 ± 1.0	---
2,3,5-TCP	----	4.8 ± 2.2	---
PCP	10.4 ± 1.2	5.8 ± 0.9	5.8 ± 2.3

Based on the 15 % acceptance criteria established for accuracy, three of the blinded unknowns were considered inaccurate: *meta*-CP and 2,4,5-TCP in Sample 1 and *meta*-CP in Sample 2.

Application to Anaerobic Reactor Samples

The laboratory scale anaerobic immobilized sludge reactor used is made of glass. Its volume is 2000 mL, the length 1 m and the diameter 5 cm. The reactor was filled with 20.8 g of polyurethane cubes (size 0.3 cm) to which the bacteria were attached to accomplish the PCP degradation. Three representative samples were analyzed to evaluate the method applicability on monitoring the performance of the reactor. The first sample was from the inlet of the reactor containing pentachlorophenol, the second was from the middle and the third sample from the outlet of the reactor. Figure 3 shows the chromatograms obtained from the analysis of the anaerobic reactor profile. Dechlorination intermediates were found in the sample from the middle of the reactor: *para*-chlorophenol, *meta*-chlorophenol, 3,5-dichlorophenol, 2,3,5-trichlorophenol, together with pentachlorophenol, which was detected in all samples. Table 3 shows the concentrations of these chlorophenols in the analyzed samples.

CONCLUSIONS

The method presented here showed good precision, recovery, and usefulness for monitoring the anaerobic reactor performance in the degradation of pentachlorophenol present in synthetic wastewaters, and the identification of intermediates of the dechlorination process.

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