DEVELOPMENT OF BIOLOGICAL PERMEABLE BARRIERS FOR REMOVAL OF 2,4,6 TRICHLOROPHENOL IN CONTAMINATED GROUNDWATER

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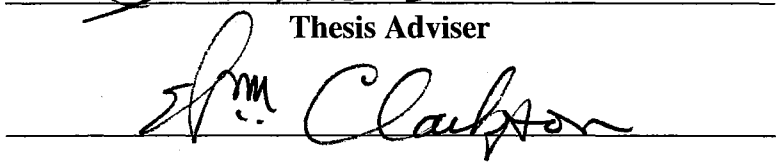

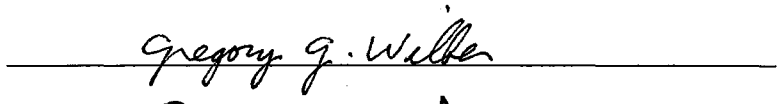

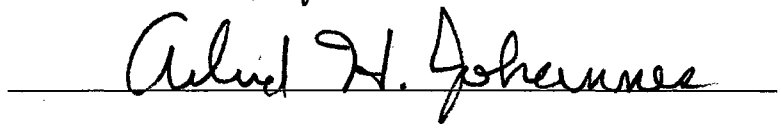

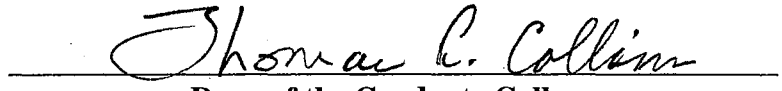

Dean of the Graduate College

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I was born not knowing

and have only had a little time to change that here and there

-Richard Feynman.

My deepest appreciation goes to my family who provided persistent encouragement, and to whom this thesis is dedicated.

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NOMENCLATURE

BOD	biochemical oxygen demand
COD	chemical oxygen demand
CP	chlorophenol(s)
DCP	dichlorophenol
DO	dissolved oxygen
ECD	electron capture detector
FID	flame ionization detector
GAC	granular activated carbon
GC	gas chromatograph
IC	ion chromatograph
ICl	inorganic chloride
M	molarity
MCP	monochlorophenol
MS	mass spectrometry
MW	molecular weight
N	normality
PAC	powered activated carbon
PCP	pentachlorophenol

PVA	polyvinyl alchohol
r_g	growth rate
r_{su}	substrate utilization rate
SEM	scanning electron microscope
TCP	2,4,6 trichlorophenol
TeCP	tetrachlorophenol
T-N	total organic carbon
VSS	voletile suspended solids

CHAPTER I

INTRODUCTION

A major concern exists over contamination of groundwater by chlorophenols. The United States Environmental Protection Agency (USEPA) included chlorophenols on its priority list of the most common hazardous substances found in the United States (52 FR 12866, April 17, 1987; 53 FR 41279, October 20, 1988) and mandated a maximum contaminant level (MCL) in drinking water of 0.1 µg/l for pentachlorophenol (40 CFR .141.61).

Chlorophenols are organic chemicals formed from phenol by substitution on the phenol ring with one or more atoms of chlorine. Significant amounts of chlorophenol can be formed and subsequently released into the environment from the chlorine bleaching process in pulp and paper-mills (>293,000 kg/yr.), the chlorination of wastewater and drinking-water (>1,000 kg/yr.), and the incineration of municipal waste (>272,000 kg/yr.) (Jones, 1984).

It has been shown that trichlorophenol and pentachlorophenol in sediments, where photolysis and apparently biodegradation are minimal, may persist for years (Pierce and Victor, 1978; DeLaune *et al.*, 1983). Levels of chlorophenols in effluents from chemical and wood preservation industries may reach several thousand µg/L, while the maximum concentrations in surface waters and groundwaters can reach several µg/L (WHO 1989). As a result of spills, isolated levels as high as 100 mg/L of chlorophenols in groundwater, and 18 mg/L in surface waters have been reported (WHO 1989).

The available information on the effects of chlorophenols on the environment centers primarily on aquatic organisms. Toxicity generally increases with the degree of chlorination of the phenol ring (WHO 1984). In long-term studies, sublethal levels of chlorophenol reduced both growth and survival of fathead minnows. In humans, symptoms associated with exposure include eye, nose, and airway irritation, and dermatitis. Abnormal liver function tests, changes in brain wave activity, and slowed visual reaction time have been reported in association with high-level exposure (WHO, 1989). A guideline value of 10 µg/L was recommended by WHO (1989) for 2,4,6-trichlorophenol in drinking water, based on animal carcinogenicity data (WHO, 1989). Due to their toxicity, tendency to bioaccumulate, and persistence in the environment, chlorophenol contamination of soil and water is of concern, and remediation is warranted.

Conventional aquifer restoration alternatives such as pump and treat or on site remediation are not generally effective. These technologies have numerous problems associated with them (Thomson, *et al.* 1991) which include:

1. management of large volumes of water,
2. potential production of undesirable by-products,
3. undesirable effect on hydraulic characteristics in uncontaminated parts of the aquifer (change in direction of water movement), and
4. labor or energy intensive.

Treatment of contaminated groundwater can be accomplished using various techniques. In situ biological treatment has various advantages when compared to other treatment techniques. Such advantages include low cleanup costs and the possibility of

complete transformation of organic contaminants to harmless end products (Thomson *et al.* 1991). A number of microorganisms from a variety of habitats can readily degrade chlorophenols under aerobic conditions (Ingols *et al.*, 1966). The relative rate of degradation of chlorophenols generally decreases as the number of chlorine atoms on the phenolic ring increases (Ingols *et al.*, 1966). In most instances, aerobic metabolism involves dechlorination and hydroxylation, which are usually followed by cleavage of the phenol ring at the ortho position and subsequent complete degradation (PCP→ TeCP→ TCP→ DCP→ MCP→ Phenol) (Jones, 1984).

Chlorophenols may be degraded in a number of anoxic environments by reductive dechloronation and subsequent transformations of the ring to carbon dioxide and methane.

An alternative to conventional groundwater treatment processes is the use of barriers which are permeable to water, but prevent the migration of contaminants. They are referred to as permeable barriers (Thomson *et al.,* 1991)

In situ permeable barriers are a relatively new cost-effective technology that can be used in groundwater remediation of shallow aquifers (Thompson *et al.*, 1991). Permeable barriers are installed as permanent, semi-permanent, or replaceable units across the flow path of a contaminant plume. Permeable barriers allow water to move passively through while precipitating, sorbing, or degrading the contaminants (Rael *et al.*, 1995). These mechanically simple barriers may contain metal-based catalysts for degrading volatile organics, chelators for immobilizing metals, nutrients and oxygen for microorganisms to enhance bioremediation, or other agents. Degradation reactions may break down the contaminants in the plume into harmless byproducts (EPA 1995).

Crushed limestone, peat, and powdered activated carbon are also several effective barrier mediums that have been used to adsorb or precipitate contaminants (Rael *et al.*, 1995).

Permeable barriers are not suitable for contaminants in deep aquifers (>30 m). Barrier media design may also be limited by uncertainties of the hydrogeology of the region. Advantages of these barriers include the following:

1) simple installation,

2) simple recovery and replacement of the material,

3) low operation maintenance,

4) less surface disruption, less labor, and less energy are required than other remedation technologies and

5) comparatively quick installation and containment of contaminants (Rael *et al.*, 1995).

A mixture of powdered activated carbon (PAC) and sand has been shown to be a successful medium for benzene removal in trench-based permeable barrier (Rael *et.al.*, 1995). They looked mostly at physical uptake of different mixtures (3% and 10%) of PAC/sand and nonadsorbant material such as sawsand and zeolite. Based on their investigation, a mixture of PAC/sand was selected as the most successful media.

A recent in situ remediation technology status report (EPA 1995) mentions a number of ongoing pilot tests on permeable barriers. In one pilot study, which was completed in 1993, a permeable barrier containing an iron-based catalyst reduced the concentration of trichloroethene (TCE) by 95% and the tetrachloroethene (PCE) concentration by 91% (EPA 1995).

This study proposes to examine the feasibility of novel permeable barriers that would create a "bio-trench' or "bio-curtain" (Figure 1) to biodegrade chlorophenols in place.

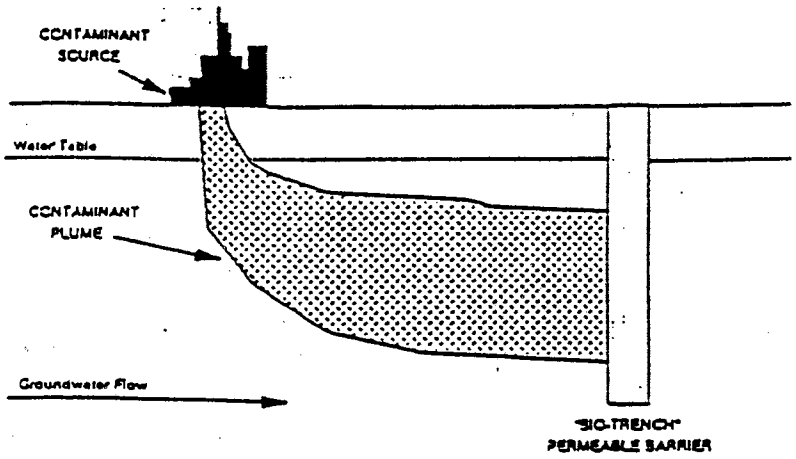

Figure 1.A. Bio-trench Concept (Thomson, *et al.*, 1991)

The 'bio-trench" concept in this study will examine PVA-immobilized cells and 3% GAC-immobilized cells/ 97% silica sand as potential biological permeable barriers.

PVA-immobilization of cells can be defined as an entrapment of microorganisms within a porous polymeric matrix which allows the diffusion of substrate to and products from the entrapped microorganisms (Wu and Wisecarver, 1992).

The work of Bettmann and Rehm (1984) determined that entrapped microorganisms were protected against toxic chemicals compared to free cells. The immobilization technique has also been recognized as a promising method for the biological removal of chlorophenols which are known to be recalcitrant (Sofer *et al.*, 1990).

GAC-immobilizition of cells can be defined as an attachment or adsorption of microorganisms on the surface of activated carbon. The activated carbon operates like a "buffer and depot": it protects the microorganisms and sets low quantities of toxicant for biodegradation (Ehrhart and Rehm, 1985).

Another investigation by Tien (1980) showed that in contrast to a nonadsorbent material such as sand, activated carbon allows storage of substances that are difficult to biodegrade. Such storage provides a longer contact time between the microbial population and the substrates and could promote microbial acclimation and subsequent biodegradation.

The work of Weber (1972) also showed that bacterial growth in activated carbon adsorption columns can significantly increase the apparent capacity of the carbon. The work of Rodman (1971) showed bioregeneration of systems in which spent carbon is regenerated by contact with an active bacterial culture.

For this feasibility study permeable barriers were tested using groundwater contaminated with TCP. This study focused on the use of immobilized cells on GAC (3%) mixed with sand and PVA-immobilized cells as permeable barriers. The use of these two media as permeable barriers to treat chlorophenol contaminated groundwater is entirely new and novel.

Objectives of the Project

This project was undertaken to investigate the potential of using PVA-immobilized cells and GAC-immobilized cells as small scale permeable barriers to clean

up groundwater contaminated with trichlorophenol (TCP). The objectives of this study are as follows:

1) To evaluate and compare two candidate permeable barrier media, (a) mixture of sand and cells immobilized on GAC, and (b) immobilized cells in PVA beads, for their ability to remove trichlorophenol (TCP), under aerobic conditions from contaminated groundwater.
2) Investigate the performance of these two permeable barriers under different operating conditions such as different concentrations (10 mg/L to 40 mg/L), and different flow rates (1 mL/min to 4 mL/min).
3) Evaluate the removal efficiency of these barriers under stressed conditions such as low dissolved oxygen and high TCP concentration (shock load of 500 mg/L).
4) Evaluate the ease of operation and cost of these two permeable barriers under the same operating conditions.

CHAPTER II

LITERATURE REVIEW

Introduction

This chapter presentss a review of the literature on different approaches to remove chlorophenols from groundwater. Secondly, physical and chemical characteristics of chlorophenols are presented to address their persistence, toxicity, mobility and biodegradation. Thirdly, different applications of immobilized cells are described here to emphasize their ability to be applied to groundwater contaminated with chlorophenol(s). Finally, permeable barriers are reviewed as to their applicability for in situ remediation along with a comparison of different mediums used.

Chlorophenols

Chlorophenols are classified as EPA priority pollutants and pose a serious threat to the environment. Nineteen congeners are possible (WHO, 1989). The following are some congeners formed from the direct chlorination of phenol and are found polluting groundwater: 2-monochlorophenol (2-MCP), 2,4-dichlorophenol (DCP), 2,4,6-trichlorophenol (TCP), 2,3,4,6-tetrachlorophenol (TeCP) and pentachlorophenol (PCP) (WHO, 1989). Common names, abbreviations, molecular formulas, and common

synonyms and trade names for MCP, DCP, TCP, TeCP, and PCP are listed in Table A.1, Appendix A.

The compound 2,4,6-trichlorophenol (TCP), the contaminant of interest in this study is widely used and has been determined to be carcinogenic to rats and mice (NCI, 1979).

Uses

Chlorophenols, particularly tetra-, and to a lesser extent, trichlorophenols, have been used as bactericides, algicides, fungicides, and mold inhibitors, and for less specific uses, such as general antiseptics and disinfectants (WHO, 1989). Large quantities of chlorophenols are used in wood preservation. They are also used as intermediates in the production of hericides, dyes and drugs (WHO, 1989).

Environmental levels

The majority (>70%) of the chlorophenols released into the Canadian environment arose from wood-treatment facilities (Jones, 1984). Large spills have been responsible for fish kills in waters contaminated with chlorophenols (Jones, 1981). The remaining third of the environmental releases, which Jones (1984) identifies as primary 2,4-dichlorophenol, is from agriculture sources.

Lindstrom and Nordin (1976) found 115 μg/L of 2,4,6-trichlorophenol in spent bleach liquors from Kraft mill pulp and noted that dichlorophenols were also present.

Environment Canada (1979) analyzed sediments in British Columbia waters

associated with wood-preservation plants. Tetrachlorophenol was present at all 11 sites, and ranged from a trace to 1600 mg/kg dry sediment.

In a water ecosystem, chlorophenols have been found to bioaccumulate in the food chain (Lu *et al.*, 1978). Two hundred micrograms of pentachlorophenol (PCP) per liter is lethal to fish and ten micrograms per liter of PCP inhibits chlorophyll synthesis in algae (Rudling, 1970).

Transport

Commercial preparations of pesticides contain chlorophenols as contaminants. Runoff from soils treated with pesticides finds its way into adjacent water bodies and groundwater. Environmental transport of chlorophenols, particularly in soils, can be affected by adsorption onto particulates. Schellenberg *et al.* (1984) determined that sorption of chlorophenols on natural sediments and aquifer materials was a combined function of the organic content of the potential sorbent and the partition coefficients (known also as soil adsorption). They found that acidic soils bind chlorophenols strongly, while adsorption is minimal under alkaline conditions.

No estimate of the rate of volatilization of chlorophenols in the environment has been published. Diffusion, a process related to volatilization, does not contribute significantly to the long-range transport of substances in either the soil or aquatic habitats (WHO, 1989).

Degradation/Biodegradation

Many, if not all, chlorophenol isomers are degraded to some extent by exposure to ultraviolet radiation. 2,4-dichlorophenol in an aqueous solution was decomposed in a matter of minutes by irradiation from a UV light (Nakagawa and Crosby, 1974).

The major biodegradation pathway involves the degradation of 2,4-dichlorophenol to 4-chlorophenol, which in turn produces 1,2,4-benzenetriol, and finally a mixture of polyquinoid humic acids (Crosby and Tutass, 1966).

Rhodococcus chlorophenolicus degrades PCP through a hydrolytic dechlorination and three reductive dechlorinations, thus producing 1,2,4- trihydroxybenzene (Haggblom *et al* 1988). The proposed aerobic degradation pathway for PCP is as follows:

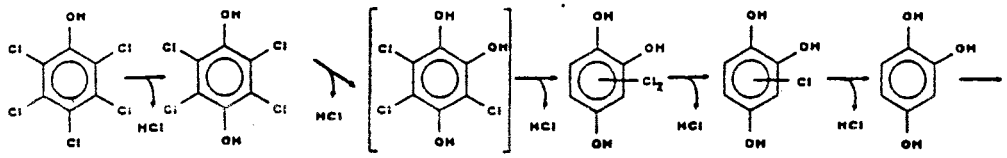

Figure 1.B. Aerobic Degradation Pathway for PCP (Haggblom *et al.* 1988).

In contrast to chlorophenol degradation under aerobic conditions, chlorophenol degradation under anaerobic conditions usually requires a sequence of transformations involving more than one organism. The PCP initially dechlorinated to 2,3,4,5-tetrachlorophenol, 2,3,4-trichlorophenol, 2,4- and 3,4-dichlorophenol. The sequential

degradation of 2,4-dichlorophenol under (methanogenic) conditions proposed by Gibson and Sulfita (1986) is as follows:

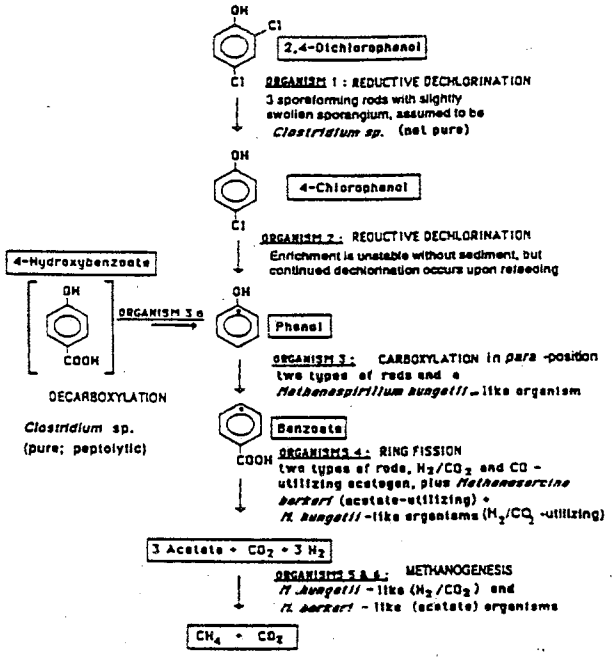

Figure 1.C. Anaerobic Degradation Pathway for DCP(Gibson and Sulfita 1986).

Although chlorophenols are quite toxic to microorganisms in general, they are nonetheless readily metabolized by a large number that occur in soils, natural waters, sediments, and sewage sludges (Tabak *et a.l.*, 1964).

Using an acclimated, activated sludge, Ingols *et al.* (1966) observed complete ring degradation of the following compounds at an initial concentration of 100 mg/L: 2-monochlorophenol (2-MCP) in 3 days, 3-MCP in 2 days, 4-MCP in 3 days, 2,4-dichlorophenol in 5 days, and 2,4,6- trichlorophenol in 3 days. In sediment cores, higher chlorinated phenols are persistent in anaerobic environments, because of the low microbial degradation of chlorophenols under such condition (Gee and Peel, 1974). However, under the right conditions, anaerobic metabolism can be substantial:

acclimated anaerobic sludge from a municipal sewage plant degraded 25 mg monochlorophenols/liter in a few days (Boyd & Shelton, 1984).

Tabak *et al.* (1964) studied 206 isolated microorganisms from a petroleum waste lagoon and observed that 46% of them were able to degrade chlorophenols as a sole source of carbon after acclimation to the particular chlorophenol. In that study up to 95% of the added 3- monochlorophenol (3-MCP) and 4- MCP (initial concentration at 250 and 300 mg/L, respectively) was consumed in 3-6 days, while the same amount of 2,4- dichlorophenol (250 mg/L) and 2,4,6-trichlorophenol (initially 300 mg/L) disappeared in 7-10 days.

Schmidt *et al.* (1983) observed phenol or a mixture of isomeric chlorophenols in synthetic sewage to be completely degraded by a defined mixed culture with *Pseudomonas* sp. strain B13 as a chlorocatechol-dissimilating member of the community.

Methods to Remove Chlorophenols

Makinen *et al.* (1994) studied the bioremediation of simulated groundwater containing 41 mg/L of 2,4,6-trichlorophenol (TCP), 33 mg/L 2,3,4,6-tetrachlorophenol (TeCP) and 19 mg/L pentachlorophenol (PCP) in a laboratory-scale aerobic fluidized-bed reactor. The authors used a hydraulic retention time of 5.0 hours and chlorophenol loading rate of 445 mg L^{-1} d^{-1}. They achieved a 99.7% reduction in chlorophenols and a corresponding 94% of the theoretical mean inorganic chloride release (ICl). They stated that expected oxygen consumption, the increase of ICl and the decrease in pH supported

mineralization. They used the Microtox acute toxicity assay, where luminescent bacteria were exposed to the effluent, to monitor the degradation of chlorophenols. They determined that toxicity increased with the degree of chlorination. To study the effect that an upset or disturbance had on the degradation performance, the aeration process was interrupted several times by withholding oxygen overnight. It took 30-40 days to overcome the upset each time the oxygen was withheld. They monitored the upset by changes in the PCP concentration in the effluent. The PCP concentrations were directly measured by gas chromatography. They concluded that the Microtox assay responded to changes in chlorophenol concentrations as low as 0.1 mg/l for PCP and that it could be used as an easy indicator for system upsets. They observed that treatment of 78-445 mg L^{-1} d^{-1} of a chlorophenols mixture in an aerobic fluidized-bed reactor removed each chlorophenol congener. The removal efficiency of over 99.4% was typical for the mixture of these chlorophenols.

Jarvinen *et al.* (1994) employed aerobic fluidized-bed treatment for chlorophenol contaminated groundwater at temperatures as low as 4°C. The authors spiked the groundwater in the flow reactors with chlorophenol concentrations of 7-11 mg/L of 2,4,6-TCP, 32-36 mg/L of 2,3,4,6-TeCP, and 1.8-2.3 mg/L of PCP. The reactors were in a controlled - temperature incubator in the dark and the influent groundwater was at 4 degree C. They concluded that bioremediation of chlorophenol (CP) contaminated groundwater resulted in over 99.9% CP mineralization with effluent concentrations of 0.003 mg/L or less, even at 4°C. They confirmed CP removal by inorganic chloride release with no chlorinated intermediates, suggesting mineralization. They also concluded that TCP and TeCP were readily biodegradable, but that the chlorophenol

degrading microorganisms needed to be acclimated for at least 1.5 months to get efficient PCP degradation. This system can be operated and maintained at low groundwater temperatures which would eliminate the expense of having to heat the groundwater.

Litchfield *et al.* (1994) used a biotreatment-train approach for in situ bioremediation of a pentachlorophenol (PCP)-contaminated site. Recovered groundwater that had been pumped to the surface was passed through an ultraviolet light/ozone system and then enhanced with nutrients. The treated groundwater was gravity fed to seepage beds. A 90% reduction of PCP was achieved. After 2 years a fluidized bed-activated carbon tower unit replaced the ultraviolet/ozone system. PCP reduction averaged 93.1%. They concluded that not only was the PCP removed by the ultraviolet system and the activated carbon tower, but that additional removal by biodegradation resulted from the simulation of indigenous microorganism by the nutrients.

Jarvinen *et al.* (1994) demonstrated that aerobic fluidized bed treatment was effective at low temperatures (4 °C) for the biodegradation of chlorophenols. The authors incubated continuous-flow reactors with nonacclimated activated sludge. The reactors were filled with nutrient-amended GW. Then they were operated on semi-batch mode at different temperatures (4-20 °C). They reduced 2,4,6-TCP and 2,3,4,6-TeCP by more than 99% and PCP by 83.5% over a period of 22 days. They concluded that an enrichment period of a few months was necessary to achieve over 99% PCP biodegradation using an aerobic fluidized bed treatment. In their study fluidized bed

treatment was effective for on-site bioreclamation of chlorophenol-contaminated groundwater at high flow rates and lower temperatures.

Hendriksen *et al.* (1992) reported 99.8% pentachlorophenol(PCP) removal, of which about 6% was found as less chlorinated - phenols in an anaerobic sludge blanket reactor. The PCP removal rate was 2.2 mg L^{-1} d^{-1}, at a 2 day HRT. In another study (Hendrikson *et al.,* 1991), the authors used an anaerobic fixed-film reactor which removed 98% of PCP when the HRT was 2.9 day or more; about 26% of PCP was found as TeCPs or TCPs.

Immobilization of Cells for Biodegradation of Chlorophenols

Cell immobilization can be defined as any technique that limits the free movement of cells (Tyagi and Vembu, 1990). Cell mobility can be restricted by aggregating the cells or by confining them into, or attaching them to, a solid support. Historically, immobilized cells have been widely used in the wastewater treatment industry, generally through the use of undefined mixed cultures immobilized by natural flocculating tendencies or as films on solid surfaces (Scott, 1987). The use of immobilized or entrapped microorganisms to degrade toxic chemicals in industrial process streams or in the environment is a rapidly evolving technology that shows great promise for the hazardous waste management industry. Following are the state of the art in immobilized cells applications to treat contaminated waters.

Yang *et al.* (1989) investigated different carriers to entrap mixed microbial cells for removal of organics from wastewater. The polymeric materials tested included

cellulose triacetate (mono-carrier), polyacrylamide, K-carrageenan and a combination of cellulose triacetate and calcium alginate (bi-carrier). The mono-carrier was used to determine long term operational performance because it had better mechanical strength. The bi-carrier was more porous and more elastic than the mono-carrier. It was determined that K-carrageenan and calcium alginate were weak in mechanical strength.

Bettmann and Rehm (1984) used immobilized *Pseudomonas* sp. in alginate and polyacrylamide-hydrazide (PAAH) to degrade phenol at initial concentrations of up to 2 g/L in less than two days. A sieve-like container within the fermenter held the immobilized cells which simulated entrapped microorganisms in a packed column. Continuous measurements of pH, optical density (OD), and oxygen concentrations were taken. The phenol degradation activity and the cell growth of the entrapped microbial cells to those of free microbial cells were compared. They found that immobilization acts as a protective cover against phenol toxicity, a conclusion previously stated by Rubelt *et al.* (1982). They showed that free cells degraded phenol up to concentration of 1.5 g/L and the entrapped cells degraded phenol up to a concentration of 3 g/L. The entrapped cells could be exposed to high concentrations of phenol without loss of cell viability.

O'Reilly and Crawford (1989) investigated the biodegradation of PCP by *Flavobacterium* cells immobilized within polyurethane. They compared PCP degradation capacities of free and immobilized cells at various initial PCP concentrations. Results showed that immobilized cells were able to degrade PCP up to a concentration of 200 mg/L, whereas free cells were unable to mineralize PCP during the four-day course of the experiment. The authors conducted experiments in batch,

semicontinuous batch, and continous-culture bioreactors. In the batch reactors with cell-free polyurethane foam, less than 0.1 % of the radioactivity was recovered in the CO_2 traps. In the semicontinuous batch reactors with the immobilized cells, a constant concentration of 10 mg/L PCP was fed and the reactors were run for 150 days. They concluded that immobilization was responsible for the maintenance of PCP degradation activity for up to 150 days. An 86% removal of PCP was achieved during the first 15 day period, but the removal efficiency decreased to 12% by the last 15 day period (days 136-150). The authors did not give an explanation for this decrease in removal efficiency. The activity of the immobilized cells in a continuous-culture reactor was tested in a BioFlow chemostat. A 93% removal of PCP was achieved in the continuous-culture bioreactors within 30 days. The PCP influent concentration varied approximately from 5 mg/L to 15 mg/L. They concluded that twice amount of PCP was degraded per gram of polyurethane in the continuous-culture reactors than in the semi-continuous batch reactors. Polyurethane was determined to be an effective immobilization matrix as indicated by its protection against toxicity.

Sofer *et al.* (1990) studied activated sludge immobilized in calcium alginate gel for biodegradation of chlorophenol. The activated sludge was a mixed microbial population that was acclimated to phenol over a period of 10 days. They were immobilized in sodium alginate and dropped into a calcium chloride solution to form 3 to 3.5 mm diameter beads. The investigator used an air-sparged reactor and a recirculation reactor to measure the physical removal of 2-chlorophenol under identical conditions as immobilized cells, but without biomass. The kinetic constants were determined by varying the flow, chlorophenol concentration, and biomass loading. The

rate constant for the physical removal, by air stripping (K), was evaluated to be 0.085 /hr for the air sparged reactor and 0.057/hr for the recirculation reactor. The temperature activity coefficient (Θ) was 1.16 indicating a high temperature dependence. The rate of biodegradation decreased as the spiked concentration of 2-CP increased. The maximum substrate utilization rate (K_m) decreased from 14.58 to 9.63 mg L^{-1} hr^{-1} corresponding to concentration increases from 50 to 100 mg/L. In the recirculation reactor, as 2-CP concentrations decreased from 110 mg/L to 0 mg/L, pH values correspondingly decreased from 5.5 to 4.2. Both the physical and biological removal data were used for determination of 2-chlorophenol removal. The authors were able to obtain a physically strong bead structure by optimizing the concentrations of sodium alginate and calcium chloride. The immobilized cells in their study showed the ability to degrade chlorophenol in various concentrations (up to 100 ppm). They used the Monod expression and developed a nonlinear regression model for substrate utilization. The model was modified to accommodate the physical removal of substrate by stripping.

Hashimoto and Furukawa (1987) developed a new method for immobilization of activated sludge known as the polyvinyl alcohol (PVA)-boric acid method. The preparation of this method involved mixing one portion of concentrated activated sludge (mixed microbial cell population) with one portion of an aqueous PVA solution. This mixture was dropped into a gently stirred saturated boric acid solution to form spherical beads. The beads were cured in the solution for 15-24 hours and then washed with tap water. The beads produced were used to determine removal rates of total organic carbon (TOC) and total nitrogen (T-N) from a synthetic wastewater. Weight changes of the PVA beads were recorded and used as the indicator of microorganism growth inside the

beads. The beads increased in weight as the loading increased, indicating growth of the microorganisms in the beads. The eventual TOC removal was 93% and T-N removal was 30-40%. The NO_3-N was denitrified in the anaerobic portion of the immobilized activated sludge beads (aerated denitrification). In conclusion they determined that the PVA-boric acid method was inexpensive compared to other methods and that it was possible to operate an immobilized cell system at 2-3 times the loading rate of conventional systems. They mentioned that since activated sludge cells become surrounded by extracellular polymer, microbial activity was not reduced during the immobilization process where the pH was 4.0 for 24 hours.

Wu and Wisecarver (1992) prepared PVA beads using a modification of the PVA-boric acid method developed by Hashimoto and Furukawa (1987), but added a small amount of sodium alginate to prevent or minimize the tendency for the beads to agglomerate. They demonstrated the viability of *Pseudomonas* immobilized cells by utilizing them in a fluidized bed bioreactor for a period of two weeks. The bioreactor achieved 100% removal when influent phenol concentrations ranging from 250 to 1300 mg/L were continuously fed through the bioreactor. They concluded that the removal of phenol was due almost entirely to biodegradation and physical processes accounted for less than 0.1% of the total phenol removed. The beads were able to withstand high shears with no sign of breakage when an 8-L fluidized bed column was sparged at an air flow rate of 1.4 L/min. The authors suggested that this technique might be applicable to a wide variety of other microorganisms.

Hanaki *et al.* (1994) investigated the application of acetate-utilizing methanogens immobilized by the PVA-boric acid method in an anaerobic treatment process. Acetate-

utilizing methanogens are known to be sensitive to influent quality, wastewater constituents, toxic materials, and pH. In order to determine the effect of toxic substances to PVA immobilized cells, various concentrations of toxic substances were added to immobilized cells under anaerobic condition. The toxic substances were phenol, sodium oleate, nickel, sulfide, propionate, and ammonia. In this study, the beads were prepared at different pH's by adjusting the pH with Na_2CO_3. They concluded that the beads made at pH 4.0 were superior to the beads made at pH 6.0 in terms of strength and durability. At pH of 4, the initial lag phase prior to the active methane production was prolonged. The lag phase shortened as pH increased. The toxic effects of phenol, oleic acid and nickel, as compared to free cells, were reduced and the authors concluded it was due to the adsorption of these substances by the bead material. The authors speculated that a pH gradient occurs within the beads, protecting the bacteria from acidic substances such as sulfide and propionic acid. The inhibitory effect of ammonia, as compared to free cells, was not reduced by either adsorption or formation of a pH gradient.

Kindzierski *et al.* (1992) investigated the use of activated carbon and two other synthetic ion-exchange resins as support materials for an anaerobic phenol-degrading consortia. The initial attachment of microorganisms on different media was studied in terms of availability of accessible pore volume and associated surface area for the colonization. The initial phenol concentration was 525 mg/L. The rapid adsorption of phenol on activated carbon without bacteria occured over the first 33 minutes; aqueous phenol concentrations decreased to 73 mg/L during this time. The adsorption of phenol on activated carbon with bacteria was 3.9 times smaller than on activated carbon without bacteria. The biological uptake of phenol over the 2 hour period was not substantial

(estimated at 5 mg L^{-1} h^{-1} from experience with similar cultures). They demonstrated that activated carbon exhibited favorable qualities as a biological support for the rapid development of attached biomass. They observed a substantial decrease in the rate of phenol adsorption by activated carbon due to the colonization of the bacteria.

Ehrhardt and Rehm.(1985) studied the adsorption of phenol as well as *Pseudomonas* sp. and *Candida* sp. on activated carbon, and the phenol degradation by these immobilized microorganisms was compared to that of free microorganisms. They observed that one gram of activated carbon adsorbed 4x10E9 *Pseudomonas* cells and 3x10E8 *Candida* cells in about 10 hours. Results of the degradation studies showed that free cells did not tolerate more than 1.5 g/L phenol, while the immobilized microorganisms survived at temporary 2.0 hour of high phenol concentrations up to 15 g/L, and they ultimately degraded about 90% of the adsorbed phenol.

Ehrhardt and Rehm 1989 studied phenol degradation in a semi-continuous and continuous reactor by *Pseudomonas* putida P8 adsorbed on activated carbon. They stated that phenol introduced into the reactor was initially removed from the media by a combination of degradation and adsorption. As the biomass in the reactor increased, adsorption decreased and the degradation rate increased. They were able to show that immobilized cells on activated carbon can tolerate high concentration of phenol up to 15 g/L. They concluded that protection in the activated carbon system was afforded by adsorption of phenol onto the immobilization substrate, which reduced the aqueous concentration to which the organisms were exposed. As the phenol in solution was degraded, desorption occurred, allowing the organisms to metabolize the substrate released from the carbon.

Permeable Barriers

Rael *et al.* (1995) evaluated candidate permeable barrier media designed to remove benzene in-situ from ground water. Effectiveness of several common material including coal, powdered-activated carbon (PAC), peat, and zeolite were evaluated in a series of batch and column studies with an initial benzene concentration of 50 mg/L. Silica sand was used as an inert matrix and was mixed with PAC to produce either 3 % (by weight) or 10 % PAC/sand mixtures. Based on their results, a mixture of PAC and sand was considered the most successful candidate. At a flow rate of 54 mL/min , breakthrough time occurred at 37 days in a column (1.52 m by 0.16 m interior diameter) with a 0.91 m reaction bed of 3 % PAC/sand mixture. The authors stated when the barrier reached its treatment capacity it can be replaced with fresh media. The barrier medium allowed the flow of contaminated water but adsorbed the contaminant preventing further migration. This technology is limited to the depth accessible by trenching equipment and therefore would be applicable in shallow aquifer systems of less than 30 m.

EPA (1995) prepared a document to describe recent field demonstrations, applications, and research on technologies that treat soil and ground water in place. In this report treatment walls were considered a cost-effective in situ water treatment alternative. Among all the field demonstrations only two had been completed by 1995 and they seem to hold the potential for great successes. In one study described in EPA (1995) document researchers tested a proprietary solid peroxide formulation (an oxygen-releasing compound) to determine whether it could provide dissolved oxygen in a

controlled steady manner to enhance biodegradation of BTEX-contaminated water. The results indicated that the oxygen releasing compound can provide oxygen to enhance biodegradation of BTEX-compounds (O'Hannesin 1994).

In another study O'Hannesin (1993) completed pilot tests using a permeable reaction wall containing an iron-based catalyst to treat contaminated groundwater with a maximum concentration of 250 mg/L TCE and 43 mg/L of PCE. In this study a rectangular cell was constructed on the surface and driven to depth of 32 feet using sealable joint sheet piling. The cell was sealed and dewatered and the native sand was replaced by the reactive material consisting of 22% by weight granular iron (zero valance iron) and 78% by weight course sand. Using coarser sand ensured that the wall would be more permeable the then surrounding sand. The sheet piling was completely removed after installing the reactive material. The wall dimensions were 18 feet long, 5.2 feet thick, and 7.2 feet deep and it was positioned 3.3 feet below the water table. The reaction wall reduced the TCE concentration by 95% and the PCE concentration by 91%. Increased chloride concentrations downstream of the wall were consistent with the quantity of TCE and PCE that had been degraded. No vinyl chloride production was detected in the samples. Concentration distributions through the wall were determined on 13 occasions over 474 days, during which there was no decline in the effectiveness of the barrier.

O'Hannesin (1995) also field tested an in situ semipassive permeable wall into which nutrient solutions that enhance biodegradation of organics are introduced. The wall was installed across the path of a plume contaminated with trichloroethylene (TCE) and carbon tetrachloride in which nutrients were introduced to enhance biodegradation.

A stable anaerobic microbial population was produced some distance downgradient from the wall. There was no transformation of TCE. The carbon tetrachloride was removed completely from groundwater by anaerobic microorganisms.

Morrison and Spangler (1993) explored the concept of chemical barriers as a passive in situ water-treatment system. The authors studied precipitation barriers (hydrated lime) and sorption barriers (ferric oxyhydroxide) for removing uranium from ground water. Chemicals used in the barrier were placed in the subsurface either by lining a disposal site, by trench and fill, or by injection. Dissolved contaminants became part of the immobile solids of the aquifer, by either precipitation or adsorption, as the contaminated groundwater passed through the chemical barrier. In a series of column studies using ferric oxyhydroxide at different pHs (5,6,7), they concluded that pH of 7 was the most effective pH in removing uranium. One major problem in precipitation barriers was reduction of hydraulic conductivity which is a dominated consideration for this type of system. They concluded that accurate groundwater characterization is more critical to determining the performance of sorption barriers than precipitation barriers, because each contaminant has its own pH for optimal adsorption.

Morrison (1995) later conducted laboratory batch and column studies on chemical reactive barriers for the purpose of evaluating the applicability for in situ remediation of uranium tailing. He examined sorption capacities of various contaminants under aquifer flow conditions on barriers containing low cost materials. It was determined that ferric oxyhydroxide can remove uranium and molybdenum up to 99% and 96%, respectively. Ferric oxyhydroxide can immobilize metals and uranium and that it is (1) inexpensive, (2) injectable, and (3) did not reduce the aquifer's permeability.

This study was described as an abiotic system and there was no investigation of the possibility of biological removal.

Thomson *et al.* (1991) examined the concept of designing permeable barriers to remove groundwater contaminants in situ. Permeable barriers constructed by trenching had two advantages: 1) accessibility of the medium placement and 2) ease of recovery of medium by re-excavation. Permeable barriers were classified as either passive or active. An active barrier required continuous operation and maintenance while a passive barrier required no operation or maintenance once the medium is in place. An example of active barrier, in situ air stripper was investigated and compared with conventional packed tower air stripping. The authors determined that 1) the trench-based stripping needed high pressure air compressors, but no water pumping equipment was needed which made the operating cost less and 2) biostimulation did occur from the oxygen, resulting in a combined air stripping and biodegradation of volatile organic contaminants. The authors described a geochemical barrier which consisted of limestone and peat for immobilizing metals from uranium milling tailings as an example of passive permeable barrier. In passive barrier, upon exhaustion the barrier was re-excavated and disposed of as a hazardous waste. It was concluded that the permeable barrier had several advantages which include reduced capital, operations and maintenance costs, improved reliability, and less volume of treatment by-products. The barrier treatment can also be operated at much lower process loading rates than conventional surface processes due to low groundwater velocities.

Thompson (1996) investigated the feasibility of using PVA-immobilized cells as a permeable barrier media for in situ biodremediation of TCP contaminated groundwater.

The author prepared plain PVA beads in accordance with the PVA -boric acid method (Hashimoto and Furkawa,1987; Wu and Wisecarver, 1992) using various molecular weights(MW) of PVA to obtain a porous, rubber-like, elastic bead for the purpose of immobilizing cells and using it as a permeable barrier medium. A bed of beads was characterized with its density, porosity, permeability, and compressibility or deformation. Batch studies were conducted to obtain necessary data to determine the rate of TCP diffusion into the PVA beads, adsorption properties of the beads, and the substrate-use of the mixed bioculture as free cells and as immobilized cells. The author conducted initial column study with a bed of PVA beads located between layers of aquifer sand to simulate a "bio-trench" and monitor the biodegradation of TCP. The author also conducted another column study with a constant concentration of 10.0 mg/L of TCP at a constant flowrate into two columns. The two columns varied in size to provide different hydraulic retention times (HRT) to show the effect on biodegradation of TCP.

The author demonstrated that PVA-immobilized cells would be a successful permeable barrier media to remove TCP at 10.0 mg/L concentration from groundwater during 15 and 45 days of column studies. The major findings of this study were :

1. The concept of a bio-trench using PVA-immobilized cells as a permeable media to remove TCP from groundwater appeared to be feasible. During 45 days of column operation, the column of PVA-immobilized cells situated between layers of sand

reached 100% removal efficiency of TCP within 14 days of operation. The column continued with the same efficiency for the remainder of the 45 day experiment. After 45 days of operation, the beads appeared to be resilient, firm, and structurally sound.

2. Dehalogenation of TCP by PVA-immobilized cells in the batch and column studies was suggested by the evidence of chloride increases and pH decreases. Dehalogenation of TCP was further supported by GC-MS analysis.

3. The compressibility study showed the bed of PVA beads had a 48% deformation from the overburden pressure at a depth of 40 feet.

4. A bed of PVA beads was characterized as to its porosity, permeability, and compressibility. The PVA beads as a permeable barrier would not impede the flow of groundwater.

The summary of the experiments and the results (Thompson 1996) are presented in Tables 1 and 2, respectively.

TABLE 1. Summary of experiments*

EXPERIMENTS	EXPECTED INFORMATION
Porosity test	density, specific gravity, porosity percent.
Falling heads permeameter test	permeablility coefficient.
Compression test	compressibility index , deformation percent.
Diffusion study	diffusion coefficient.
Adsorption study	adsorption capacity, adsorption intensity.
Kinetic study	substrate utilization rate, growth rate.
Tracer study	reactor dispersion number, actual flow rate.
Column study	biodegradation of TCP by the continuous flow reactor, effect different hydraulic retention times on biodegradation of TCP.

* Thompson (1996).

TABLE 2. Summary of results obtained on PVA-immobilized cells*

EXPERIMENTS	RESULTS
Porosity test	specific gravity =1.008 ,density = 0.9869 g/cm^3, porosity = 25%
Permeability test	permeability coefficient = 0.1425 cm/s
Compression test	deformation % of beads = 48 %, compressibility index = 4.08 X 10^{-3} m^2/kN
Diffusion test	diffusion coefficient = 3.1 X 10^{-6} cm^2/s
Adsorption study	adsorption capacity = 5.01 X 10^{-15} L/g , adsorption intensity = 11.1
Kinetic study	1) free cells substrate use rate = 1.11 mg L^{-1} hr^{-1}, growth rate = 3.9 mg VSS L^{-1} hr^{-1}, ICl released = 8.0 mg/L. 2) immobilized cells substrate use rate= 0.14, 0.47, and 2.0 mg $L^{-1}hr^{-1}$, ICl released = 7.0 mg/L
Column studies	1) initial column study (10 cm of beads), 100% removal, ICl increase 7.0 mg/L, pH decreased (8.3 to 7.8), DO decreased (8.6 mg/L to 2.5 mg/L). 2) final column study: column #1 (10.0 cm of beads) TCP removal 100% within 10.0 days, ICl increased 6 mg/L, pH decreased (8.3 to 7.5), DO decreased (8.6 mg/L to 6.6 mg/L) 3) final column study: column #2 (20.0 cm of beads) TCP removal 100% within 8 days, ICl increased 6 mg/L, pH decreased (8.3 to 7.5), DO decreased (8.6 mg/L to 7.5 mg/L).

*Thompson (1996).

Several investigations have been done on the biodegradation of chlorophenols or phenol using immobilized cells. The application of immobilized cells as potential biological permeable-barrier media to remove chlorophenols from in-situ groundwater has been investigated by Thompson (1996). The work done by Thompson (1996) focused on PVA-immobilized cells as a permeable barrier for removal of TCP from groundwater during 45 days and 15 days continuous operations. The following are the issues, which need to be addressed:

1) For the chlorophenols compound, most research has been conducted using aerobic fluidized-bed, fixed-film reactor, and an anaerobic sludge blanket reactor. The use of 3%GAC immobilized cells mixed with silica sand as a permeable barrier media for biodegradation of TCP contaminated groundwater has never been investigated.

2) For the chlorophenols compound, most research has been conducted for on-site bioremediation of chlorophenol-contaminated groundwater. In order to investigate biological permeable barrier as an in-situ remediation technology, it is necessary to design biological permeable barrier media in the laboratory scale to assess their performance.

3) There has been some research on permeable barrier concerning the use of metal-based catalysts for degrading volatile organics, chelators for immobilizing metals, nutrients and oxygen for microorganisms to enhance bioremediation. The use of PVA-immobilized cells beads as a biological permeable barrier media has never been tested under variety of flow rates and concentrations. The 3%GAC-immobilized cells mixed with sand as another candidate biological permeable barrier media has never been investigated.

4) Although most barriers are designed to operate for years with minimal maintenance, the stability of aging barriers has not been established. It is necessary to investigate the stability and the removal efficiency of any permeable barrier under different operating conditions for extended period of time.

The above existing issues need to be addressed for development of biological permeable barrier as a cost-effective technology to treat chlorophenols in groundwater.

The aims of this study were to evaluate permeable barrier technology as an in situ groundwater remediation tool using PVA-immobilized cells and 3%GAC-immobilized cells mixed with silica sand as permeable barrier media by:

1) studying the physical characteristics of 3% GAC-immobilized cells/sand mixture,

2) studying the ability of 3% GAC-immobilized/sand mixture to biodegrade TCP under aerobic condition,

3) providing the data of the removal efficiencies of PVA-immobilized cells and 3%GAC-immobilized cells /sand mixture under different operating conditions (i.e. flow rate, TCP concentration),

4) studying the stability and the performance of PVA-immobilized cells and 3% GAC-immobilized cells /sand mixture over extended period of time,

5) studying the tolerance and removal efficiencies of PVA-immobilized cells and 3%GAC immobilized cells/sand mixture under toxic shock load and deficiency of dissolved oxygen, and

6) comparing PVA-immobilized cells with 3%GAC-immobilized cells /sand mixture on the bases of the ease of operation, the abilities to biodegrade TCP, the stability and the tolerance under the same operating conditions.

CHAPTER III

MATERIALS AND METHODS

The main objectives of this study are to demonstrate biodegradation of 2,4,6 trichlorophenol (TCP) using polyvinyl alcohol (PVA)-immobilized cells and granular activated carbon (GAC)-immobilized cells functioning as two new permeable barrier media. This study also focused on evaluating these barriers under a variety of conditions such as different flow rates and different contaminant influent concentrations. These barriers were compared on the basis of removal efficiency, relative ease of operation , and capital cost.

Experimental Approach

This study focused on evaluating PVA-immobilized cells and GAC-immobilized cells as two new permeable barrier media for in situ biodegradation of TCP contaminated groundwater under aerobic conditions. Beads were prepared in accordance with the PVA-boric acid method (Hashimoto and Furukawa, 1987: Wu and Wisecarver, 1992) using various molecular weights (MW) of PVA to obtain porous, rubber-like, elastic beads for the purpose of immobilizing cells and using then as a permeable barrier medium. A bed of beads was characterized with its density, porosity, permeability, and compressibility or deformation.

Batch studies were conducted to obtain necessary data to determine the rate of TCP diffusion into the PVA beads, adsorption properties of the beads, and substrate-use rate of the mixed bioculture as free cells and immobilized cells. The initial column studies on PVA-immobilized cells were conducted to evaluate aerobic biodegradation of TCP (10 mg/L) and flow rate of 1 mL/min. The above experiments were conducted by Thompson (1996) in conjuction with the following experiments on 3% GAC-immobilized cells /sand mixture and PVA-immobilized cells.

A bed of 3% GAC and silica sand was also characterized utilizing density, porosity, and permeability. GAC-immobilized cells were prepared in accordance with the method used by Ehrhardt and Rehm (1985). Batch studies were conducted to obtain equilibrium time, adsorption capacity of GAC, impact of immobilization time on cell retention, and kinetics of TCP adsorption on GAC with and without immobilized cells.

Column studies were conducted to evaluate aerobic biodegradation of TCP (theoretical value of 0.89 mg of O_2 uptake/mg TCP) under various operating conditions such as different flow rates (i.e. 1 to 4 ml/min.) and different TCP concentrations (i.e. 10 and 40 mg/L). The barriers also were compared under shock load conditions, oxygen deficient conditions and changes in C:N:P ratio.

Chemicals

Polyvinyl alcohol was obtained from Scientific Polymer Products, Inc., Ontario, NY. Granular Activated Carbon (GAC) of 20-40 US. Standard Sieve Size was obtained from Atochem Inc., Tulsa, OK. 2,4,6-trichlorophenol (TCP) was obtained from Fluka

Chemical Corp., Ronkonkoma, NY. Ethyl acetate and methanol were obtained from Fisher Scientific, Fair Lawn, NJ,. Potassium bromide was obtained from J.T Baker Chemical Co., Phillipsburg, NJ. All chemicals used in this study were reagent grade.

Groundwater Analysis

Groundwater used in this was obtained from a water well located in the NE/4 NE/4 NE/4 Section of 9-T16N-R2E, Lincoln County, Oklahoma. The groundwater was initially analyzed by the State of Oklahoma, Department of Environmental Quality, Water Laboratory, and the total organic carbon was analyzed by The Stover Group, Analytical/Toxicology Laboratories, Stillwater, Oklahoma. Standard EPA analytical methods were used in accordance with federal regulations (40 CFR 136). The groundwater analysis is given in Table A.2, Appendix A.

Silica Sand

Silica sand of 20-40 mesh size was obtained from U.S.Silica, Ottawa.IL. The sand was washed and dried completely before use.

Microorganisms

Activated sludge was obtained from the Georgia-Pacific Leaf River Pulp Mill, New Augusta, Mississippi. The activated sludge was obtained from the recirculation

line where there is a high cell concentration. The mill operation included a bleaching process which would unintentionally produce some chlorophenols. The microorganisms from this mill were assumed to have had some exposure to chlorophenols which should allow quicker acclimation for the purpose of this project. The microorganisms were further acclimated by feeding them TCP (10 mg/L) as their sole carbon source with continuous aeration and additional nutrients (*phosphate buffer solution, magnesium sulfate solution, calcium chloride solution, ferric chloride solution.*). One mL of each of the following nutrient solutions was added to each liter of (13 liter volume) activated sludge every day which provided the microorganisms the weight ratio of C:N:P of 100:18:188 (Standard Methods, 1975: Method 507):

- *Phosphate buffer solution.* 8.5 g KH_2PO_4, 21.75 g K_2HPO_4, 33.4 g $Na_2HPO_4.7H_2O$, and 1.7 g NH_4Cl were dissolved in distilled water and then diluted to 1 liter.
- *Magnesium sulfate solution.* 22.5 g $MgSO_4.7H_2O$ were dissolved in distilled water and then diluted to 1 liter.
- *Calcium chloride solution.* 27.5 g $CaCl_2$ were dissolved in distilled water and then diluted to 1 liter.
- *Ferric chloride solution.* 0.25 g $FeCl_3$. $6H_2O$ were dissolved in distilled water and then diluted to 1 liter.

The weight ratio of C:N:P of 100:18:188 was kept unchanged for free cells and column studies 1-3. Since a standard ratio of the weights of carbon (C), nitrogen (N), and phosphorous (P) for C:N:P was 100:10:3 (Beltrame *et al.*, 1984). The amounts of carbon, nitrogen and phosphorous provided to the microorganisms was changed from

C:N:P 100:18:188 to 100:10:3 from column study No.4 - 9 to avoid unnecessary addition of nutrient.

Preparation of PVA blank and PVA immobilized Cells Beads

The polyvinyl alcohol (PVA) boric acid method developed by Hashimoto and Furukawa (1987) and modified by Wu and Wisecarver (1992) was used to prepare "blank" beads. These beads were used in the adsorption and diffusion studies. The details of PVA- blank and immobilized cells preparation are described by Thomson (1996).

Characterization of a Packed Bed of PVA Beads

A bed of beads was characterized with respect to its density, porosity, permeability, and compressibility or deformation by Thompson (1996).

Compressibility Study on PVA -Plain and PVA -Immobilized Cells Beads:

An oedometer (or consolidation test apparatus) was used to determine the compression behavior of a packed bed of PVA beads. The compressibility test was done on plain PVA beads by Thompson at the beginning of this project. The details of this test were reported by Thompson (1996).

PVA Beads Batch and Column Studies

Batch studies were also conducted to obtain necessary data to determine the rate of TCP diffusion into the plain PVA beads, adsorption properties of the beads, and the substrate-use rate of the mixed bioculture as free cells and as immobilized cells. Column studies were conducted using two columns of different depths of PVA immobilized cells to test the effect of different hydraulic retention times (HRT) on biodegradation rates.

The column study was conducted as aerobic, continuous flow packed-bed reactors. The feed solution containing TCP (10 mg/L) and nutrients was pumped upflow into the columns (10.0 cm and 20.0 cm) at 1.0 mL/min. Samples of the influent and effluent were taken for dissolved oxygen (DO), TCP, chloride (ICl), and pH determinations. The detail of the experiments on PVA beads can be found in the work by Thompson (1996).

Characterization of Packed Bed of 3% GAC/Sand

Specific Gravity

The density (mass/unit volume) of a bed of (3%)GAC/silica sand and water were determined at 22° C by weighing 100 mL of (3%) GAC/sand and 100mL of water. The expression to determine the specific gravity of the (3%) GAC/sand was given as (Smith, 1979):

$$\text{Specific gravity} = \frac{\text{Density of Substance}}{\text{Density of Water}} \qquad (1)$$

Porosity

Both silica sand and GAC were sieved to 40 U.S Standard Sieve Size particles. The porosity of a bed of 3% GAC/silica sand was determined by packing 160 g of 3% GAC/sand mixture into a 100 mL volume in a graduated cylinder. A volume of 100 mLs of water was added to the graduated cylinder. The water and GAC/sand were displaced to 170 mLs. The expression to determined the percent porosity of the bed of GAC/sand was given as (Smith, 1979):

$$\% \text{ Porosity} = \frac{(V_1 + V_2) - V_3}{V_1}(100) = \frac{\text{Total Void Volume}}{\text{Total Volume}}(100) \qquad (2)$$

Where

V_1 = volume of GAC/sand

V_2 = volume of water

V_3 = volume of displacement

Permeability Study

A falling head permeameter test was used to determine the permeability coefficient (K) of a packed bed of GAC/sand (Smith, 1979). GAC/sand was packed into the bottom portion of a column at a density of 1.6 g/cm^3 to simulate actual densities found in aauvial aquifers (Mandel and Shiftan, 1981) for the length of sample (L). Two (2) copper screens were placed above and below the layer of GAC/sand. A shallow layer of washed gravel was placed on top of the GAC/sand to hold the grains in place. A graduated cylinder was placed below the column to catch the flow of water. Water was

added to the column to a certain height (h_1) and after a measured time (t), the height to which the water had fallen (h_2) was determined. The permeability coefficient (K) was determined by the expression (Smith, 1979):

$$K = 2.3\frac{L}{t}\text{Log}_{10}\frac{h_1}{h_2} \quad (3)$$

Where

K= permeability coefficient

L= length of sample

t= time

h_1= height of water

h_2= height to which water level has fallen

Batch Studies

Batch studies were conducted to obtain necessary data to determine equilibrium time for carbon adsorption, the adsorption capacity of GAC and silica sand, the kinetics of substrate use rate by the mixed bioculture as free cells, impact of immobilization time on cell retention, and kinetics of TCP adsorption on GAC with or without immobilized cells.

Equilibrium Study

This study was conducted by using 2.5 grams of GAC and 500 mL of a 20 mg/L TCP solution. The solution was covered to prevent photolytic degradation and shaken on the shaker table for period of 24 hours. Samples were taken at different time intervals (0.0, 3.0, 5.0, 8.0, 10.0, 20.0, 24.0 hours) and analyzed by gas chromatograph (GC) to determine the liquid phase TCP concentration.

Isotherm Study

Granular activated carbon (GAC) was washed with distilled water and dried at 103 °C before use. The adsorption study was conducted by adding different masses of GAC to five Erlenmeyer flasks of 250 mL volume. Two hundered (200) mL of solution, with a TCP concentration of 20.0 mg/L, was poured into each flask. Flasks were covered to prevent photolytic degradation and were shaken on the shaker table for 24 hours. Twenty five (25) mL samples were taken from each flask at 0.0 hours and at 24 hours to measure the TCP concentration. The data from this experiment were used to estimate adsorption potential using an isotherm model.

An adsorption study was conducted on the silica sand used in the GAC columns. A Erlenmeyer flask (300 mL volume) containing 100 g sand and 200 mLs of a 20.0 mg/L TCP solution was shaken on the shaker table for 24 hours. Two (2.0) mL samples were taken at 0.0, 3.0, 7.0, 12.0, and 24.0 hours to measure the TCP concentration.

An adsorption study was also conducted on the 200-sieve mesh copper screen used in column studies. The copper screen served to retain the media and was placed at the top and bottom of each column. A 5.0 cm diameter circle of copper screen was

placed in a Erlenmeyer flask (500 mL volume) of 200 mLs of a 10.0 mg/L TCP solution and was shaken on the shaker table for 24 hours. Samples volume of 1 mL were taken at 0.0, 3.0, 7.0, 12.0, and 24.0 hours to measure TCP concentrations.

Immobilization Time

In order to quantify the extent of cell attachment to the carbon , 10 grams of GAC and 10 grams of centrifuged biomass were placed with 500 mLs distilled water in 800 mLs Erlenmeyer flask. An International Equipment Co. Clinical Centrifuge was used to centrifuge the sludge at 4000 rpm for 10.0 minutes. The flask were shaken vigorously on the shaker table for 24.0 hours. Samples were taken at different time intervals and processed (diluted) for membrane plate count. The number of cells attached on carbon were calculated by the difference of initial cell concentration and concentration of cells in the solution over time. This experiment defined the number of cells attached on the GAC and the required time to reach certain cell concentrations.

Kinetic Study

A batch of culture of activated sludge obtained from Georgia-Pacific had been acclimated for 60 days by feeding it 10 mg/L of TCP and nutrients each day. The solids in this batch were controlled by wasting 1.0 L every day and adding 1 liter with tap water. The culture was continuously aerated. Prior to begining the kinetic study on the free cells an initial volatile suspended solids (VSS) analysis was conducted according to Method 208E, *Standard Methods* (1975). As a result of a VSS analysis of 4376 mg/L VSS, it was determined that the activated sludge needed to be diluted for the kinetic

study. A 1.0 L volume of activated sludge was poured into an 8.0 L bottle and diluted by adding 2.0 L of tap water. Three (3.0)L of the diluted batch culture was aerated continuously and fed 10.0 mg/L TCP and nutrients. A 100.0 mL sample volume was taken at 0.0 hour, 1.0, 3.5, 5.0, 12.0, and 24.0 hours. Each 100 mL sample was filtered under vacuum using a Whatman glass fiber (GF/A) filter. A 25.0 mL volume of the filtered sample was used for TCP concentration for GC analysis. A 50.0 mL volume of the filtered sample was used for ICl analysis. The remaing filtered sample of 25 mL was used for VSS analysis. The analytical methods used for TCP concentration, ICl, and VSS are further described later in this chapter under "Analytical Methods".

Kinetics of TCP Adsorption on GAC

In this study TCP adsorption on activated carbon with or without microorganisms was evaluated. A portion of activated sludge from the continuously maintained batch culture was centrifuged to obtain 10.0 g (wet weight) of biomass. The wet centrifuged biomass was placed along with 10.0 g of GAC in 200 mL of distilled water and shaken on the shaker table for 24.0 hours. This allowed cell to be immobilized on the GAC. Settled GAC (10 gm), which contained immobilized bacteria, was placed in a flask containing 500 mL of a 500 mg/L solution TCP. Another 10.0 g of GAC (plain), without cells, was placed in another flask which also contained a 500 mg/L TCP solution. The flasked were stirred gently over 120 minutes. Samples were taken at different times and analyzed using the GC for TCP concentration during 120 minutes (Ehrhardt 1985).

Preparation of GAC-Immobilized Cells for Column Studies

Granular activated carbon (GAC) were washed with distilled water several times and dried completely in 103°C oven before use. A portion of activated sludge, from the continuously maintained batch culture, was centrifuged at 4000 rpm for 10.0 minutes to obtain the desired amount of biomass (wet weight). The amount of biomass used for immobilization on both permeable barriers (GAC and PVA beads) was 43.7 grams for short columns and 86.0 grams for the long columns. The amount of GAC for short and long column were 21.0 and 10.5 grams, respectively, for the 3% mixture of GAC/sand. The biomass and GAC were then agitated vigorously in 100 mLs distilled water for 24 hours. The GAC that settled by gravity was mixed with sand and used in column studies (3% GAC /sand mixture).

Column Studies on PVA and GAC Immobilized Cells

The experiments are carried out in total of four acrylic columns, and were set up as aerobic, continuous flow packed-bed reactors. Columns #1 and #2 consisted of 10 and 20 cm beds of PVA beads (3-5 mm), respectively prepared by Thompson (1996). Columns #3 and #4 consisted of 10 and 20 cm beds of 3% GAC immobilized cells and 97% clean silica sand. These columns have an inside diameter of 5.0 cm. A 5.0 cm diameter 200-sieve mesh copper screen was placed at the top and bottom of each of the columns. The TCP-spiked groundwater was prepared in 25.0 liter bottles and covered to prevent photolytic degradation (Figure 2.).

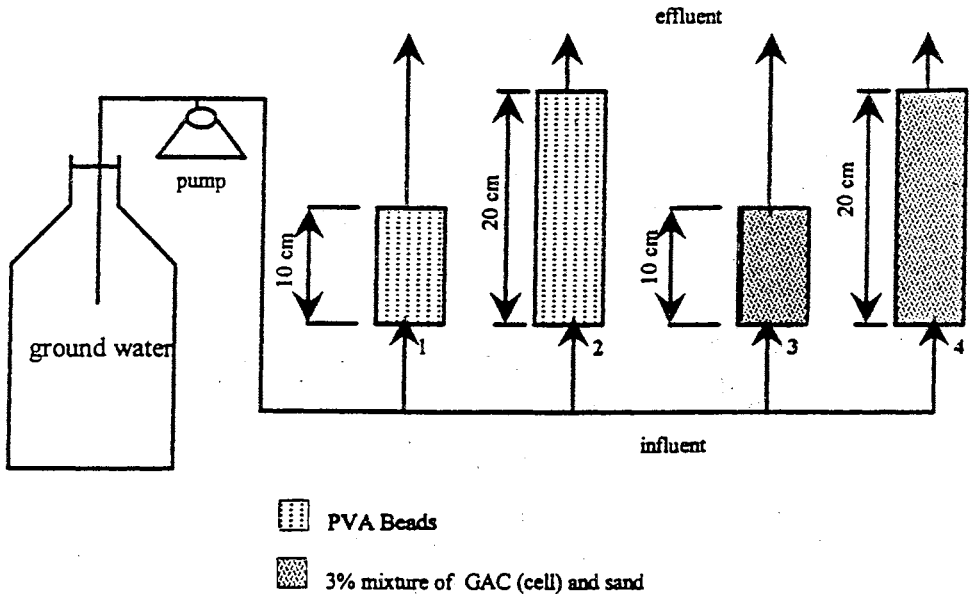

Figure 2. Schematic Diagram of Column Study

The following nutrient solutions were added to the TCP- spiked groundwater: phosphate buffer solution; magnesium sulfate solution; calcium chloride solution; and ferric chloride solution. A peristaltic pump (Cole-Parmer 7553-30) with four heads (Model 7013) and tygon tubing was used to pump the groundwater into the base of the columns (upflow mode). The experimental conditions conducted during column studies are presented in Table 3.

Table 3. Experimental Conditions for Column Studies (1-8)

Column Experiments	Experiment No. 1	Experiment No. 2	Experiment No. 3	Experiment No. 4	Experiment No. 5	Experiment No. 6	Experiment No. 7	Experiment No. 8
Influent Concentration (mg/L)	10.0	20.0	20.0	20.0	30.0	20.0	20.0	40.0
Influent Flow Rate (mL/min.)	1.0	1.0	1.0	1.0	1.0	2.0	4.0	4.0
Residence Time(min)	Columns #1=49 #2=98 #3=59 #4=118	Columns #1=49 #2=98 #3=59 #4=118	Columns #1=49 #2=98 #3=59 #4=118	Columns #1=49 #2=98 #3=59 #4=118	Columns #1=49 #2=98 #3=59 #4=118	Columns #1=24.5 #2=49 #3=29.5 #4=58.9	Columns #1=12.3 #2=24.5 #3=14.8 #4=29.5	Columns #1=12.3 #2=24.5 #3=14.8 #4=29.5
C:N:P Ratio	100:18:188	100:18:188	100:18:188	100:10:3	100:10:3	100:10:3	100:10:3	100:10:3
Dissolved Oxygen (mg/L)	8.0-9.0	8.0-9.0	above 20.0	above 20.0	above 27.0	above 20.0	above 20.0	above 30.0
Loading Rate g $L^{-1}.d^{-1}$	Columns #1,#3=.074 #2, #4=.037	Columns #1,#3=0.15 #2, #4=.074	Columns #1,#3=.15 #2,#4=.074	Columns #1, #3=0.15 #2,#4=.074	Columns #1,#3=0.22 #2,#4=.11	Columns #1,#3=0.3 #2,#4=0.148	Columns #1,#3=0.6 #2,#4=0.3	Columns #1,#3=1.2 #2,#4=0.6

Final Column Study No. 9

In this study, the columns were no longer studied in parallel sets. The effects of external disturbances such as a high shock load and low DO were evaluated on PVA and GAC immobilized cells systems. Columns #2 and #4 were subjected to a high influent TCP concentration (500 mg/L), C:P:N ratio of 100:10:3, flow rate of 2.0 mL/min, and dissolved oxygen above 30.0 mg/L for 50.0 hours. The experimental condition of columns #1 and #3 were adjusted to a very low dissolved oxygen (DO) around 2~3 mg/L, TCP concentration of 40.0 mg/L, and flow rate of 2mL/min. for 50.0 hours. During these 50.0 hours influent and effluent samples were taken to determine TCP concentration, DO, pH, and Cl^- concentrations. After 48.0 hours experimental condition were adjusted back to TCP= 40.0 mg/L, DO= above 25.0 mg/L, flow rate=2 mL/min, and C:P:N ratio of 100:10:3. The columns were monitored in terms of TCP concentration, DO, pH, and Cl concentration until all the columns reached steady state (where there is no change in effluents concentration). Once the columns reached steady state, the stress conditions (shock load and low DO) on specified column were repeated one more time for another 50.0 hours. After 50.0 hours of the shock load on columns (2,4) and low DO on columns (1,3), once again all the columns were subjected to TCP=40.0 mg/L, DO ~ 30.0 mg/L, and flow rate of 2.0 mL/min. In this study, the effects of external disturbances such as high shock load and low DO were evaluated on PVA and GAC immobilized cells systems.

Tracer Studies

A pulse tracer study for non-ideal flow (Levenspiel, 1962) was conducted on the PVA-immobilized cells by Thomson (1996). A pulse tracer study was also conducted to two GAC-immobilized cells columns. This study was used to predict the flow behavior of each column as a reactor. The actual flow rates and dispersion coefficients were determined for all four columns.

The tracer used in this study was potassium bromide (KBr). Groundwater was added to 297.9 mg of KBr to make 1.0 liter of 200 mg/L bromide tracer solution. Two (2.0) mL of this solution was injected to GAC columns (#2 and #4) while the feed of groundwater was interrupted to allow the tracer to be injected into the columns. After 2.0 mL of tracer was gone, the influent tubing was then placed back into the feed bottle. Samples were taken every 10.0 minutes for at least three HRTs to ensure that all of the bromide tracer solution was recovered. Samples were collected over a period of 360.0 minutes and analyzed for Br concentrations using a Dionex Series 2000i/SP ion chromatography.

Analytical Techniques

Contaminant Concentration

Multiple extraction methods and gas chromatograph (GC) analytical techniques were used in analyzing the chlorophenol concentrations.

ENVI-Chrom P SPE tubes (6 mL/250 mg) were used for solid phase extraction of chlorophenols. The ENVI-Chrom PSPE tubes were obtained from Supelco, Inc., Bellefonte, PA. The resin in the tubes was composed of nonionic, highly crosslinked styrene-divinylbenzene copolymer, with a particle size range of 8-160 μm, a surface area of 800-950 m^2/g, and a mean pore size of 110-175 A° A 25 mL sample was added to ENVI-Chrom P each time after SPE columns had been previously conditioned sequentially by washing with 6.0 mLs of each ethyl acetate, methanol, and dionized water with vacuum suction. The tubes were dried for 5 minutes with the vacuum on. The vacuum was turned on and the tube was washed with an additional 2 mL and 1 mL of ethyl acetate until 5 mLs of eluent was collected in volumetric flask. A series (0.1 mg/L; 5.0 mg/L; 10.0 mg/L; 20.0 mg/L)of standard were prepared by a series of dilution of 10.0 g/L stock solution of TCP. A four-point standard curve (with correlation coefficient of above 0.98) was developed using a Hewlett-Packard 5890 Series II Gas Chromatography (GC) equipped with a column containing GP 10% SP-2100 on 100/120 mesh SUPELCOPORTcolumn for chlorophenols analysis. Since the GC response differs slightly with every usage, a series of standards were analyzed and a standard curve was developed before any samples were injected. The oven temperature was programmed (isothermal) for 200°C. The injection and detector temperatures were both 275° C and helium gas at a flow rate of 20 mL/min served as the carrier gas. Two μL extracted sample in ethyl acetate was injected into the GC

Breakdown Products

To identify any breakdown products analyses were performed using a GC-mass spectrometry (MS); Hewlett-Packard 5890-5970 MSD. The GC-MS was equipped with a DB-1 capillary column (30 m long, 0.25 mm i.d., 0.25 μm film thickness). Helium was the carrier gas with a linear velocity of approximately 40 cm/s. The injection volume was 1μ L. The injector was at 250 °C, and the detector was at 250°C. The oven temperature was set at 65 °C for 3.0 minutes, then ramped at 10 °C/minute up to 230 °C, for a total run time of 20 minutes. The samples were analyzed by Dr. Dilip Sensharma, Mass Spectrometry Laboratory, Department of Chemistry, Oklahoma State University, Stillwater, Oklahoma.

Bromide Concentration

Bromide concentrations were determined with a Dionex Series 2000i/SP ion chromatography (IC) with an Ionpak AS4A-SC 4 mm analytical column and an Ionpak AG4A-SC 4 mm guard column. Effluent sample volumes of 0.4 mLs were injected into the IC. The IC eluent consisted of 1.8 mM Na_2CO_3 and 1.7 mM $NaHCO_3$ while the regenerant was 25 mM H_2SO_4. A series of standards were analyzed (1.0 mg/L; 2.0 mg/L 3.0 mg/L; and, 4.0 mg/L) and a four-point calibration curve (with a correlation coefficient of 0.98) was developed.

Inorganic Chloride

Inorganic chloride ion concentration were determined by a chloride electrode (Type 15 213 3000, Fisher) and an Ingold Ag/AgCl reference electrode (Type 373-90-

WTE-ICE-S7) using an Orion pH/mV meter (Orion Research Inc.Model SA 720). To each 50 mL of sample, 1mL of 5 M $NaNO_3$ was added as an ionic strength adjuster. Measurements were carried out at 20 °C.

Dissolved Oxygen

Dissolved oxygen (DO) for effluent samples (collected under nitrogen blanket) was measured with an ORION Research Analog pH meter/model 301 and an ORION model 97-08-00 O_2 electrode. For influent samples, where a high concentration of DO (above 14 mg/L) potentially existed, the Winkler Method was used according to Standard Method (APHA, 1975).

pH Measurement

The pH was measured with a Fisher Scientific Accumet 900 pH meter and probe, model No. 13-620-108.

Volatile Suspended Solids

The procedure described in section 208F. of Standard Methods (APHA, 1975) was used to determine the volatile suspended solids (VSS) of bioculture. Total Volatile and Fixed Residue at 550 °C were determined in accordance with methods in section and of *Standard Methods*,(APHA, 1975).

Bacterial Population Counts

The standard plate count method presented by Benson (1967) was followed to perform the bacterial population counts of the cell titer in the suspension before and after the adsorption on activated carbon. One mL of sample was taken at different time intervals during immobilization of cells on GAC and processed (diluted) and filtered for the standard plate count. In addition, 7-mL of nutrient agar was poured into each 100 X 15 sterilized-disposable plastic Petri dish (Fisher Scientific, Fair Lawn, NJ), which were incubated at a room temperature(~25 °C) for 48 hours. The bacteria were counted at the end of 48.0 hours.

Electron Micrographs

The structure and growth of microorganisms on GAC and PVA beads were examined by scanning electron microscopy (SEM). SEM samples of GAC's for scanning electron microscopy were fixed with 0.1 M phosphate buffer (pH 7.2) containing 1.6 % glutaraldehyde at room temperature for 2 hour, washed with 0.1 M phosphate buffer, (pH 7.2) for 3 x 20 min. each. Adhered carbon to poly-1-lysine coverslips, then dehydrated through a graded series of ethanol solutions (25, 50, 75, and 100% ethanol). The samples then critical point dried and coated with gold. A JEOL JSM 35 U scanning electron microscope operating at 25 kV was used for examination of the samples. PVA-boric acid samples were prefixed with 1.6% (v/v) glutaraldehyde in 0.1 M phosphate buffer (pH 7.2) for 2.0 hours at room temperature and washed 3 times in 1.6 % glutraldehyde in phosphate buffer (pH= 7.2). The samples were postfixed for 2.0 hours

in a buffered 1% (w/v) osmium tetroxide (OsO_4) solution and dehydrated in graded series of ethanol. The speciments were mounted on aluminum specimen stubs and coated with gold and palladium. The samples were prepared and examined by Ginger Baker, Electron Microscopy Lab Manager, College of Veterinary Medicine, Oklahoma State University, Stillwater, Oklahoma.

Summary

Tables 4 and 5 summarize the studies and experiments conducted, specific objectives for each study, information expected from each study, and how that information relates to evaluation of permeable barrier technology using PVA and GAC immobilized cells.

Table 4. SUMMARY OF EXPERIMENTS/STUDIES

Experiment/ Study	Specific Objectives	Expected Information	How expected results relate to evaluation of permeable barrier technology
Porosity	Measure the capacity of the bed to allow water pass through its pore	* specific gravity * density *porosity percent	The porosity percent is needed to determine whether or not the flow of the water would be impeded.
Falling head permeameter test	Measure the ability of the packed bed to transmit water	* permeable coefficient	Permeability is needed to determine if the water will flow through the bed and the bed would not act as a dam.
Equilibrium study	Measure the equilibrium time	* equilibrium time	It is important to know how long will take for GAC to adsorb TCP and reach equilibrium.
Adsorption study	Measure the capacity of GAC , Sand, and copper screen to adsorb. TCP	* adsorption capacity (K) * adsorption intensity (1/n)	It is important to know how adsorbable TCP is.

Table 4. Continued.

Immobilization Time	Measure the time for maximum adsorption of cells on GAC	* immobilization Time(hr)	To quantify cells attached on GAC, this information needed to determine the quantity of cells immobilized on GAC.
Kinetic study of free cells	Measure the amount of TCP degraded, DO consumed and Cl release by the free cells.	* substrate utilization rates of the free cells * growth rate of the free cells	The batch study shows the free cells are active, growing and utilizing the TCP as carbon source prior to immobilizing them. The ICl increased, DO consumed indicates aerobic dehalogenation.
Kinetics of TCP adsorption on GAC w/wo cells	Measure the capacity of GAC w/wo immobilized cells	* effect of immobilization on adsorb. capacity of GAC.	This study shows that once cells are immobilized on GAC, fewer adsorption sites are available for TCP
Tracer study	Measure the flow rate and residence time in the columns	* flowrate (Q) * reactor dispersion number (D/μL)	This study shows the flow behavior and amount of time that the feed solution is in contact with the immobilized cells for degradation of TCP.

Table 5. SUMMARY OF COLUMN STUDIES

Experiment/ Study	Specific Objectives	Specific Objectives	How expected results relate to evaluation of permeable barrier technology
Column Studies Experiment No.1 | | | | | No.8	Simulate "bio-trench" using PVA and GAC Immobilized cells under variety of conditions.:	*the extend of biodegradation of TCP by continuous flow reactor. * effect of different operating conditions described in Table 3. on removal efficiency of these columns.	These column studies will show the applicability of PVA and GAC immobilized cells as two novel permeable barriers and their capability to biodegrade TCP under under variety of conditions such as different flow rates(1-4 mL/min), different TCP concentration(10-40 mg/L), different C:N:P ratio, and availability of DO (8.0-30.0 mg/L).
No.9	Subject the columns to shock load and low DO.	Effect of shock load and low DO on TCP degradation	This study evaluate the removal efficiency of the columns under low DO and high TCP conc.

CHAPTER IV

RESULTS AND DISCUSSION

The main objective of this study was to evaluate the use of PVA-immobilized cells and 3%GAC-immobilized cells/sand mixture as two novel candidate technologies to biodegrade TCP contaminated groundwater. The batch and initial column experiments on PVA-immobilized cell beads were conducted to determine physical characteristics of the PVA beads and the capabilities of these beads to serve as a permeable barrier. The details of these experiments and their results were reported by Thompson (1996). As a reference, results of these experiments were summarized and presented at the end of Chapter II in Table 2.

Based on the experimental design described in Chapter III, experiments were conducted to determine the capabilities of these beads to serve as a permeable barrier medium under a variety of different operating conditions.

In order to evaluate the (3%) GAC-immobilized cells mixed with silica sand as a permeable barrier medium, batch experiments were conducted to determine physical characteristics of the (3%) GAC/sand mixture; and its ability as a biological carrier system. These experiments were:

- characterization of a packed bed - to evaluate the applicability of (3%) GAC/sand mixture packed bed as a medium in a permeable barrier by measuring its porosity and permeability,

- equilibrium study - to measure the time required for GAC to adsorb the maximum amount of TCP in water,
- adsorption study - to measure the adsorption capacity of GAC, silica sand, and copper screen used in the column studies,
- kinetics study - to determine the ability of free cells to degrade TCP by the determination of the substrate utilization rate and growth rate,
- immobilization time - to measure the time required for GAC to reach its maximum capacity of adsorb cells,
- equilibrium of TCP adsorption - to measure and compare the adsorption capacity of plain GAC and GAC-immobilized cells for TCP,
- tracer study - to determine a dispersion coefficient, and residence time for each GAC column (10 cm and 20 cm),
- column studies - to simulate a "bio-trench" using PVA immobilized cells and 3%GAC-immobilized cells/sand mixture to biodegrade TCP at four TCP concentrations (10, 20, 30, 40 mg/L), three flow rates (1, 2, 4 mL/min), and two C:N:P ratios (100:18:188, 100:10:3),
- final column studies - to evaluate the effects of organic shock loadings and the deficiency of dissolved oxygen (DO) on PVA -immobilized cells, and 3%GAC-immobilized cells /sand mixture columns, and
- finally, compare GAC-immobilized cells and PVA-immobilized cells on the basis of the removal efficiency, ease of preparation and operation, durability, survival under stress conditions (low DO, shock load of TCP), and capital cost.

Characterization of 3% GAC/Sand

Silica sand and GAC were washed with distilled water and oven dried at 103°C separately. The two materials were then blended to achieved the desired weight ratio. The (3%) GAC/sand mixture was packed into the column with a density of 1.6 g/cm^3 to simulate actual densities found in alluvial aquifers (Mandel and Shiftan, 1981). The results of the characterization of a packed bed of (3%) GAC/sand mixture along with characterization of the PVA beads are presented in Table B.1, Appendix B. The porosity of the bed, at 22° C, was 30% which was comparable to an average porosity of aquifer sand (Linsely, *et al.*, 1982). The permeability coefficient obtained for the 3%GAC/sand mixture was 0.0162 cm/s which was comparable to a medium grained sand (Smith, 1979). The compressibility index (Cc) for medium grained sand is reported to be 2.87 X 10^{-5} m^2/kN (Smith, 1979). The GAC/sand mixture (40.0 mesh size) is compareable to a medium grained sand, and was assumed to have similar value of compressibility.

Batch Studies

Equilibrium Study

An equilibrium study was conducted on 2.5 g of GAC in a continuously stirred (magnetic stirring bar) 500 mL volume of a 20.0 mg/L TCP solution. Samples of the solution were taken at various times during a 24 hour period to analyze TCP concentrations. The result of this study is illustrated in Figure 3. It can be seen that the aqueous TCP concentration reached a value of 0.5 mg/L within 8.0 hours after the start

of the experiment. For the samples taken at 20.0 hours and 24.0 hours, the value of TCP concentration in solution was 0.0. Therefore, the time required for GAC to adsorb TCP and reach equilibrium was around 20.0 hours. The raw data are shown in Table C-1., Appendix C.

Adsorption studies

GAC: As a results of the equilibrium study, the adsorption study for GAC was conducted for 24.0 hours. This study consisted of adding varying masses of GAC (0.01, 0.1, 0.5, 1.0, 5.0 g) to 5 glass flasks of 250 mL volume. Two hundred (200) mL of solution with a TCP concentration of 20.0 mg/L, was added to each flask. The samples were taken and analyzed by GC for TCP remaining in solution. The data were plotted and the resulting Freundlich isotherm is shown in Figure 4. A regression analysis of the data resulted in a linear fit with the slope (1/n) of 3.868 and an intercept (k) of 0.0894. The raw data and calculation of the physical adsorption capacity of the GAC are listed in Table C-2., Appendix C.

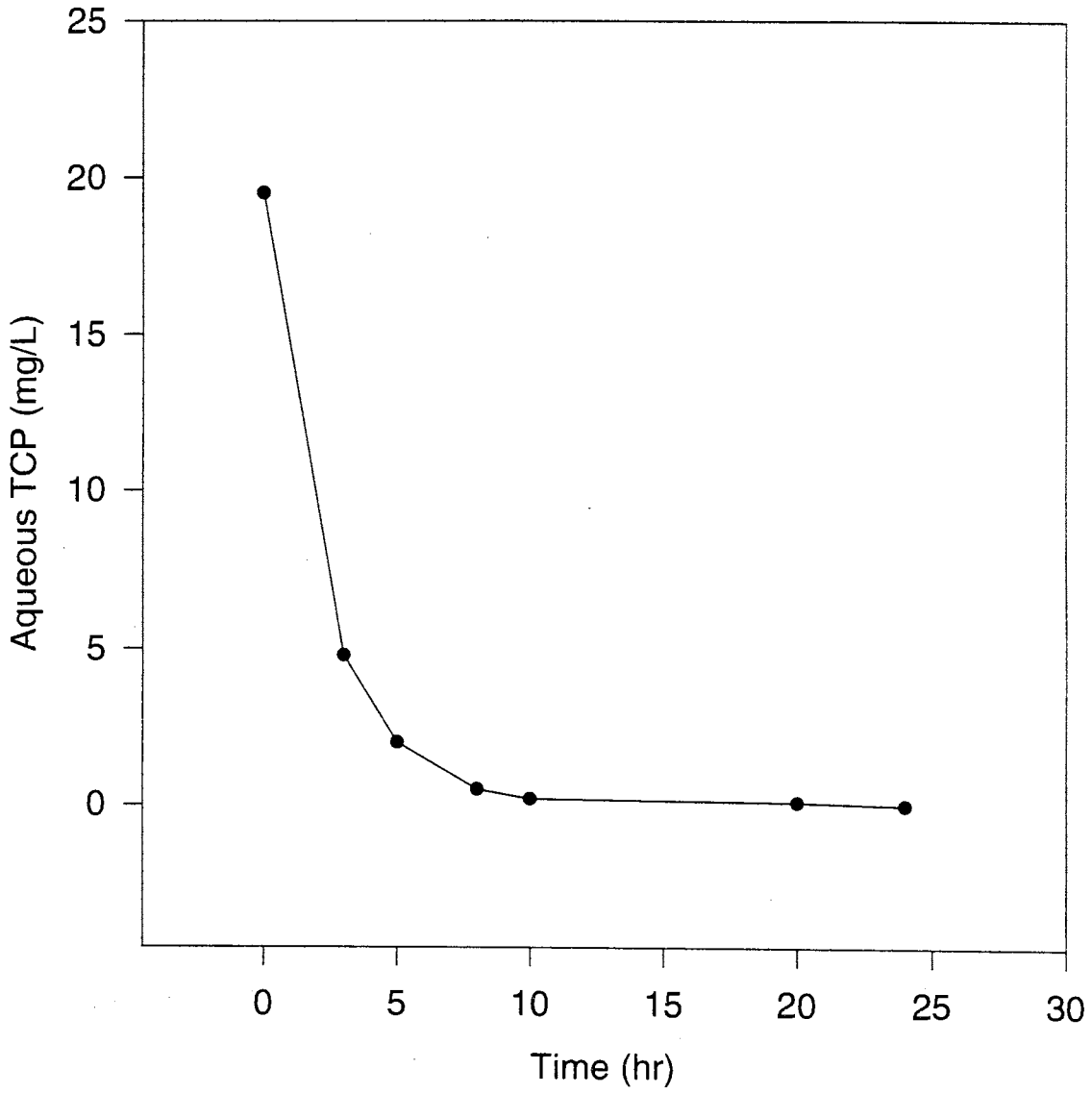

Figure 3. Equilibrium Study for GAC and TCP.

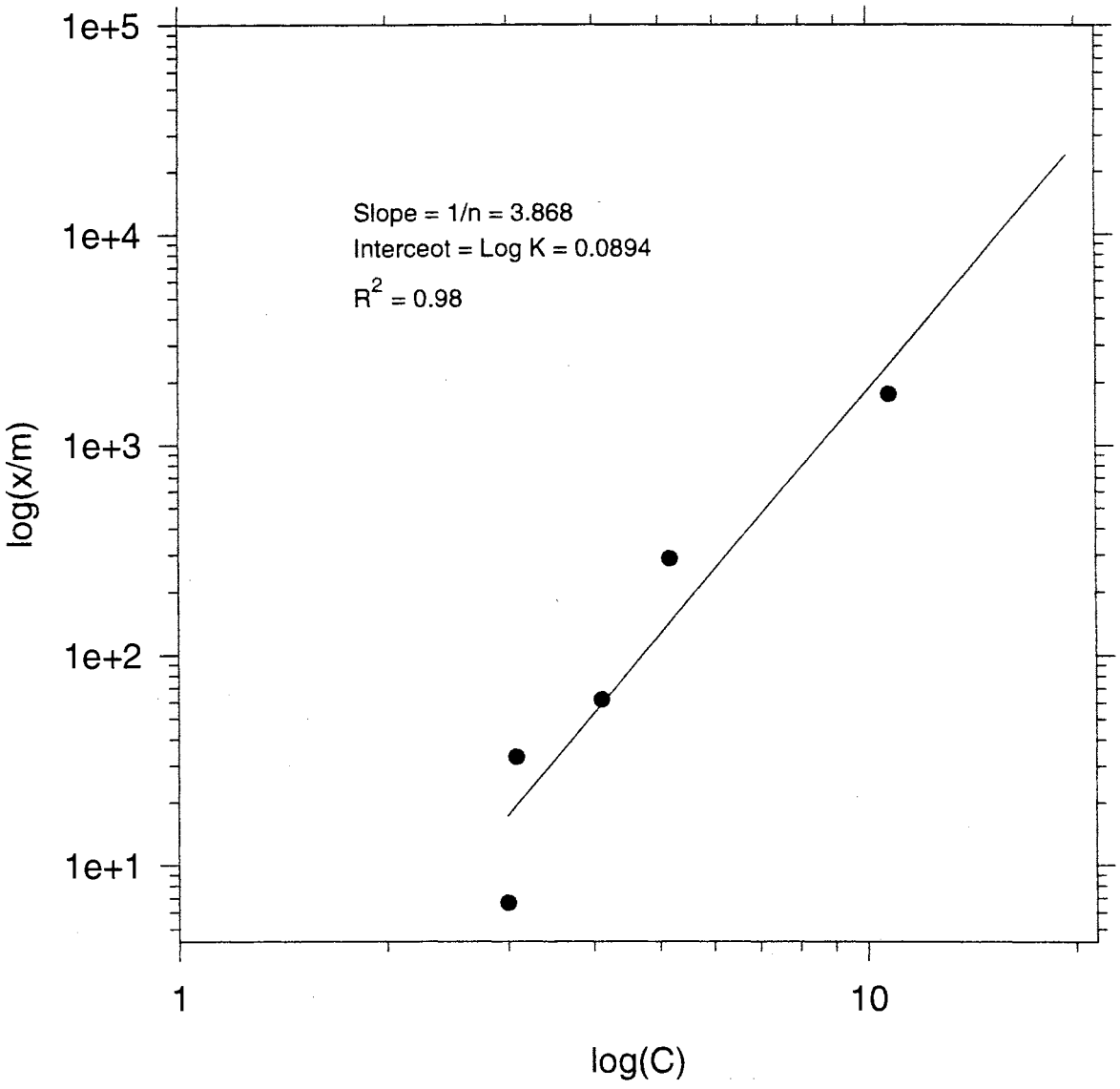

Figure 4. Freundilich Isotherm for Adsorption of TCP onto GAC.

Silica Sand: The TCP concentration in solution in contact with the silica sand was 20.0 mg/L. Samples of the TCP solution were taken at 0.0, 3.0, 7.0, 12.0, and 24.0 hrs. The effect of sorption of TCP onto the silica sand is shown in Figure 5. It can be seen that about 0.8 mg/L of TCP was adsorb in an elapsed time of 3.0 hours. For the samples taken after 3.0 hours, the TCP concentration in solution increased back to 20.0 mg/L by the 24.0 hour sample, indicating that the silica sand did not physically remove any TCP. The raw data are shown in Table C-3., Appendix C

Copper Screen: The TCP concentration in the solution in contact with a piece of copper screen was 10.0 mg/L. The copper screen had a dimeter of 1.0 inch and weighted 0.77 mg. Samples taken over 24.0 hours showed no reduction in TCP concentration, indicating that the copper screen did not adsorb any TCP over 24.0 hours. The result is shown in Figure 6. The raw data are presented in Table C-4., Appendix C.

Immobilization Time

The quantity of immobilized cells attached to the GAC was calculated by difference between the initial cell concentration in suspension and cell concentration at times 0.0, 1.0, 3.0, 7.0, 10.0, 13.0, 16.0, 20.0, and 24.0 hours. The result of this study is shown in Figure 7. Initial cell concentration in the solution was 6.4×10^6. As seen in Figure 7 the quantity of immobilized cells attached to GAC is dependent on immobilization time. After about 13.0 hours an adsorption balance was reached between free and immobilized cells. The raw data are presented in Table C-5., Appendix C.

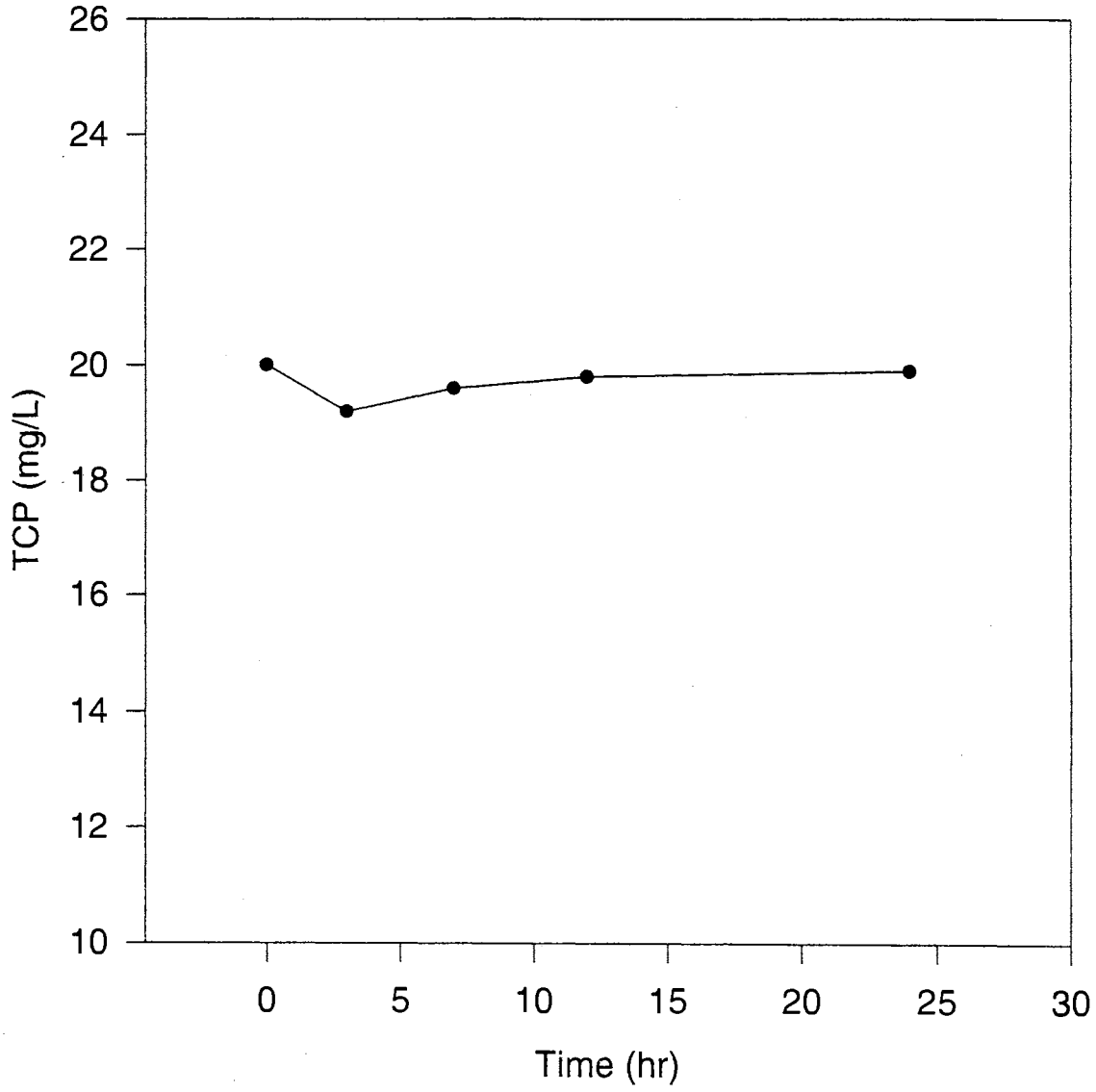

Figure 5. Sorption of TCP onto Silica Sand.

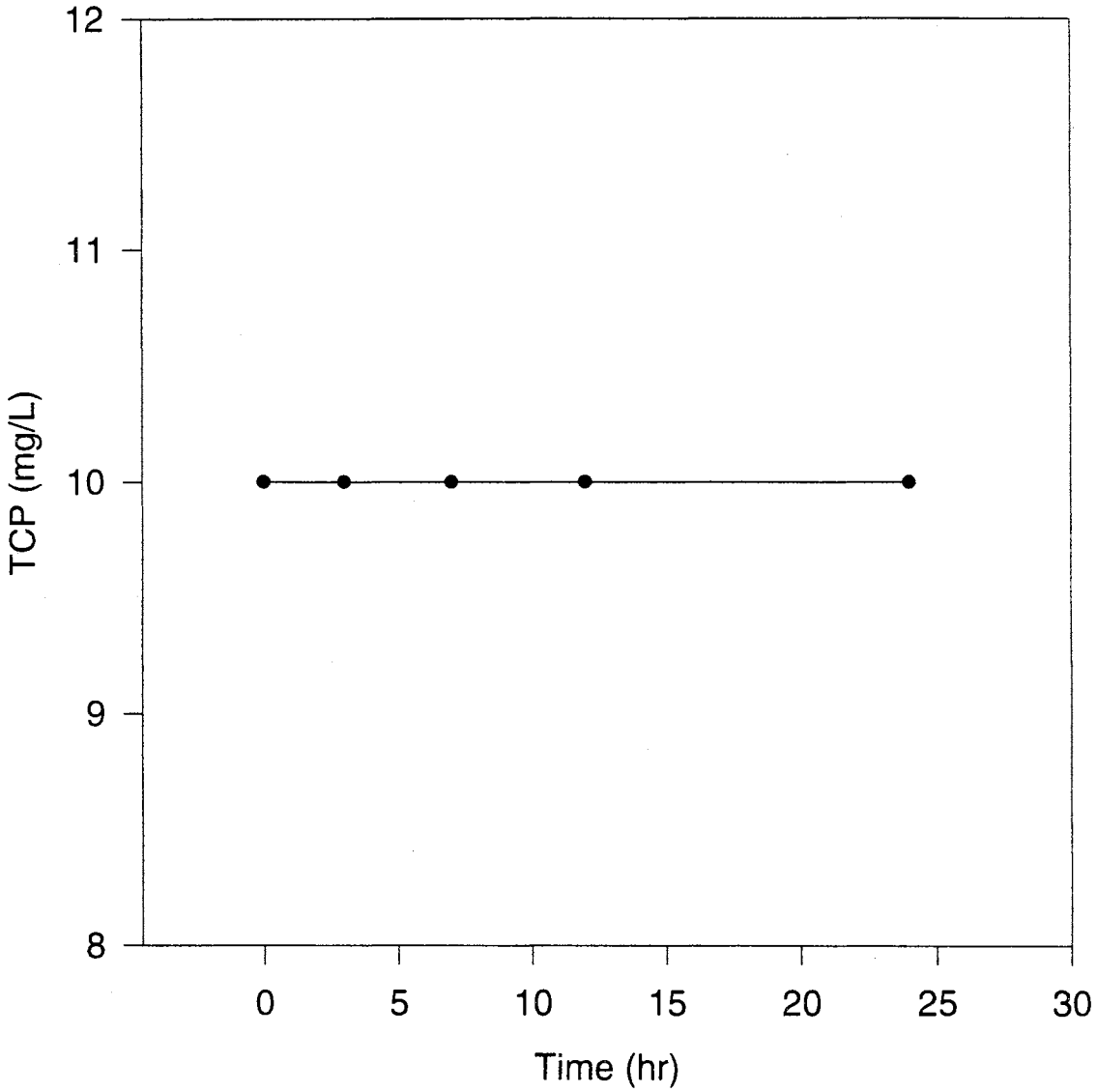

Figure 6. Sorption of TCP onto Copper Screen.

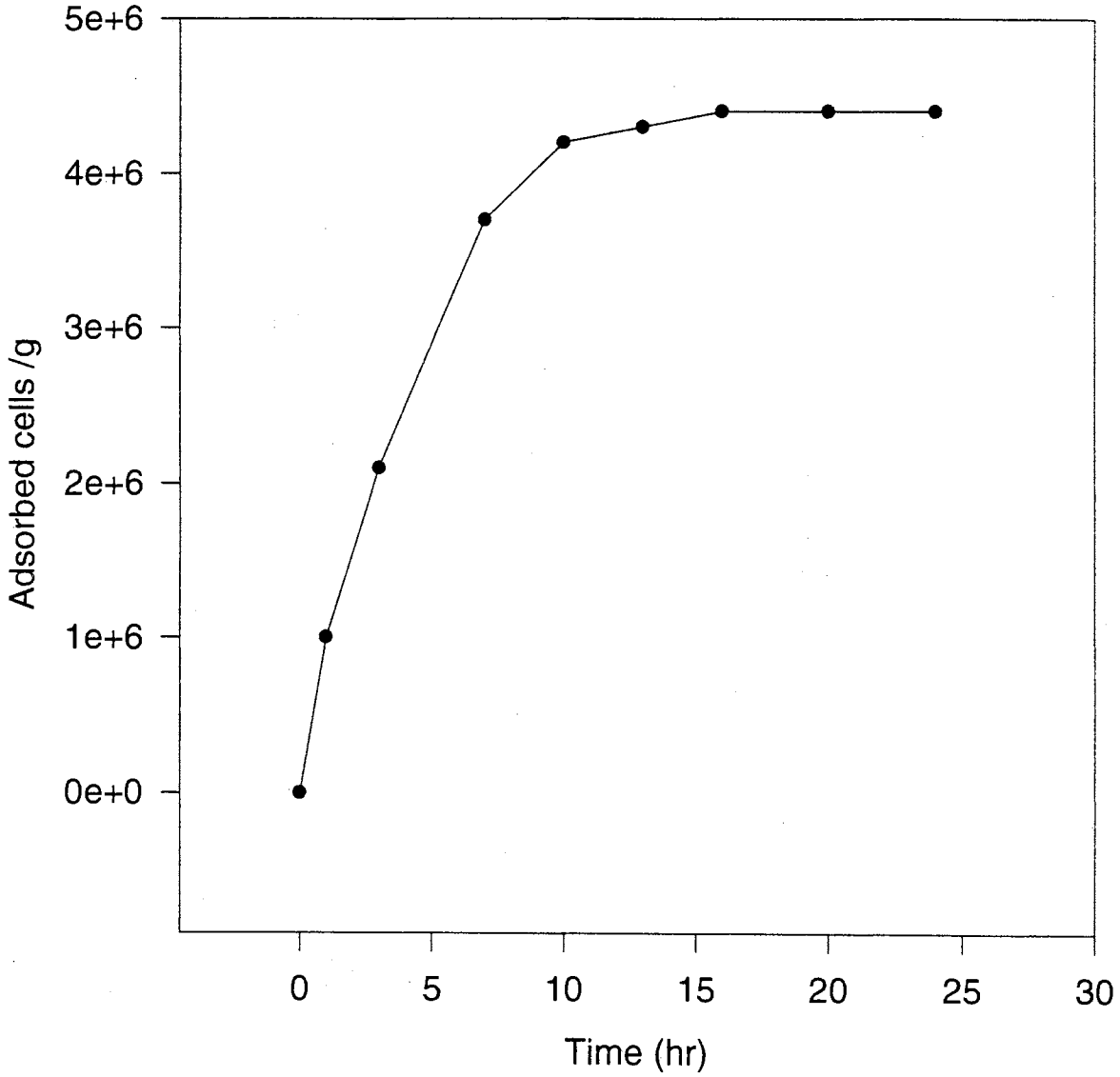

Figure 7. Time-dependence of immobilization.

Kinetic Study of Free Cells

The kinetic study was conducted over a period of 24.0 hours. Samples taken over the 24.0 hour period were analyzed for TCP concentration, VSS, and chloride release. The raw data and detailed calculations of substrate utilization rate (r_{su}) and growth rate (r_g) are presented in Table C-6.,Appendix C. The TCP concentrations over 24 hours are plotted in Figure 8. As seen in Figure 8 the free cells removed 71% of TCP within 3.5 hours and 100% removal within 5.0 hours, giving a substrate utilization rate of 2.0 mg L^{-1} hr^{-1}. The results of the VSS analysis (Table C-6) showed that the VSS increased from 1035 mg VSS/L to 1075 mg VSS/L within 5.0 hours. This gave a growth rate (r_g) of 8.0 mg L^{-1} hr^{-1}.

The theoretical increase of chlorides was predicted to be 5.39 mg/L for complete dehalogenation of 10.0 mg/L of TCP. Measured chloride releases resulted in an increase of 7.0 mg/L over 5.0 hours in which 10.0 mg/L of TCP was removed. The measured chloride releases and theoretical inorganic chloride releases are plotted in Figure 9. The theoretical and the measured inorganic chloride increases were close in value, but with the measured values being slightly higher.

The kinetic study was conducted multiple times. The initial kinetic study by Thompson (1996) showed that free cells consumed 10.0 mg/L of TCP within the first 9.0 hours resulting in chloride production of 8.0 mg/L.

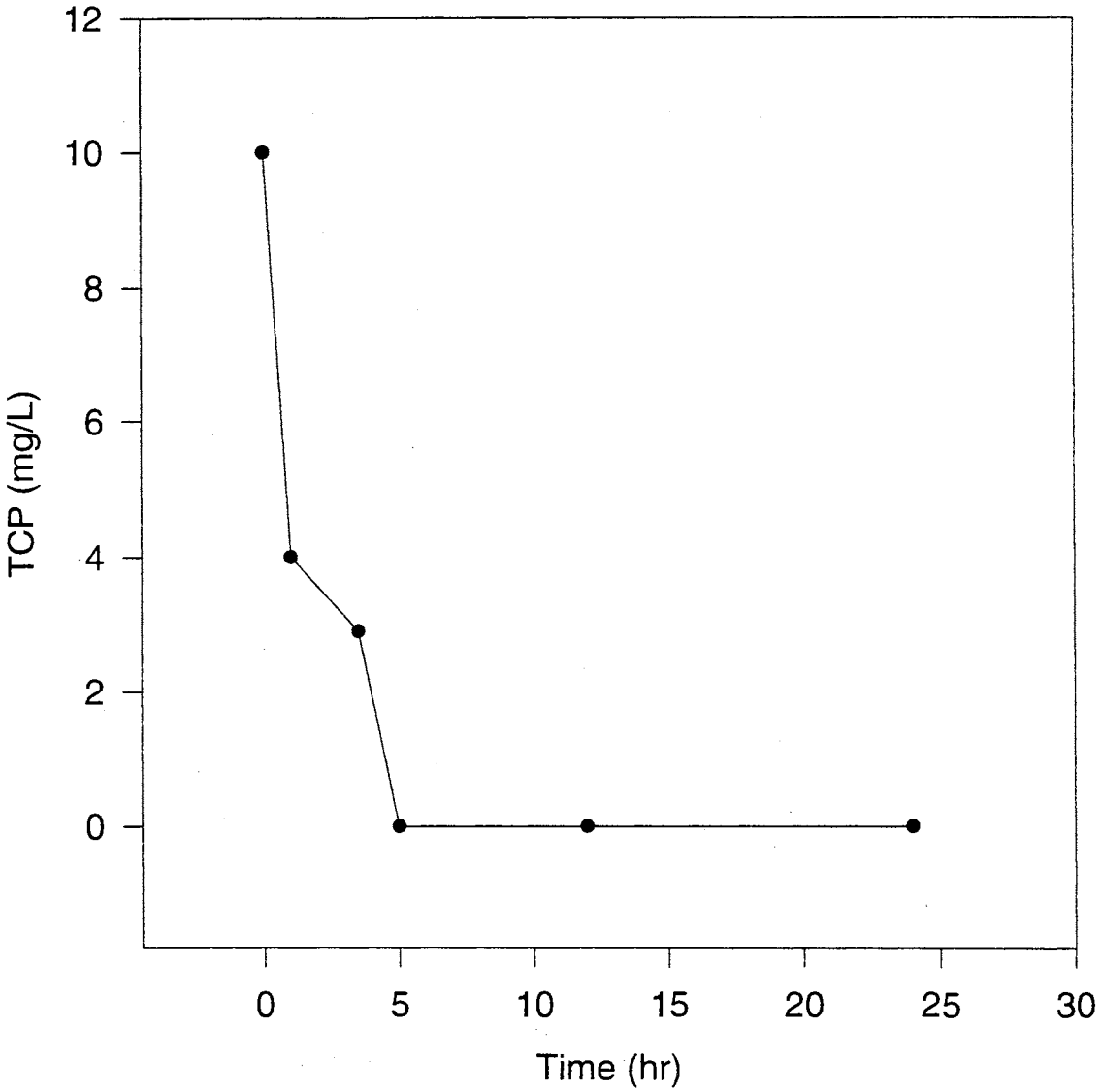

Figure 8. Biodegradation of TCP by Free Cells.

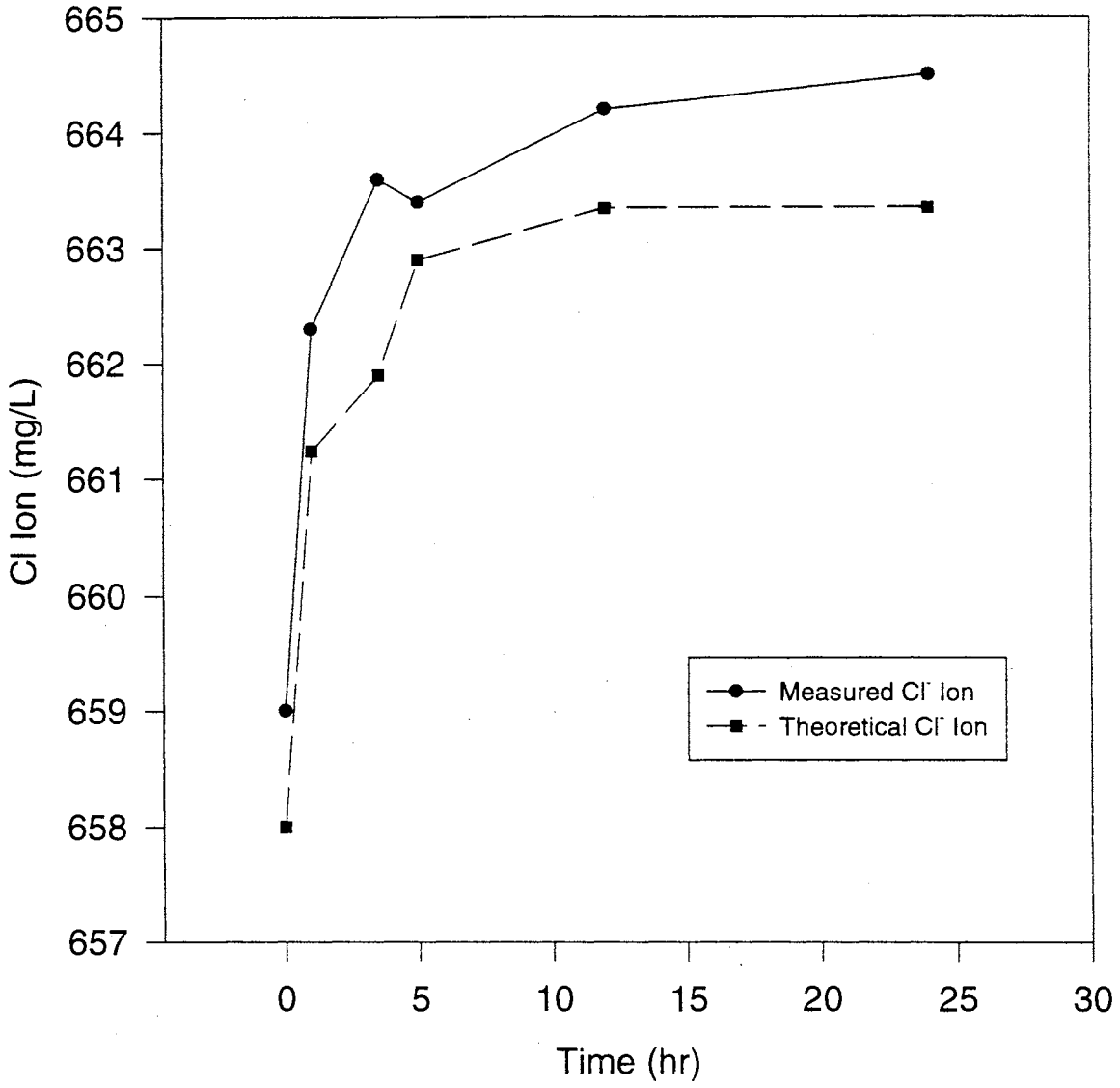

Figure 9. Inorganic Chloride Released by Free Cells.

Kinetics of TCP Adsorption on GAC

The adsorption of TCP to GAC in the absence and presence of free cells is shown in Figure 10. The initial TCP concentration in both the GAC-immobilized cells and plain GAC flasks was 500 mg/L. Figure 10 shows that rapid adsorption of TCP occurred over the first 30.0 minutes for plain GAC; the aqueous TCP concentration decreased to about 50.0 mg/L during this time. For GAC-immobilized cells, aqueous TCP concentrations decreased from 500 mg/L to 300 mg/L during the first 20.0 minutes of experiment. TCP levels were further reduced to 250 mg/L over the next 40.0 minutes. Comparison of TCP adsorption on GAC-immobilized cells and plain GAC, indicated that a smaller amount of TCP was adsorbed on the GAC immobilized cells system versus the plain GAC. This difference could be attributed to fewer adsorption sites are available in GAC-immobilized cells for TCP. This results is consistent with an earlier observation by Kindzierski *et al.* (1992). The authors reported the capacity of activated carbon for phenol adsorption decreased from 1.4 L/g for fresh activated carbon to 0.16 L/g for activated carbon with bacteria attached. The authors suggested the rate constant for adsorption with bacteria was 3.9 times smaller than on fresh activated carbon, which suggested an effective diffusivity over 35 times smaller for activated carbon with bacteria as compared to fresh activated carbon. The raw data are presented in Table C-7, Appendix C.

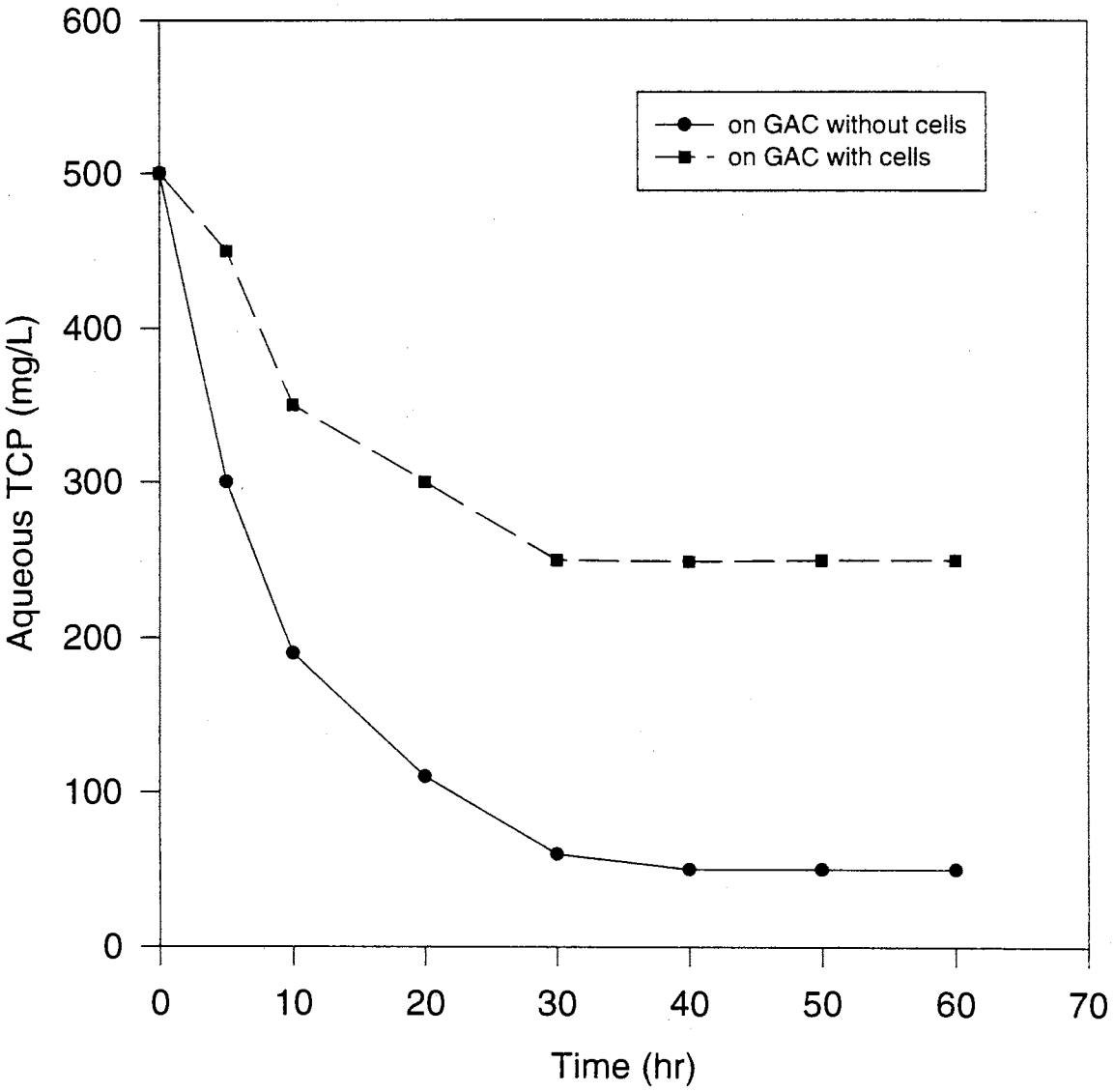

Figure 10. TCP Adsorption onto GAC-Immobilized Cells and Plain GAC.

Tracer Study

A pulse tracer study for non-ideal flow was conducted on the PVA-immobilized cells columns #1, #2 (Thompson, 1996) and GAC columns #3, #4. The specifications for columns #1-# 4 were as followed (Table 6.):

Table 6. The specification of the PVA and GAC columns.

Column	Height(cm)	Diameter(cm)	Volume(cm^3)	Porosity	HRT*(min)
(PVA) # 1	10.0	5.0	196	25 %	49.0
(PVA) # 2	20.0	5.0	392.7	25 %	98.0
(GAC)# 3	10.0	5.0	196	30 %	58.9
(GAC) # 4	20.0	5.0	392.7	30 %	117.8

• at flow rate of 1 mL/min.

The bromide concentrations analyzed from the pulse tracer study for GAC columns (#3 and #4) are presented in Table D-1 and D-2, Appendix D. Column #3 had a volume of 196 cm^3 and column #4 had a volume of 392 cm^3. The volumetric flow rates for GAC columns (#3 and #4) were determined by taking the volumes of the effluent collected during the tracer study and dividing by the number of minutes. Column #3 and #4 had average flowrates of 0.99 and 1.01 mL/min, respectively.

The following expression gives the total amount of bromide tracer in the pulse input (Levenspiel, 1962):

$$\Sigma C\,\Delta t \qquad (4)$$

where

C = bromide concentration (mg/L)

t = time (min)

The mean residence time was determined from the expression (Levenspiel, 1962):

$$\tau = \frac{\Sigma tC}{\Sigma C} \tag{5}$$

where

τ = mean residence time (min)

and

$$\theta = \frac{t}{\tau} = \text{reduced time} \tag{6}$$

and

$$E = \frac{\tau C}{\Sigma C \Delta t} \tag{7}$$

E, $\Sigma C\ \Delta t$, τ, and θ were calculated and the values are listed in Table D-1and D-2, Appendix D. The vessel dispersion number $D/\mu\ L$ was calculated from the following expression (Levenspiel, 1962):

$$\sigma^2 = (\Sigma\theta^2/\Sigma E) - 1 \tag{8}$$

and

$$\sigma^2 = 2(D/\mu\ L) - 2(D/\mu\ L)^2\ (1 - e^{-\mu L/D}) \tag{9}$$

The second term on the right of the equation 9 was ignored because its value was very small and an approximation was made:

$$D/\mu L \approx \sigma^2/ 2 \qquad (10)$$

Correcting for the term ignored, the value of D/μ L was found by trial and errorThe dispersion number $D/\mu L$ for each column was determined by using the plot of E vs. θ for columns #3 and #4 in Figures 11 and 12. The dispersion number D/μ L for column #3 and #4 were 0.043 and 0.036, respectively. Column #3 and #4 had small amount of dispersion as shown in Figure 11 and 12 (Levenspiel, 1962). The walling effect was evidenced in both column by the low slope fluctuating about 0.6 and 0.8 E value for column #3 and #4, respectively. The steep symmetry about the point where θ equals 1.0 is similar to that of a plug-flow reactor.

To determine the recovery efficiencies of the tracer (bromide), effluent bromide concentrations vs. effluent volumes for each GAC column were plotted and are shown in Figure 13 and 14. The areas under the curve represents the amount of Br recovered during the tracer study. The resulting area equalled 0.31 mg and 0.3 mg, for column #3 and column #4, respectively. Column #3 had a 75% Br recovery and column #4 had a 77.5 % Br recovery. The % recoveries in this tracer study means that bromide is a inert tracer.

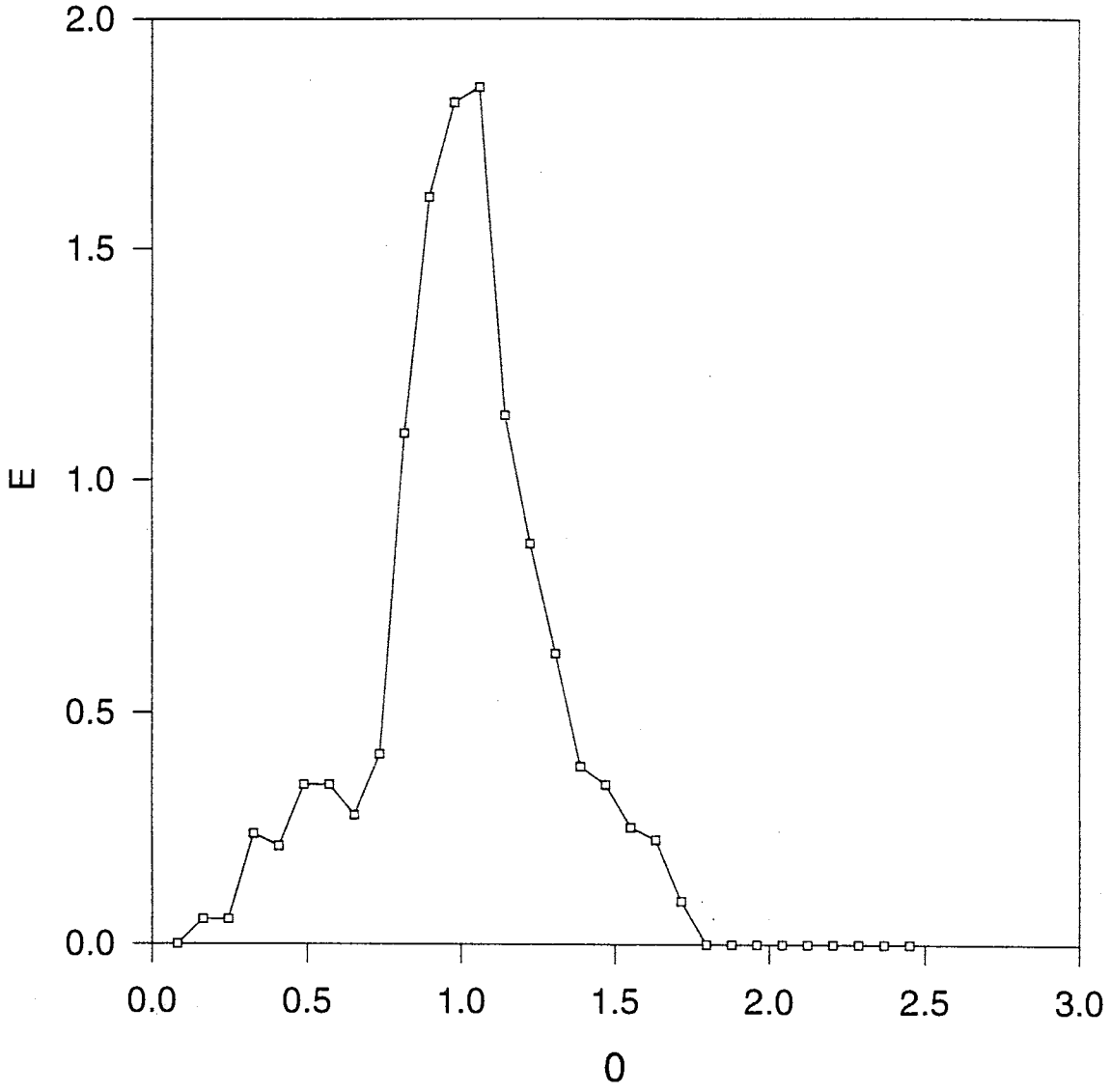

Figure 11. Tracer study for column #3 - E curve

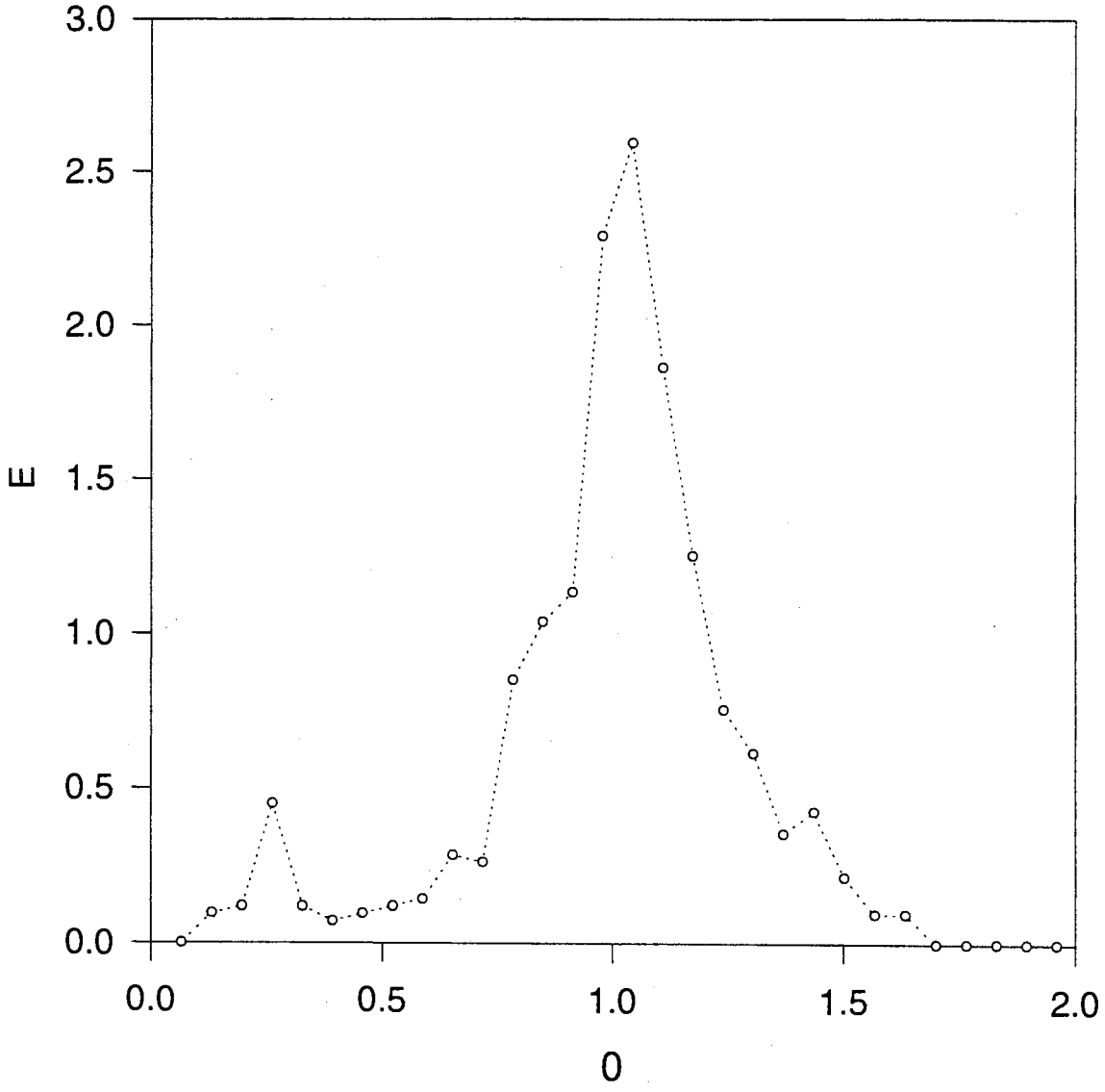

Figure 12. Tracer study for column #4 - E curve

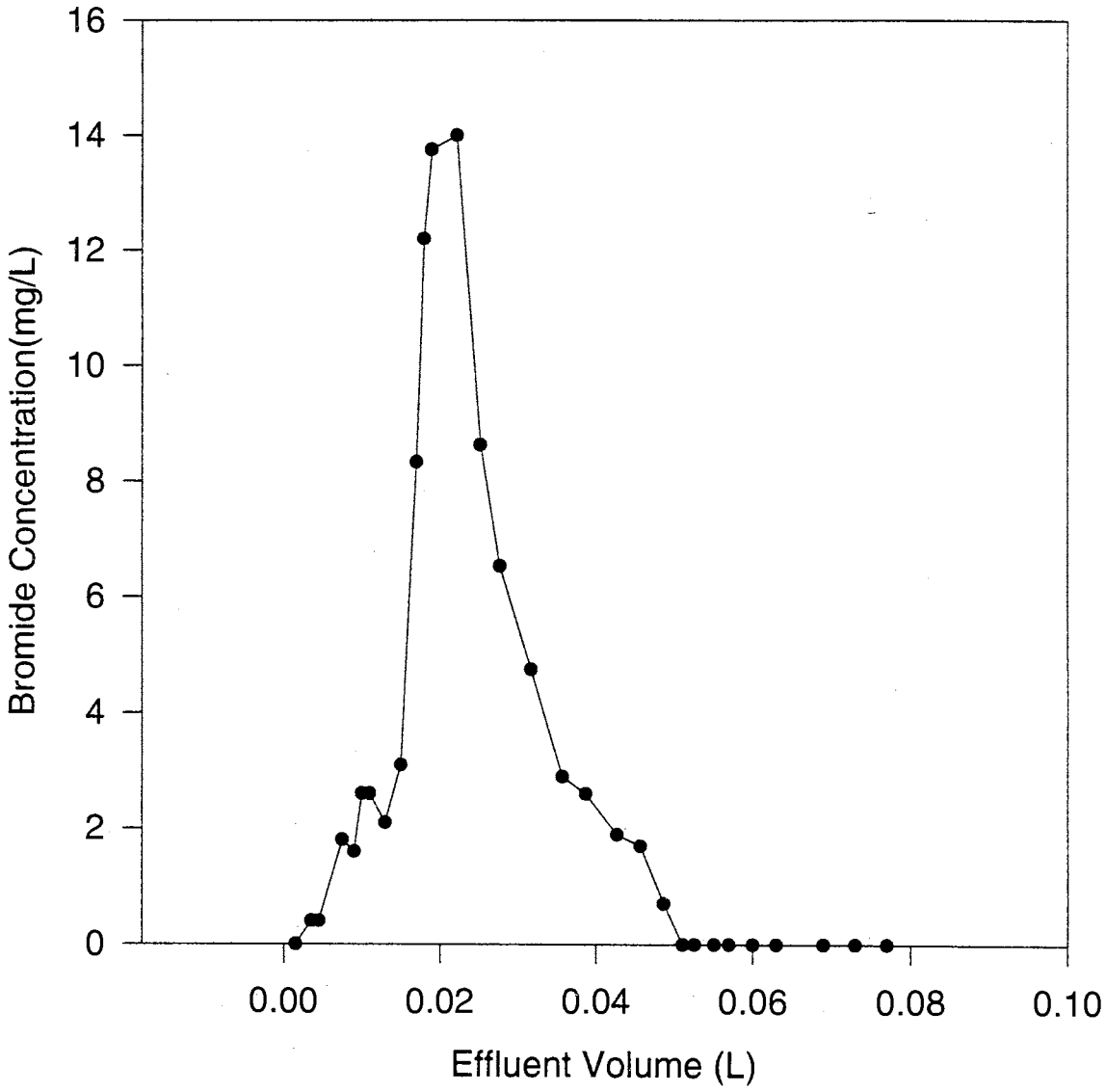

Figure 13. Tracer study - Bromide concentration vs volume collected for column #3.

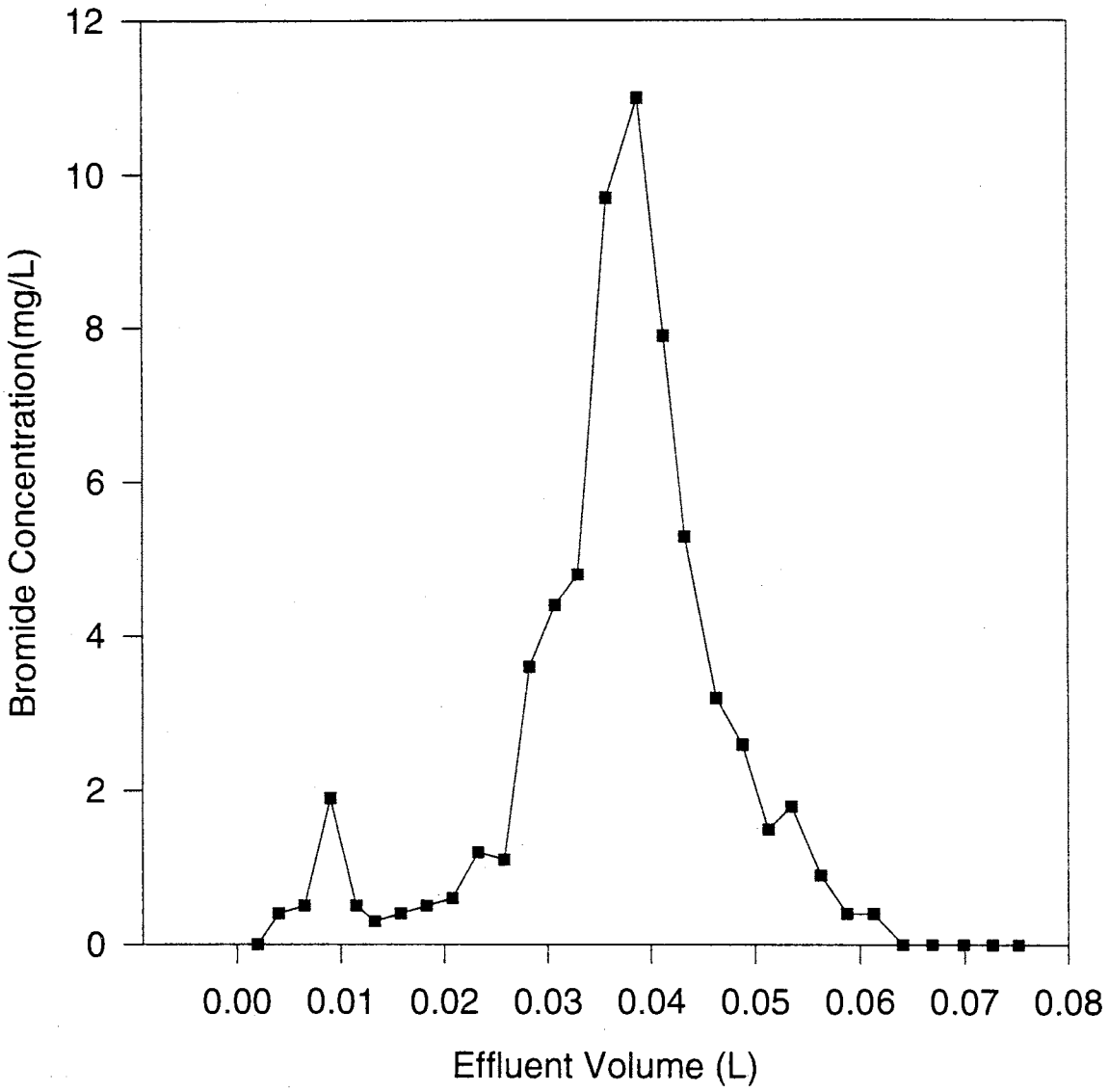

Figure 14. Tracer study - Bromide concentration vs volume collected for column #4

Column Studies

Column studies were set up to evaluate and compare aerobic biodegradation of TCP by PVA-immobilized cells columns (#1,#2) and GAC-immobilized cells columns (#3,#4) under varying operational conditions.

PVA columns #1 and #2 contained 10.0 cm and 20.0 cm beds of 3-5 mm PVA-immobilized cells beads, respectively. The GAC columns #3 and #4 contained 10.0 cm and 20.0 cm bed of (3%) GAC-immobilized cells/silica sand (97%) mixture, respectively

A minimum two week experiment period was considered adequate to collect the required data. During the experiments, between 100-300 mL of influent and effluent samples were collected every other day and tested for TCP concentration, DO, chloride release, and pH. The data were collected during the transition and steady state periods.

Aerobic dehalogenation of TCP was expected to reduce the pH value in the effluent due to the production of HCl (Makinen *et al.*1993). The production of HCl has the potential to be used to cross-checking, the chlorine release data collected. To allow for this cross checking a titration curve (pH curve) was prepared by titrating a 1.0 L sample of 10.0 mg/L TCP influent feed solution with 0.1 N HCl solution. The pH measurements of the feed solution were plotted against the milliliters of 0.1 N HCl used in the titration and are shown in Figure 15. Each mL of titrant contained 3.55 mg Cl^-/mL (0.1 N HCl = 0.1 mole/L (35.5 g Cl^-/mole HCl) = 3.55 mg Cl^-/mL of titrant). A drop in the pH value can be correlated to the corresponding amount of titrant used to obtain the same reduction in pH and end up at the column effluent pH. The amount of titrant used reflects the concentration of Cl^- in the effluent. It should be mentioned that other acids can be produced as the result for bacteria metabolism which could also impact the

effluent pH to some extent. The raw data for pH curve are presented in Table E-1, Appendix E. The experimental conditions for column studies 1-8 were as follows (Table 7):

Table 7. Experimental conditions for column studies 1-8.

Column Study No.	Influent Flow Rate (mL/min)	Influent Conc. (mg/L)	C:N:P
1	1	10	100:18:188
2	1	20	100:18:188
3	1	20	100:18:188
4	1	20	100:10:3
5	1	30	100:10:3
6	2	20	100:10:3
7	4	20	100:10:3
8	4	40	100:10:3

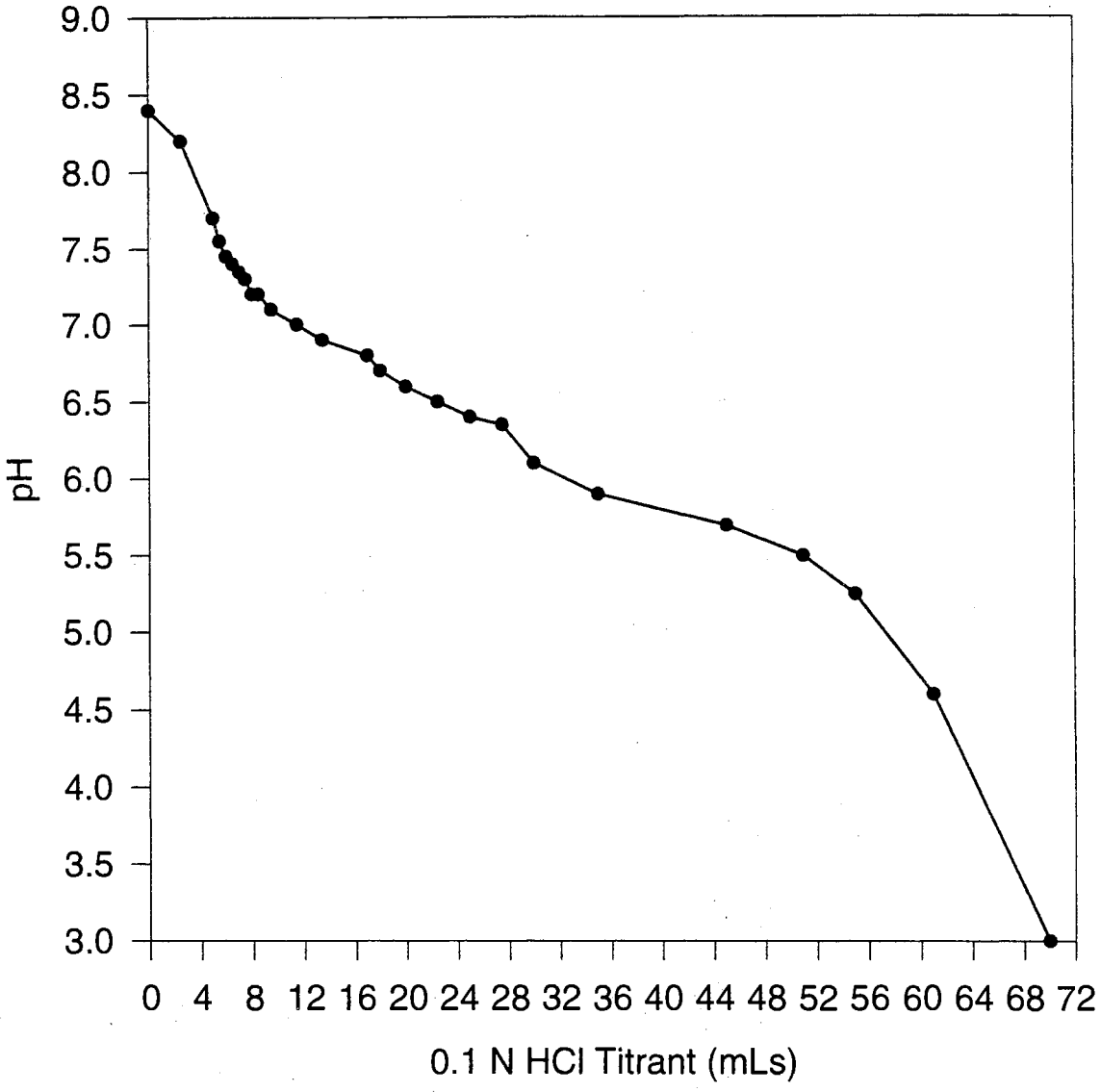

Figure 15. Titration curve of influent feed solution containing 10.0 mg/L TCP.

Column Study No. 1

The purpose of this experiment was to establish the removal mechanism of TCP from groundwater by PVA-immobilized cells (columns #1, #2) and 3%GAC-immobilized cells /sand mixture (#3,#4).

During this study the average influent TCP concentration was 10.50 ± 0.83 mg/L. The C:N:P ratio was kept at 100:18:188 by adding nutrients to the influent feed solution. The influent solution was aerated with laboratory compressed air 24.0 hours a day to maintain the DO above 8.4 mg/L. The flow rate for all four columns was 1.0 mL/min. Applied loading in this experiment for columns #1, and #3 and for columns #2, and #4 were 0.074 and 0.037 g L^{-1} d^{-1}, respectively. The raw data are presented in Appendix F.

TCP Concentrations: TCP influent and effluent concentrations for the PVA and GAC columns are shown in Figures 16 and 17. PVA columns #1 and #2 reduced the influent TCP concentration to zero on day 17 and 13, respectively. PVA column #2 with a 20.0 cm bed height provide longer contact time between cells and TCP than PVA column #1 with a 10.0 cm bed height. As seen in Figure 16, both columns maintained 100% TCP removal for remaining time of this experiment. As Figure 17 shows, no TCP was ever detected in the effluents of GAC columns over entire course of this experiment. The explanation might be that during this period, TCP was removed initially by adsorption on GAC that is no leakage was seen, and later by biodegradation (supported by change in DO and Cl release data).

Dissolved Oxygen (DO): For aerobic mineralization of each mg of TCP, 0.89 mg of oxygen is expected to be consumed by bacteria. Figures 18 and 19 show that all four columns consumed oxygen to about the same extent (2.5 mg/L), except PVA #1 which

had a slightly higher consumption (3.3 mg/L). The reduction in DO in the effluent is an indication of the biodegradation process that is going on in these columns. During the 5.0 hour required to collect the effluent sample (300 mLs), the samples were exposed to the air, which probably yield a residual oxygen concentration different than that expected based on stiochiometry.

Inorganic Chloride Released: The chloride ion (ICl) concentrations were measured and are shown in Figures 20 and 21. The Cl^- concentrations in the effluents of all four columns showed an increase of about 6.0 - 8.0 mg/L. The increase in chloride concentration support aerobic dehalogenation of TCP. For the idea of aerobic dehalogenation of each mg of TCP, 0.54 mg of chloride is expected to be release based on stiochiometry.

pH Change: Aerobic dehalogenation of TCP produced HCl which would cause the drop in effluent pH. The influent feed solution had a pH range from 8.1- 8.3. Figures 22 and 23 show the approximate average pH in the effluents from columns #1, #2, #3, #4 are 7.7, 7.7, 7.9, 7.6, respectively. The estimated amounts of Cl^- concentration in the effluents of columns #1, #2, #3, and #4 needed to cause the observed drops in pH are 10.6, 10.6, 7.1, and 12.4 mg/L, respectively. The Cl^- concentrations obtained from the pH curve are (15-35 %) higher than Cl^- concentrations measured which ranged from 6.0 -8.0 mg/L chloride. The possible explanation could be the formation of acids other than HCl. The drop in pH tends to support the dehalogenation of TCP.

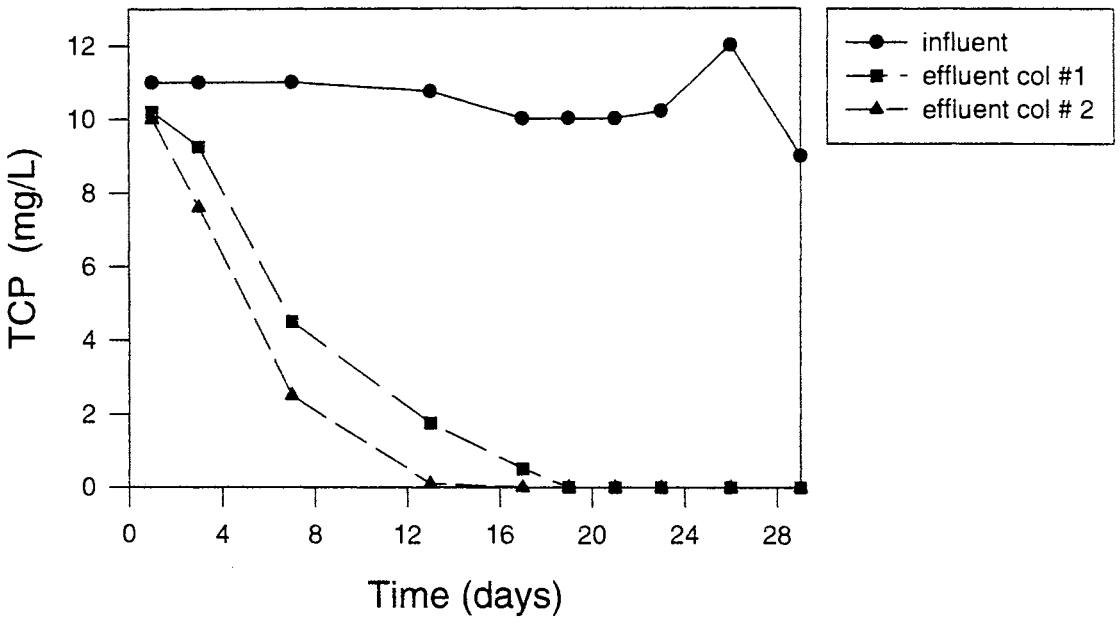

Figure 16. Removal of TCP by PVA columns #1 (10.0 cm) and #2 (20.0 cm) during column study No.1.

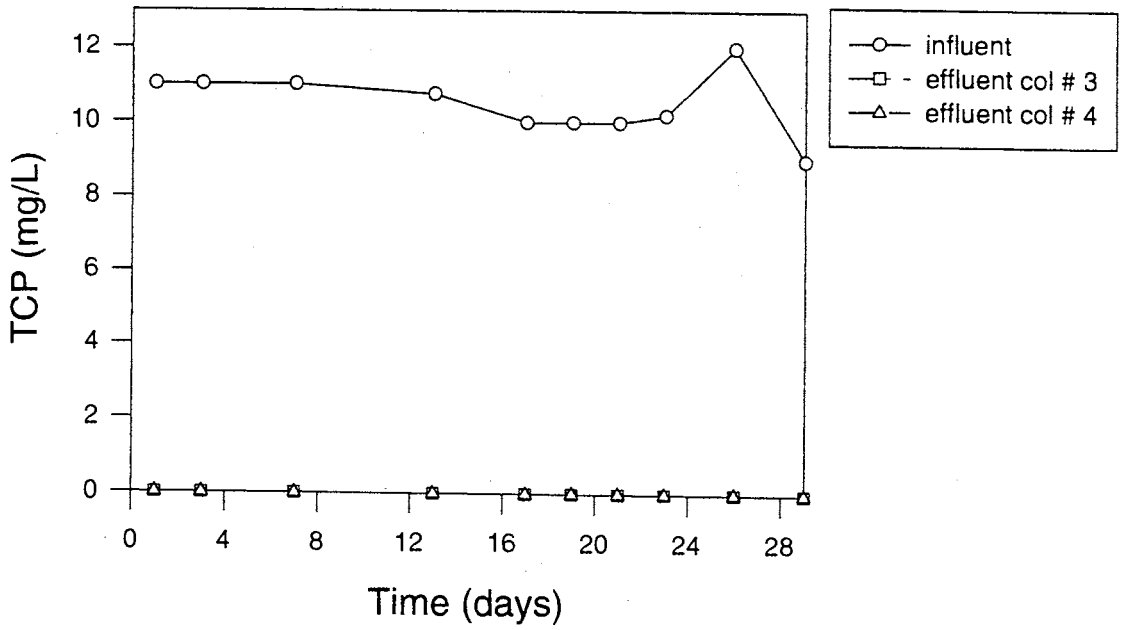

Figure 17. Removal of TCP by GAC columns #3 (10.0 cm) and #4 (20.0 cm) during column study No. 1.

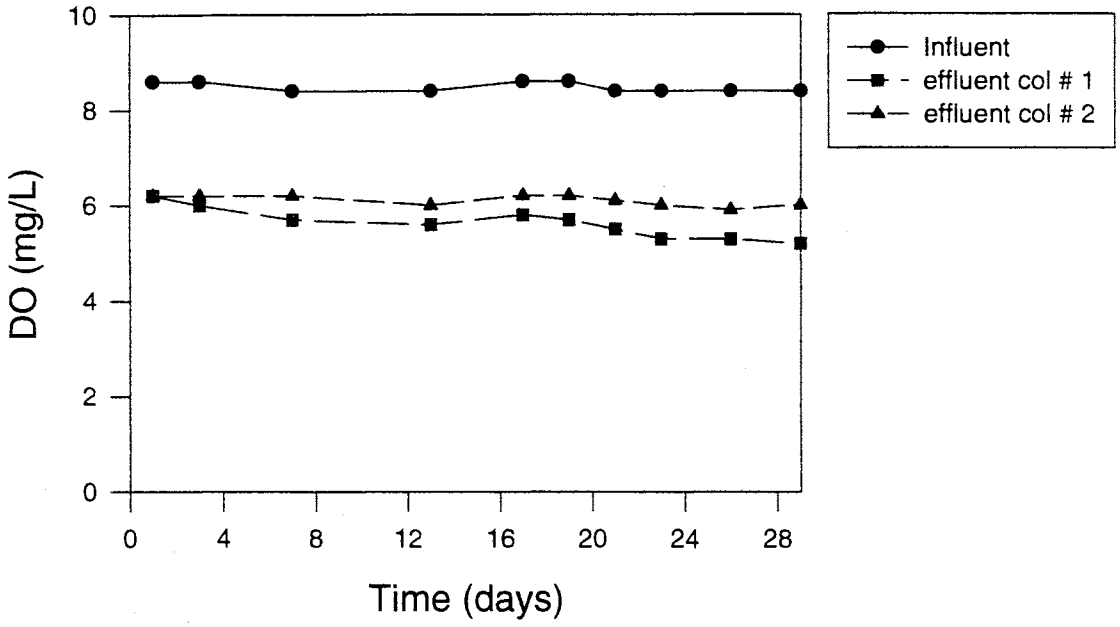

Figure 18. Comparison of DO-- PVA column #1 (10.0 cm) and #2 (20.0 cm) during column study No. 1.

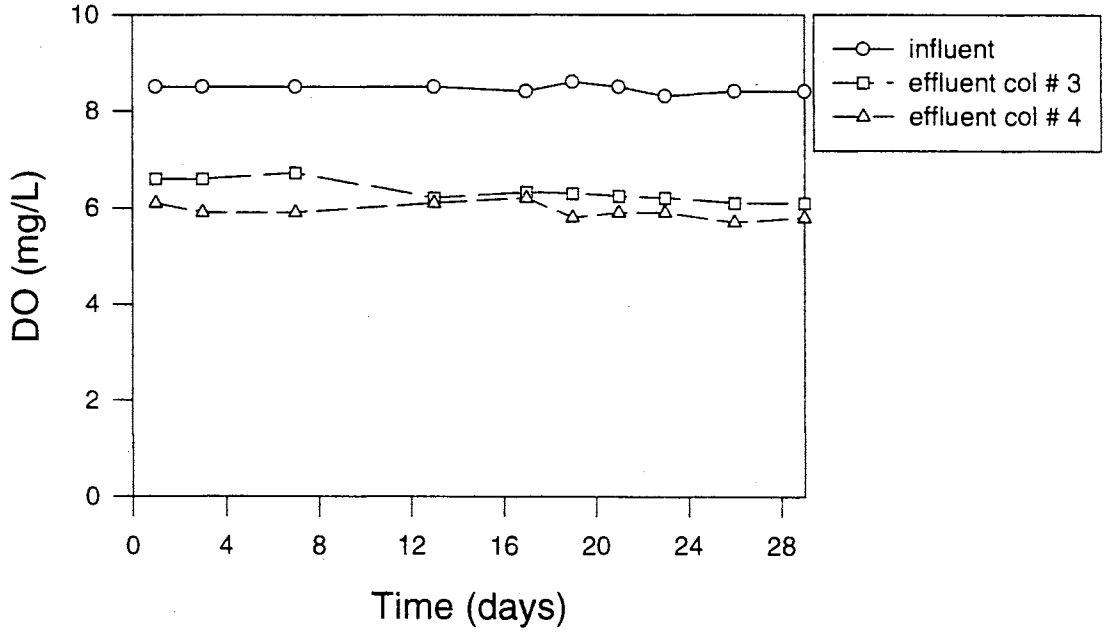

Figure 19. Comparison of DO-- GAC columns #3 (10.0 cm) and #4 (20.0 cm) during column study No.1.

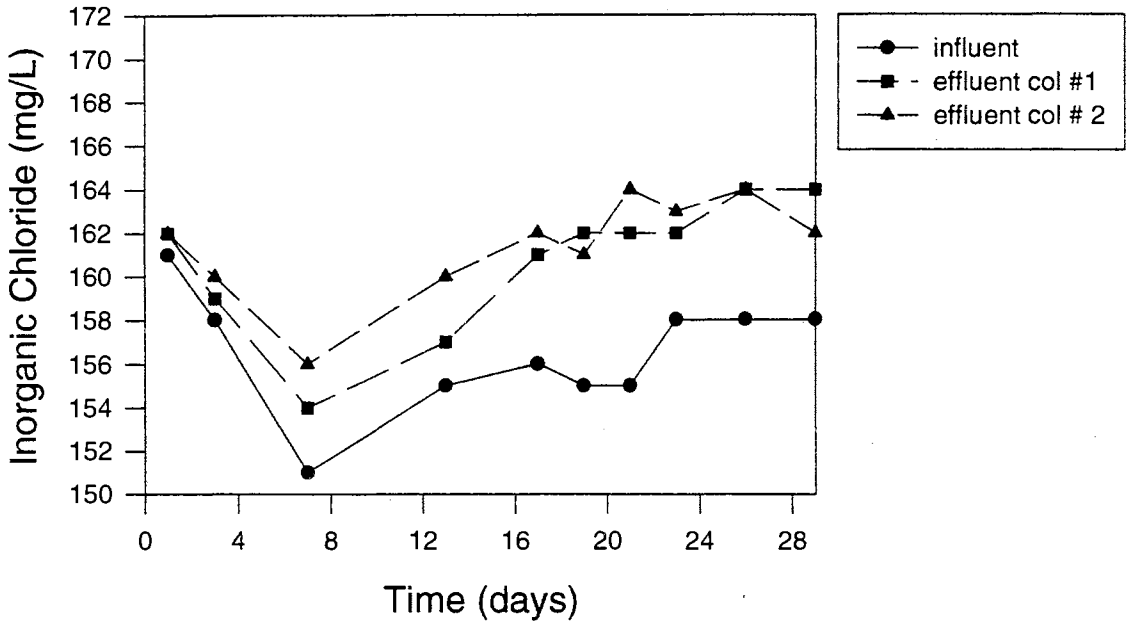

Figure 20. Comparison of Cl^- --PVA columns #1 (10.0 cm) and #2 (20.0 cm) during column study No.1.

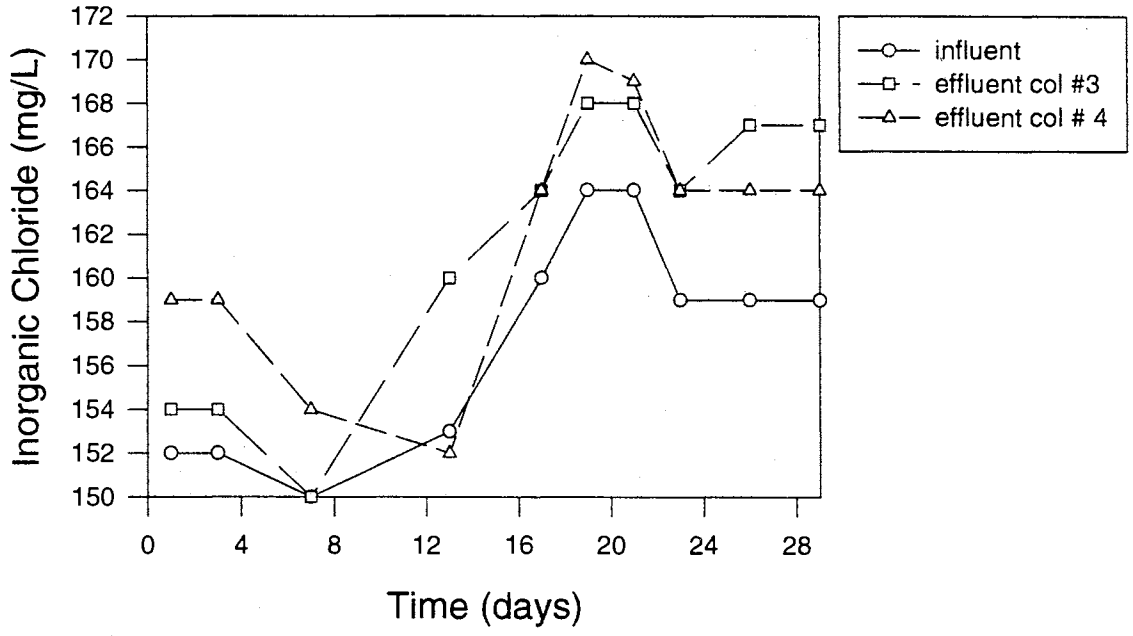

Figure 21. Comparison of Cl^- -- GAC columns #3(10.0 cm) and #4 (20.0 cm) during column study No.1

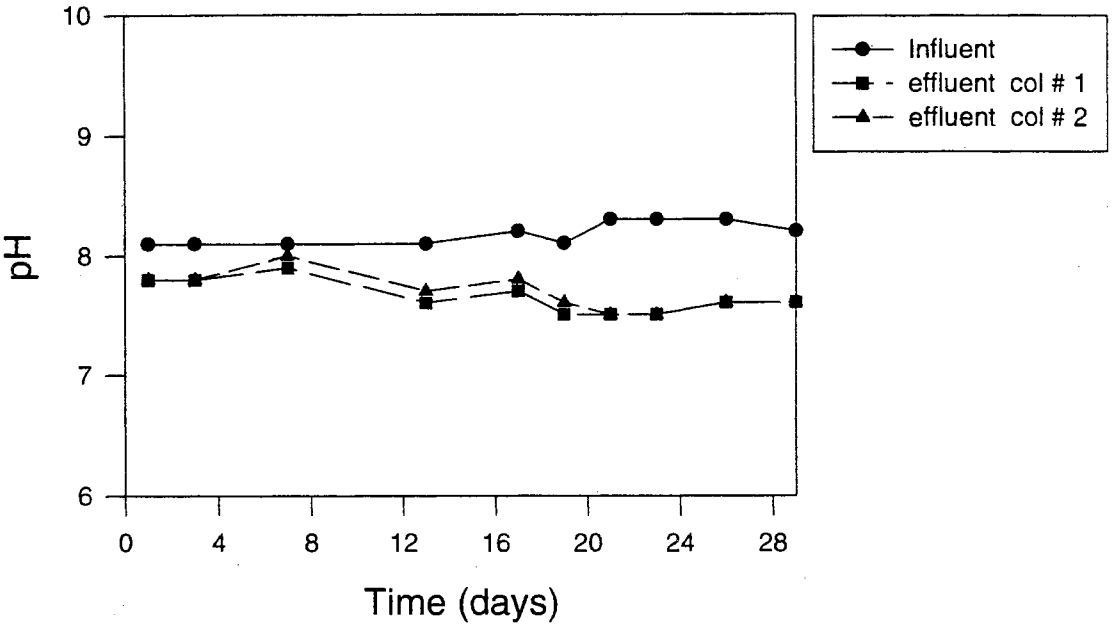

Figure 22. pH Comparison--PVA columns #1 (10.0 cm) and #2 (20.0 cm) during column study No.1.

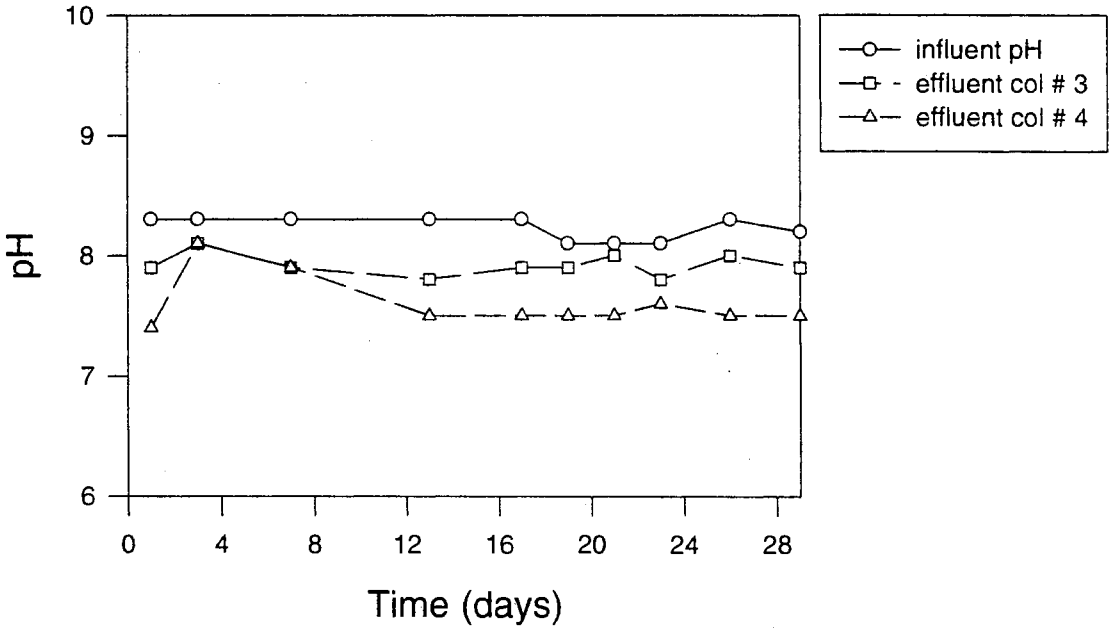

Figure 23. pH Comparison--GAC columns #3 (10.0 cm) and #4 (20.0 cm) during column study No.1.

Column Study No.2

The purpose of this experiment was to evaluate the performance of all four columns where the average TCP influent concentration changed from 10.5±0.83 mg/L to 20.34±0.45 mg/L. The flow rates for all columns remained at 1.0 ml/min. The applied loading for columns (#1, #3) and (#2, #4) were 0.15 and 0.074 g L^{-1} d^{-1}, respectively. The addition of nutrients to the influent feed solution kept the C:N:P ratio at 100:18:188. The influent solution was aerated with compressed laboratory air 24.0 hours a day to maintain DO levels above 8.4 mg/L. The amount of DO provided for this experiment was less than the DO needed for complete degradation of 20.0 mg/L of TCP. This experiment lasted 25 days.

The 100 mLs effluent samples were collected under nitrogen blanket. The samples were analyzed for TCP concentration, DO, chloride release, and pH measurement. The 300 mLs influent sample was pumped out and collected in BOD bottle for DO analysis. Another 100 mLs influent sample was also collected and analyzed for TCP concentration, chloride release, and pH. The raw data for this experiment are presented in Appendix G.

TCP Concentrations: The TCP influent and effluent concentrations for PVA and GAC columns are shown in Figures 24 and 25. Both PVA columns were able to remove TCP from the influent for up to a week. On day 40 TCP was detected in the effluent of both PVA columns. As seen in Figure 26 the effluent TCP concentration in column #1 was 1.2 mg/L on day 40. The TCP effluent concentration in column #1 continued to rise and reached 6.5 mg/L on day 58. The TCP effluent concentration in column #2 also starts to rise on day 40 and reached its maximum concentration of 4.9 mg/L on day 58.

The removal efficiencies of column #1 and #2 reduced from 100% to 68.0 % and 76.0 %, respectively. The explanation for this occurrence during this period is that the DO was insufficient for complete biodegradation of 20.0 mg/L of TCP. For aerobic mineralization, each mg of TCP would require 0.89 mg of oxygen to be consumed by the bacteria. For aerobic mineralization of 20.0 mg/L of TCP, 17.2 mg/L of DO was needed. The average DO provided during this experiment was 8.7 mg/L.

As Figure 27 shows no TCP was ever detected in the effluents of GAC columns #3 and #4 over the entire period of this experiment. An explanation for this might be that TCP removal was occurring by both adsorption and biodegradation.

Dissolved Oxygen (DO): As seen in Figure 26 the effluents of PVA columns #1 and #2 have an average DO of 2.9 ± 0.5 mg/L and 3.1 ± 0.4 mg/L, respectively. This was a clear indication of biological activity occurring in both PVA columns. The cells in PVA columns #1 and #2 were able to consume about 66% and 64% of the average 8.6 mg/L DO in the influent, respectively. The average DO provided for this experiment is about 50% less than the DO needed for aerobic mineralization of 20.0 mg/L TCP.

Figure 27 shows that the effluents of GAC columns #3 and #4 have an average DO of 3.0 mg/L and 3.1 mg/L, respectively. The cells in GAC columns #3 and #4 were also able to consume about 66% and 64%, respectively, of the average 8.6 mg/L DO in the influent.

Inorganic Chloride Released: The ICl concentrations of influent and effluents were measured and are shown in Figures 28 and 29. All four columns show an increase in the chloride concentrations in their effluent which supports the idea that dechlorination of the TCP is occurring. Average chloride releases were 10.8 mg/L, 9.2

mg/L, 8.8 mg/L, 10.1 mg/L for PVA(#1), PVA(#2), GAC(#3), GAC(#4), respectively. For complete dehalogenation of 20.35±1.2 mg/L TCP, an average of 10.99 mg/L inorganic chloride release was expected. During the first 11 days of this experiment, the average chloride releases for columns #1, #2, #3, and #4 were 12.4, 10.1, 9.7, and 11.5 mg/L, respectively. From day 44 to day 58, the average chloride releases were reduced to 7.9, 8.1, 7.8, and 8.7 mg/L for columns #1, #2, #3, and #4, respectively. The reduction in chloride releases in all four columns tends to support the reduction in biodegradation of TCP because of insufficient DO for complete mineralization of TCP.

pH Changes: As seen in Figures 30 and 31, the drop in pH for the first 11 days were greater than for the last 14 days for all four columns. An approximate average effluent pHs for the first 11 days of the experiment for columns #1, #2, #3, and #4 were 7.6, 7.4, 7.7, and 7.6, respectively. For the last 14 days of the experiment, the effluent pHs for all four columns were 8.1. The drop in pH tends to support the concept of dehalogenation of TCP and formation of HCl in the effluents. The smaller drop in pHs of all four columns from day 11 to day 58 correlates well with the smaller chloride release measured possibly due to insufficient DO for complete mineralization of 20.0 mg/L TCP.

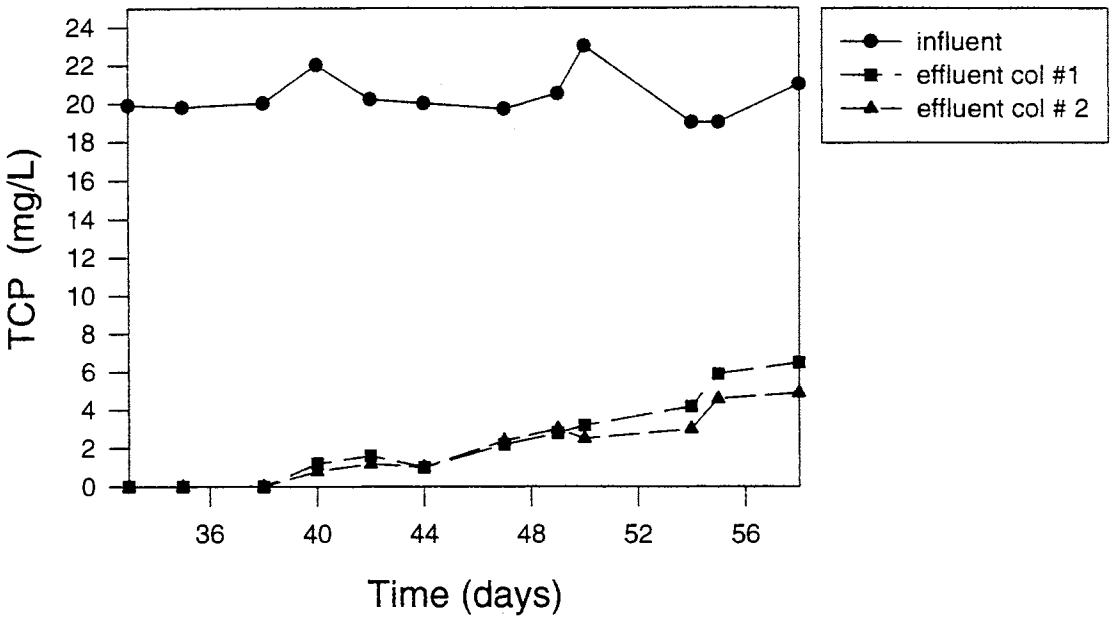

Figure 24. Removal of TCP by PVA columns #1 (10.0 cm) and #2 (20.0 cm) during column study No.2.

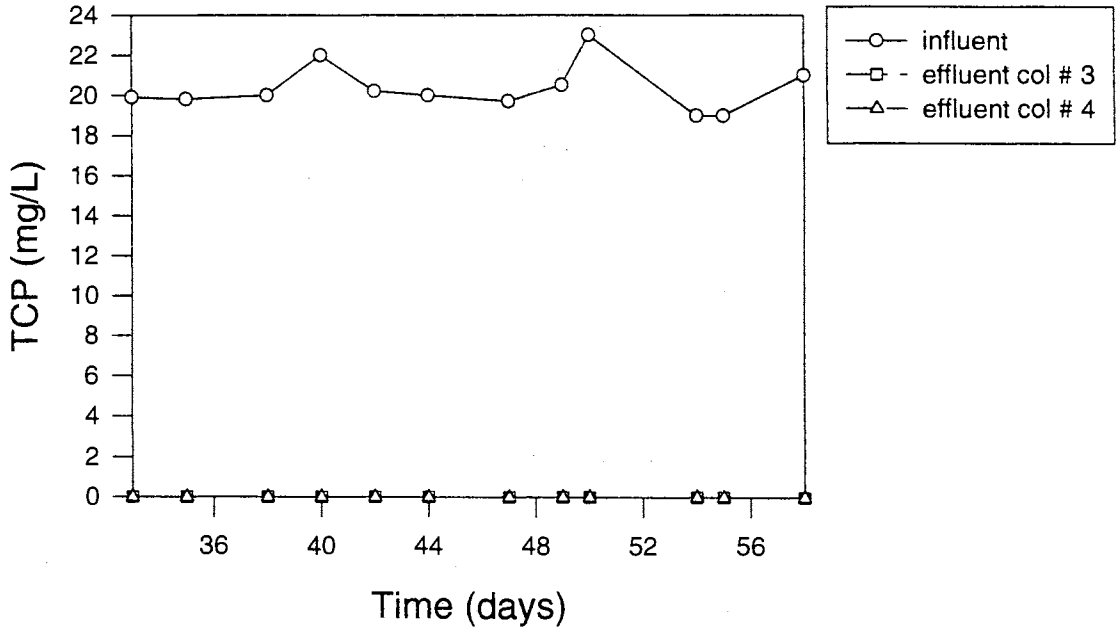

Figure 25. Removal of TCP by GAC columns #3 (10.0 cm) and #4 (20.0 cm) during column study No. 2.

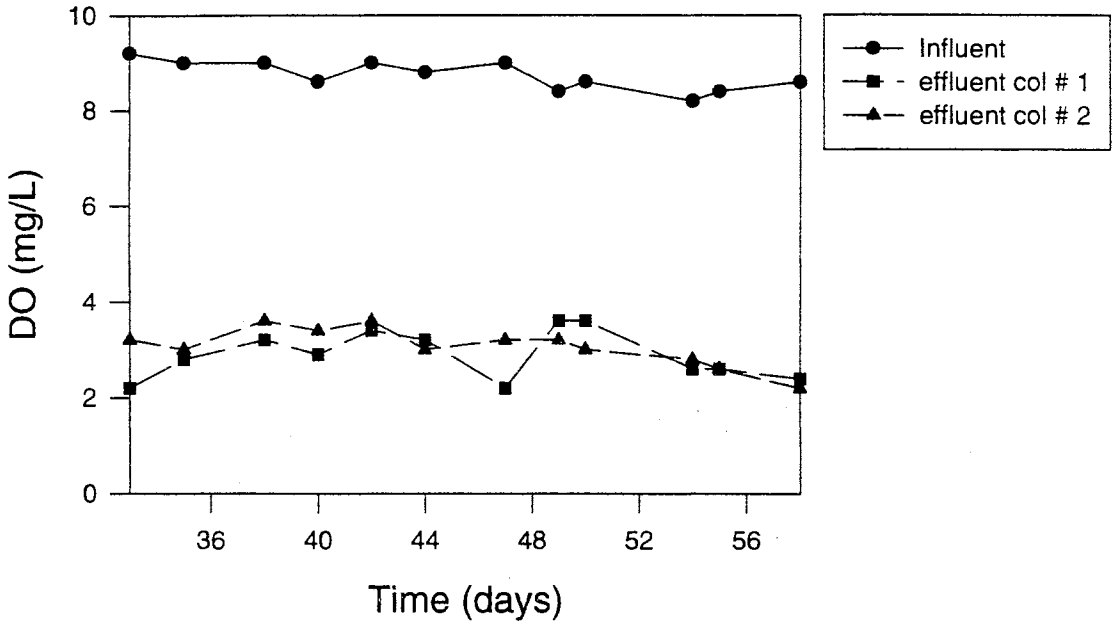

Figure 26. Comparison of DO-- PVA column #1 (10.0 cm) and #2 (20.0 cm) during column study No.2.

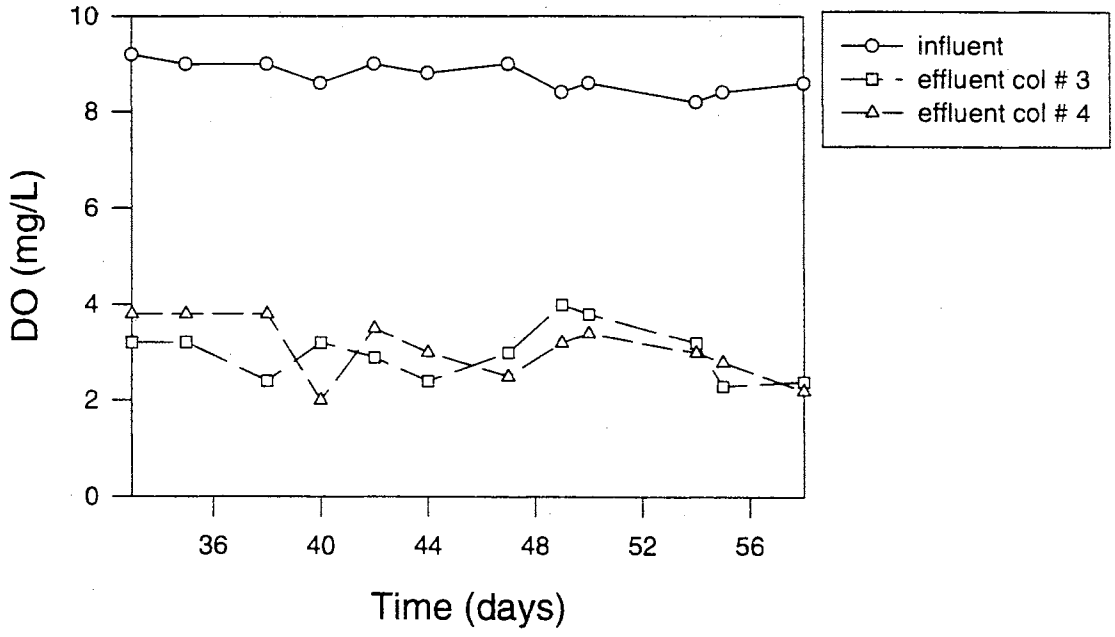

Figure 27. Comparison of DO-- GAC columns #3 (10.0 cm) and #4 (20.0 cm) during column study No.2.

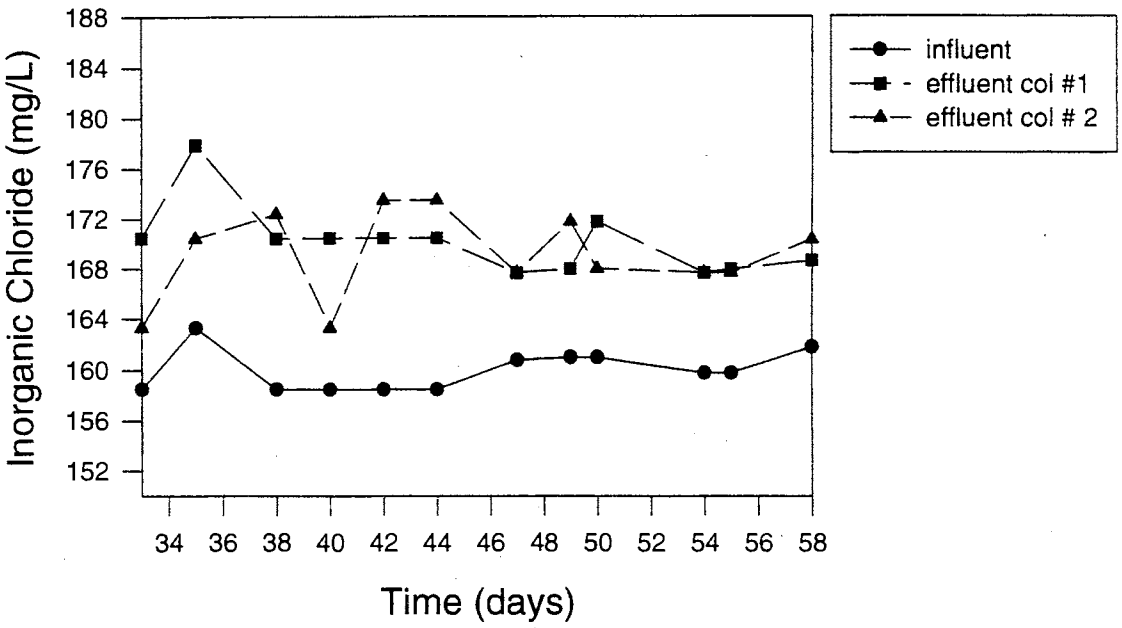

Figure 28. Comparison of Cl^- --PVA columns #1 (10.0 cm) and #2 (20.0 cm) during column study No.2.

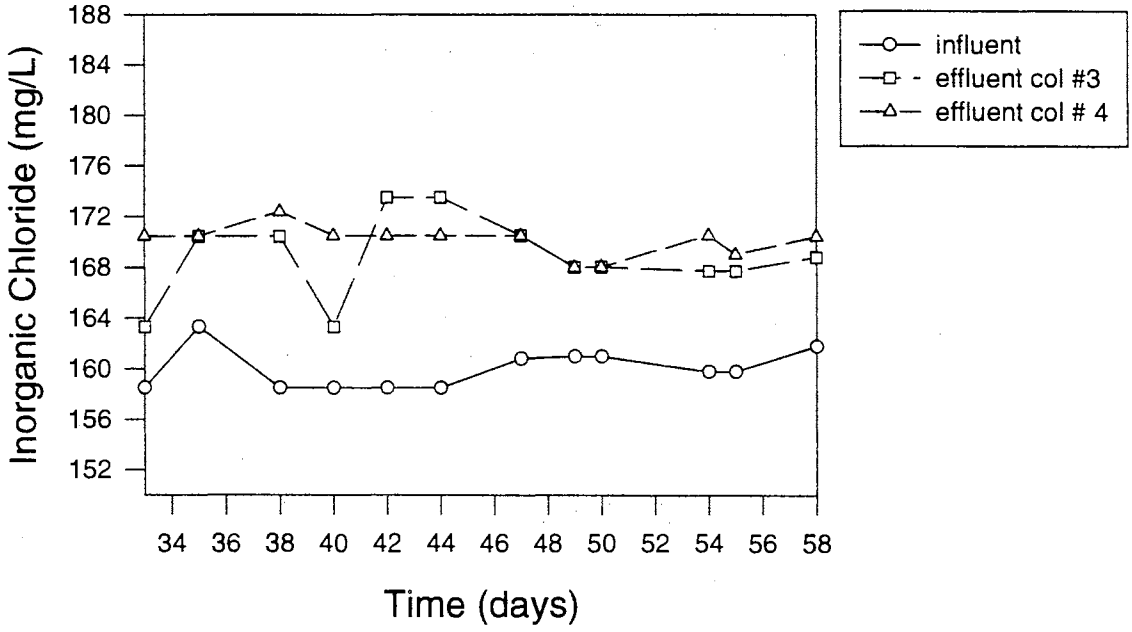

Figure 29. Comparison of Cl^--- GAC columns #3(10.0 cm) and #4 (20.0 cm) during column study No.2.

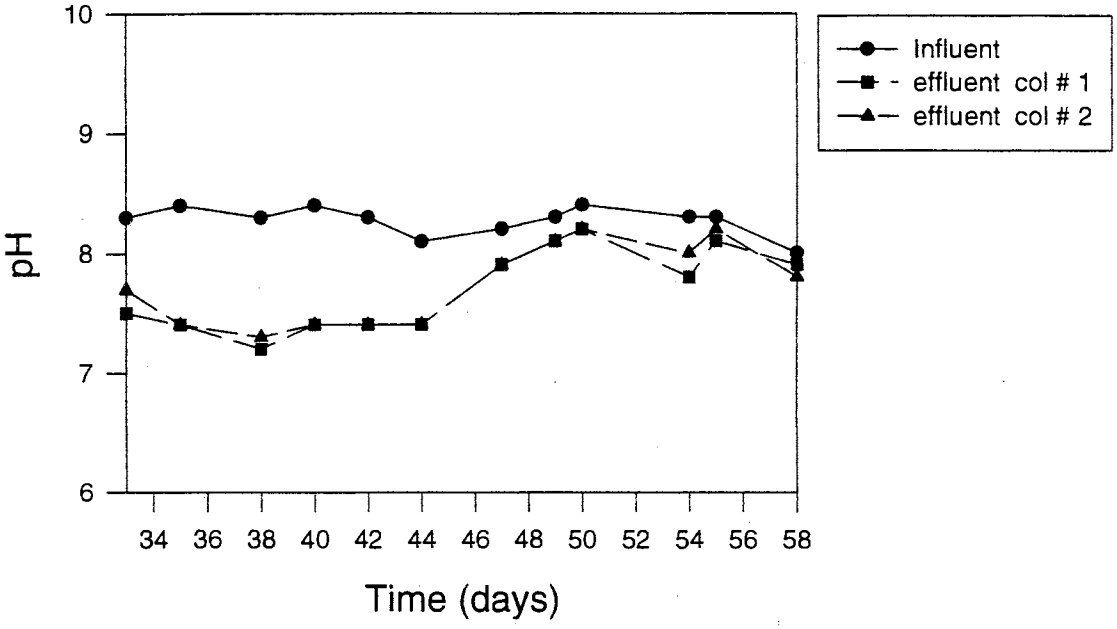

Figure 30. pH Comparison--PVA columns #1 (10.0 cm) and #2 (20.0 cm) during column study No.2.

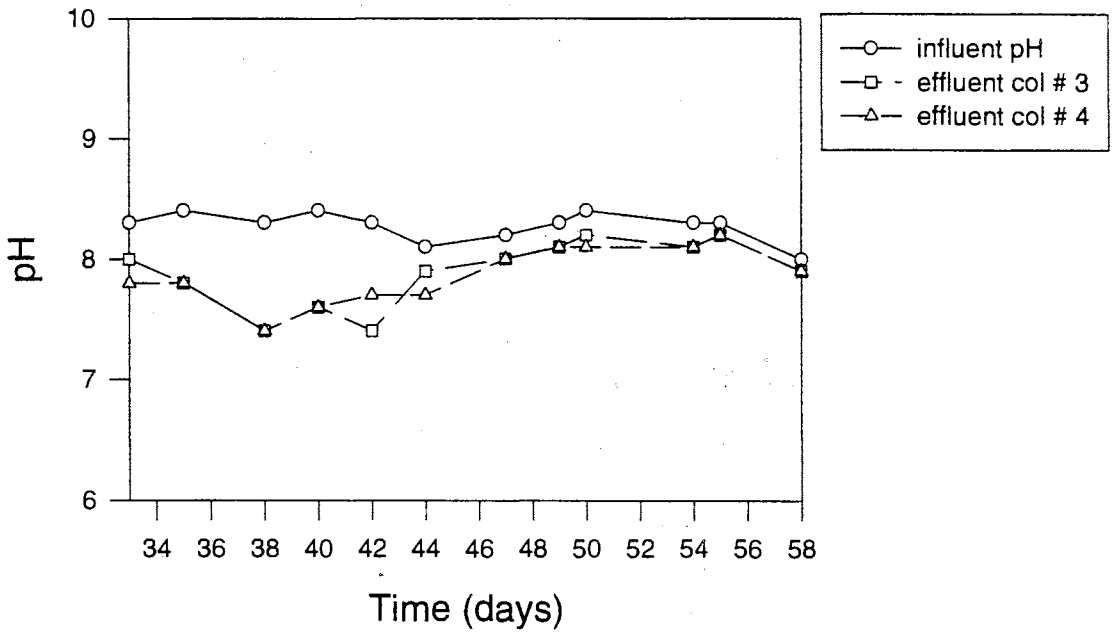

Figure 31. pH Comparison--GAC columns #3 (10.0 cm) and #4 (20.0 cm) during column study No.2.

Column Study No. 3

The purpose of this experiment was to improve the performance of all four columns observed during the later stages of column study No. 2 by providing additional oxygen. For aerobic biodegradation of 20.0 mg/L of TCP, at least 17.8 mg/L of DO are needed. In order to provide sufficient DO for the bacteria, the influent feed solution was oxygenate with pure oxygen for at least 10.0 minutes every day (begining day 63) during the course of this experiment. The influent bottle was almost completely capped to reduce loss of oxygen.

The C:P:N ratio was maintained at 100:18:188 by addition of appropriate nutrients to the influent feed solution. The influent flow rates for all four columns were 1.0 mL/min. The raw data are presented in Appendix H.

TCP Concentrations: Figures 32 and 33 reflect the TCP influent and effluent concentrations for all four columns. As can be seen the PVA and GAC columns reduced the average influent TCP concentration of 20.0 mg/L to zero during the entire period of the experiment. The removal efficiencies of all four columns were 100% which can be due to the sufficient DO provided for the bacteria.

Dissolved Oxygen (DO): As can be seen in Figures 34 and 35, an average influent DO of 23.5 ± 3.5 mg/L was reduced to an average effluent value of 2.9 ± 1.4, 3.2 ± 1.7, 3.0 ± 1.1, and 2.9 ± 1.2 mg/L by columns #1, #2, #3, and #4, respectively. This was a clear indication of microbial activity present in both the PVA (#1,#2) and GAC (#3,#4) columns.

Inorganic Chloride Released: Inorganic chloride concentration in the influent and effluent of PVA columns (#1,#2) and GAC columns (#3,#4) are presented in Figures

36 and 37. Average chloride releases in the PVA columns #1, #2 and GAC columns #3, #4 are 9.19 ± 2.11, 10.25 ± 1.97 and 10.73 ± 2.5, 11.1 ± 1.8 mg/L, respectively. The measured chloride concentration in both PVA and GAC columns effluents are very close in value to the theoretical chloride release expected for dehalogenation of 20.0 mg/L of TCP.

pH Change: The pH values were measured in the influent and effluent of all four columns, and are shown in Figures 38 and 39. The influent feed solution had an average pH of 8.0. Columns #1, #2, #3, and #4 had an approximate average effluent pH values of 7.5, 7.5, 7.6., and 7.5, respectively. From the pH curve, Figure 15, the drop in pH from an influent of 8.0 to 7.5 in the effluents shows that a 2.8 mL volume of 0.1 N HCl would be required. This is a 10.3 mg/L chloride concentration (2.9 mL/L X 3.55 mg/mL) which is similar in value to the chloride concentration of 10.8 mg/L expected based on the stoichiometric dehalogenation of 20.0 mg/L TCP. This tends to support the dehalogenation of TCP.

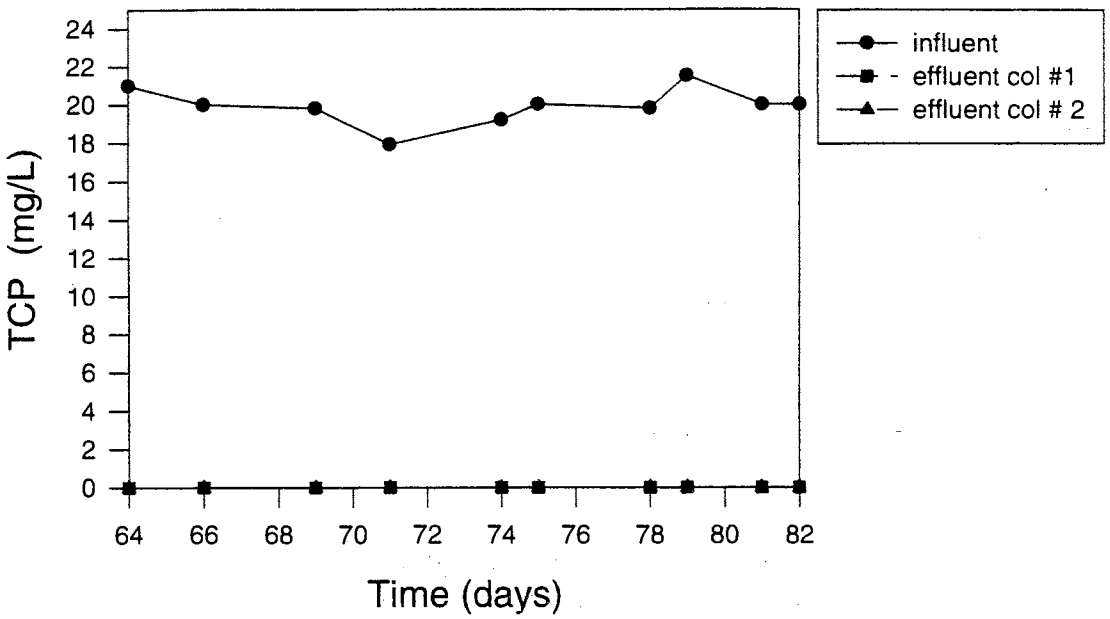

Figure 32. Removal of TCP by PVA columns #1 (10.0 cm) and #2 (20.0 cm) during column study No.3

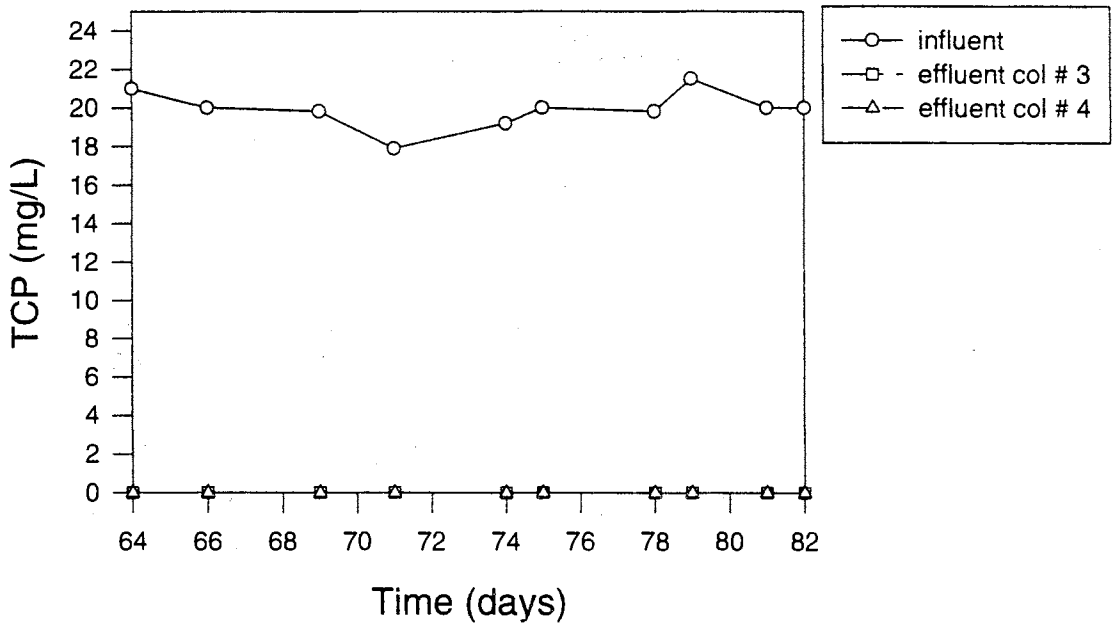

Figure 33. Removal of TCP by GAC columns #3 (10.0 cm) and #4 (20.0 cm) during column study No.3.

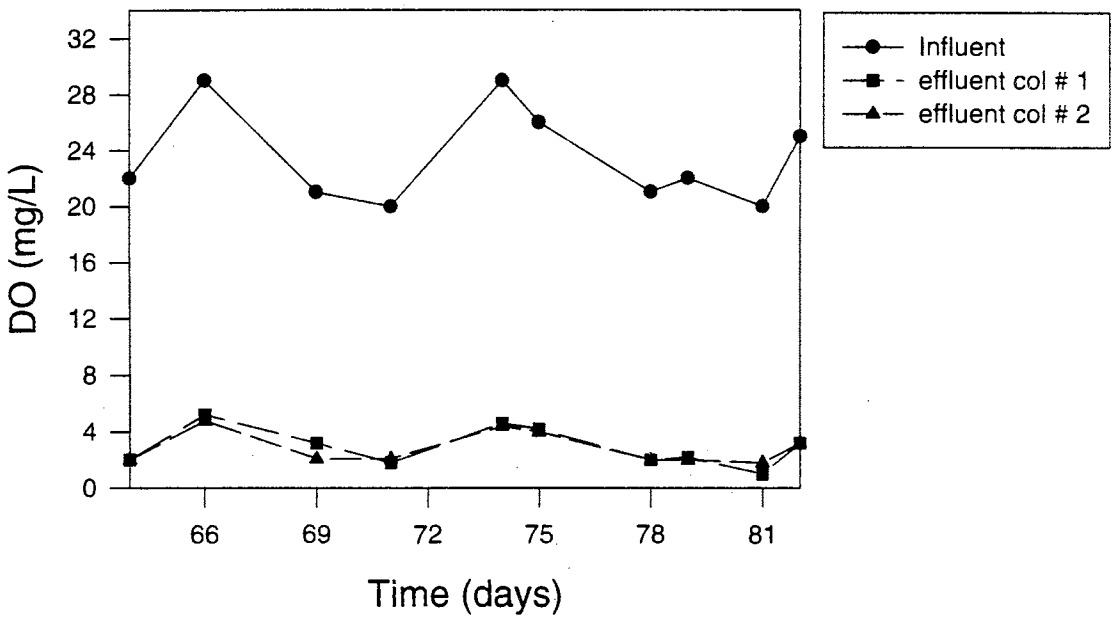

Figure 34. Comparison of DO-- PVA column #1 (10.0 cm) and #2 (20.0 cm) during column study No. 3.

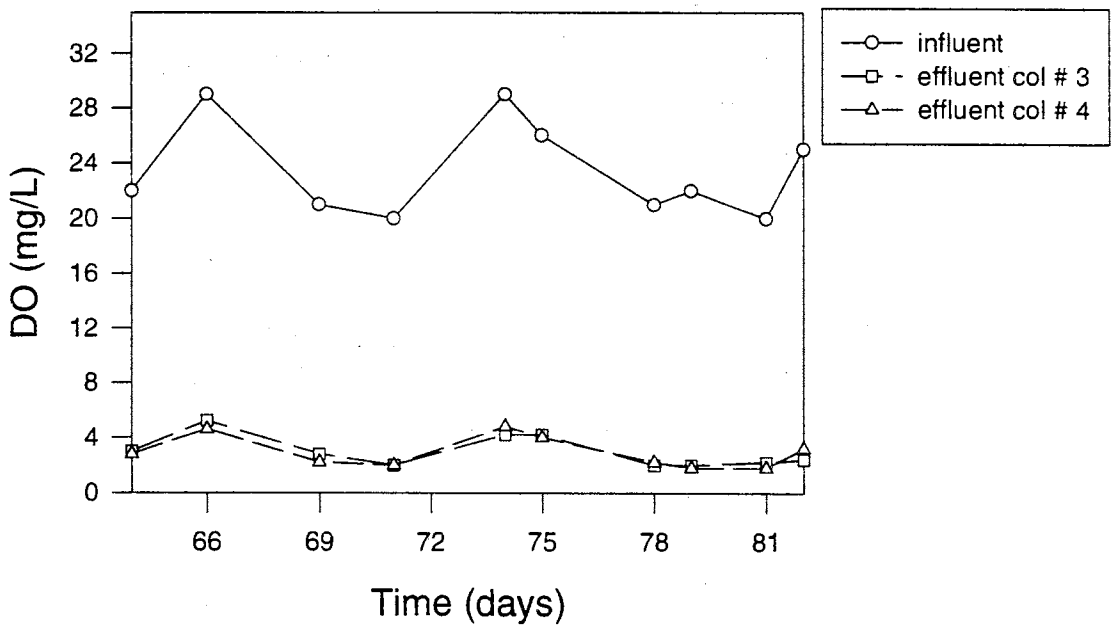

Figure 35. Comparison of DO-- GAC columns #3 (10.0 cm) and #4 (20.0 cm) during column study No.3.

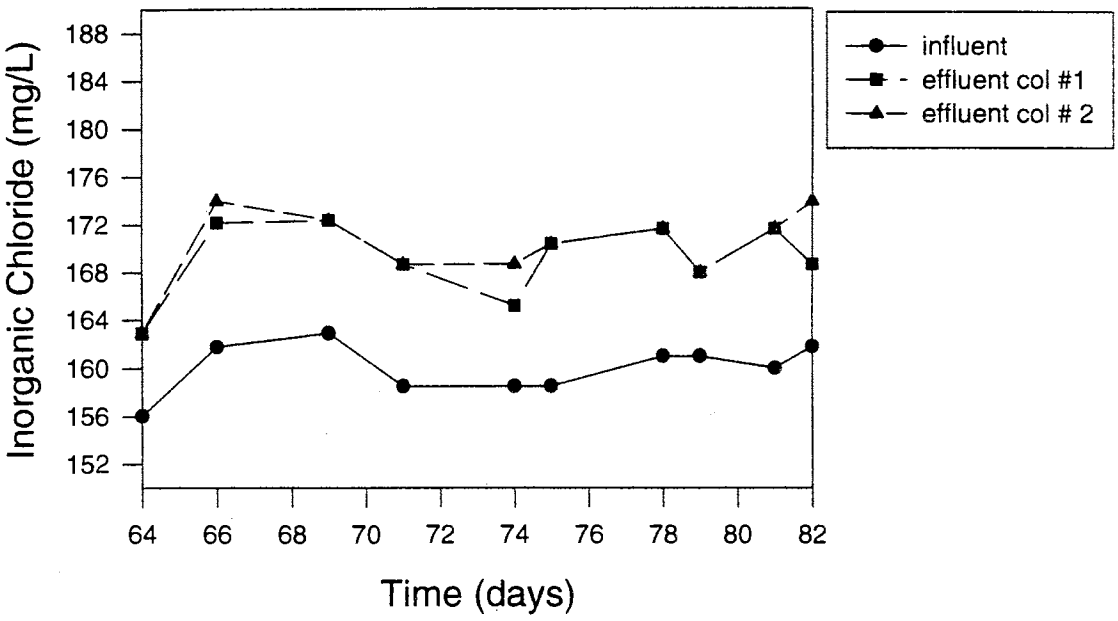

Figure 36. Comparison of Cl^- --PVA columns #1 (10.0 cm) and #2 (20.0 cm) during column study No.3.

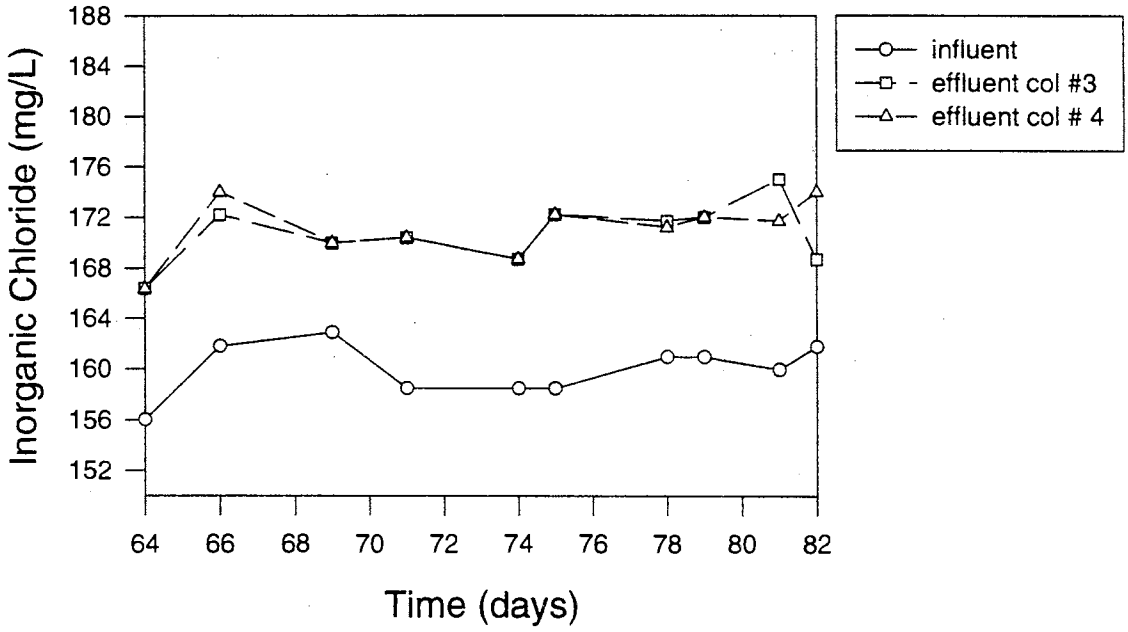

Figure 37. Comparison of Cl^- -- GAC columns #3(10.0 cm) and #4 (20.0 cm) during column study No. 3.

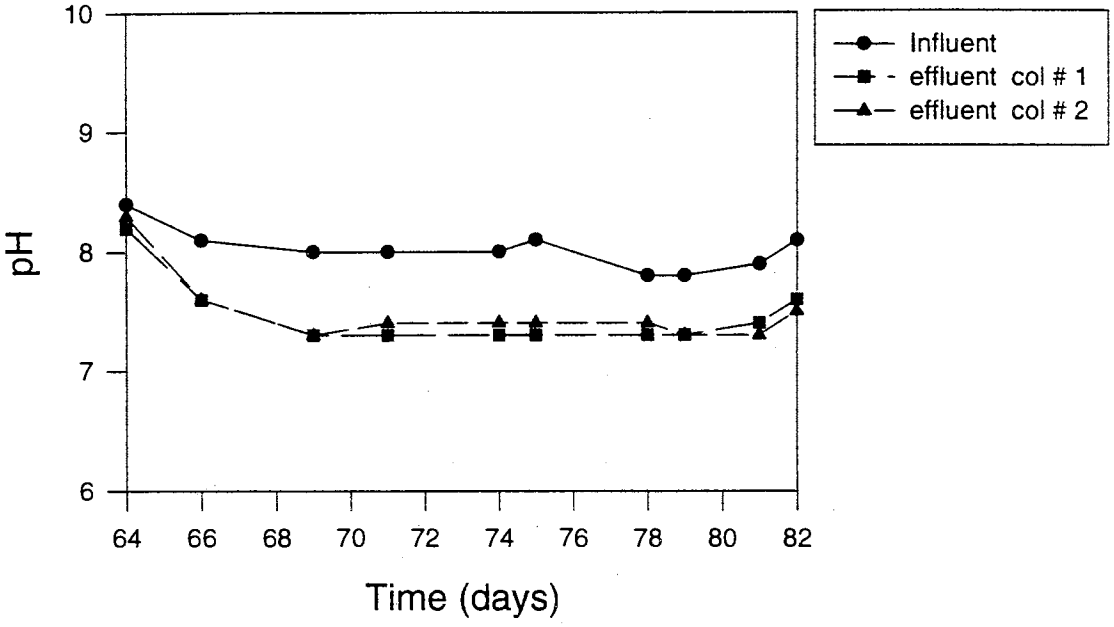

Figure 38. pH Comparison--PVA columns #1 (10.0 cm) and #2 (20.0 cm) during column study No.3

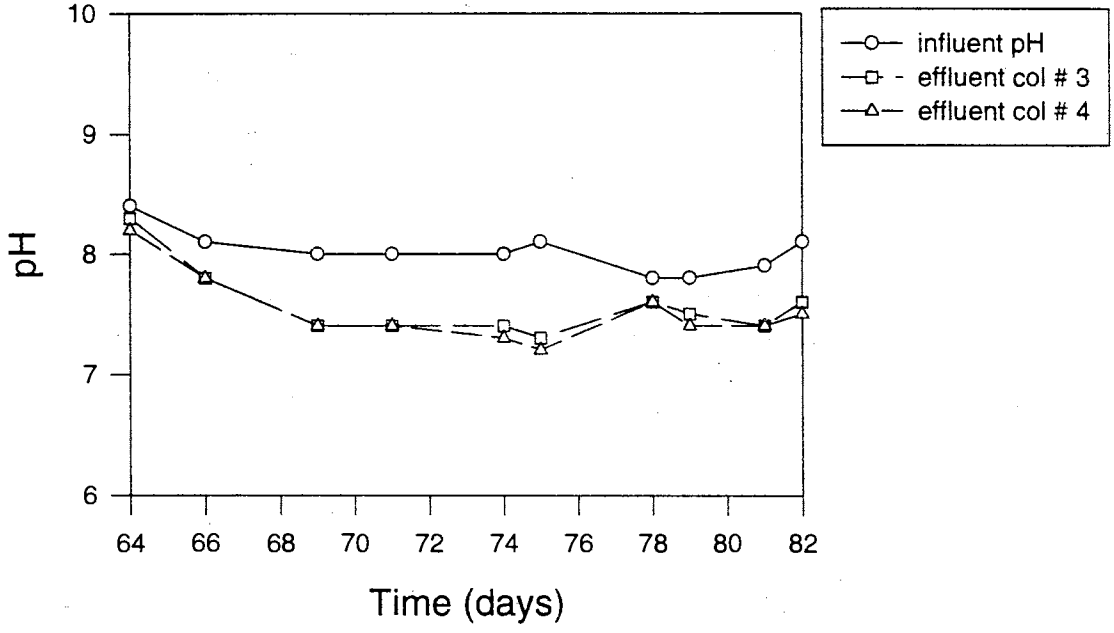

Figure 39. pH Comparison--GAC columns #3 (10.0 cm) and #4 (20.0 cm) during column study No. 3.

Column Study No. 4

The C:N:P nutrient ratio used in the first three column studies was 100:18:188. The standard ratio for C:N:P for microorganisms to grow is 100:10:3 (Beltrame *et al.* 1984). In order to avoid unnecessary addition of nutrients, the C:N:P ratio was adjusted from 100:18:188 to 100:10:3. The effect of varying the C:N:P ratio on biodegradation of 20.0 mg/L TCP was evaluated in this experiment. The flow rates for all columns remained at 1 mL/min. The applied loading for all the columns remained the same as column study No. 2. and 3. The influent feed solution was aerated with pure oxygen for 10.0 minutes every day to maintain a DO above 20.0 mg/L. The raw data for this experiment are presented in Appendix I.

TCP Concentration: The TCP influent and effluent concentrations for the PVA and GAC columns are shown in Figures 40 and 41. No TCP was detected during the entire period of this experiment. It is clear that the change in C:N:P ratio did not negatively effect the removal of 20.0 mg/L TCP by both PVA and GAC columns. The results of this experiment can be directly compare to column study No. 3.

Dissolved Oxygen (DO): The dissolved oxygen for the influent and effluents of PVA columns (#1,#2) and GAC columns (#3,#4) were measured and presented in Figures 42 and 43. All four columns (#1,#2,#3,#4) continued to reduced the DO of 22.8 $\pm$ 3.3 mg/L in feed solution to effluent value of 4.0 $\pm$ 0.7, 4.0 $\pm$ 0.51, 4.0 $\pm$ 0.6, and 3.9 $\pm$ 0.7 mg/L, respectively. The consumption of DO is a clear indication of biological activity in the columns. The cells in all four columns were able to use DO efficiently and remove 20.0 mg/L of TCP during the entire course of this experiment.

Inorganic Chloride Release: Figures 44 and 45 represent the measured chloride concentrations in the influent and effluents of PVA and GAC columns. The average chloride increase in the effluents for columns #1,#2,#3, and #4 are 11.1± 1.9, 11.6± 1.8, 11.3 ± 3.5, and 11.2 ± 2.2 mg/L, respectively. Aerobic dehalogenation of 20.0 mg/L TCP should theoretically release 10.8 mg/L inorganic chloride, which is very close to measured inorganic chloride of both PVA and GAC columns.

pH Change: Figures 46 and 47 show pH measurements of influent and effluent the PVA and GAC columns, respectively. The influent feed solution had an approximate average pH of 7.9. The approximate average of the effluent pH for both PVA columns #1 and #2 was 7.5. The approximate average of the effluent pH for both GAC columns #3 and #4 was 7.6. According to the pH curve, the drop in pH from 7.9 to 7.5 shows that a 2.9 mL volume of 0.1 N HCl would be required. This is a 10.3 mg/L chloride concentration (2.9 mL/L X 3.55 mg/mL) which is similar in value to the theoretical chloride concentration of 10.8 mg/L expected from the complete dehalogenation of 20.0 mg/L TCP. This tends to support the theory of complete dehalogenation of TCP.

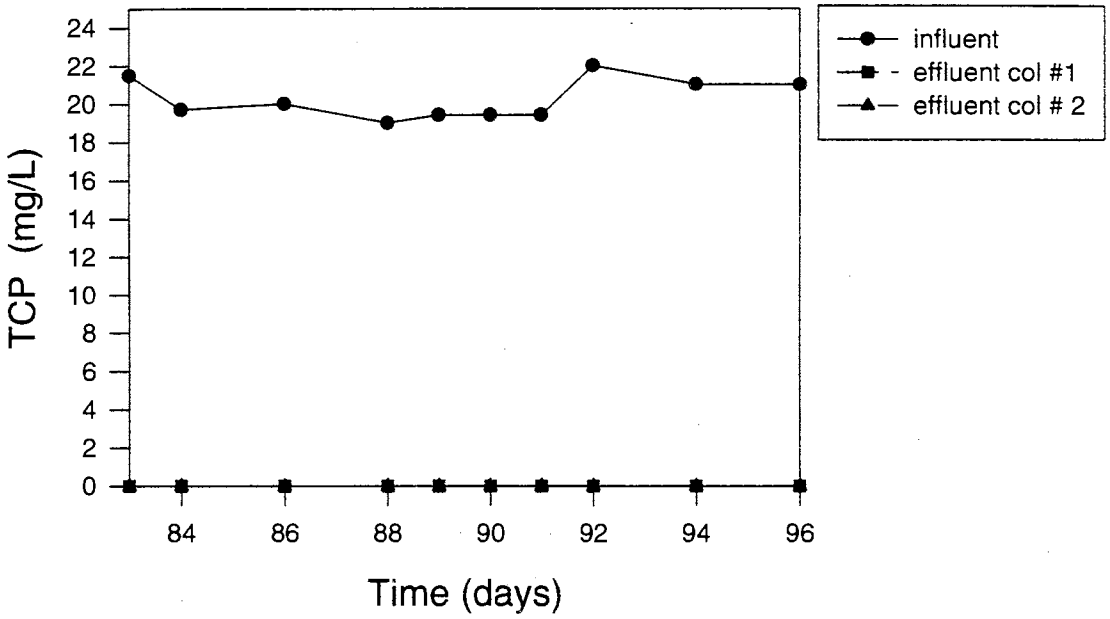

Figure 40. Removal of TCP by PVA columns #1 (10.0 cm) and #2 (20.0 cm) during column study No.4.

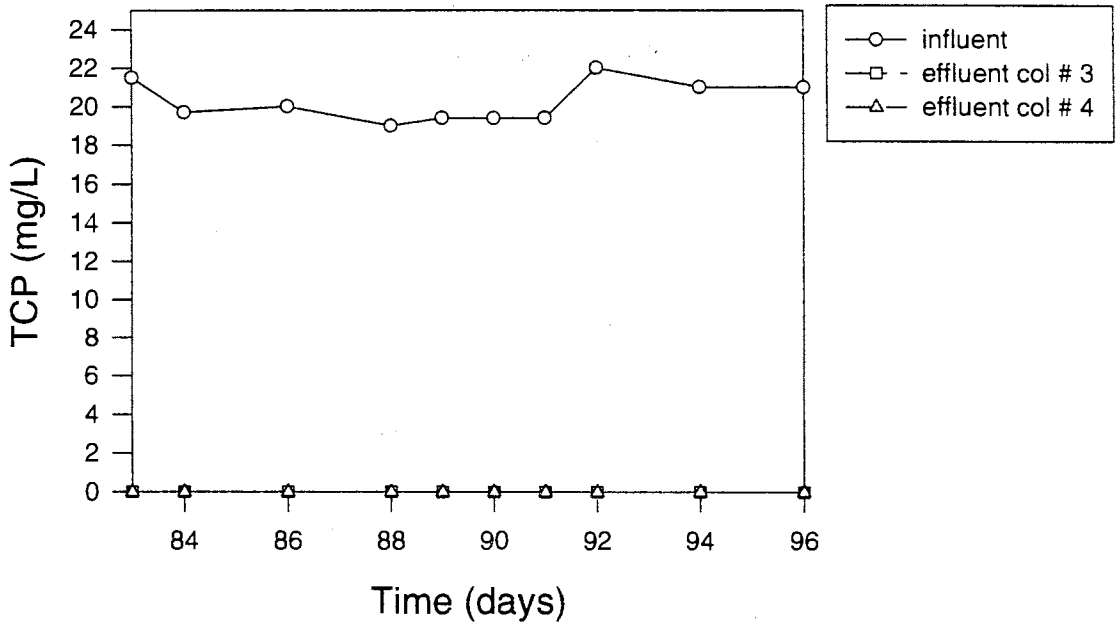

Figure 41. Removal of TCP by GAC columns #3 (10.0 cm) and #4 (20.0 cm) during column study No.4 .

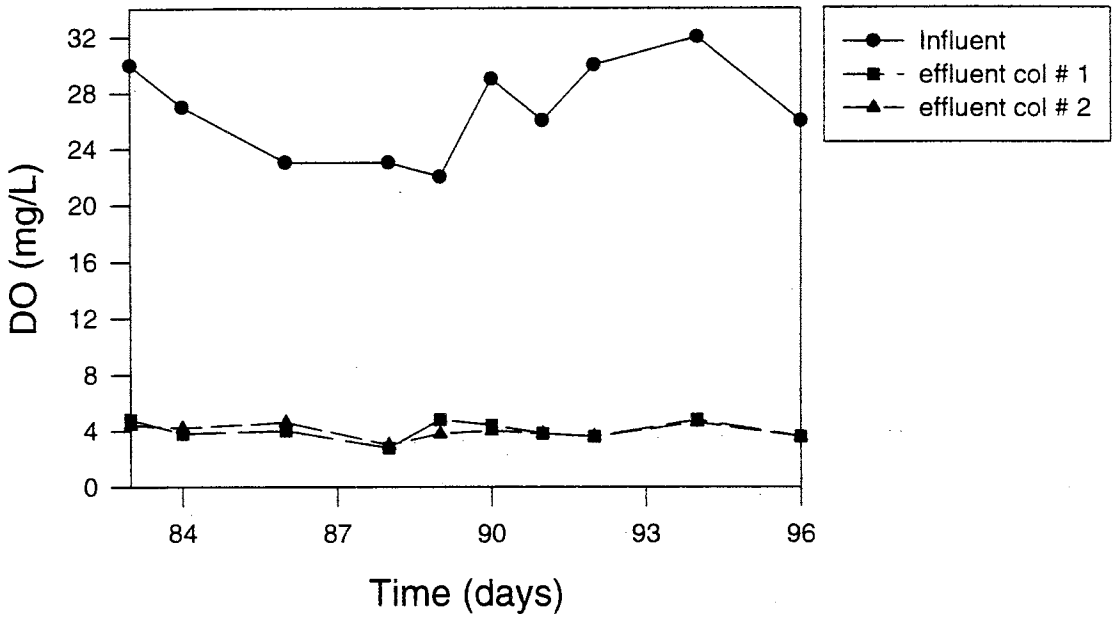

Figure 42. Comparison of DO-- PVA column #1 (10.0 cm) and #2 (20.0 cm) during column study No. 4.

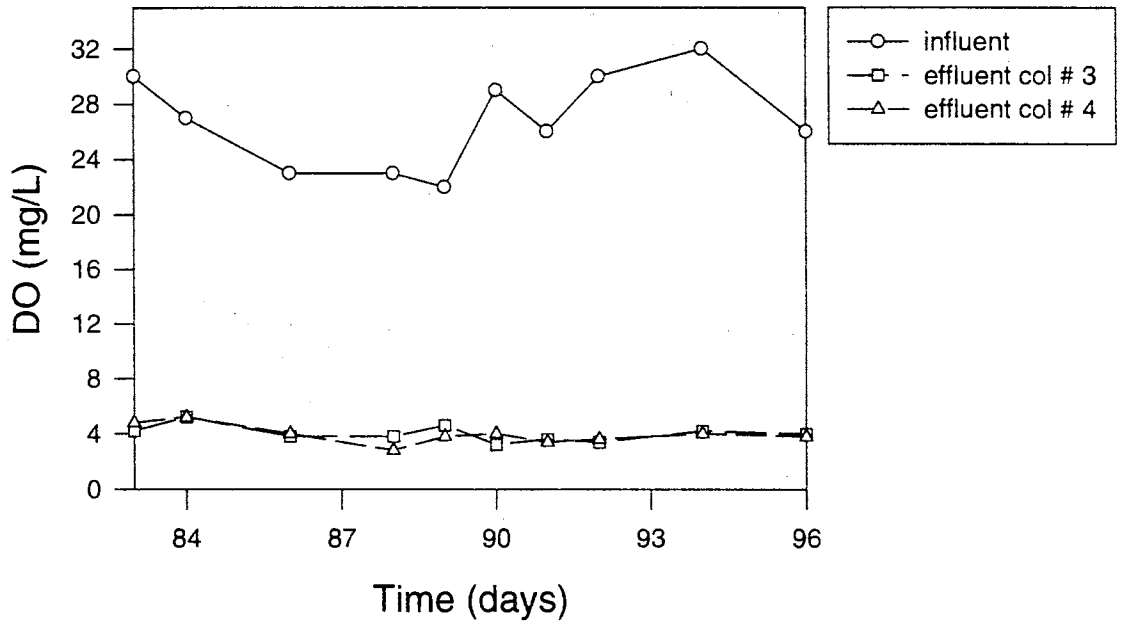

Figure 43. Comparison of DO-- GAC columns #3 (10.0 cm) and #4 (20.0 cm) during column study No.4.

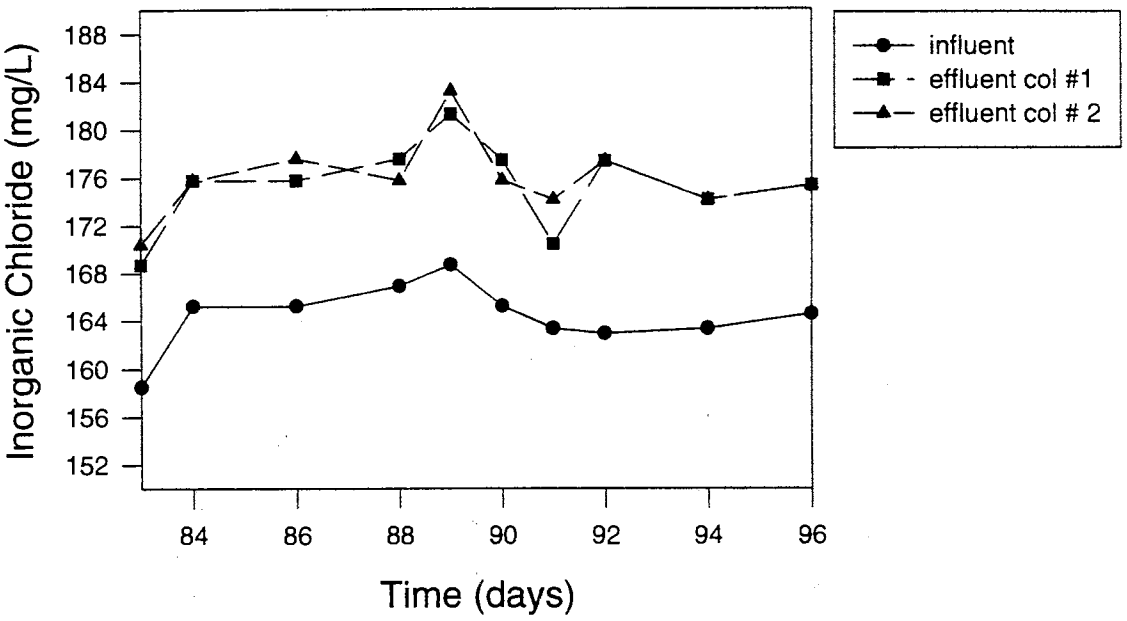

Figure 44. Comparison of Cl^- --PVA columns #1 (10.0 cm) and #2 (20.0 cm) during column study No.4.

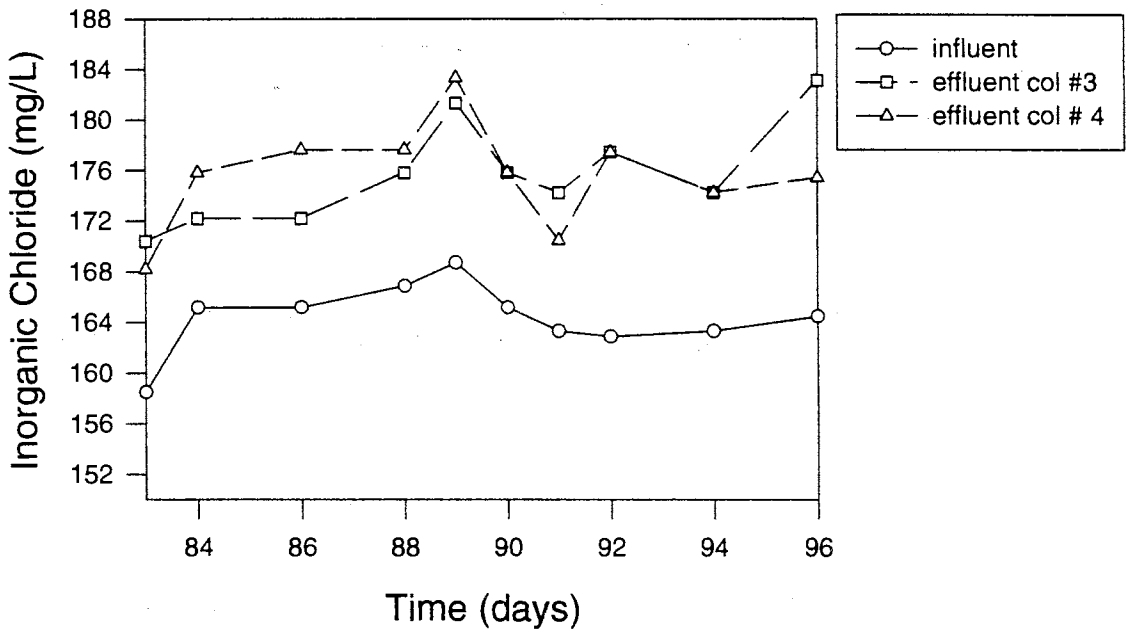

Figure 45. Comparison of Cl^- -- GAC columns #3(10.0 cm) and #4 (20.0 cm) during column study No.4.

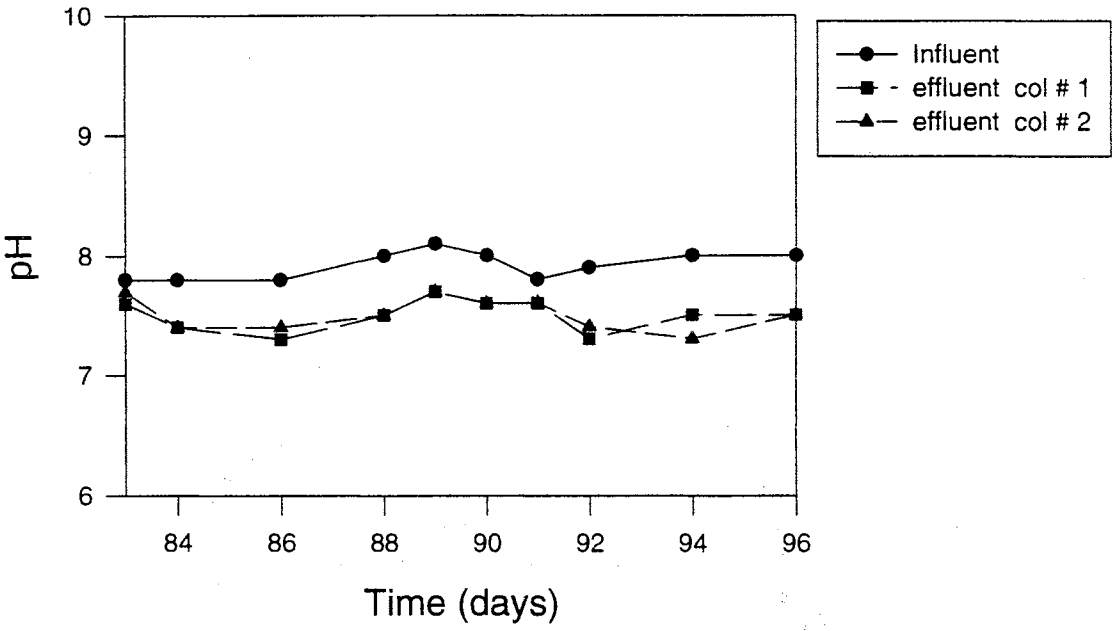

Figure 46. pH Comparison--PVA columns #1 (10.0 cm) and #2 (20.0 cm) during column study No.4.

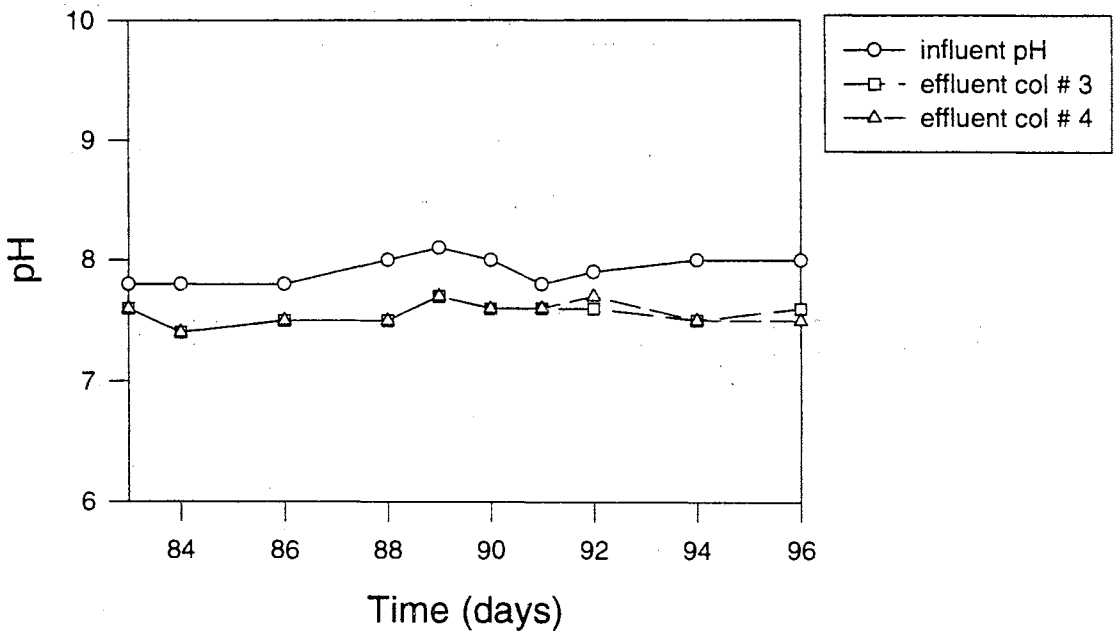

Figure 47. pH Comparison--GAC columns #3 (10.0 cm) and #4 (20.0 cm) during column study No.4.

Column Study No. 5

The purpose of this experiment was to evaluate the removal efficiency of all four columns when TCP concentration increases to 30.0 mg/L. The flow rate for all columns remained at 1 mL/min. The applied loading for the columns (#1,#3) and (#2, #4) were 0.22 g L^{-1} d^{-1} and 0.11 g L^{-1} d^{-1} , respectively. The influent feed solution was aerated with pure oxygen for 10.0 -15.0 minutes every day to maintain DO of above 27.0 mg/L. The influent bottle was almost completely capped to reduce loss of oxygen. The raw data for this experiment are presented in Appendix J.

TCP Concentration: The TCP influent and effluent concentrations for PVA and GAC columns are shown in Figures 48 and 49. The TCP removal efficiency for PVA columns #1 and #2 was 98-100%. The removal efficiency of both GAC columns was 100% during the entire period of the experiment. It is clear that the increase in TCP concentration did not effect the removal efficiencies of both the PVA and GAC columns.

Dissolved Oxygen (DO): The dissolved oxygen for the influent and effluents of the PVA columns(#1,#2) and GAC columns(#3,#4) were measured and presented in Figures 50 and 51. All four columns (#1,#2,#3,#4) continued to reduce the influent DO of 30.3 ± 1.6 mg/L in feed solution to 3.0 ± 0.6, 2.9 ± 0.7, 2.9 ± 0.5, and 2.4 ± 0.5 mg/L, respectively, was an indication of biological activity in the columns. The cells in all four columns were able to use DO efficiently and removed 30.0 mg/L of TCP during the entire course of this experiment.

Inorganic Chloride Release: Figures 52 and 53 represent the measured chloride concentrations in the influent and effluent of the PVA and GAC columns. Average chloride concentrations in the effluent for columns #1,#2,#3, and #4 were 15.7± 4.5, 17.1

± 4.9, 18.1 ± 3.3, and 18.9 ± 3.2 mg/L, respectively. Aerobic dehalogenation of 30.0 mg/L of TCP should release 16.2 mg/L chloride. The measured values for all four columns are close to theoretical chloride release for 30.0 mg/L TCP.

pH Change: Figures 54 and 55 show pH measurements of influent and effluents of the PVA and GAC columns, respectively. The influent feed solution had approximate average pH of 8.0. The approximate average effluent pH for both PVA column #1, #2 was 7.2. An approximate average effluent pH for both GAC columns #3 and #4 was 7.3. According to the pH curve, the drop in pH from 8.0 to 7.2, and 7.3 show that 5.0 mL and 4.0 mL volume of 0.1 N HCl would be required. This is a 17.8 mg/L chloride concentration (5.0 mL/L X 3.55 mg/mL) for the PVA columns which is close to the chloride concentration of 16.2 mg/L expected from the dehalogenation of 30.0 mg/L TCP. According to the pH curve, both GAC columns #3 and #4 were expected to release 14.2 mg/L chloride (4.0 mL/L X3.55 mg/mL).

The drop in pH supports inorganic chloride release which resulted from dehalogenation of TCP and formation of HCl.

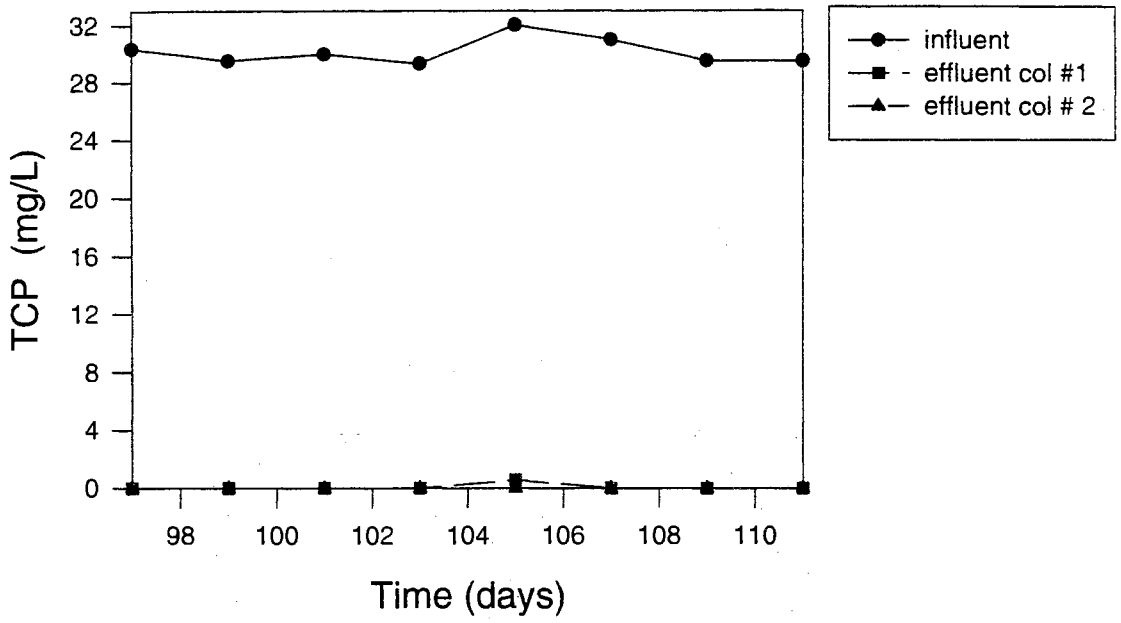

Figure 48. Removal of TCP by PVA columns #1 (10.0 cm) and #2 (20.0 cm) during column study No.5.

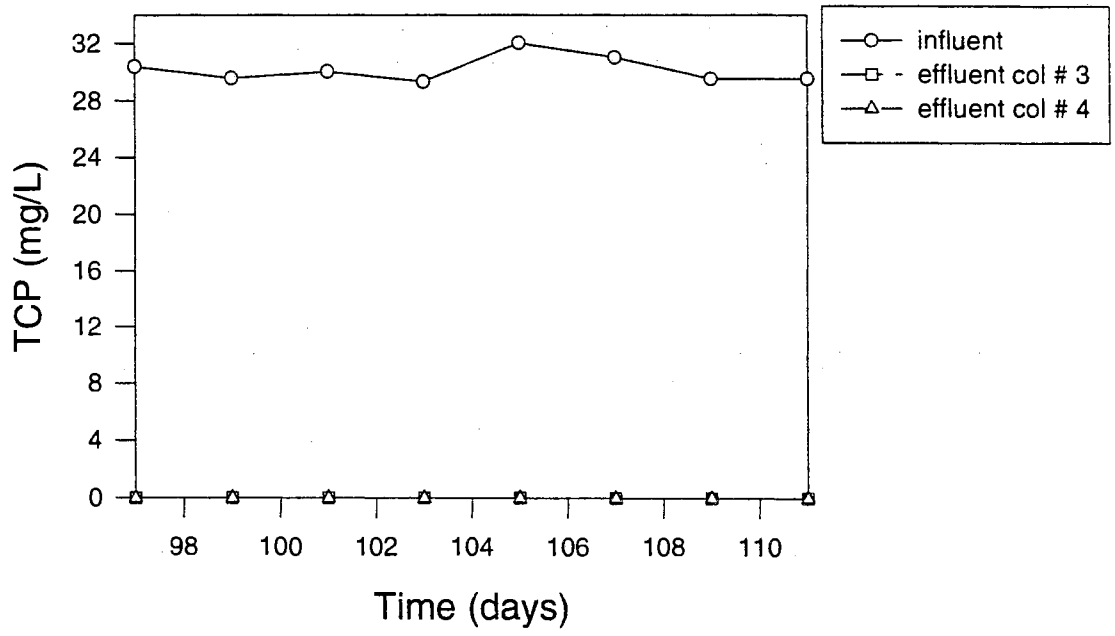

Figure 49. Removal of TCP by GAC columns #3 (10.0 cm) and #4 (20.0 cm) during column study No.5.

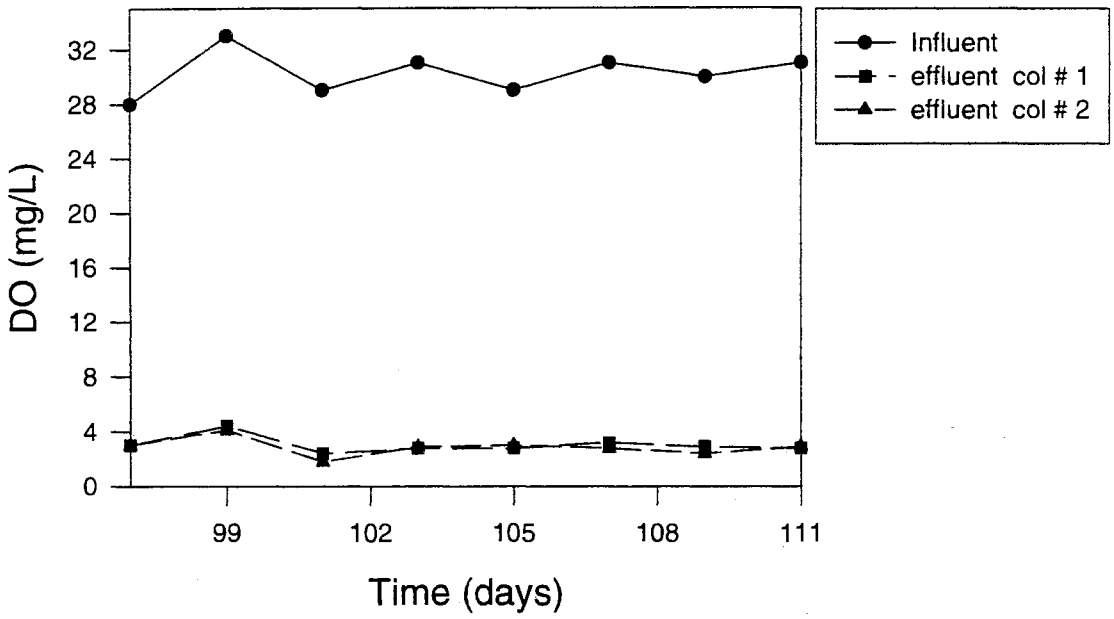

Figure 50. Comparison of DO-- PVA column #1 (10.0 cm) and #2 (20.0 cm) during column study No.5.

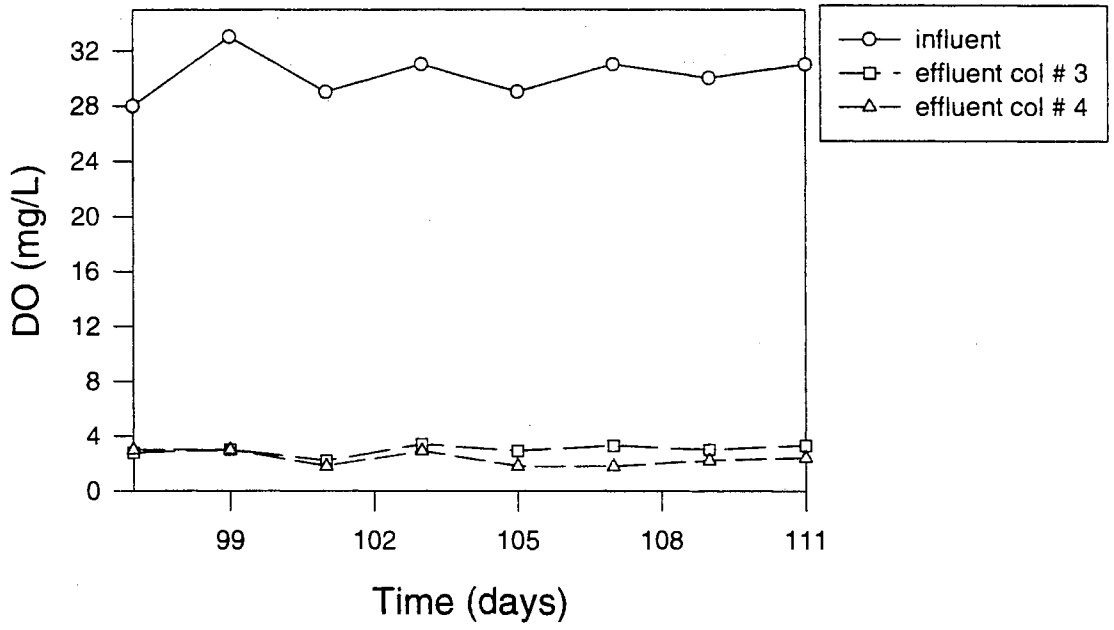

Figure 51. Comparison of DO-- GAC columns #3 (10.0 cm) and #4 (20.0 cm) during column study No.5.

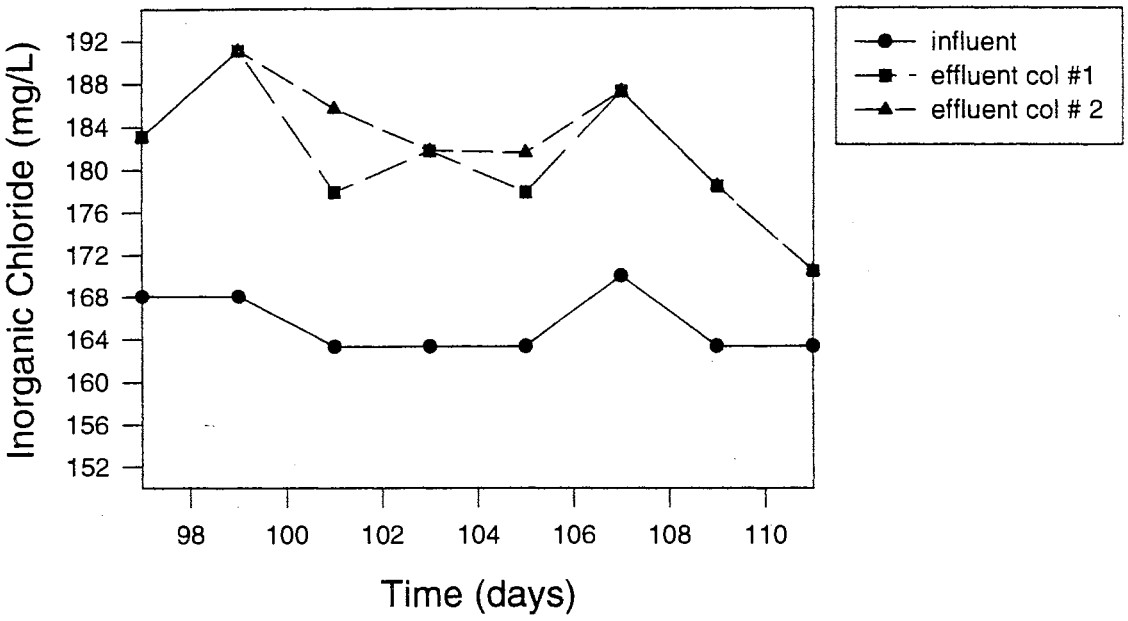

Figure 52. Comparison of Cl^- --PVA columns #1 (10.0 cm) and #2 (20.0 cm) during column study No.5.

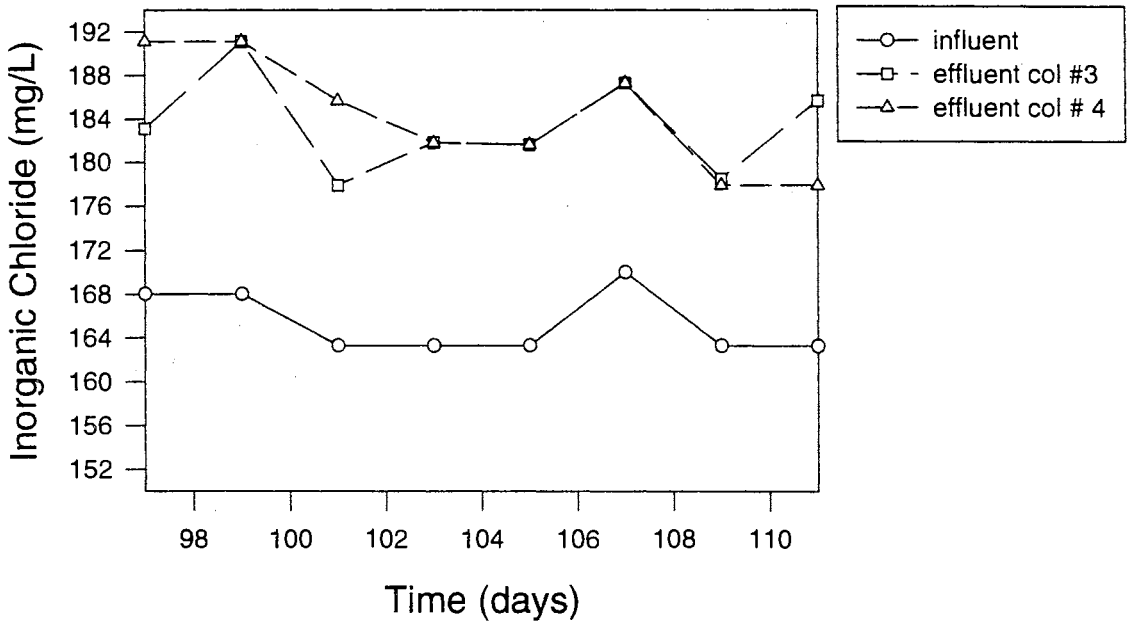

Figure 53. Comparison of Cl^--- GAC columns #3(10.0 cm) and #4 (20.0 cm) during column study No.5.

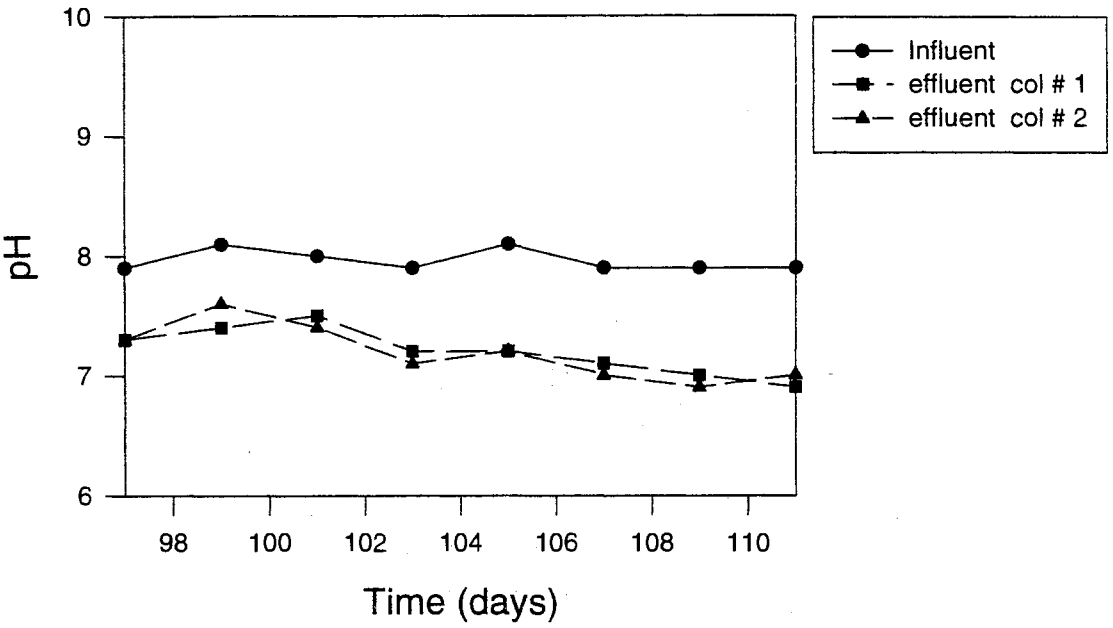

Figure 54. pH Comparison--PVA columns #1 (10.0 cm) and #2 (20.0 cm) during column study No.5.

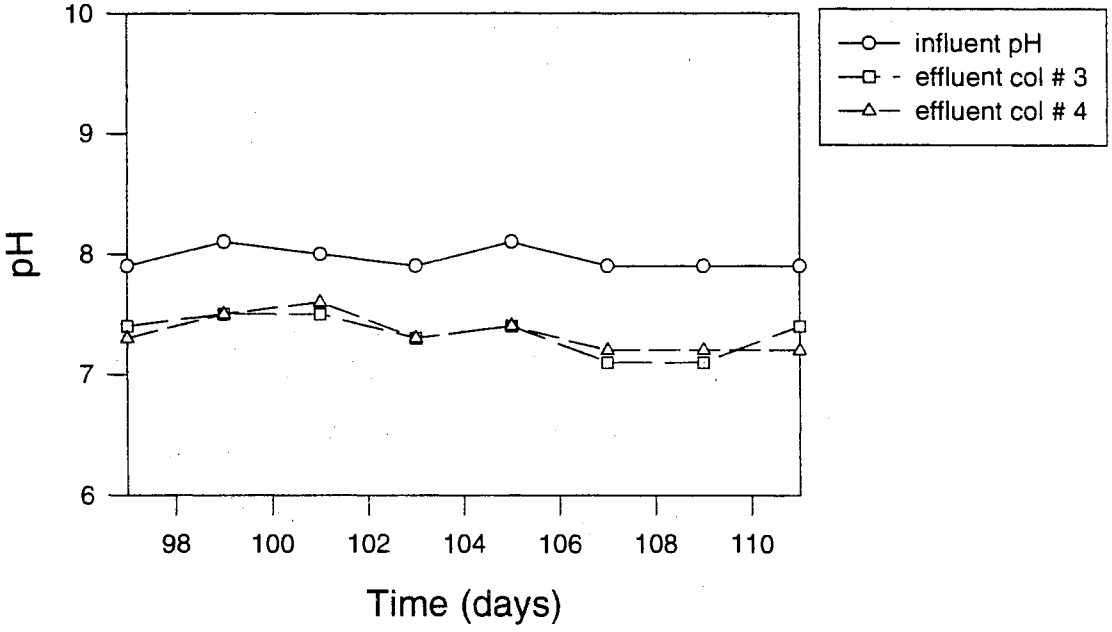

Figure 55. pH Comparison--GAC columns #3 (10.0 cm) and #4 (20.0 cm) during column study No.5.

Column Study No. 6

The purpose of this experiment was to evaluate the effect of increased flow rate on biodegradation of TCP in both PVA and GAC columns. The average influent feed concentration was 22.0 mg/L. The flow rate was increased to 2 mL/min. The applied loading for the columns (#1, #3) and columns (#2,#4) were 0.3 and 0.148 g L^{-1} d^{-1}, respectively.

In this experiment the flow rate increase to 2 mL/min which reduced the HRTs for columns #1-#4 to 24.5, 49.0, 29.5, and 58.9 minutes, respectively. The effect of contact time for the cells with TCP was evaluated in this experiment. The raw data are presented in Appendix K.

TCP Concentration: TCP influent and effluent concentrations for the PVA and GAC columns are shown in Figures 56 and 57. As seen both PVA columns (#1, #2) reacted to the change in HRT. It took at least 8-10 days for PVA column #1, with an HRT of 24.5 minutes to reach steady state and reduce the TCP concentration to zero. PVA column #2, with an HRT of 49.0 minutes, took 6.0 days to reduce the TCP concentration to zero. There was no TCP detected in both PVA columns throughout out the end of the experiment (after day 123). In the effluent of the GAC columns, no TCP was ever detected during the entire period of this experiment. It is clear that the change in flow rate affected both PVA columns. The increase in TCP concentration in the effluent of PVA column #1 shows the impact of short residence time on this column.

Dissolved Oxygen (DO): The dissolved oxygen for the influent and effluents of PVA columns (#1,#2) and GAC columns (#3,#4) were measured and are presented in Figures 58 and 59. The high DO readings during the transition state (days 113-119) for

PVA column #1 is due to incomplete TCP removal, which is the result of a possible upset caused by the flow increase. All four columns (#1,#2,#3,#4) continued to reduced the DO of 25.6± 2.9 mg/L in feed solution to 4.7 ± 1.8, 4.9 ± 1.4, 4.1± 1.4, and 4.3 ± 1.7 mg/L, respectively.

Inorganic Chloride Release: Figures 60 and 61 represent the measured inorganic chloride concentrations in the influent and effluents of the PVA and GAC columns. During the transition period (days 113-119), the average effluent chloride concentration for PVA column #1 was 5.2 mg/L only. This is also consistent with the incomplete TCP removal and high DO reading in the PVA column #1. During steady state conditions (days 119-127), the average effluent inorganic chloride concentration for columns #1,#2,#3, and #4 were 10.3 ± 4.2, 10.6 ± 2.8, 10.6 ± 1.6, and 12.3 ± 1.6, respectively. Aerobic dehalogenation of 20.0 mg/L should release 10.8 mg/L of inorganic chloride which is close to measured inorganic chloride in the effluent of all four columns.

pH Change: Figures 62 and 63 show pH of the influent and effluent values for the PVA and GAC columns, respectively. The influent feed solution had an average pH of 7.9. The approximate average effluent pH for PVA column #1 during the transition period (days 113-119) was 7.5. According to pH curve, a drop in pH from 7.9 to 7.5 shows that 2.0 mL volume of 0.1 N HCl would be required. This is a 7.1 mg/L chloride concentration (2.0 mL/L X 3.55 mg/mL). This was expected due to partial TCP removal and high DO reading during the transition period. An approximate average pH value for columns #2, #3, and #4 was 7.3. According to the pH curve, the drop in pH from 7.9 to 7.3 shows that a 3.0 mL volume of 0.1 N HCl would be required. This equals 10.7 mg/L

of chloride (3.0 mL/L X 3.55 mg/mL) which is similar to the theoretical chloride concentration of 10.8 mg/L expected from the dehalogenation of 20.0 mg/L TCP. The drop in pHs tends to support the concept of dehalogenation of TCP and release of chloride (HCl).

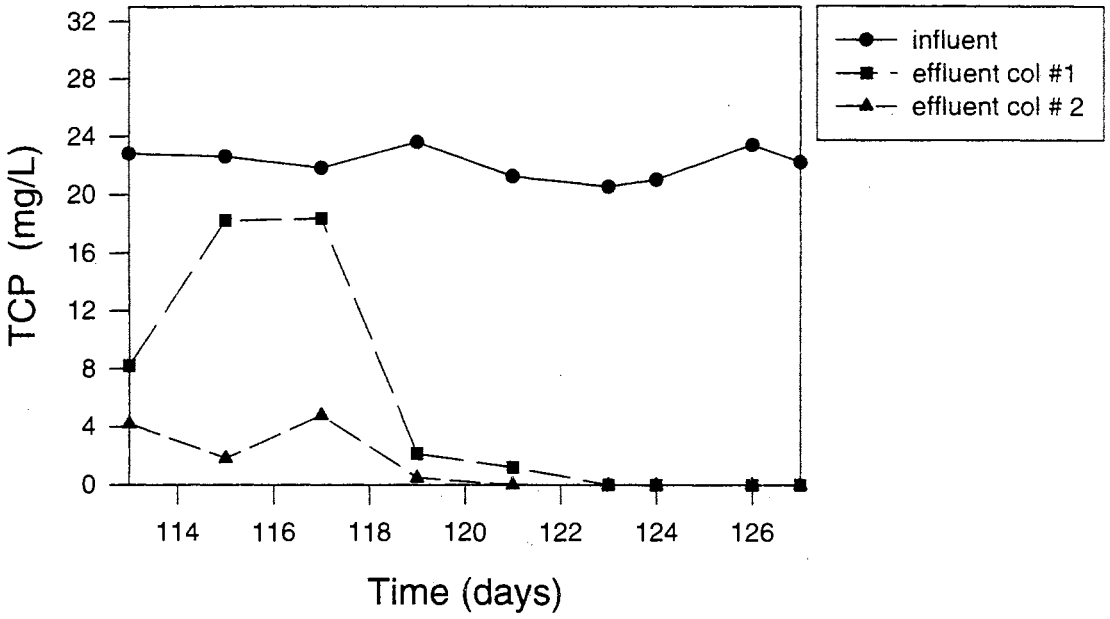

Figure 56. Removal of TCP by PVA columns #1 (10.0 cm) and #2 (20.0 cm) during column study No.6.

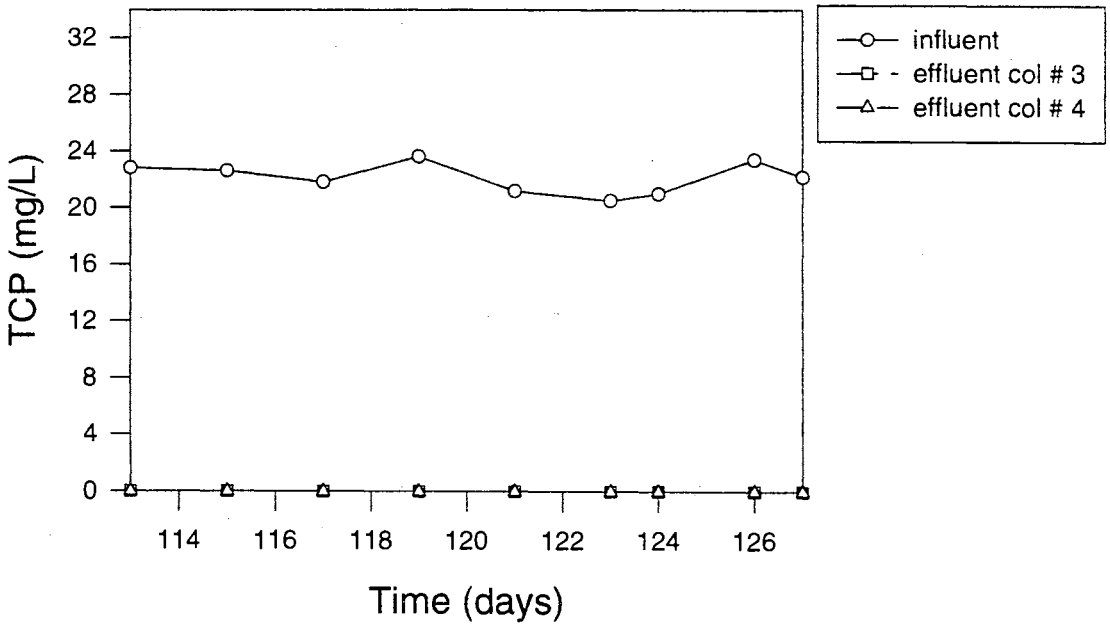

Figure 57. Removal of TCP by GAC columns #3 (10.0 cm) and #4 (20.0 cm) during column study No.6.

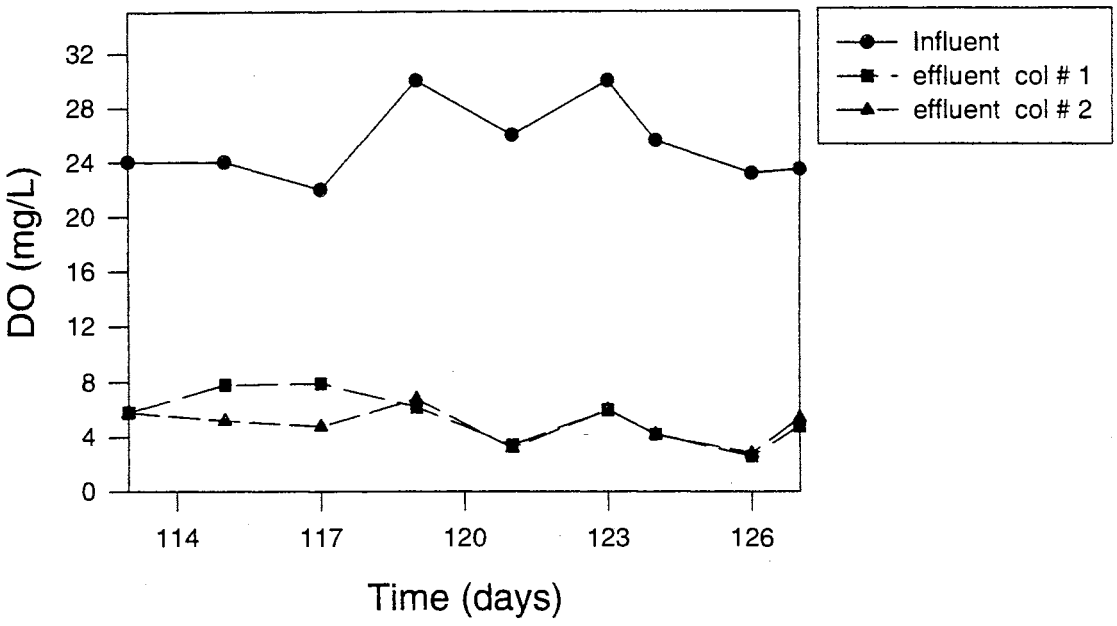

Figure 58. Comparison of DO-- PVA column #1 (10.0 cm) and #2 (20.0 cm) during column study No.6.

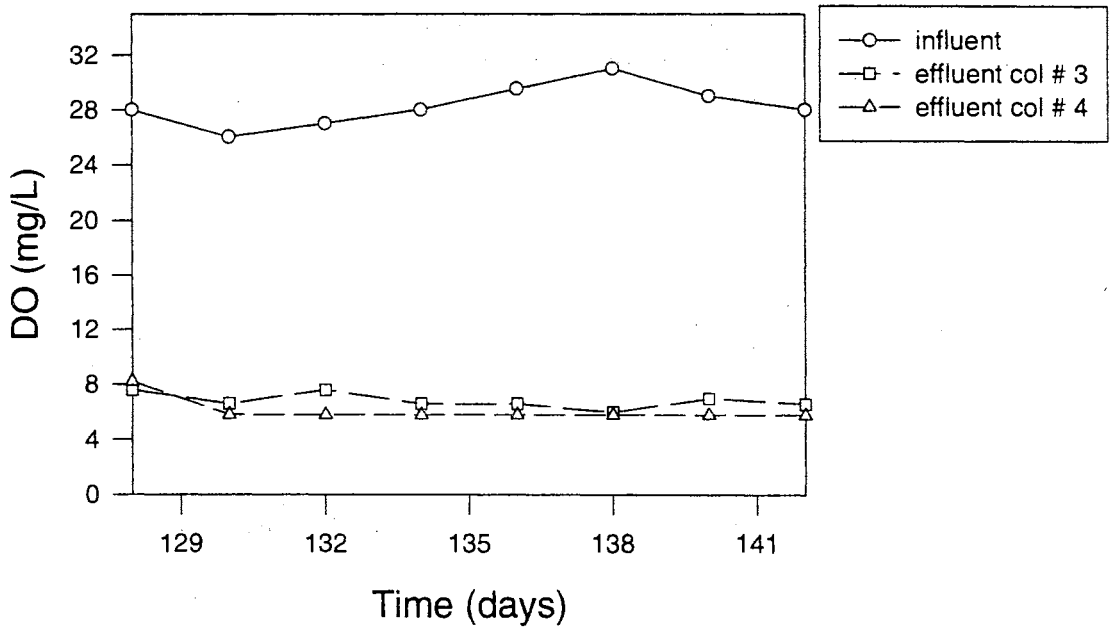

Figure 59. Comparison of DO-- GAC columns #3 (10.0 cm) and #4 (20.0 cm) during column study No.6.

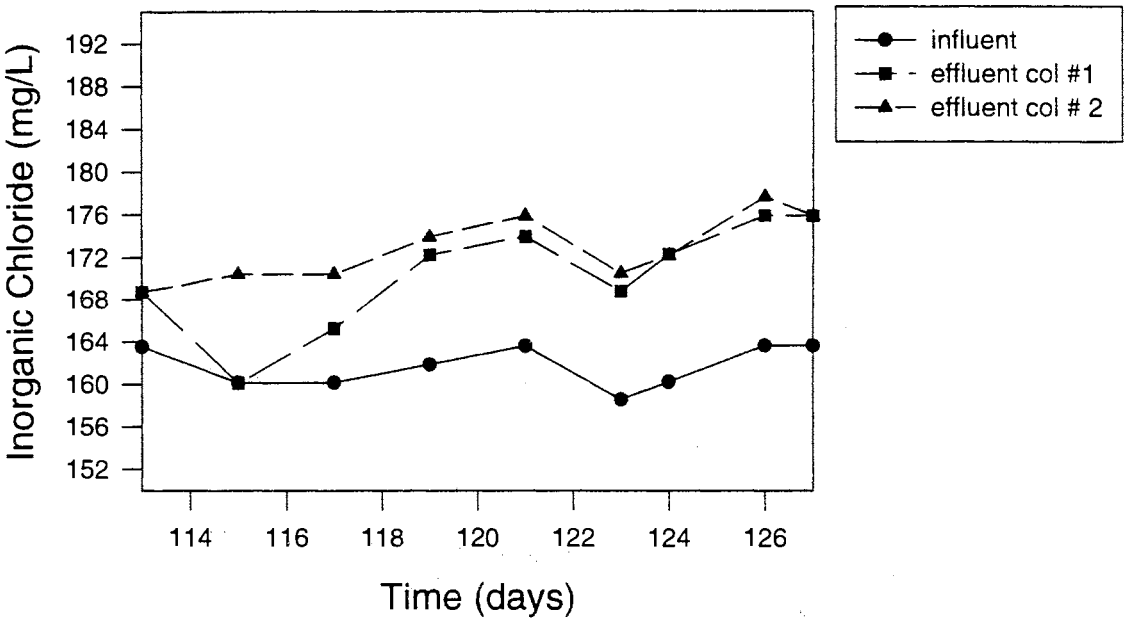

Figure 60. Comparison of Cl^- --PVA columns #1 (10.0 cm) and #2 (20.0 cm) during column study No.6.

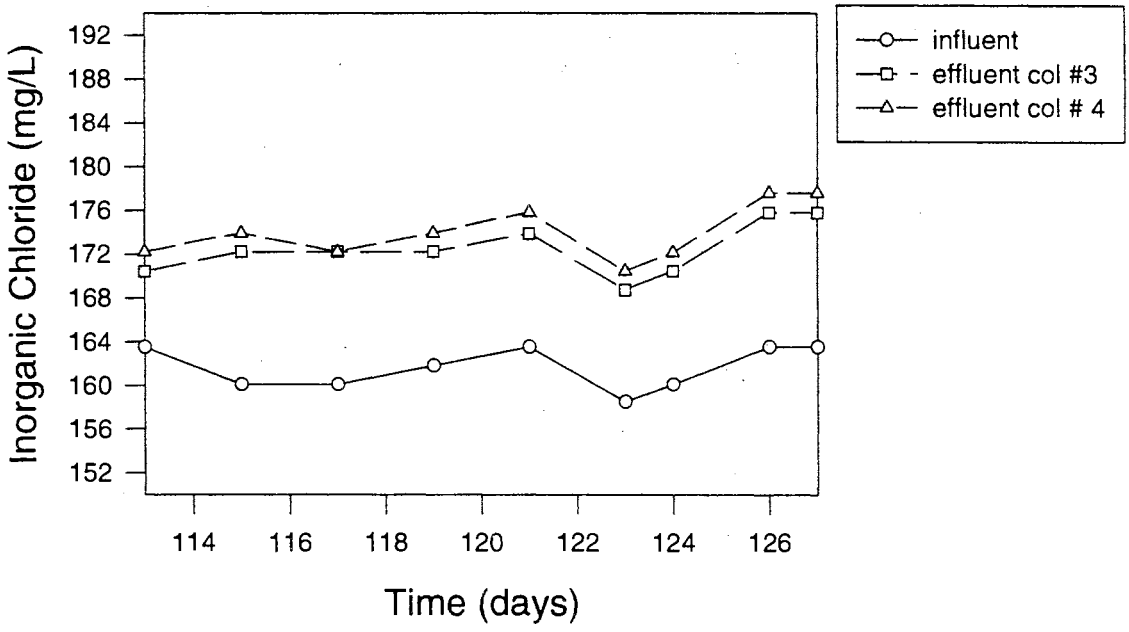

Figure 61. Comparison of Cl^- -- GAC columns #3(10.0 cm) and #4 (20.0 cm) during column study No.6.

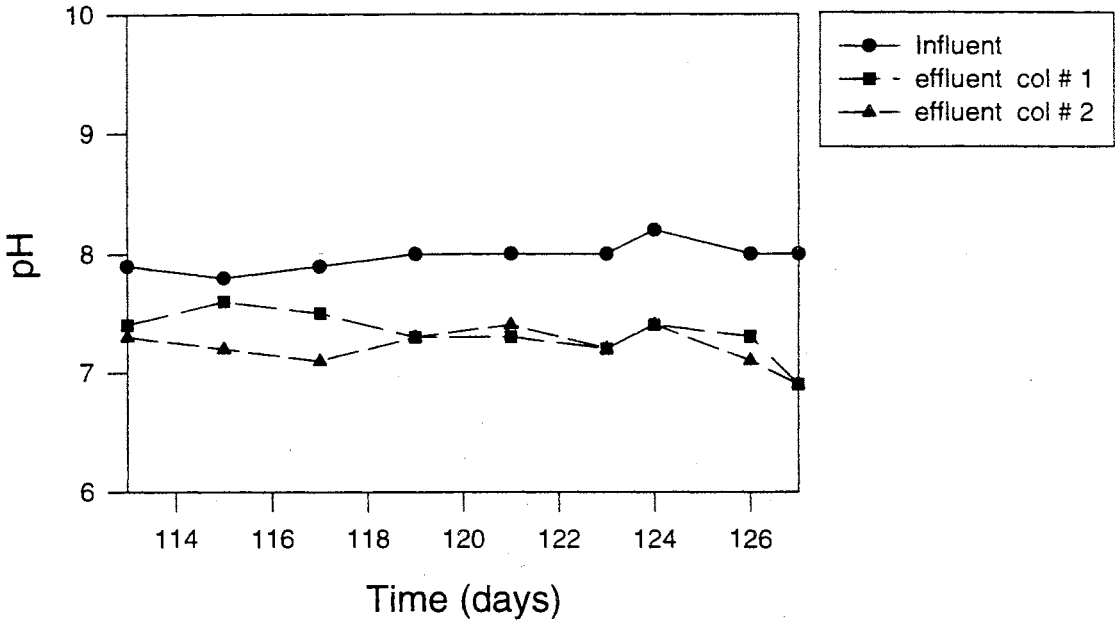

Figure 62. pH Comparison--PVA columns #1 (10.0 cm) and #2 (20.0 cm) during column study No.6

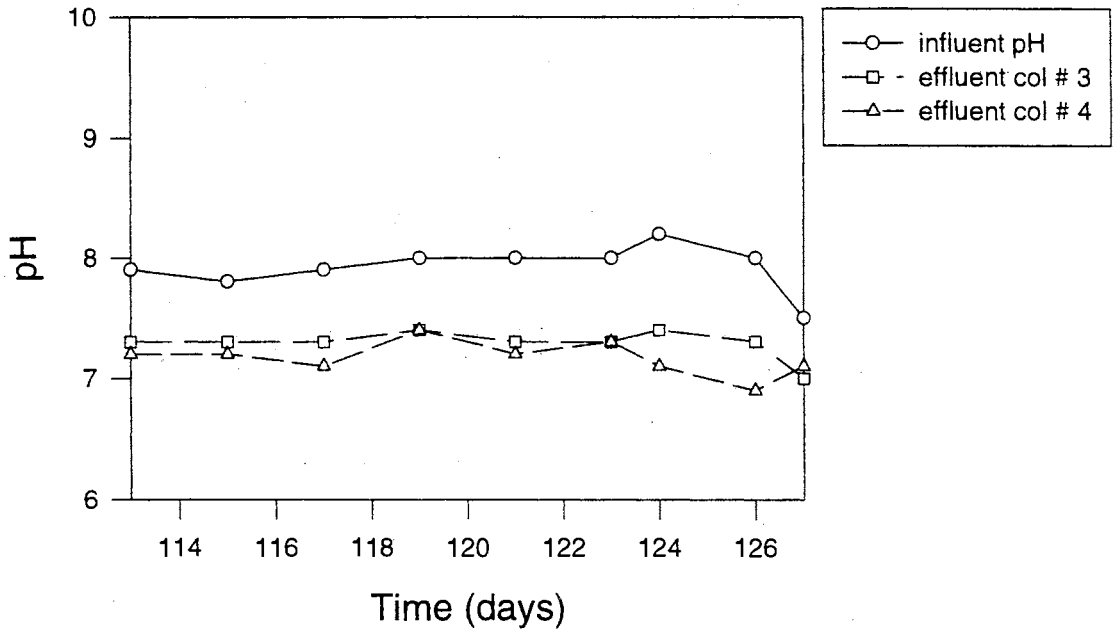

Figure 63. pH Comparison-- GAC Columns #3(10.0 cm) and #4(20.0 cm) during column study No.6.

Column Study No. 7

The purpose of this experiment was to evaluate another change in influent flow rate on the biodegradation of TCP (20.0 mg/L) in both the PVA and GAC columns. The average influent feed concentration was 21.0 ± 0.9 mg/L. The applied loading for columns (#1, #3) and columns (#2, #4) are 0.6 and 0.3 g L^{-1} d^{-1}, respectively. The influent bottle was aerated with pure oxygen for 10.0 minutes every day to maintain DO of around 27.0 mg/L. The influent bottle was completely capped to prevent the loss of oxygen. In this experiment the flow rate increased to 4 mL/min which reduced HRTs for column #1-#4 to 12.3, 24.5, 14.7, and 29.5 minutes, respectively. The effect of contact time was evaluated in this experiment. The raw data are presented in Appendix L.

TCP Concentration: The TCP influent and effluent concentrations for the PVA and GAC columns are shown in Figures 64 and 65. Figure 64 shows both PVA columns (#1, #2) reacted to the change in HRT starting on the first day of the experiment. It took almost 8 days for PVA column #1 with HRT of 12.3 minutes to reduce the TCP concentration to 2.3 mg/L. Average removal efficiency during the transition period (days 128-134) for this column was about 78%. The removal efficiency of PVA column #1 increased to 91% once the column reached steady state (day 8 of the experiment). The PVA column #2 with an HRT of 24.5 minutes had a TCP removal efficiency of 93% during the first four days of this experiment. The TCP removal efficiency of PVA column #2 increased to 100% once the column reached steady state (day 6 of the experiment). In the effluent of GAC columns, no TCP was ever detected during the entire period of this experiment. It is clear that the change in flow rate affected both

PVA columns removal efficiency. The change in flow rate had greater impact (in terms of removal efficiency) on PVA column #1 than PVA column #2.

Dissolved Oxygen (DO): The dissolved oxygen for the influent and the effluents of the PVA columns (#1,#2) and GAC columns (#3,#4) were monitored and the results are presented in Figures 66 and 67. All four columns (#1,#2,#3,#4) continued to reduced DO of 27.9 ± 1.5 in the feed solution to 8.1 ± 0.8, 7.5 ± 0.9, 6.8 ± 0.6, and 6.4 ± 1.1 mg/L, respectively. The results indicate that the effluent DO of all four columns were higher than compared to previous experiments. The DO provided in this experiment was around 27.9 mg/L, which is higher than the theoretical DO (around 18.9 mg/L) needed for complete biodegradation of 20.0 mg/L of TCP in influent. The consumption of DO is a clear indication of biological activity in the column(s).

Inorganic Chloride Release: Figures 68 and 69 represent the measured inorganic chloride concentrations in the influent and effluents of the PVA and GAC columns. The average inorganic chloride release for columns #1,#2,#3, and #4 were 8.3 ± 4.2, 10.6 ± 2.8, 10.6 ± 1.6, and 12.3 ± 1.6, respectively. Aerobic dehalogenation of 20.0 mg/L releases 10.8 mg/L inorganic chloride which is close to the measured inorganic chloride in the effluent of all four columns.

pH Change: Figures 70 and 71 show pH measurements of the influent and effluents of the PVA and GAC columns, respectively. The influent feed solution had an approximate average pH of 7.9. An effluent pH for the PVA columns #1 and #2 was 7.2 and 7.1. The approximate average of effluent pH for GAC columns #3 and #4 was 7.1 and 6.9 . According to the pH curve, Figure 15, the effluent pH drop from 7.9 to 7.2, 7.1, and 6.9 showed that 4.0, 5.0, and 8.0 mL volume of 0.1 N HCl would be required,

respectively. These equal 14.2, 17.8,17.8, and 28.4 mg/L chloride concentration in columns #1-#4 effluent respectively. The effluent chloride concentration measured in all four columns was higher than expected especially in PVA column # 2 and GAC column #3 and #4. This increase over theoretical was 40%, 40%, and 65%, for PVA column #1, GAC column #3, and GAC column #4, respectively. The change in flow rate might washed out some inorganic chloride that had retained in the columns. It is also possible that the columns had some anoxic zones which might dehalogenate TCP and release chloride.

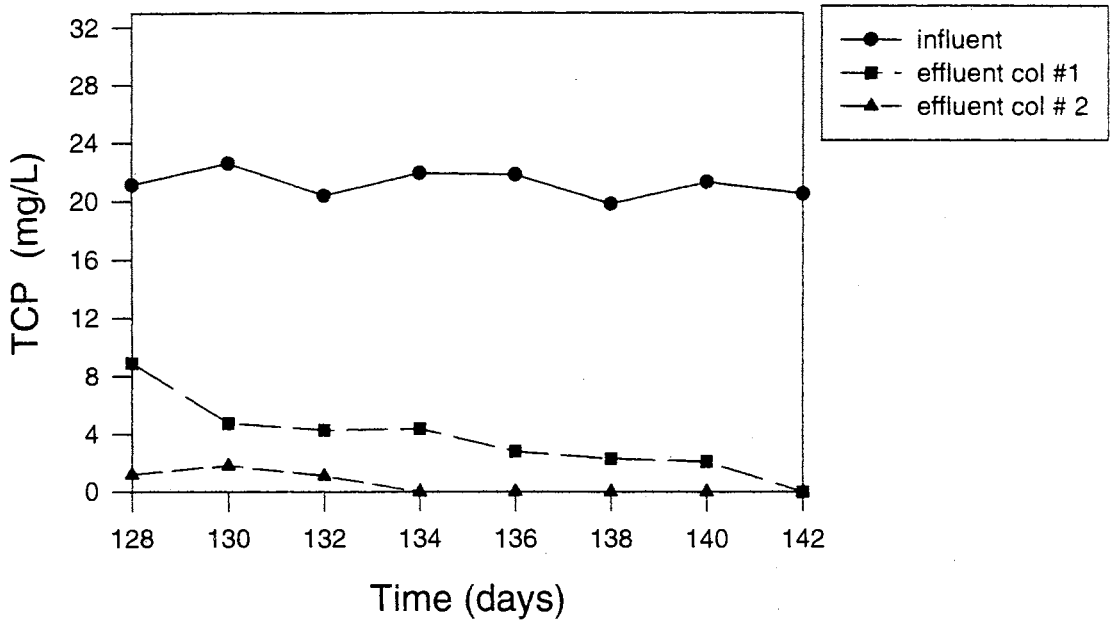

Figure 64. Removal of TCP by PVA columns #1 (10.0 cm) and #2 (20.0 cm) during column study No.7

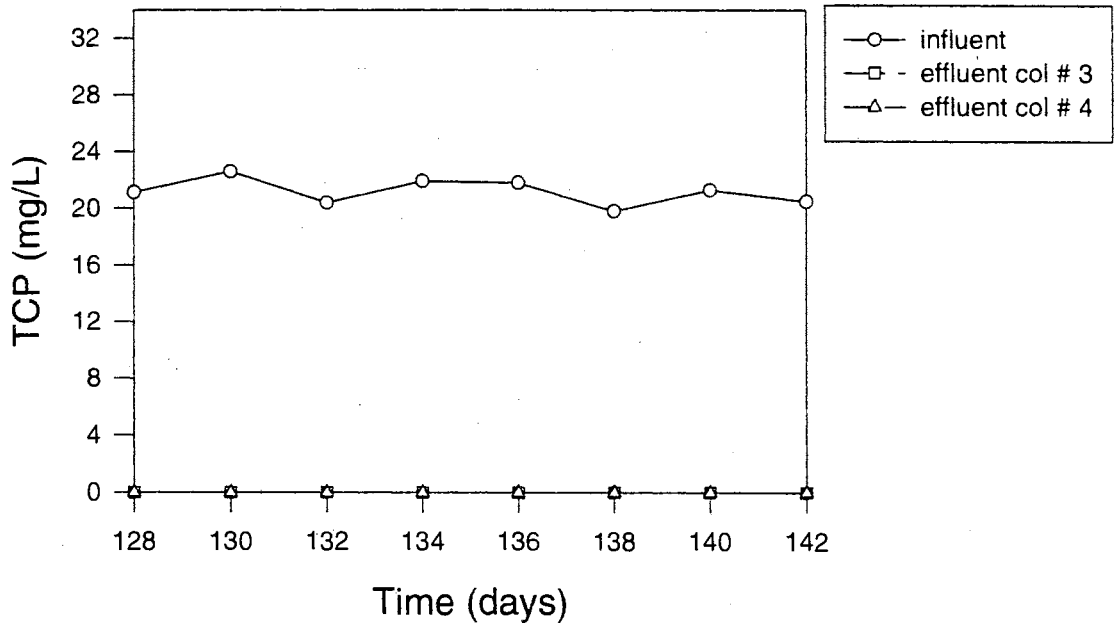

Figure 65. Removal of TCP by GAC columns #3 (10.0 cm) and #4 (20.0 cm) during column study No.7.

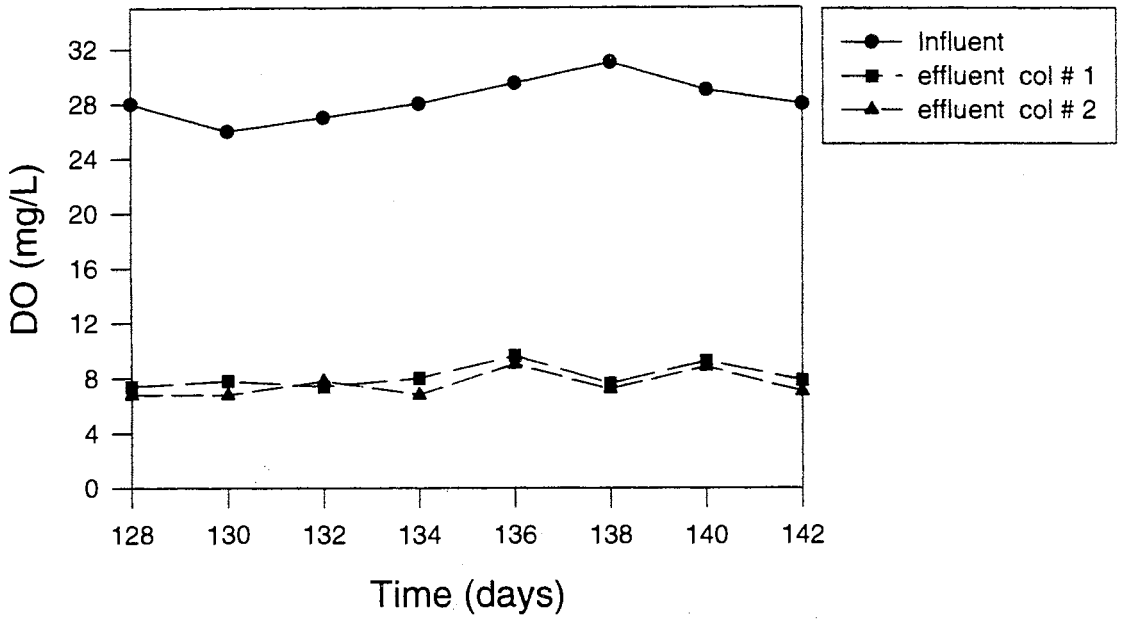

Figure 66. Comparison of DO-- PVA column #1 (10.0 cm) and #2 (20.0 cm) during column study No.7.

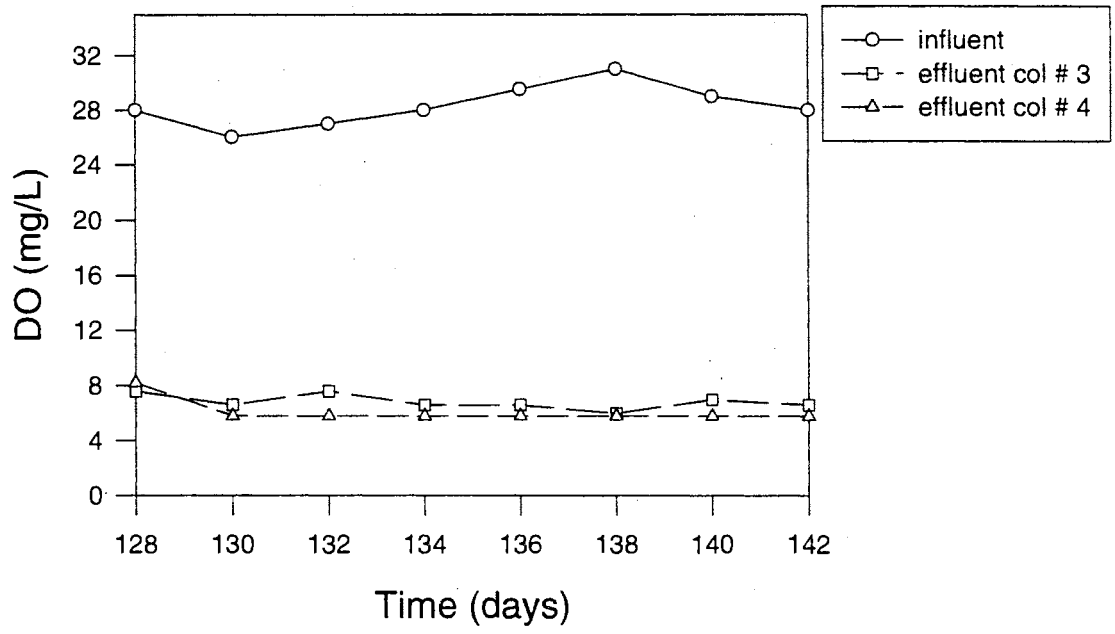

Figure 67. Comparison of DO-- GAC columns #3 (10.0 cm) and #4 (20.0 cm) during column study No.7

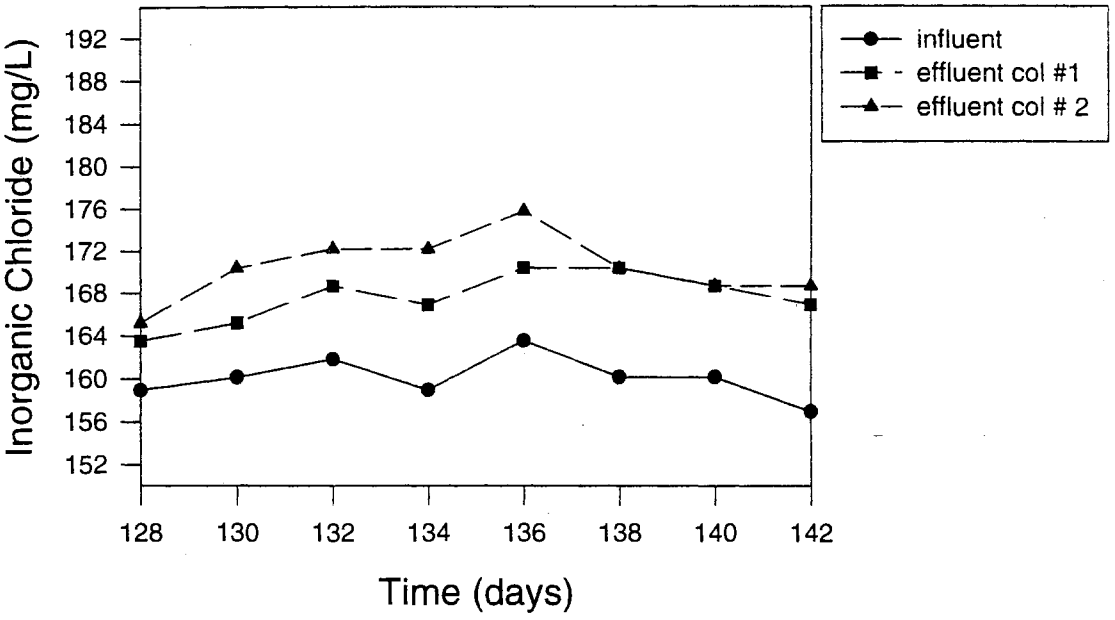

Figure 68. Comparison of Cl^- --PVA columns #1 (10.0 cm) and #2 (20.0 cm) during column study No.7.

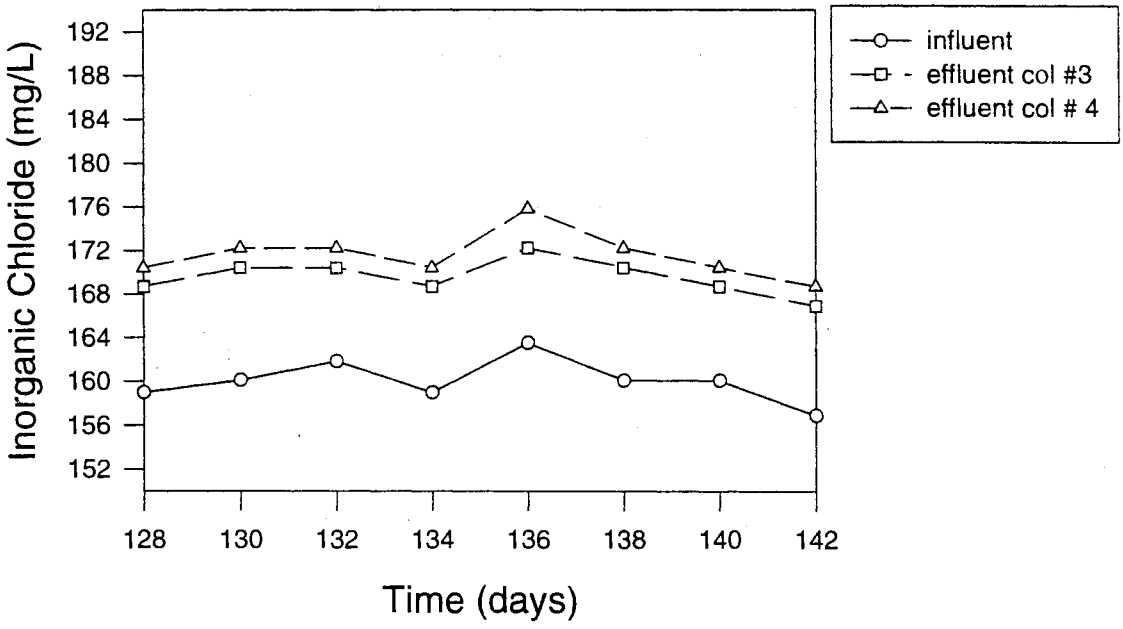

Figure 69. Comparison of Cl^- -- GAC columns #3(10.0 cm) and #4 (20.0 cm) during column study No.7.

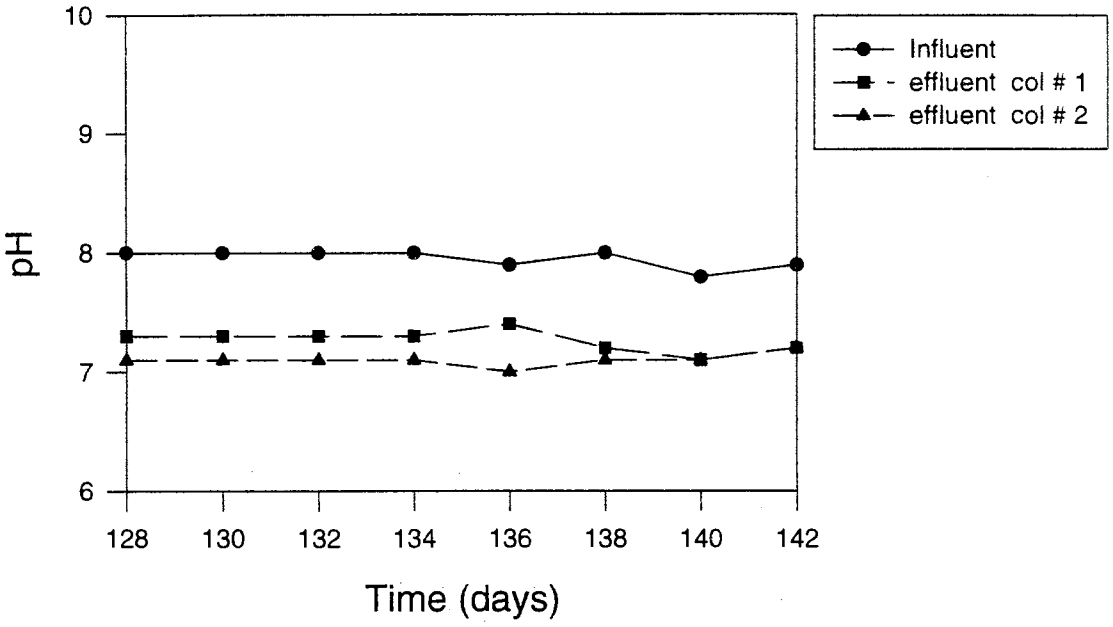

Figure 70. pH Comparison--PVA columns #1 (10.0 cm) and #2 (20.0 cm) during column study No.7.

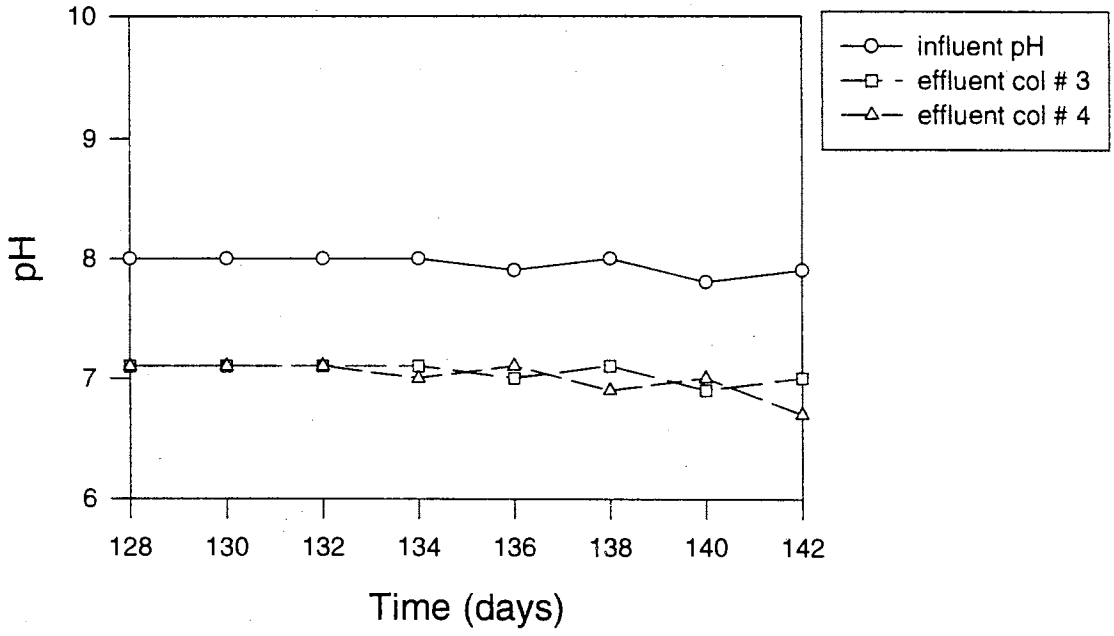

Figure 71. pH Comparison-- GAC Columns #3(10.0 cm) and #4(20.0 cm) during column study No.7.

Column Study No. 8

The purpose of this experiment was to evaluate the removal efficiency of all four columns where the TCP concentration was increased to 40.0 mg/L. The flow rate for all columns remained at 4 mL/min. The applied loading for columns (#1, #3) and columns (#2, #4) was 1.2 and 0.6 g $L^{-1}d^{-1}$, respectively. The influent feed bottle was aerated with pure oxygen for 10.0 -15.0 minutes every day to maintain DO of around 27.0 mg/L. The influent bottle was completely capped to prevent the loss of oxygen. The raw data for this experiment are presented in Appendix M.

TCP Concentration: The TCP influent and effluent concentrations for PVA and GAC columns are shown in Figures 72 and 73. The average influent feed TCP concentration was 40.6± 0.71 mg/L. An average effluent TCP concentration for PVA columns #1 and #2 was 15.5 ± 3.6 and 8.9 ± 1.2 mg/L, respectively. As seen the change of influent TCP concentration had an great impact on both PVA columns TCP removal efficiency. For the first time during the column studies, the overall removal efficiency of PVA columns # 1 and # 2 was decreased to 61% and 80%, respectively. TCP removal by PVA column #1 improved over the course of this experiment. TCP removal efficiency for PVA column #1 over the first week was 54% and increased to 67% during the last 10 days of the experiment. PVA column #2 had a removal efficiency of 76% in the first week which was increased to 81% during the last 10 days of the experiment. The removal efficiency of both GAC columns was 100% during the entire period of this experiment. It is clear that the increase in TCP concentration affected the removal efficiency of the PVA columns to a much greater extent than the GAC columns.

Dissolved Oxygen (DO): The dissolved oxygen for the influent and effluents of PVA columns (#1,#2) and GAC columns (#3,#4) were measured and are presented in Figures 74 and 75. All four columns (#1,#2,#3,#4) continued to reduced the DO of the feed solution 27.8±1.2 mg/L to 9.9 ± 1.2, 6.0 ± 1.7, 4.6± 1.1, and 4.1± 1.4 mg/L, respectively. The consumption of DO by microorganisms is a clear indication of biological activity in the columns. The effluent DO in both PVA columns was higher than in both GAC columns. All four column consumed DO available to them, but the PVA columns consumption of DO was lower than GAC columns consumption. It should be noted that for aerobic dehalogenation of 40.0 mg/L of TCP, the cells need at least 35.6 mg/L. Dissolved oxygen provided was around 27.8 mg/L which is about 22 % less than DO needed. This may have had an impact on the PVA columns removal efficiency of 40.0 mg/L TCP.

Inorganic Chloride Release: Figures 76 and 77 represent the measured inorganic chloride concentrations in the influent and effluents of the PVA and GAC columns. The average inorganic chloride release for columns #1,#2,#3, and #4 was 12.5± 1.9, 18.6± 2.9, 21.3 ± 2.6, and 20.7± 4.8 mg/L, respectively. Aerobic dehalogenation of 40.0 mg/L of TCP should theoretically releases 21.6 mg/L inorganic chloride. The average measured values for inorganic chloride release in the GAC columns effluent are close to theoretical chloride release. The average measured values for inorganic chloride release in the PVA columns #1 and #2 effluent are 42% and 14% less than the theoretical chloride release. The PVA column #1 also had lowest DO usage. This supports the observed TCP removal efficiencies of these columns.

pH Change: Figures 78 and 79 show pH measurements of influent and effluents of PVA and GAC columns respectively. The influent feed solution had an approximate average pH 8.2. An approximate average pH for PVA columns #1 and #2 was 7.0 and 6.9, respectively. An approximate average pH for the GAC columns #3 and #4 was 6.7 and 6.9, respectively. According to the pH curve, the drop in pH from 8.2 to 7.0, 6.9, 6.7 shows that an 8.0, 8.0, and 11.0 mL volume of 0.1 N HCl would be required, respectively. According to the pH curve the chloride concentration in columns #1, #2, #3, and #4 should be 28.4, 28.4, 28.4, and 42.6 mg/L, respectively, which is about 24 - 50 % higher than the theoretical chloride release. The anaerobic activity might be present as locolized pockets (since DO in the effluent was between 4 and 9 mg/L) in the columns which would also cause the release of acids. The release of acids should show up in effluent pH value.

The drop in pH support inorganic chloride release resulting from dehalogenation of TCP and the formation of HCl.

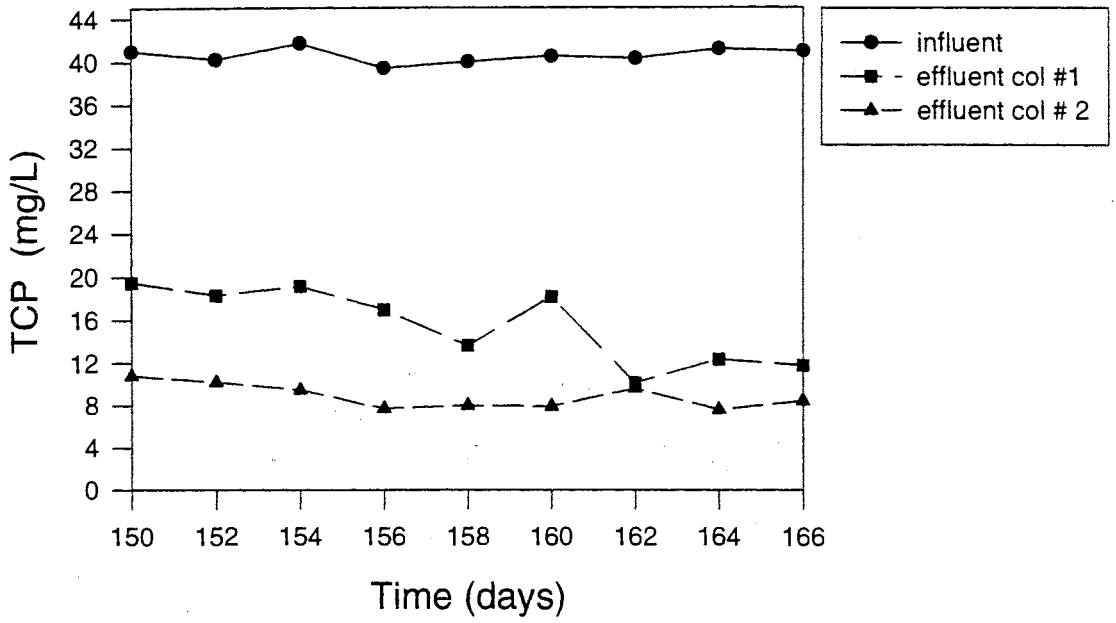

Figure 72. Removal of TCP by PVA columns #1 (10.0 cm) and #2 (20.0 cm) during column study No.8.

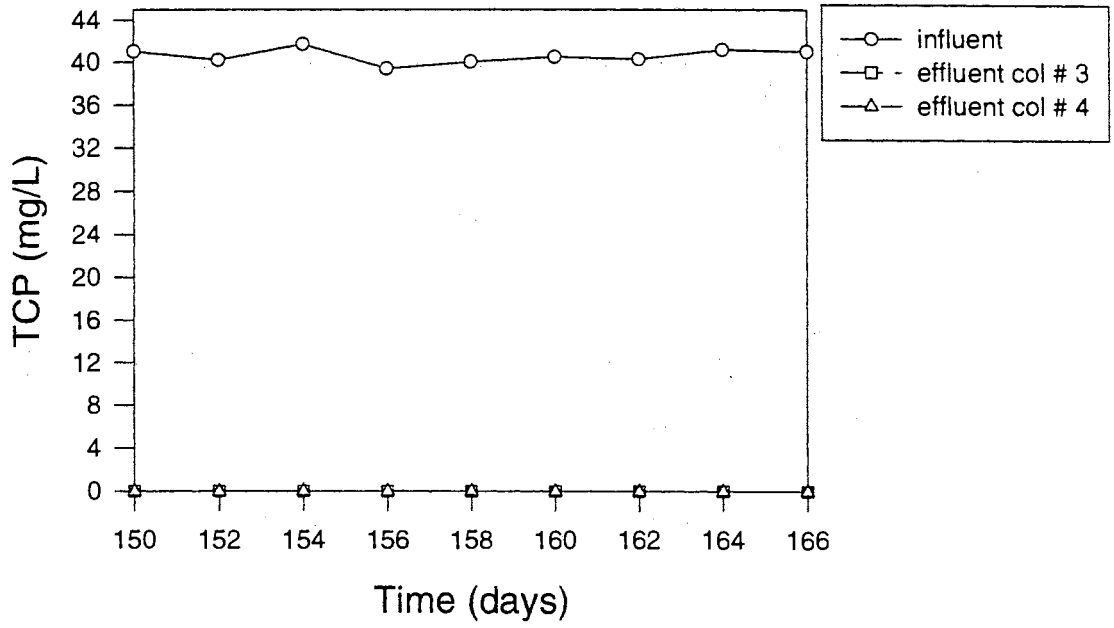

Figure 73. Removal of TCP by GAC columns #3 (10.0 cm) and #4 (20.0 cm) during column study No.8.

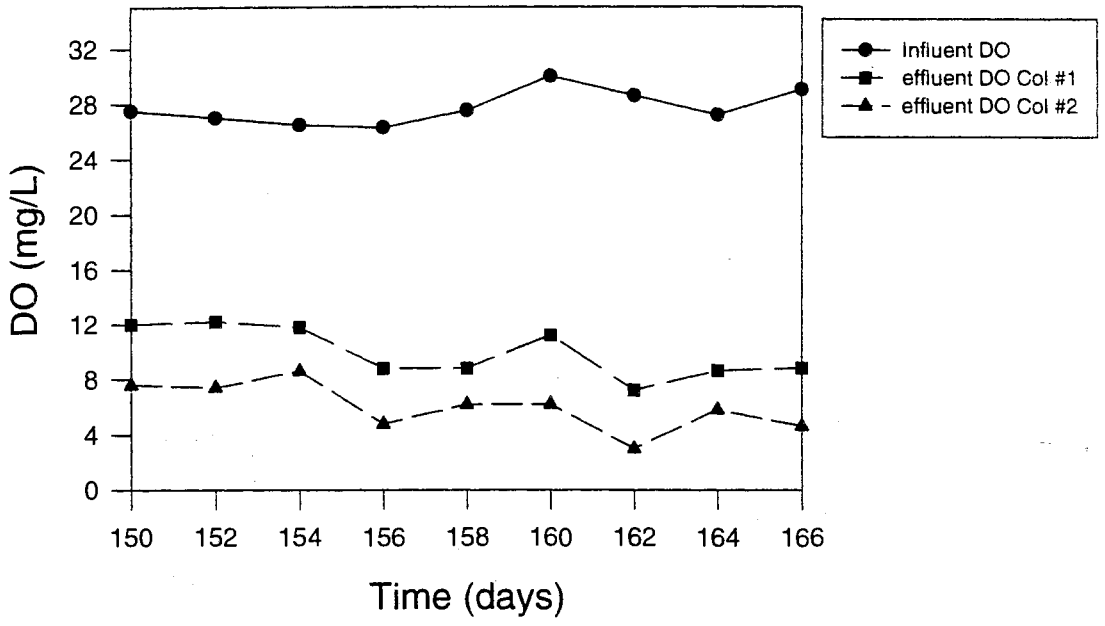

Figure 74. Comparison of DO-- PVA column #1 (10.0 cm) and #2 (20.0 cm) during column study No.8.

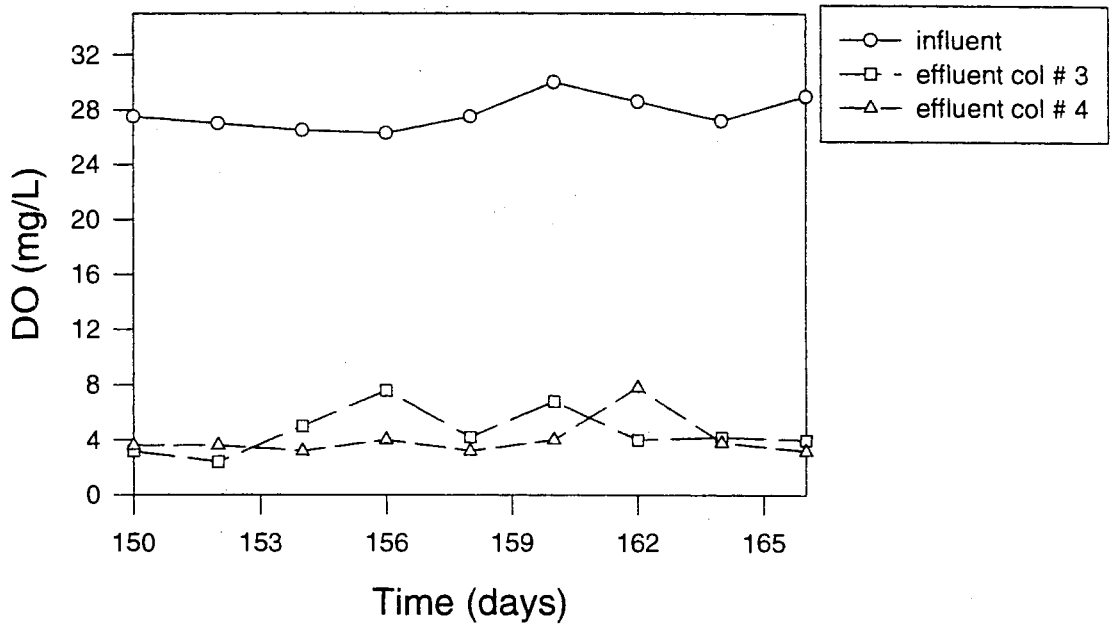

Figure 75. Comparison of DO-- GAC columns #3 (10.0 cm) and #4 (20.0 cm) during column study No.8.

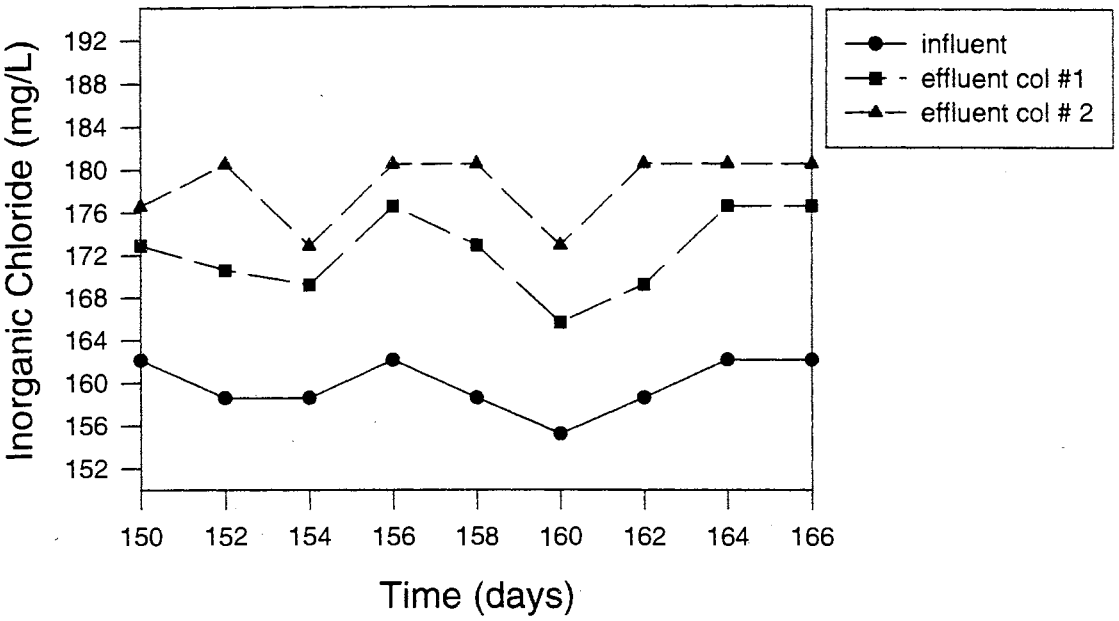

Figure 76. Comparison of Cl^- --PVA columns #1 (10.0 cm) and #2 (20.0 cm) during column study No.8.

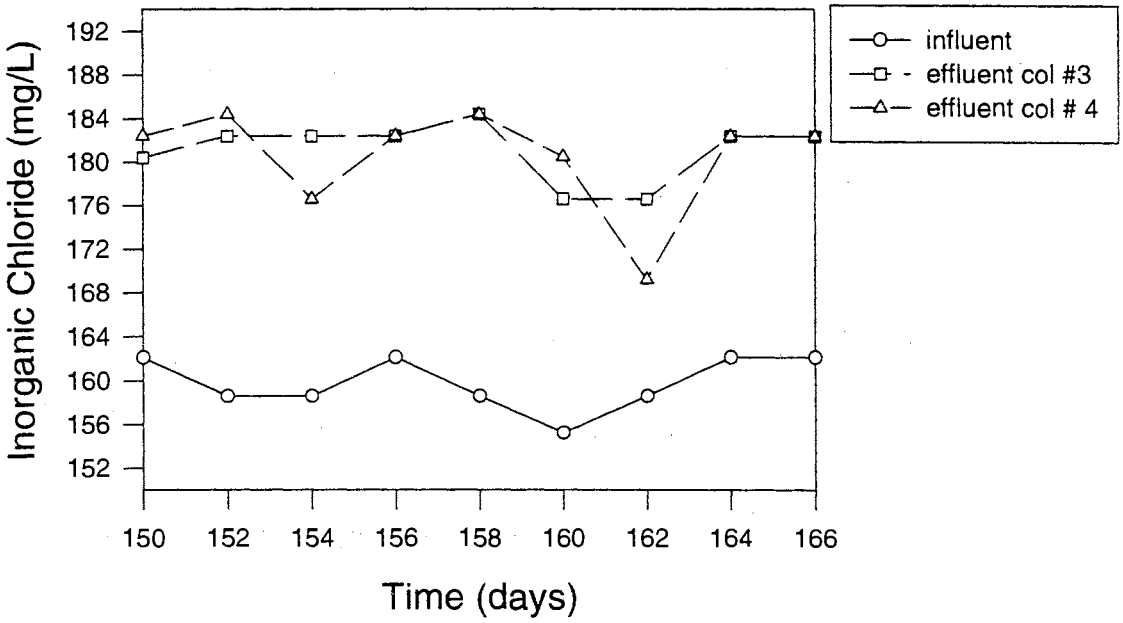

Figure 77. Comparison of Cl^--- GAC columns #3(10.0 cm) and #4 (20.0 cm) during column study No.8.

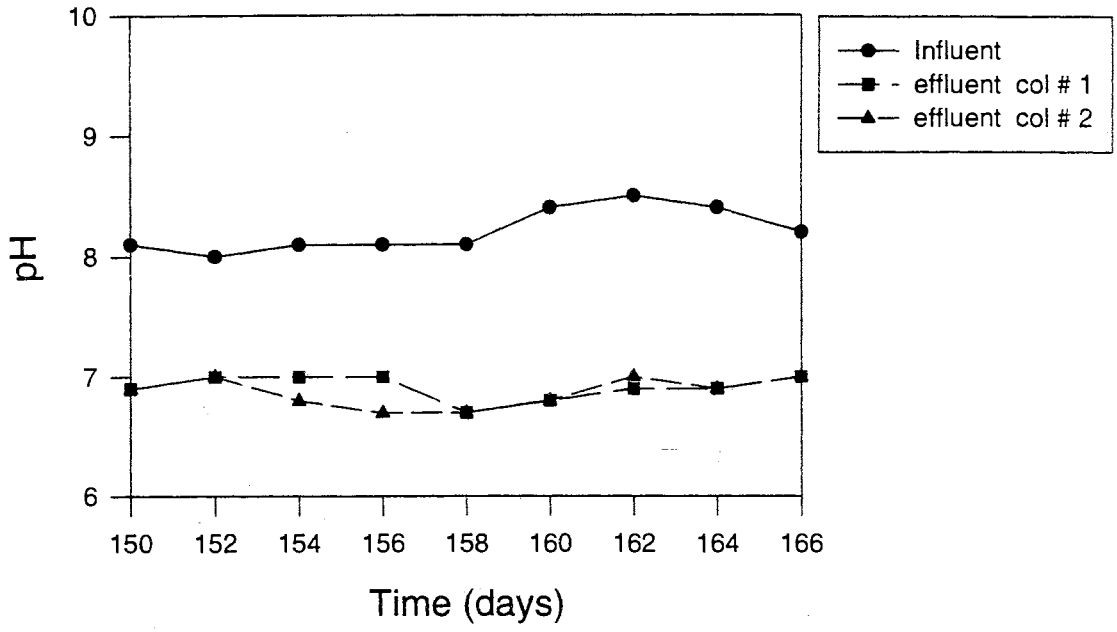

Figure 78. pH Comparison--PVA columns #1 (10.0 cm) and #2 (20.0 cm) during column study No.8.

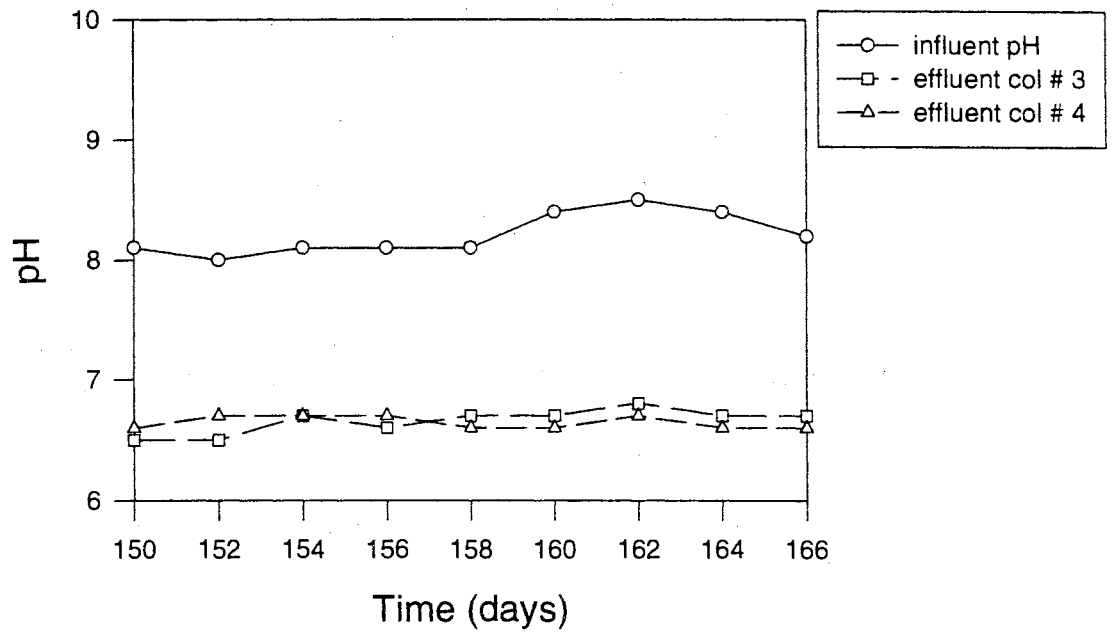

Figure 79. pH Comparison-- GAC Columns #3(10.0 cm) and #4(20.0 cm) during column study No.8.

Columns Performance During 166 Days of Continuous Operation:

The column experiments were designed to account for any significant changes in removal efficiency due to hydraulic retention time (HRT), applied loading, availability of dissolved oxygen, and nutrient C:N:P ratio added to groundwater.

Effluent and influent TCP concentrations were monitored during 166 days of continuous operation, and the results are presented in Figures 80-83. The effect of short HRTs can clearly be seen in Figure 80. The TCP concentration in the effluent of PVA column #1 was higher than PVA column # 2 which had a longer HRT compared to PVA column #1. The increase in influent flow rate on days 113 and 128 had the greater impact on PVA column #1 effluent quality than any other column. Based on the results presented in Figures 80-83, both PVA columns were affected by changes in the influent flow rate. The effect of TCP loading rate on effluent quality of the PVA columns (#1, #2) and GAC columns (#3, #4) are show on days (97, 113, 128, 150)that the loading changed. The TCP loading increase on day 97 had no effect on any of the columns. On day 113, a partial breakthrough of TCP was observed in PVA column #1 after increasing the flow rate from 1 to 2 ml/min, with a corresponding increase of TCP loading rate from 0.22 to 0.3 g L^{-1} d^{-1}. PVA column #2 showed an increase in TCP concentration in the effluent on day 113, after increasing the TCP loading rate from 0.11 to 0.15 g L^{-1} d^{-1}. The increase in the flow rate on day 113, had greater impact on PVA column #1 than PVA column #2. Both PVA columns (#1, #2) showed an increase in TCP concentration in their effluents after increasing the flow rate from 2 to 4 mL/min, with the corresponding increase in TCP loading rate from 0.3 to 0.6 g L^{-1} d^{-1} and from 0.15 to 0.3 g L^{-1} d^{-1}, respectively. On day 150, both PVA columns #1 and #2 experienced the

highest loading rate, 1.2 g $L^{-1}d^{-1}$ and 0.6 g $L^{-1}d^{-1}$, respectively during the entire 166 days of operation. The removal efficiency of the PVA columns #1 and #2 reduced to 67% and 81%, respectively, during days 150-166. The GAC columns #3 and #4 remained unaffected by any increase in loading rate during entire 166 days of continuous operation. The effect of loading rate on the elimination rate of TCP are presented in Figures 84 - 87. During period 2 (column study 2), all of the columns experienced the shortage of dissolved oxygen. The PVA columns #1 and #2, reacted to insufficient DO, which resulted to higher TCP concentration in their effluents. The elimination for PVA columns #1 and #2 reduced from 100% to 68% and 76%, respectively.

The dissolved oxygen in the influent(s) and effluent(s) was monitored and the results are presented in Figures 88-91. Based on Figures 88 and 89, the dissolved oxygen consumption of the PVA columns decreased on day 113 due to flow rate increase and partial TCP removal. The oxygen consumption of both PVA columns and GAC columns decreased by increasing applied loading during periods 6-8 (column study 6-8). The impact of high loading on the PVA column #1 oxygen consumption was greater than the PVA column #2. The decrease in oxygen consumption of both GAC columns (#3 and #4) during periods 6-8 (column study 6-8) had no impact on their elimination capacities. The consumption of dissolved oxygen by the columns is clear indication of biological activity under aerobic conditions.

Dehalogenation of TCP was monitored in terms of chloride release in the columns effluent. The chloride concentration in the influent(s) and effluents during the 166 days of operation are presented in Figures 92-95. During period 1(0-29 days), the chloride released by the GAC columns was less than the PVA columns, which indicate

TCP removal by adsorption rather than biodegradation. During periods 2 - 6 (33-113 days), the chloride release increased in proportion to increasing TCP concentration. This gave further evidence of TCP biodegradation in both PVA and GAC columns. During periods 6-8 (113-166 days), the chloride release by both PVA columns decreased with increasing TCP loading, which support the partial removal of TCP by the PVA columns. The chloride released by the GAC columns (Figures 94, 95) increased as the applied loading increased during periods 6 and 7 (113-150 days), regardless of HRT, and applied loading. The chloride release increase with the corresponding increase in TCP concentration during period 8 (days 150-166), despite insufficient DO in the influent indicates the possibility of anaerobic dehalogenation of TCP by both GAC columns.

To obtain mass balances, measured ICl releases were compared to those calculated from GC measurements (of TCP) for all four columns. The results are presented in Figures 96-99. Measured chloride releases by all four columns agreed well with those calculated from GC measurements.

Theoretically, aerobic mineralization of 1.0 mg/L TCP releases 0.54 mg/L of chloride and uses 0.89 mg/L oxygen. In order to account for any significant removal due to anaerobic degradation during the 166 days, mass balances of chloride releases (measured, calculated) along with expected oxygen consumption by mineralization of TCP are presented in Table 8.

Table 8. Comparison of measured values of TCP, DO, and Cl^- to their calculated values from GC results.

Column No,	Influent TCP (mg/L)	Effluent TCP (mg/L)	TCP removal %	Cl^- measured (mg/L)	Cl^- calculated (mg/L)	DO uptake (mg/L)	DO. needed (mg/L)
PVA #1	22.7	3.6±5.8	86±22.4	9.8±4.1	10.3±3.7	16.0	17.0
PVA #2	22.7	1.83±3.2	93±14.9	11.4±1.5	11.3±1.1	17.0	18.0
GAC #3	22.7	0.0	100	12.3±4.5	11.6±5.6	14.5	20.2
GAC #4	22.7	0.0	100	12.3±5.3	12.3±4.5	14.7	20.2

According to Table 6 the average measured chloride releases by all four columns agreed with those calculated (average) from GC. The average DO uptake by both PVA columns are close to the average theoretical DO needed for complete aerobic mineralization of the average TCP (influent). Both GAC columns needed more DO to mineralize influent TCP under aerobic condition and release corresponding chloride measured or calculated. Therefore, some of the TCP in the influent must biodegrade under deficiency of oxygen by anaerobic degradation present in both GAC columns, and release chloride. It should be noted that all TCP removal in this study was confirmed by inorganic chloride release and no chlorinated intermediates or phenol compound were found by GC/MS (Figures 120-124).

Evolution of H^+ by HCl production in the effluents of all four columns gave further evidence of TCP dehalogenation. The influent(s) and effluents pH were monitored during the 166 days of the operation, and the results are presented in Figures 100-103. The influent(s) pHs dropped for all four columns. During days 150-166, GAC

columns continued to decrease the effluent pH, regardless loading. Unlike the GAC columns, the PVA columns were affected by high loading and partial TCP removal resulted in a smaller pH drop in the effluents.

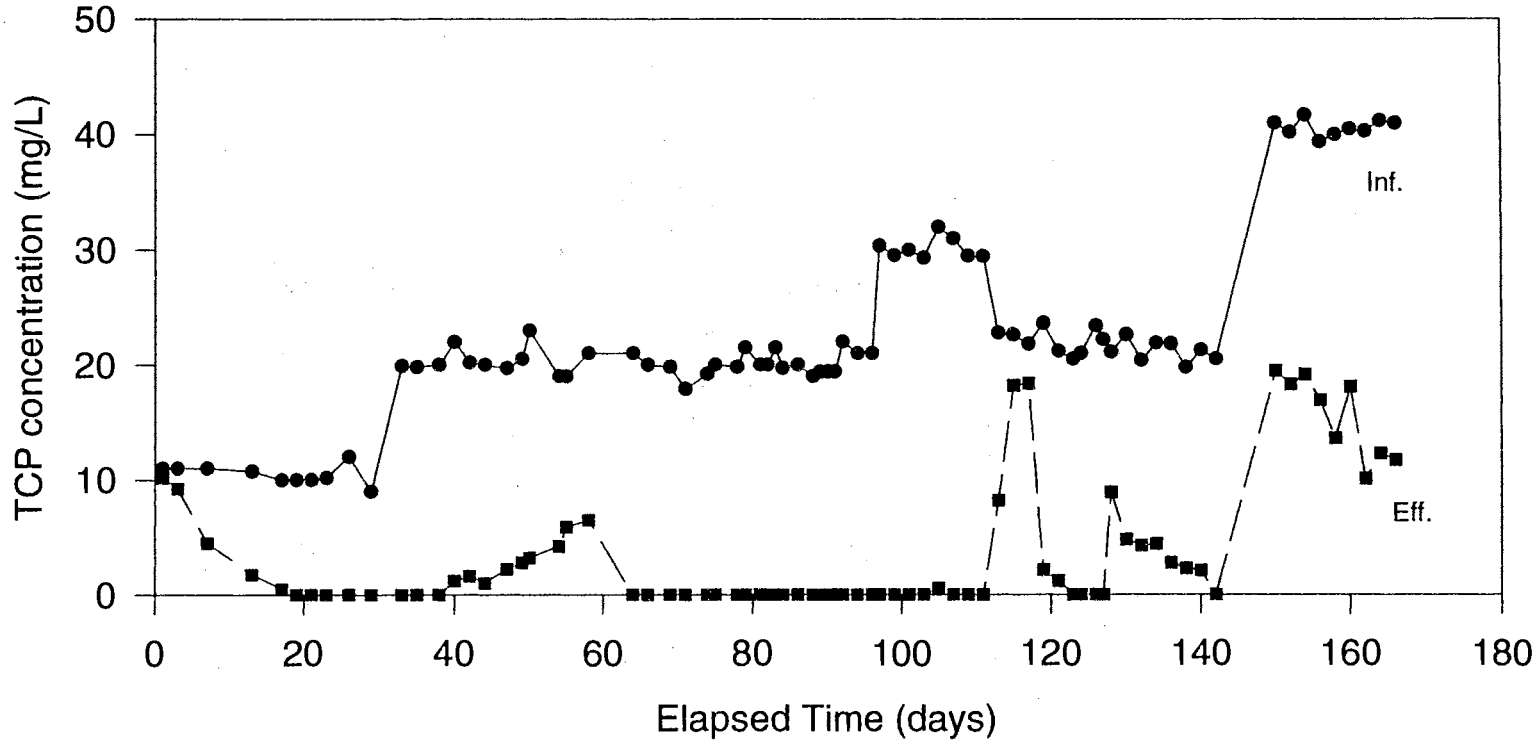

Figure 80. TCP removal by PVA (10 cm) column #1.

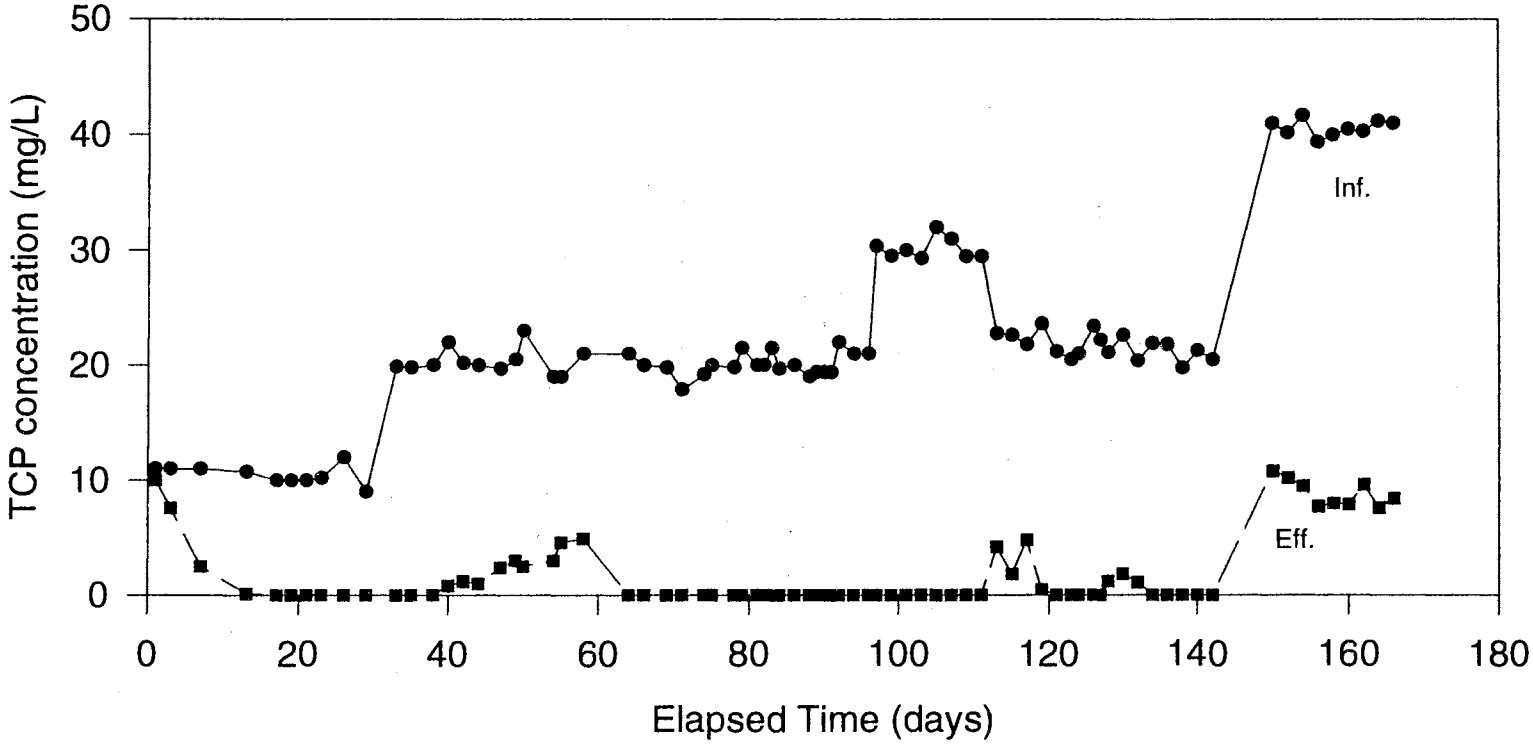

Figure 81. TCP removal by PVA (20 cm) column #2.

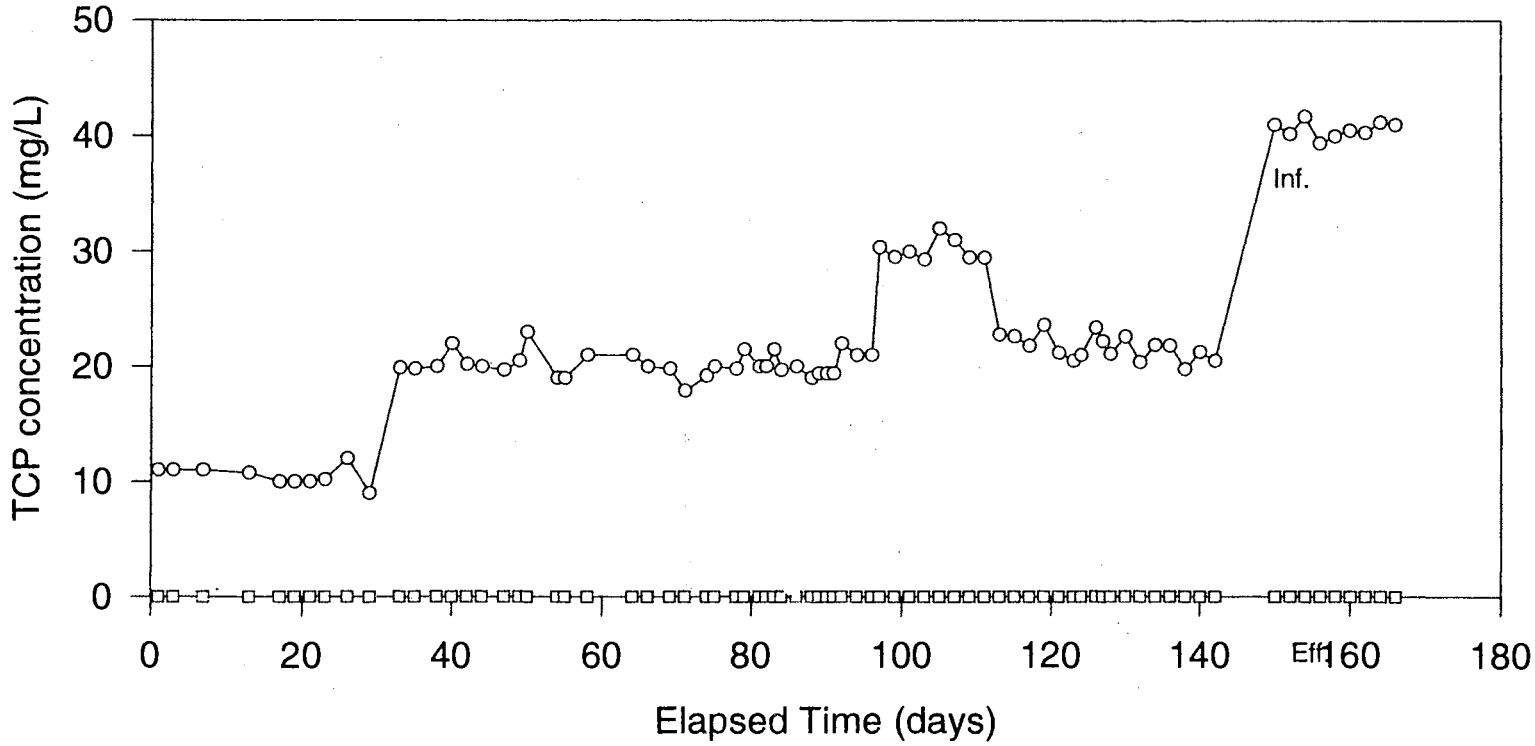

Figure 82. TCP removal by GAC (10 cm) column #3.

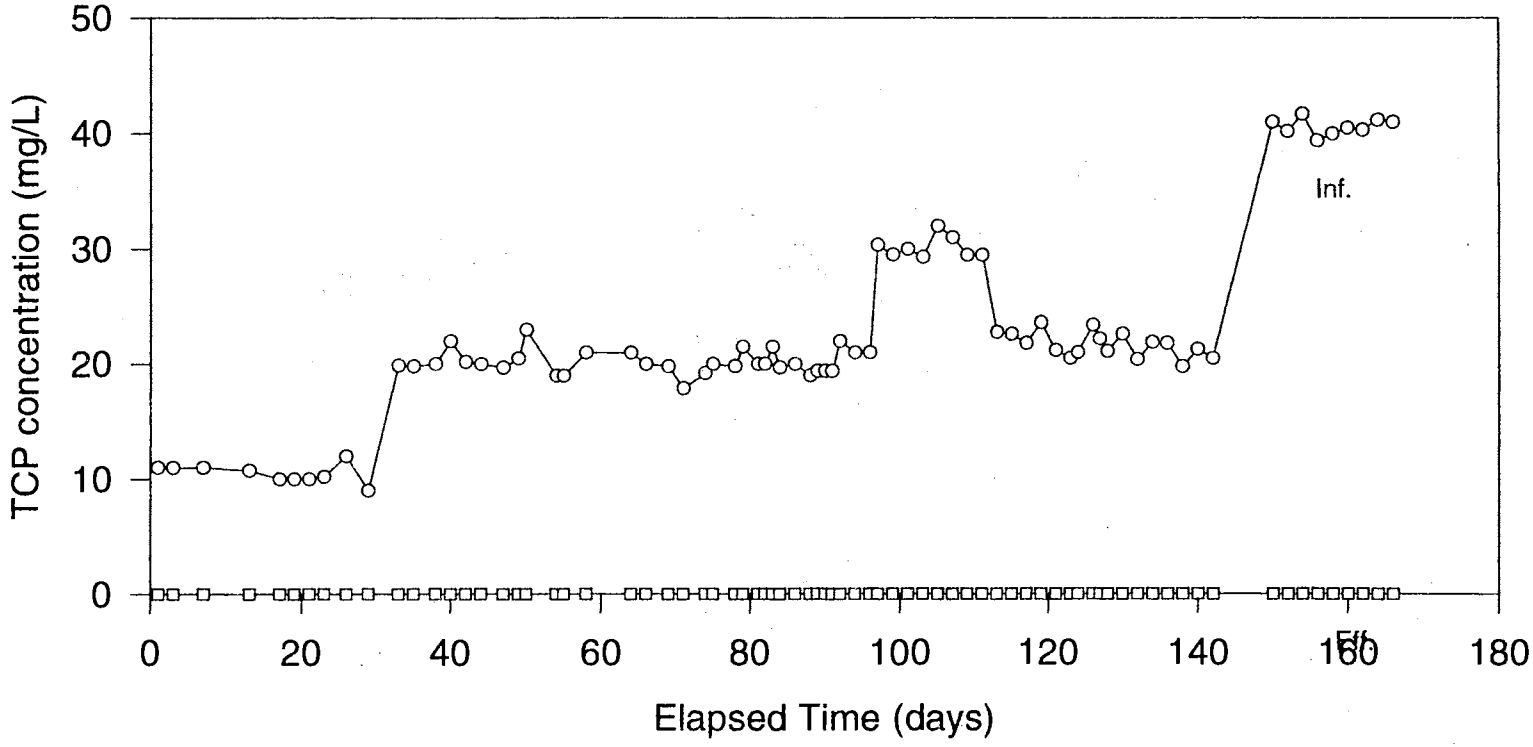

Figure 83. TCP removal by GAC (20 cm) column #4.

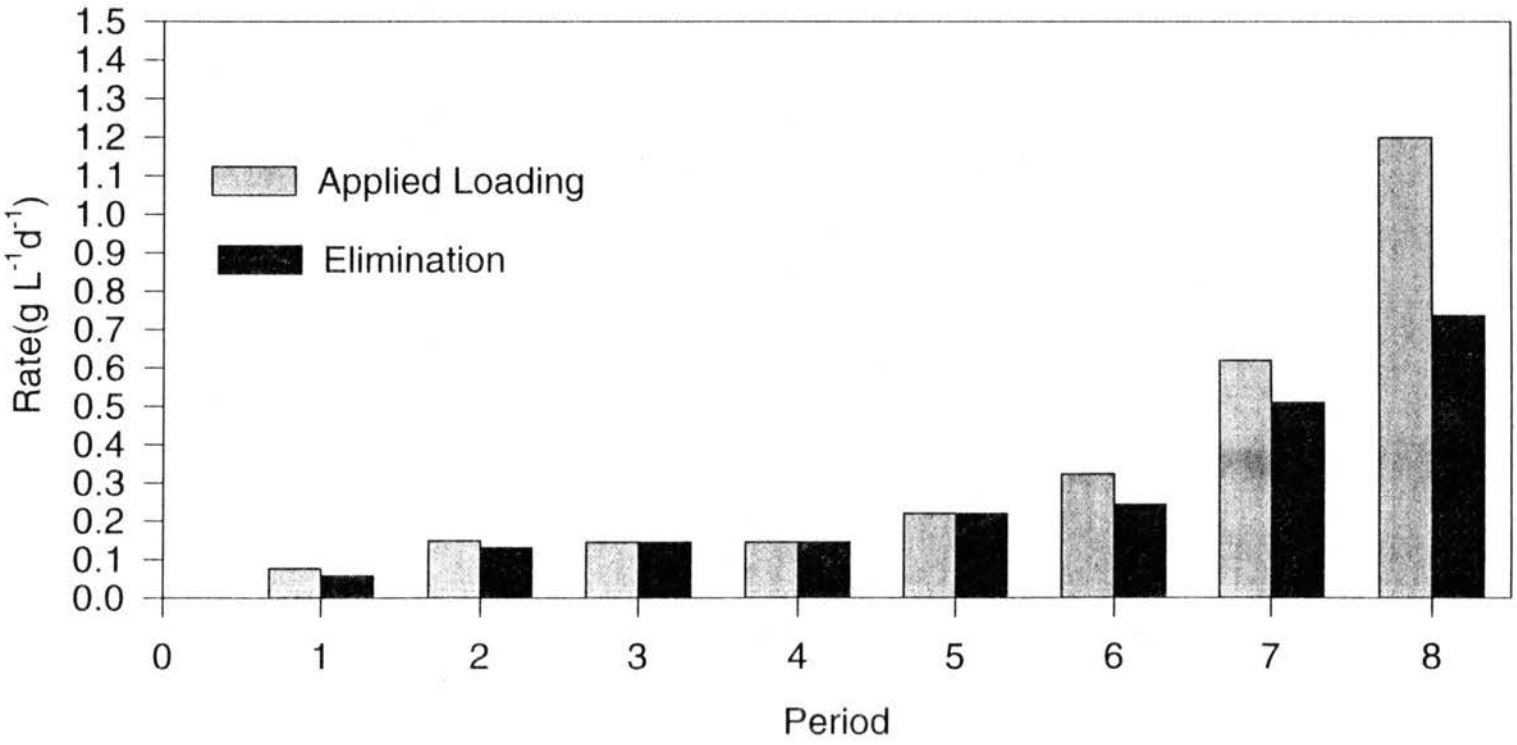

Figure 84. Elimination efficiency of PVA (10 cm) column #1.

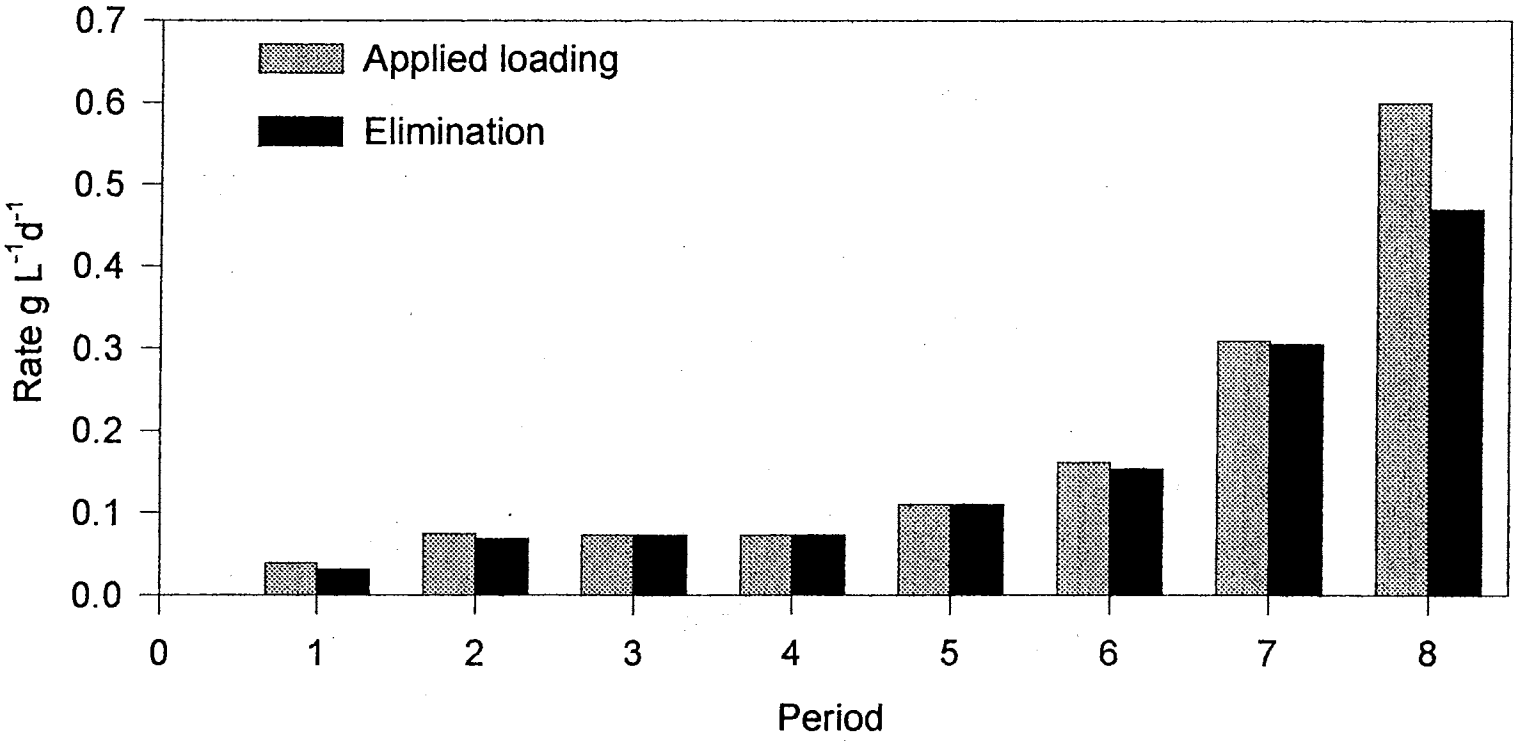

Figure 85. Elimination efficiency of PVA (20 cm) column #2.

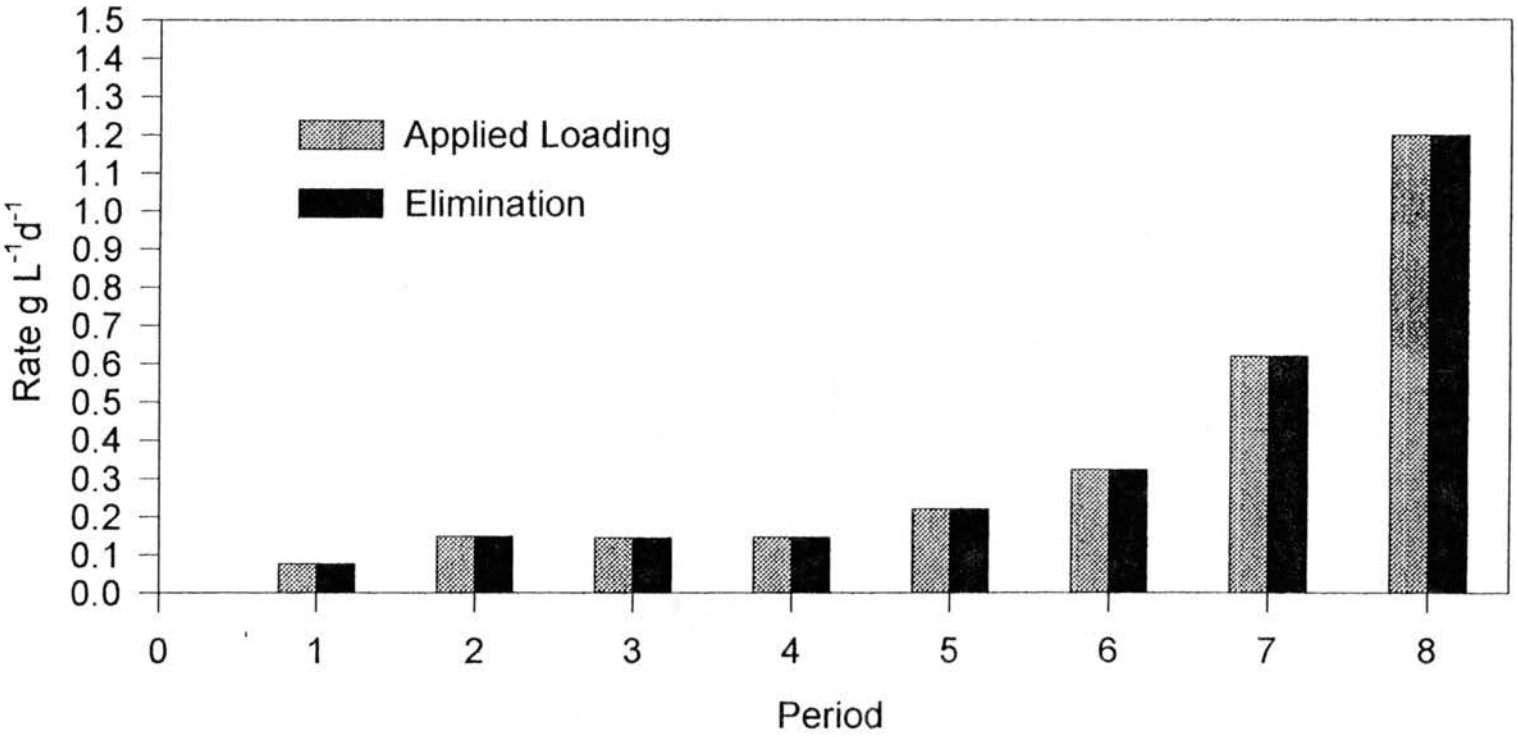

Figure 86. Elimination efficiency of GAC (10 cm) column #3.

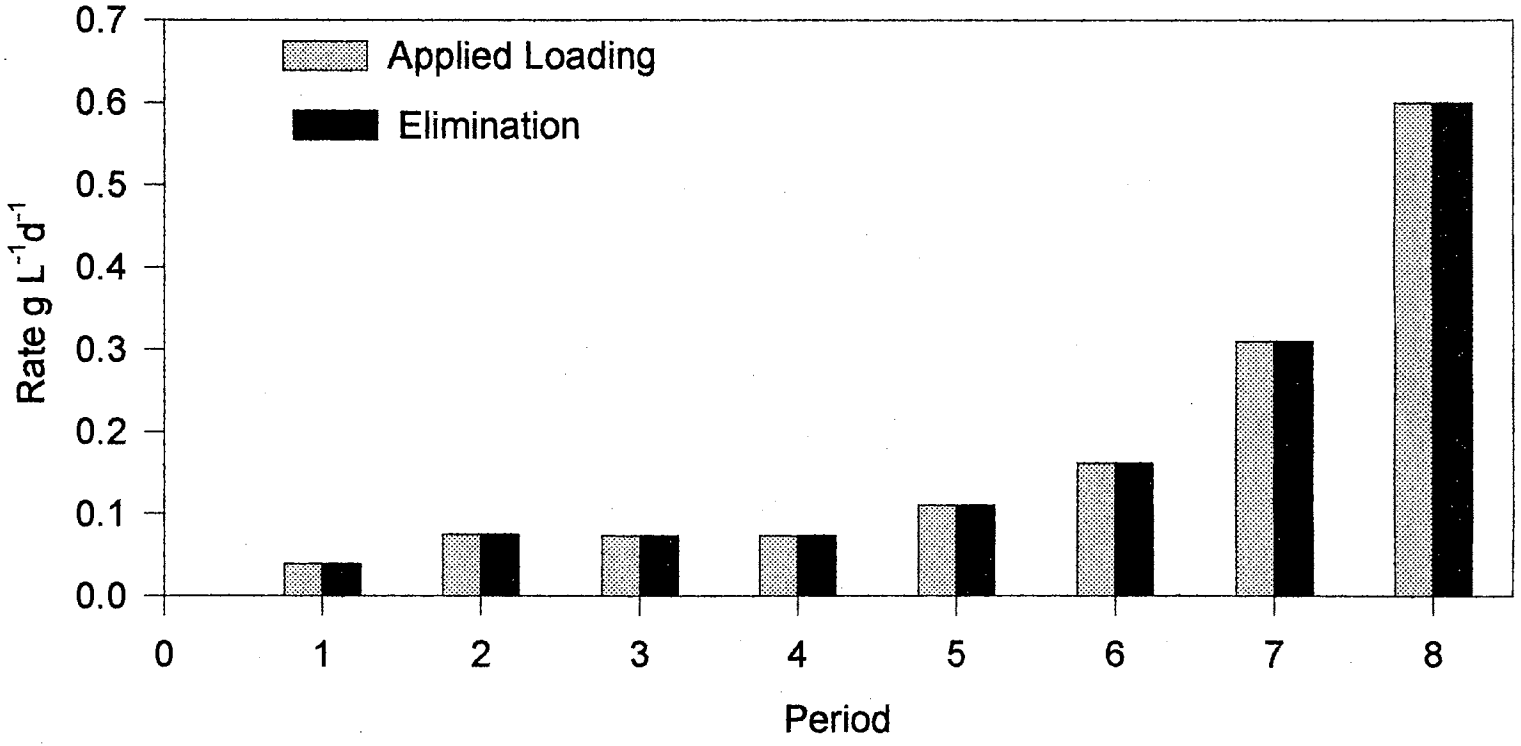

Figure 87. Elimination efficiency of GAC (20 cm) column #4.

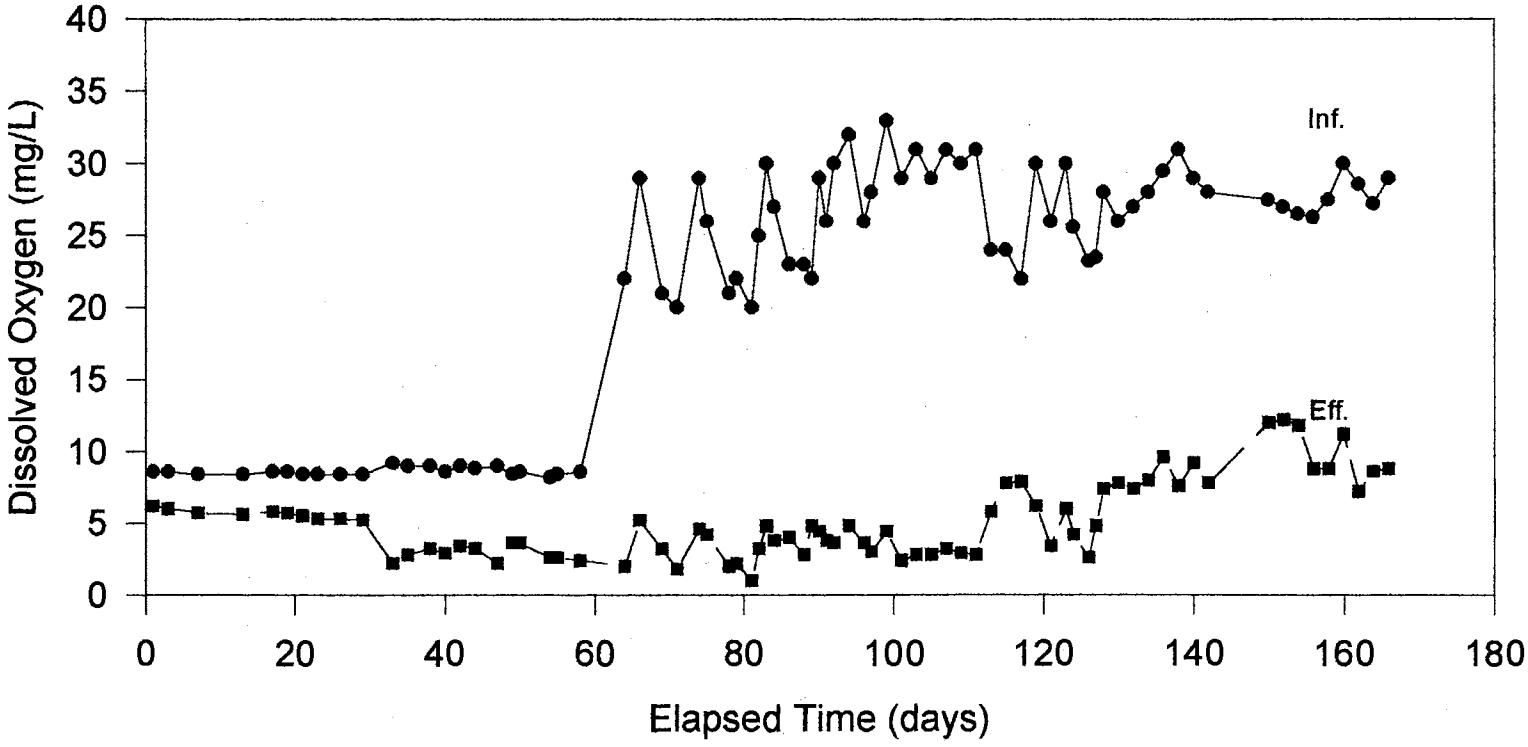

Figure 88. DO uptake by PVA (10.0 cm) column #1.

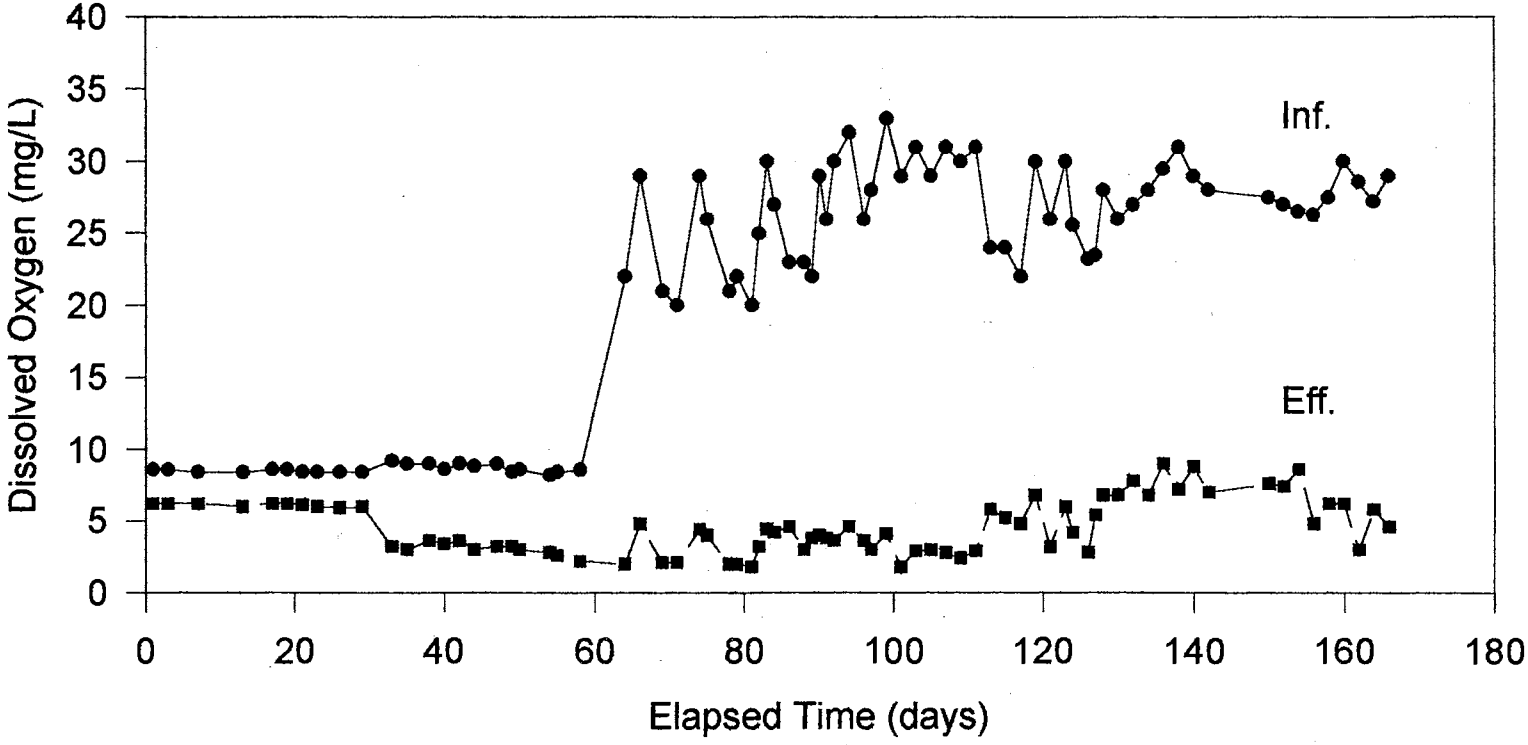

Figure 89. DO uptake by PVA (20.0 cm) column #2.

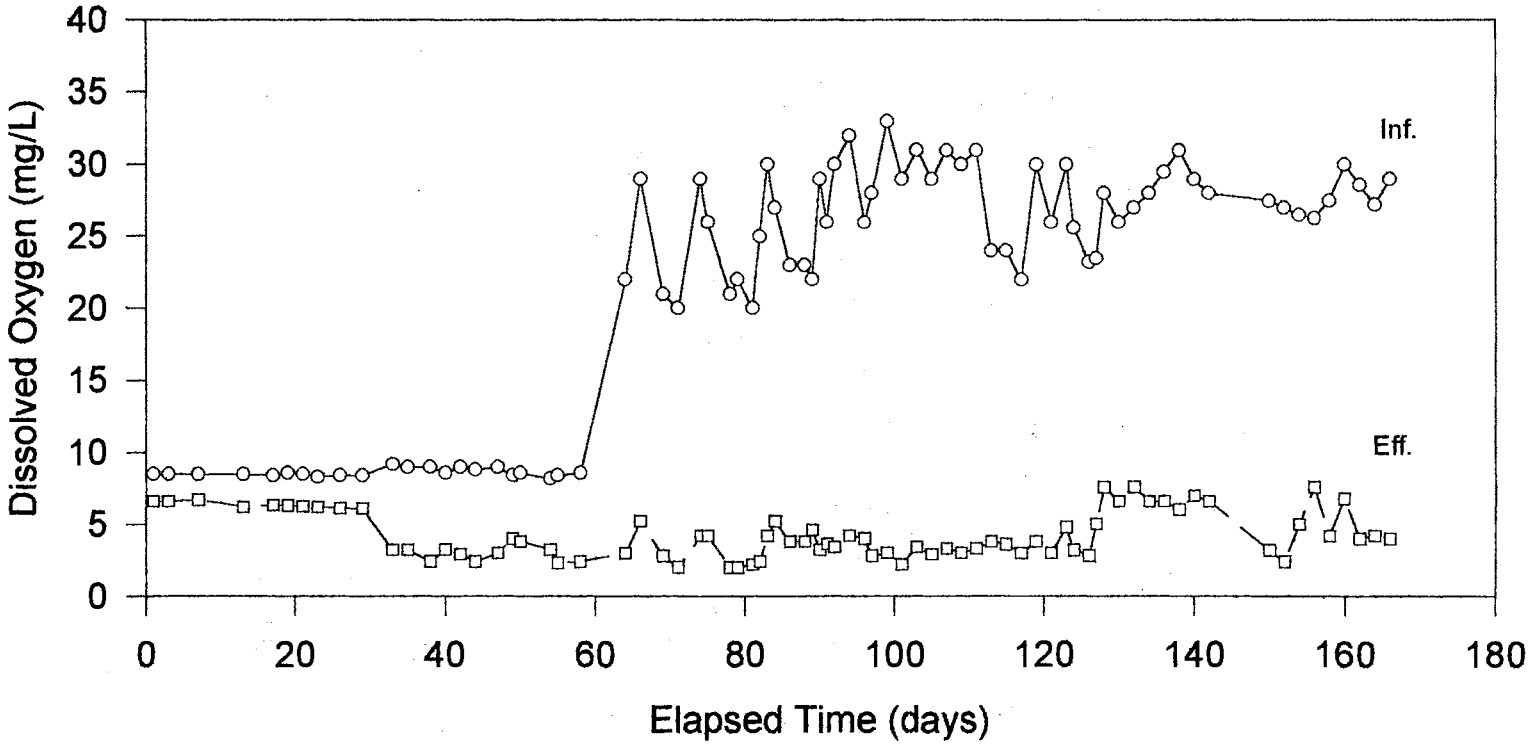

Figure 90. DO uptake by GAC (10.0 cm) column #3.

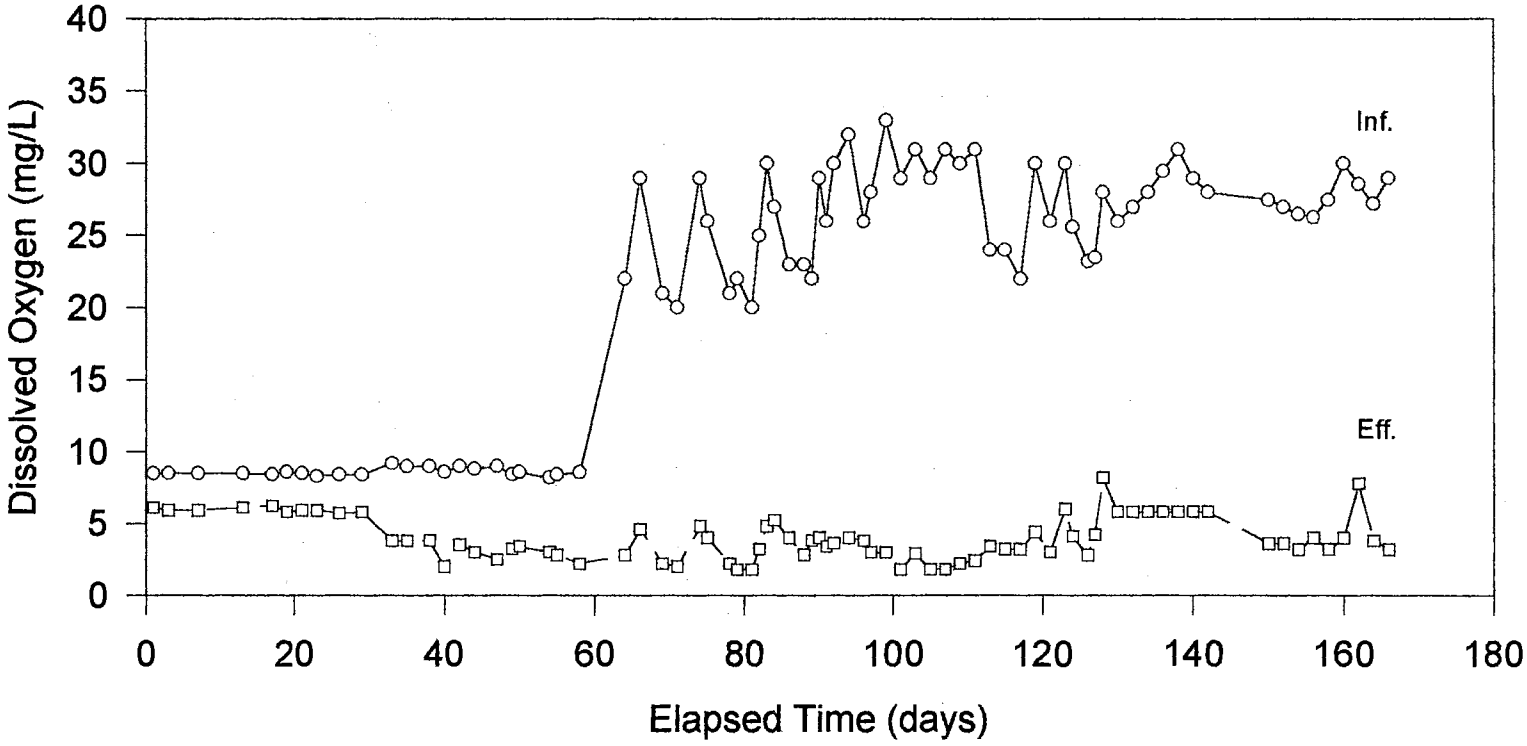

Figure 91. DO uptake by GAC (20.0 cm) column #4.

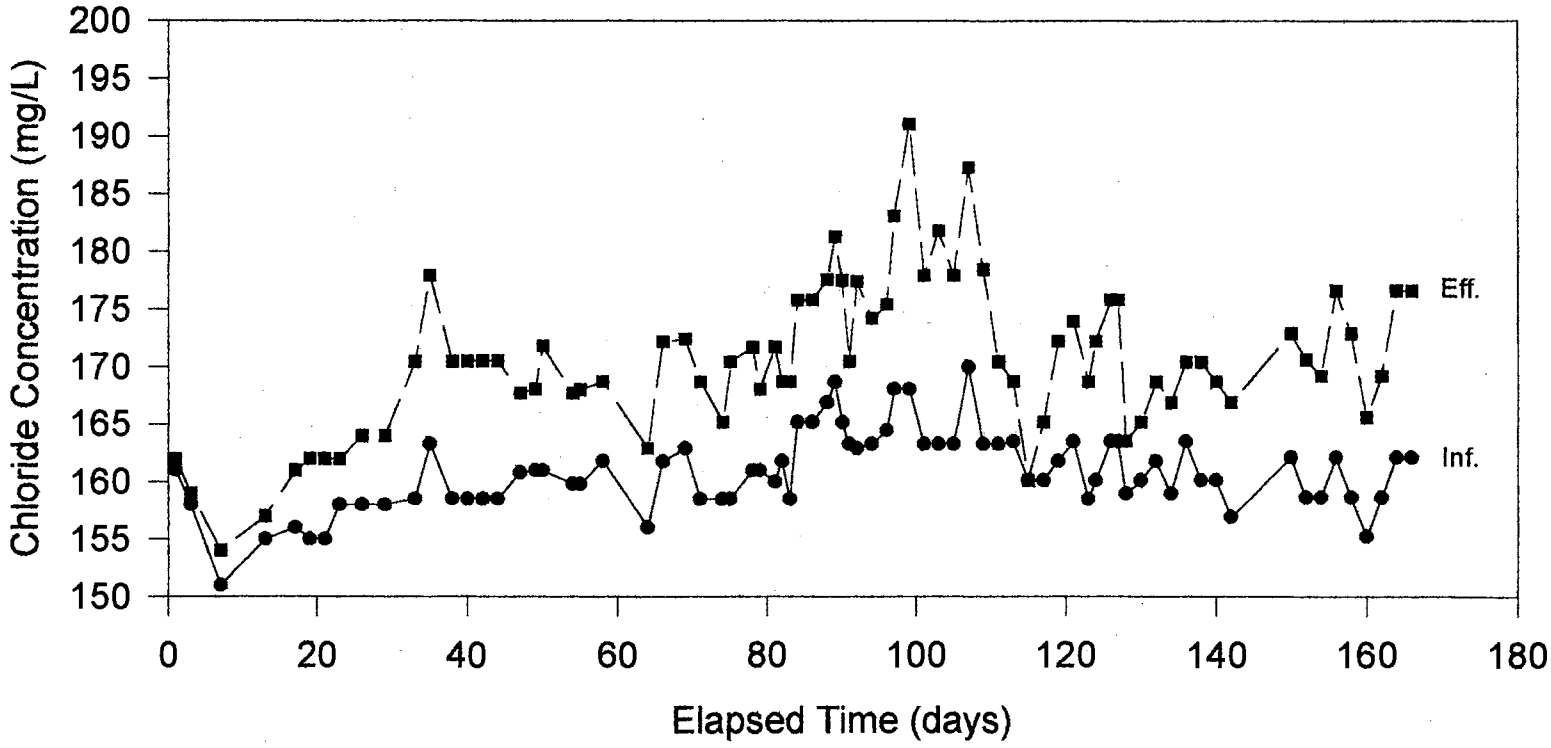

Figure 92. chloride release by PVA (10.0 cm) column #1.

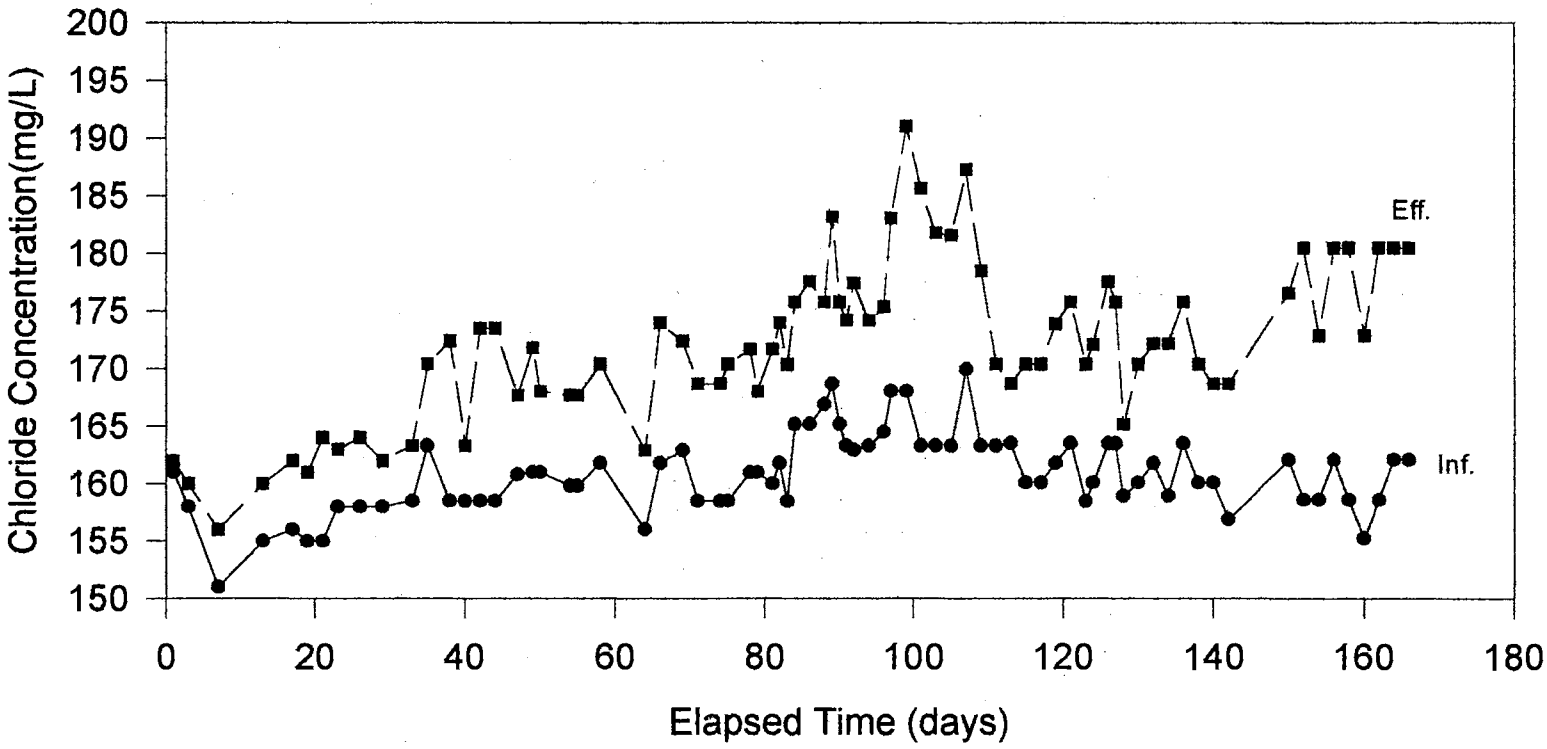

Figure 93. chloride release by PVA (20.0 cm) column #2.

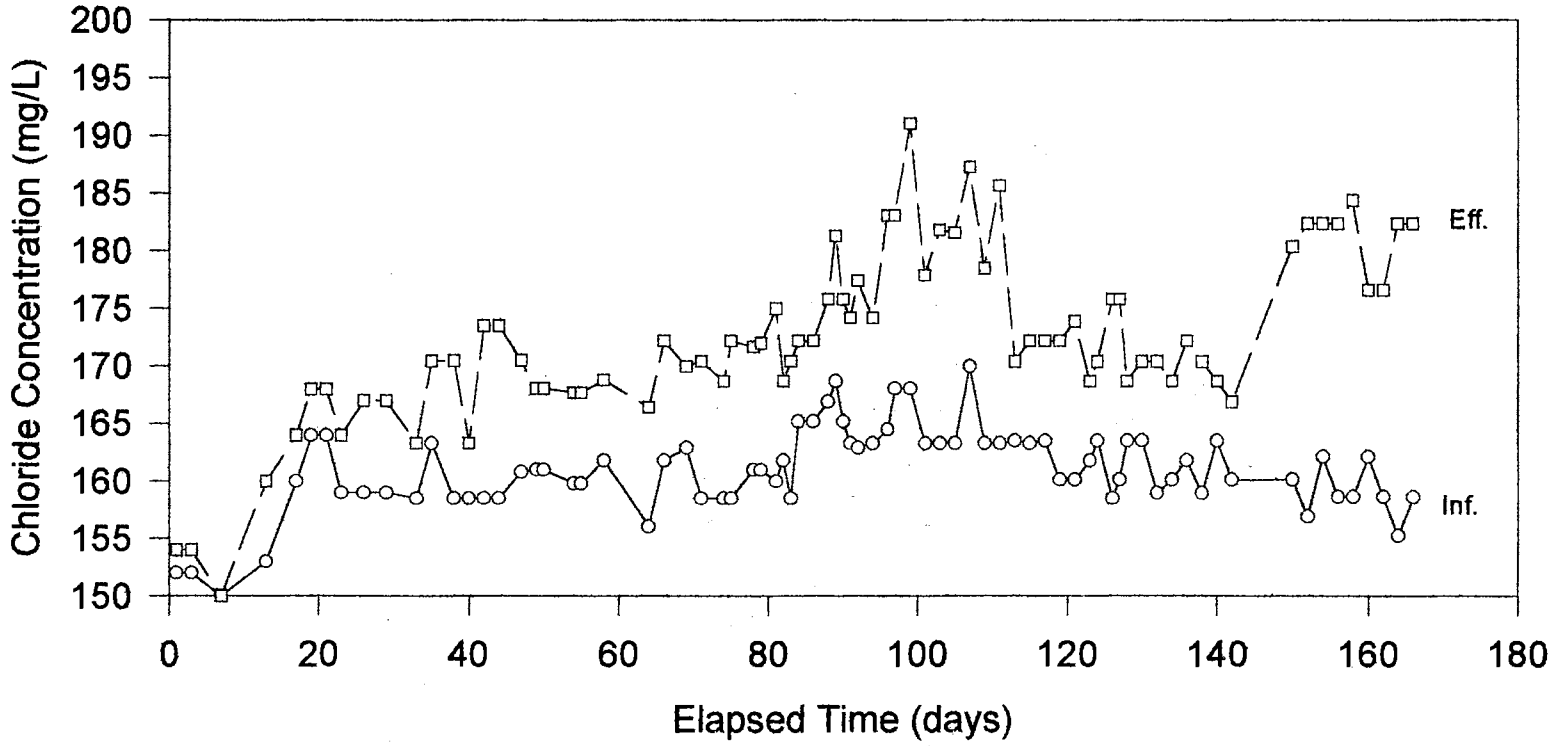

Figure 94. chloride release by GAC (10.0 cm) column #3.

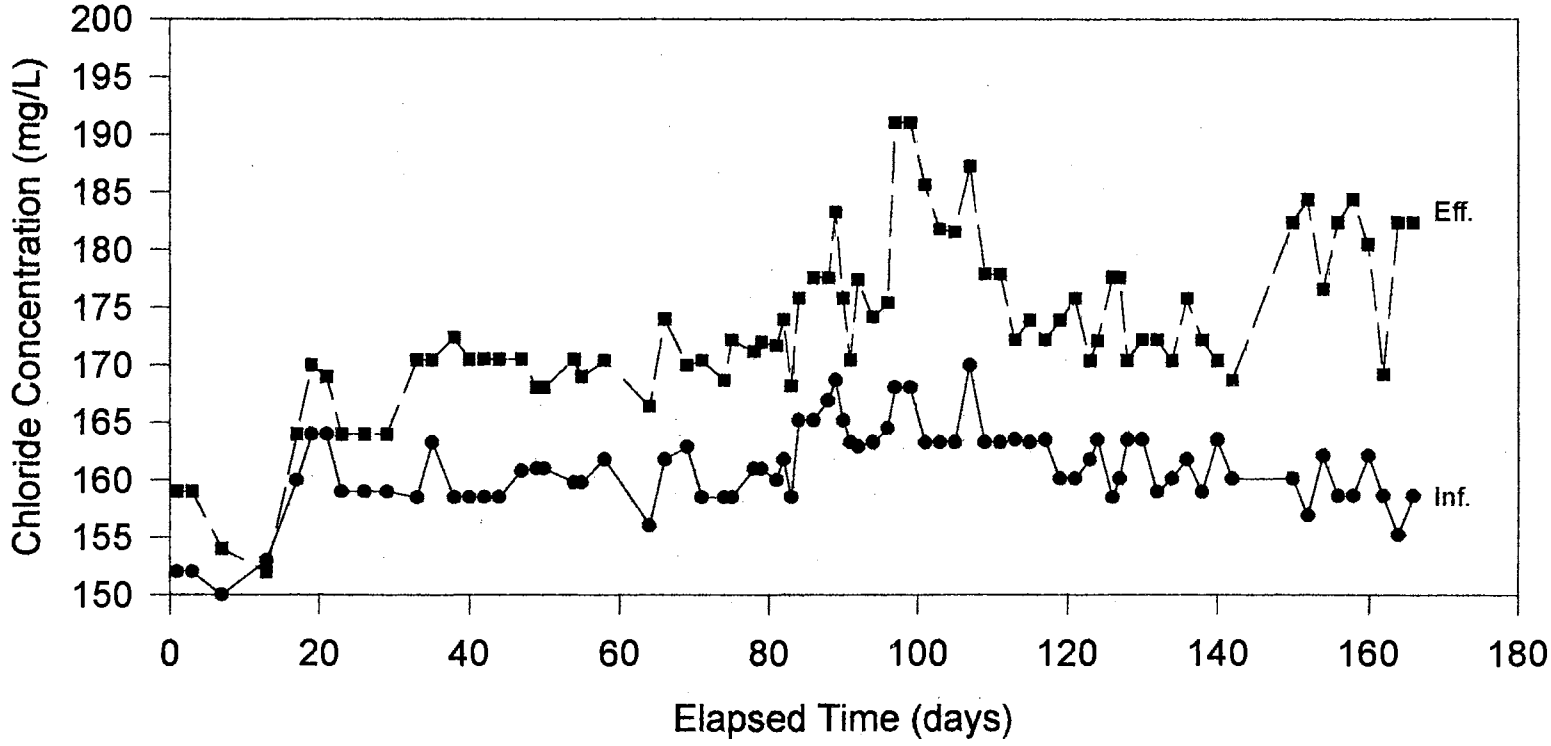

Figure 95. chloride release by GAC (20.0 cm) column #4.

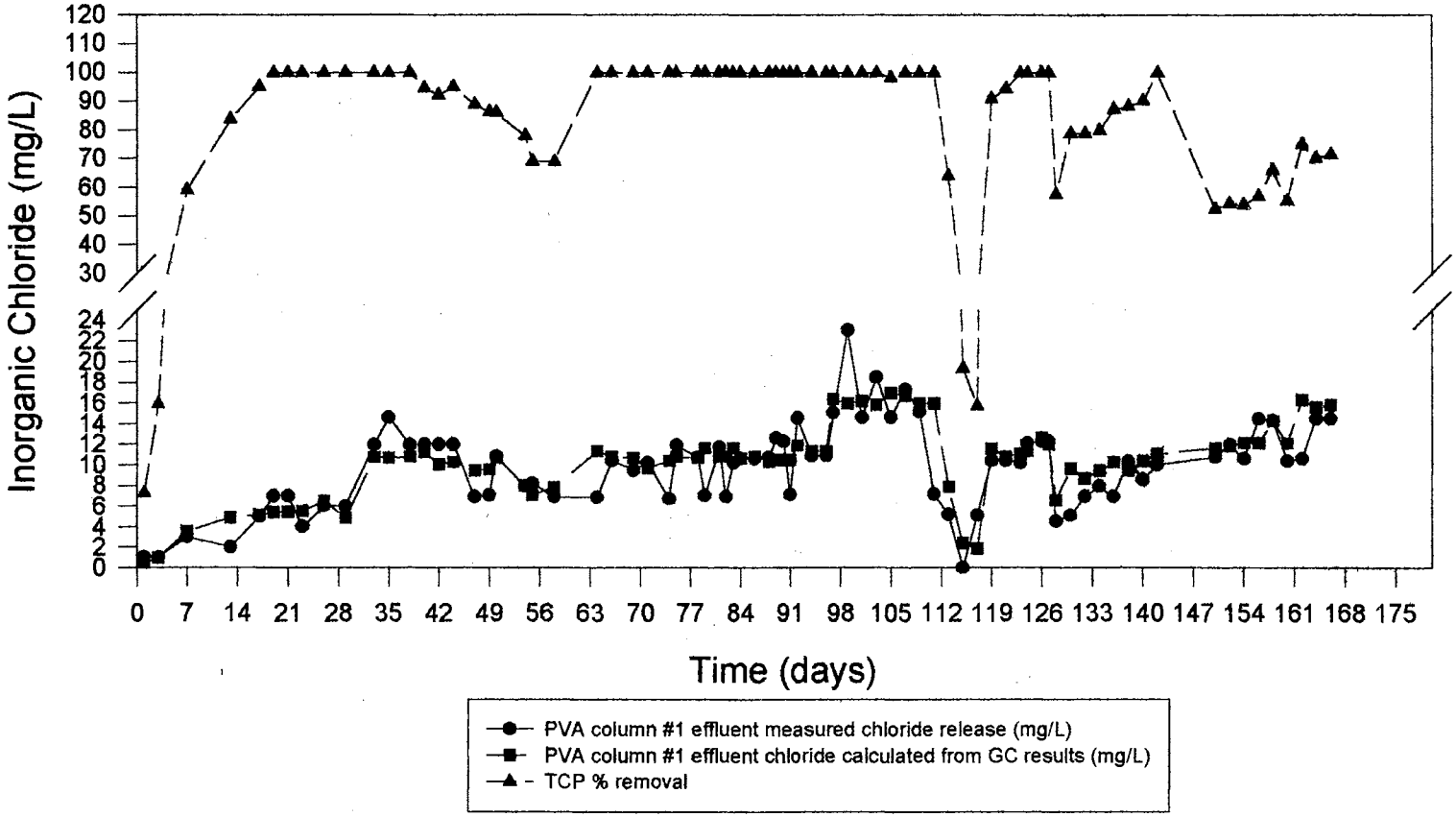

Figure 96. PVA (10.0 cm) column #1 comparison of %TCP removal, measured and calculated from GC results.

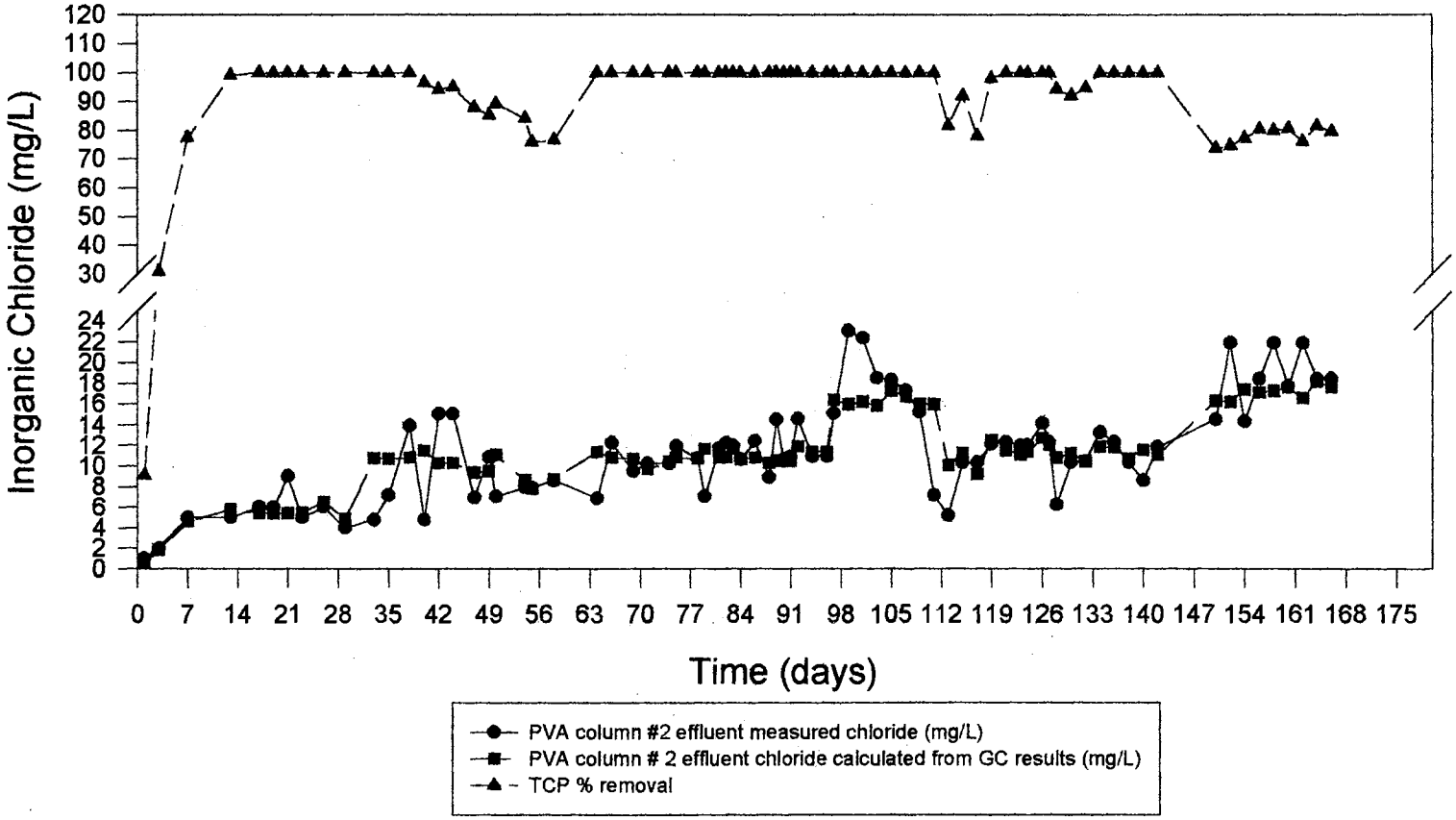

Figure 97. PVA (20.0 cm) column #2 comparison of %TCP removal, measured and calculated from GC results.

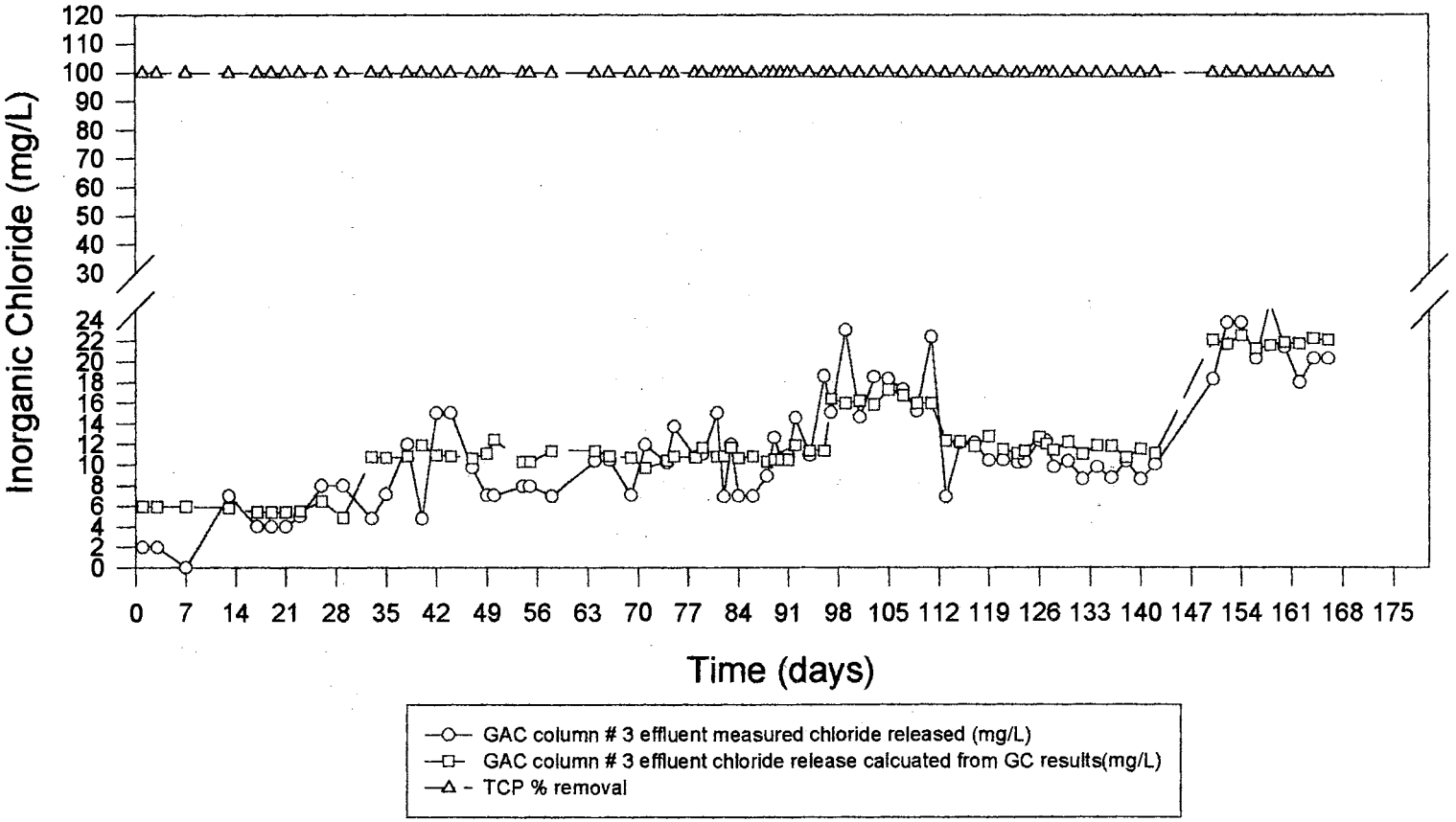

Figure 98. GAC (10.0 cm) column #3 comparison of %TCP removal, measured and calculated from GC results.

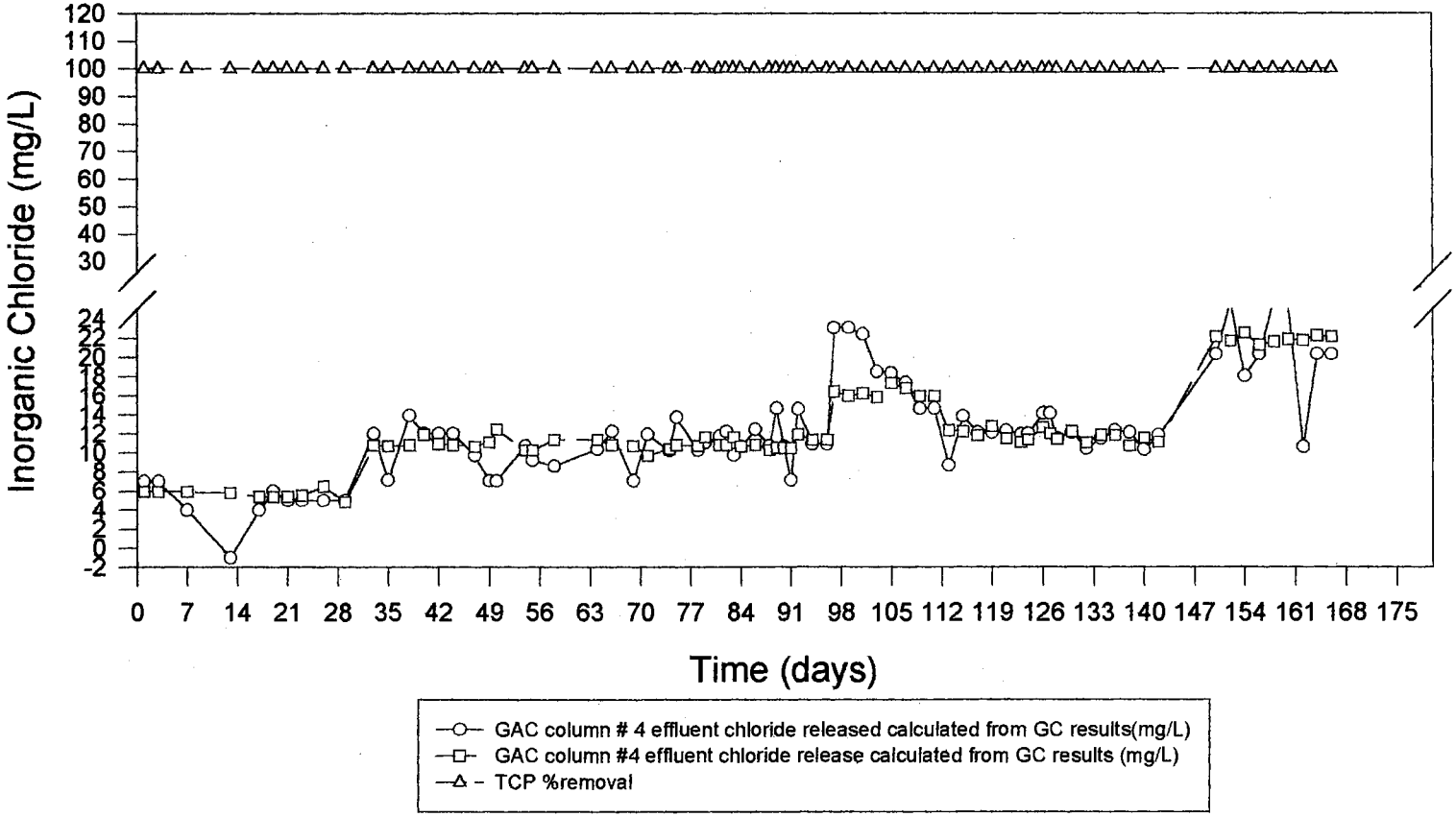

Figure 99. GAC (20.0 cm) column #4 comparison of %TCP removal, measured and calculated from GC results.

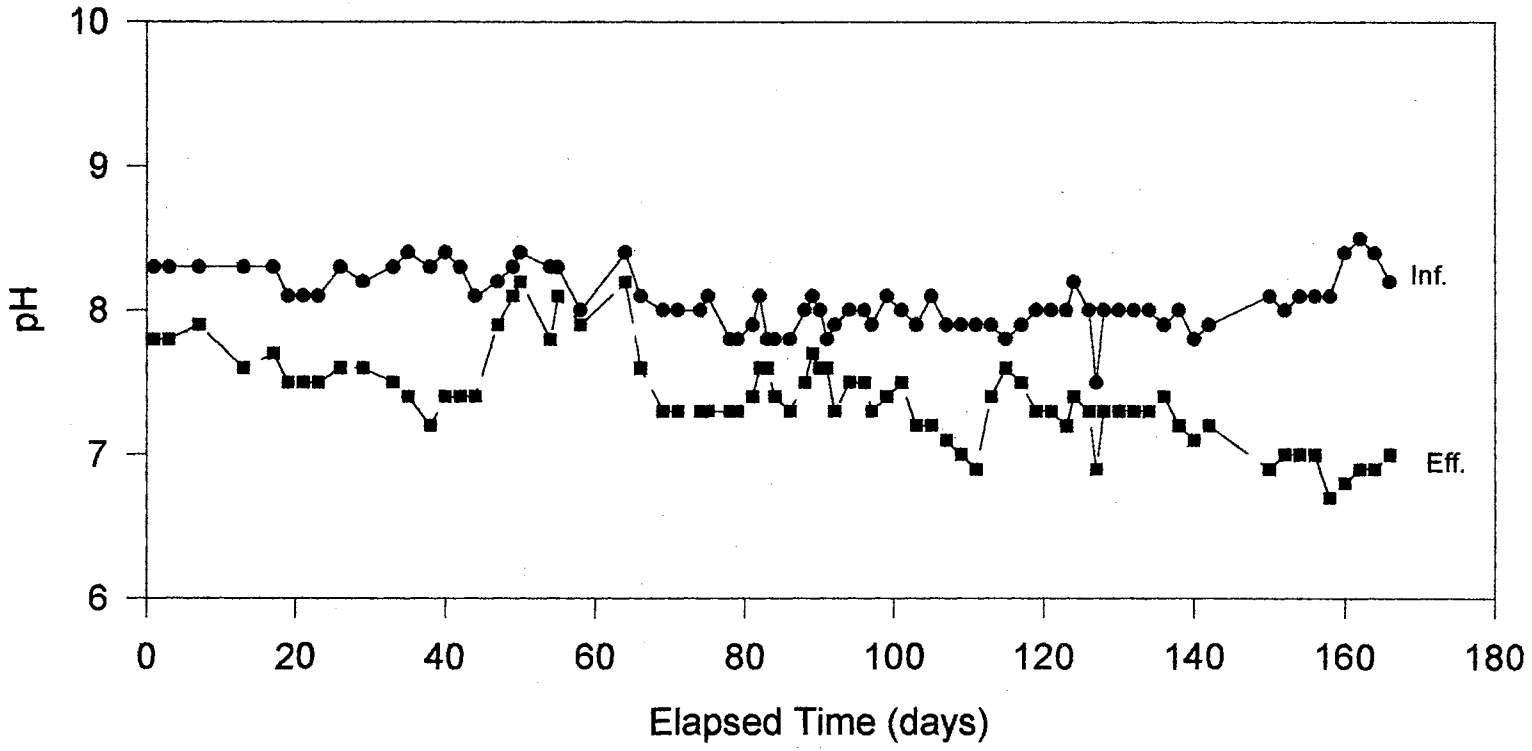

Figure 100. pH drop by PVA (10.0 cm) column #1.

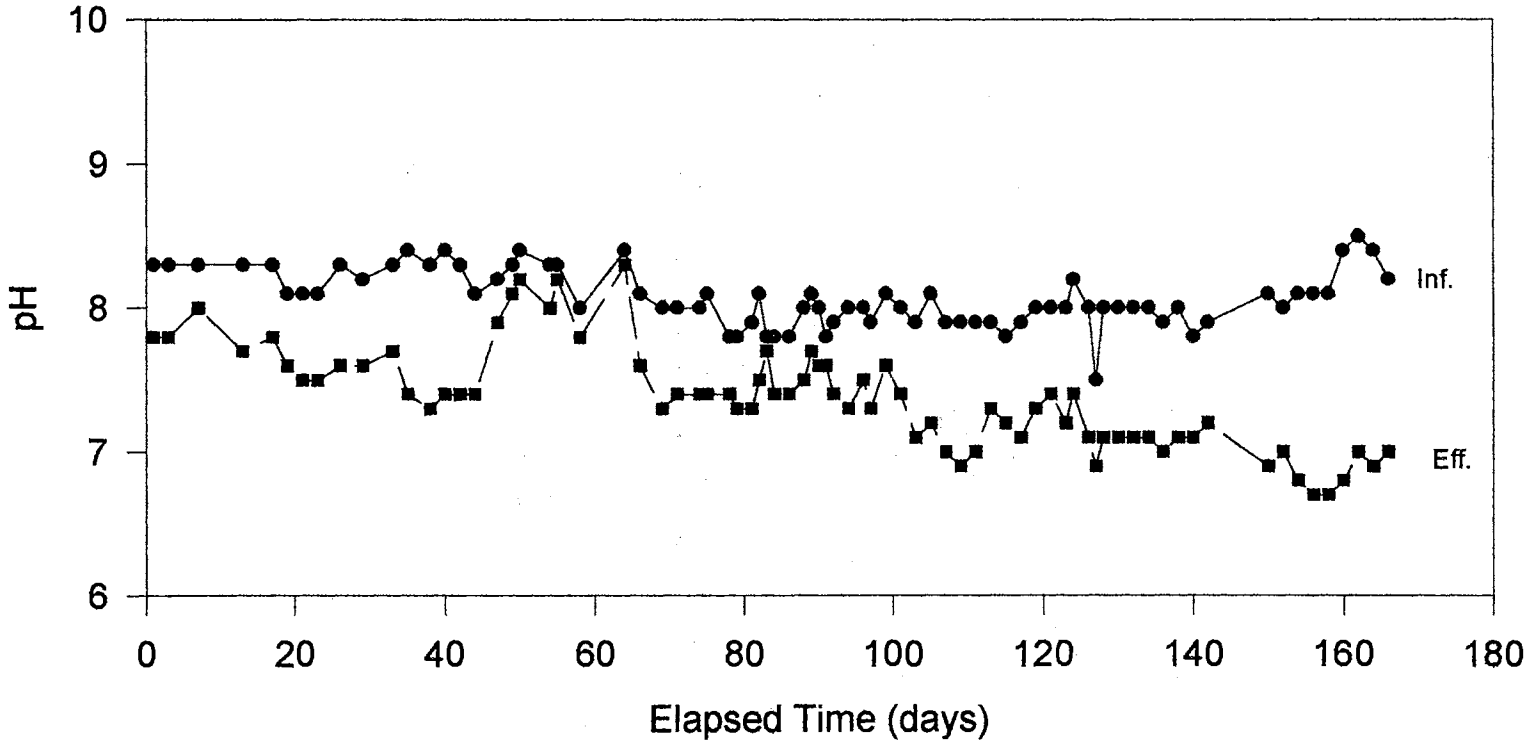

Figure 101. pH drop by PVA (20.0 cm) column #2.

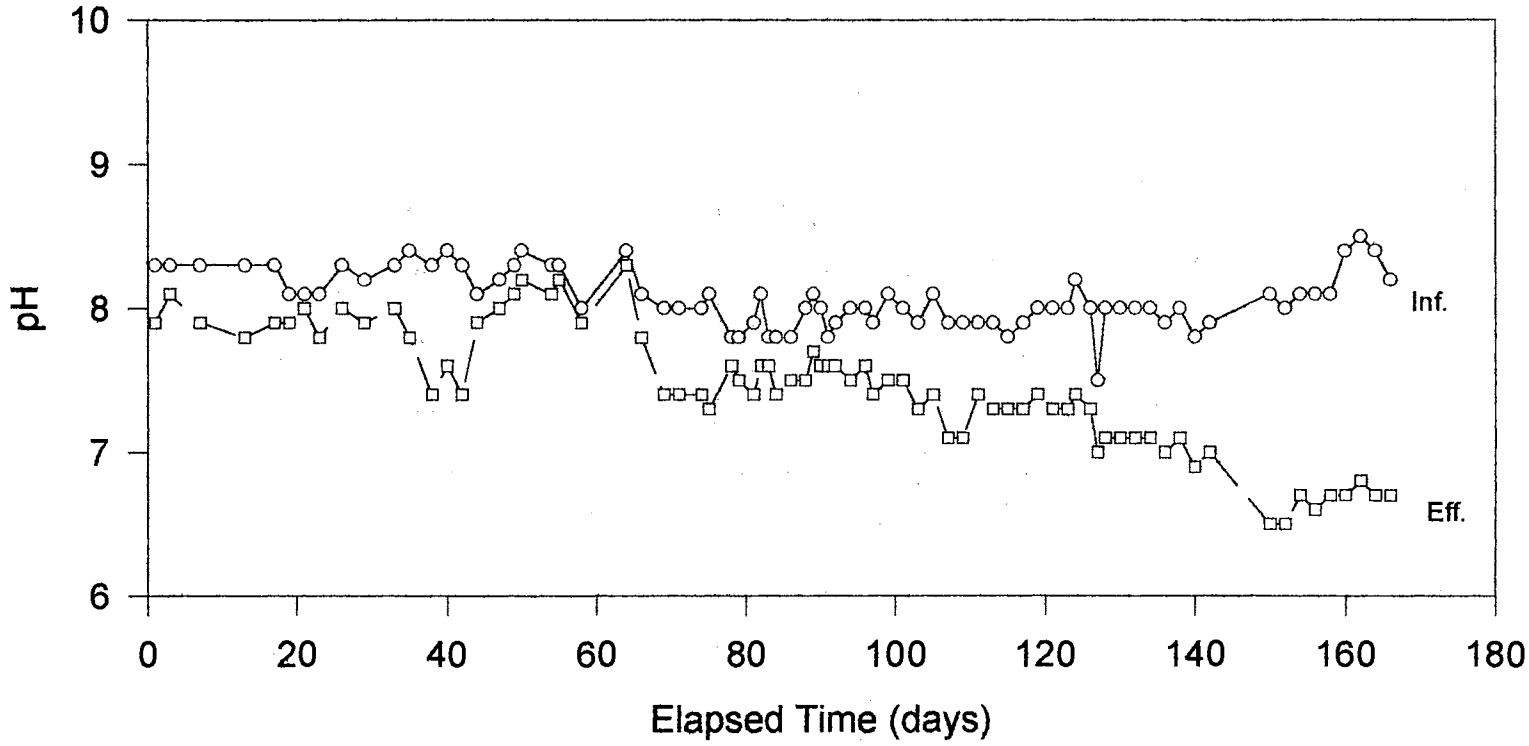

Figure 102. pH drop by GAC (10.0 cm) column #3.

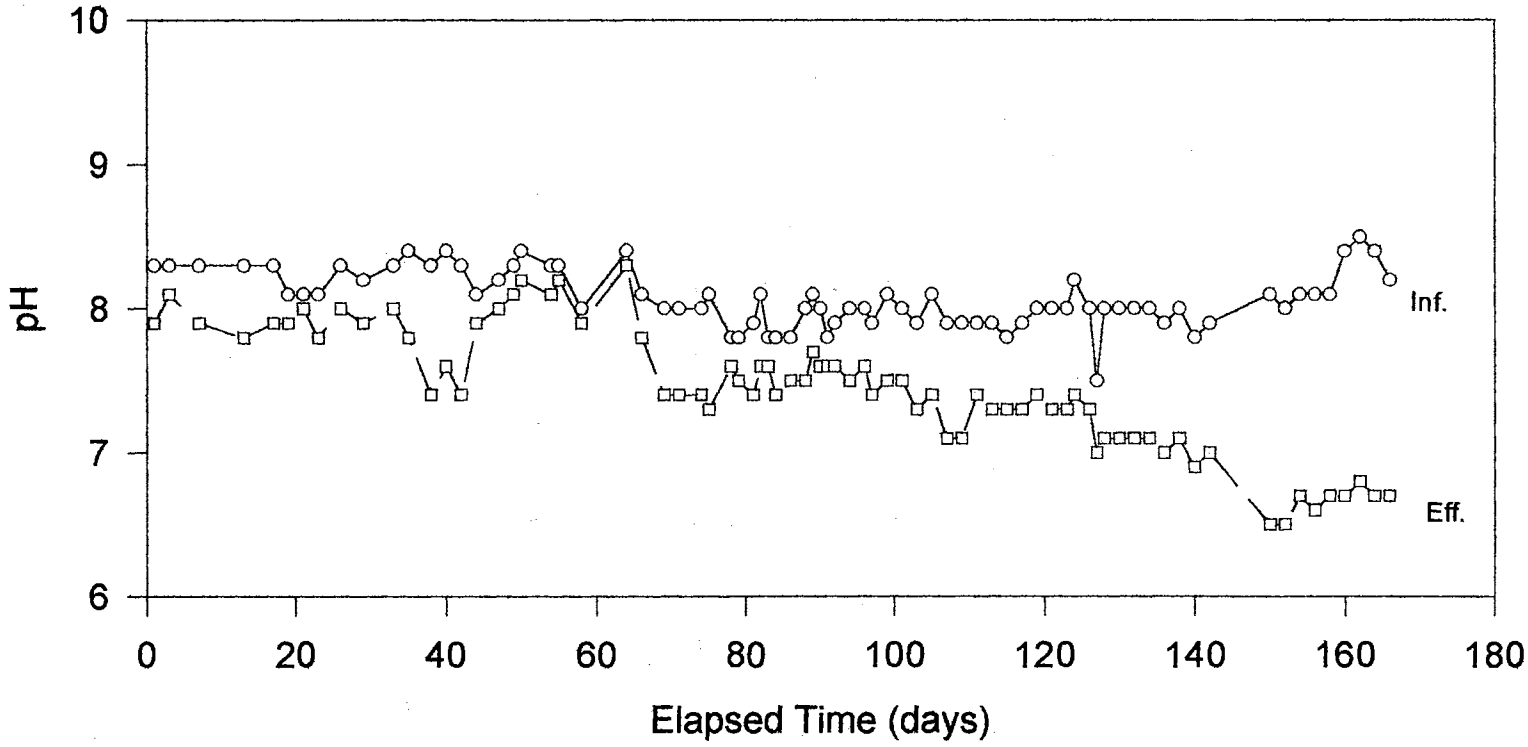

Figure 103. pH drop by GAC (20.0 cm) column #4.

Column Study No. 9

To study the effects of a external disturbance such as a TCP shock load on the PVA and GAC columns removal performance and recovery, the PVA (long) column #2 and GAC (long) column #4 were subjected twice (at two different times) to a high concentration (> 500 mg/L) of TCP for 50.0 hr. During this 50.0 hr period, the PVA (short) column #1 and GAC (short) column #3 were subjected to low DO (~ 2.0 mg/L) conditions. This external disturbance study lasted 70 days. The TCP concentration, DO, chloride release, and pH of the influents and effluents were monitored and are presented in Appendix N.

The effects of high shock load(s) on the PVA column #2 and GAC column #4 are presented in Figures 104 - 111. During the steady state process monitoring periods (days 168-179), (days 182 - 223), and (days 228 - 240), the TCP influent feed concentration was around 40.0 mg/L. With a flow rate of 2 mL/min, this resulted in the TCP loading of 0.3 g $L^{-1}d^{-1}$ for both columns. The feed bottle was oxygenated by bottled pure oxygen everyday (during 70 days) for at least 15 minutes. The influent bottle was capped to prevent oxygen loss.

Figures 104 and 105 show both columns responded to high concentration of TCP in the influent. During the 50.0 hr shock loading, the degradation of TCP by the immobilized cells in the PVA column #2 was susceptible to the high shock load. The removal efficiency of the PVA column #2 reduced from 90% before shock load to 0% during shock load. The applied loading during the first 50 hours high shock load was 4.12 g $L^{-1}d^{-1}$. The PVA column #2 recovered within 16 days as seen by the decreasing TCP concentration in the effluent. When the next shock load (50 hr) was applied on day

224, TCP concentration in the effluent of PVA column #2 increased and decreased in the same pattern as in the first shock load. This time, the recovery time was much shorter. The recovery time of the PVA column #2 from the second shock load was about 5 days.

These results demonstrated that the cells entrapped inside PVA column #2 tolerated high shock load and were protected to a certain extent by immobilization. The minimum concentration of TCP, which completely inhibited the growth of free cells (0.0 TCP removal) was found earlier to be 20.0 mg/L. Based on the TCP mass balance (influent -effluent) the cells entrapped inside PVA column #2, were not able to consume any TCP during the first shock load. This is in conformity with our observations on DO, chloride release, and pH of the PVA column #2 effluent shown in Figures 106, 108, and 110. As seen in Figure 106, there was a rise in the effluent DO during the first shock load along with no change of the effluent chloride concentration or pH (Figures 108, 109).

The mass balance on TCP (influent-effluent) during the second shock load indicate that the cells were active and biodegrade 169 mg of TCP which is about 15% of total 1154 mg of influent TCP (Figure 104). As seen in Figures 106, 108, and 110, the cells in the PVA column remained active indicated by DO uptake, chloride release, and pH drop in the effluent during the second shock load. The results also indicate that the process recovered within 5 days as seen by 100% removal of TCP in the effluent. Simultaneous oxygen uptake, chloride releases, and pH drop of the effluent gave further support to the occurrence of TCP biodegradation by PVA column #2.

The effects of shock loads on GAC column #4 are presented in Figures 105, 107, 109 and 111. Based on the TCP mass balance(influent - effluent), the GAC column was

able to take 33% (368.4 mg) and 96% (1143 mg) of influent TCP (1132 mg, 1154.3 mg) during the first and second shock load, respectively. During the first shock load, limited removal of TCP took place. This indicates that the GAC adsorption capacity was virtually exhausted (influent TCP = effluent TCP), and the biological activity was low as shown (Figures 107, 109, 111) by the rise of effluent DO, low chloride release, and small drop of pH in the effluent. On day 183, the effluent from the GAC column contained a higher TCP concentration than incoming influent. This indicates that desorption was taking place in the GAC column. The increased oxygen uptake, chloride release, and pH drop in the GAC column effluent between day 194 and day 213 seemed to be caused mostly by biodegradation of desorbed TCP. Dehalogenation of 40.0 mg/L TCP should release about 21.6 mg/L of chloride. During this period the average chloride release was about 57 mg/L in the effluent. The 60% extra chloride release is believed to be mostly as the result of biodegradation of desorbed TCP (bioregeneration). Between day 26 and day 45, the average daily influent TCP loading rate was 116 mg TCP/d (40.0 mg/L TCP at 2 mL/min), whereas the effluent TCP was zero. The average daily chloride release rate expected to be 62.6 mg Cl^- /d for complete dehalogenation of 116 mg TCP/d. The average daily chloride production rate was 160 mg Cl^- /d. Therefore, approximately 97.4 mg Cl^- /d extra chloride release was obtained that was not accounted for by the influent TCP. This extra chloride release must come from dehalogenation of TCP already adsorbed by carbon. Approximately 3427.0 mg TCP was removed from GAC (bioregenerated) between two shock loads (days 194-213). The effect of this extra chloride release and production of HCl can be seen in Figure 111. The average GAC column effluent pH between day 26 and day 45 was 6.6. According to pH curve (Figure

15), the effluent average pH drop from 8.1 to 6.6 show that 23 mL volume of 0.1 N HCl would be required to have the same pH drop. This is 60.4 mg/L Cl^- concentration which was close to the chloride measured (57 mg/L) during this period. The pH drop in the effluent along with chloride release (measured) supports complete dehalogenation of TCP. Aerobic mineralization of 40.0 mg/L TCP requires at least 35.6 mg/L of DO to release 21.6 mg/L chloride subsequently. It is clear that the DO provided was insufficient to biodegrade TCP already adsorbed by the carbon and released an average 35.5 mg/L extra chloride during days 194-213. Therefore, the dehalogenation of TCP already adsorbed was believed to be mostly the result of anaerobic biodegradation.

As shown in the Figures 109 and 111, the immobilized cells in GAC column #4 continued to biodegrade already adsorbed TCP until day 213. The samples taken on day 217 indicated that there was no extra chloride release in the effluent which was consistent with rise of the effluent pH. Therefore, the cells remained active and survived the shock load and continued bioregenerate the carbon completely during 19 days under DO deficiency (anaerobic condition).

During the second shock load (day 224 and 225), the GAC column #4 adsorbed a total of 3120 mg TCP out of 3237 mg TCP applied in the influent. The immobilized cells remained very active during the second shock load and continued to dehalogenate TCP as seen in Figures 107, 109, and 111. The cells were able to biodegrade(aerobic condition) approximately 32.0 mg/L of TCP with the corresponding DO usage (Figure 107) , chloride release (Figure 109), and effluent pH drop (Figure 111). Within the first week after the second shock load, the immobilized cells started to biodegrade TCP already adsorbed on GAC column during the second shock load, evident from Figure

109 and 111. The extra chloride released by GAC column and the effluent pH drop during 233-240, followed the same pattern as seen during days 194-213. The immobilized cells in GAC column #4, remained active during the second shock load and continued to biodegrade TCP under both aerobic and anaerobic conditions.

The presence of CO_2 and methane gas determined by GC/MS gave further evidence of the existence of both aerobic and anaerobic activity present in GAC column #4. The results of GC/MS are presented at the end of this chapter.

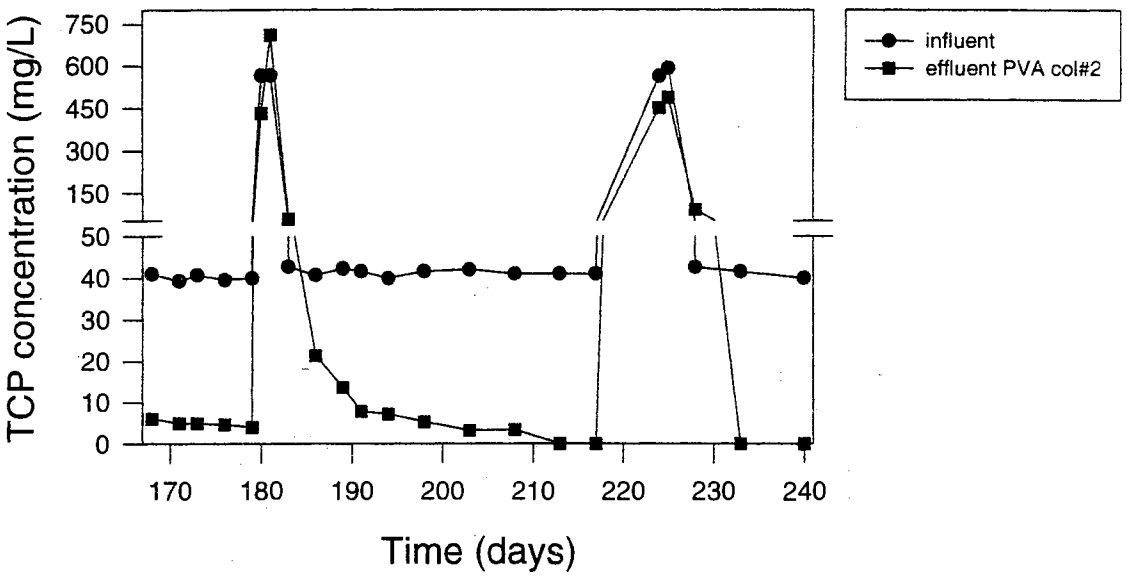

Figure 104. PVA column #2 TCP concentrations response to the high shock loads.

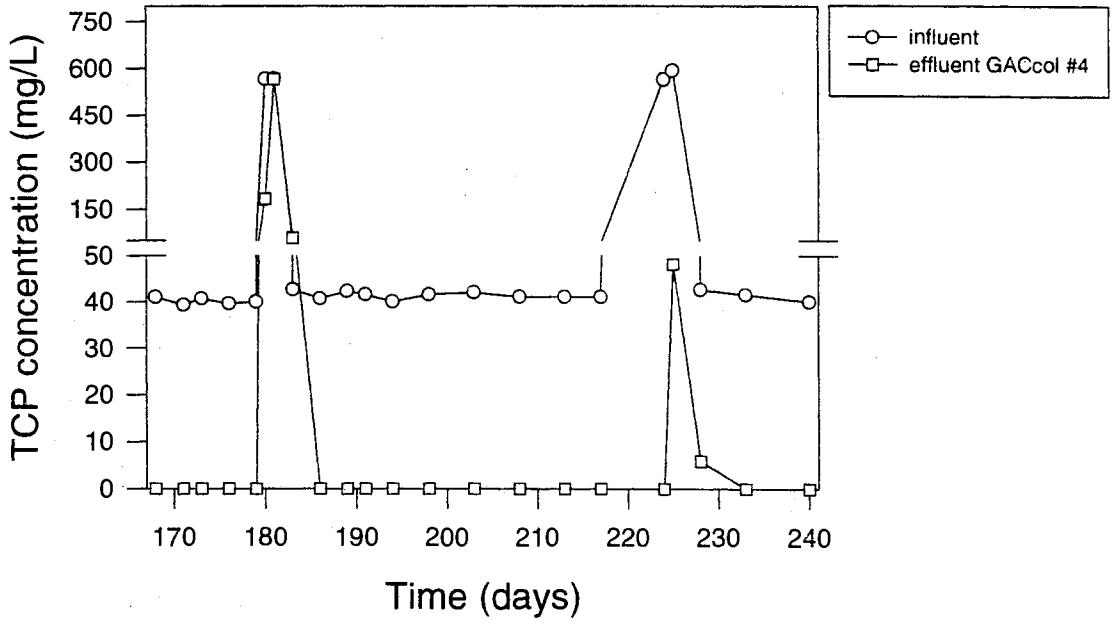

Figure 105. GAC column #4 TCP concentrations response to the high shock loads.

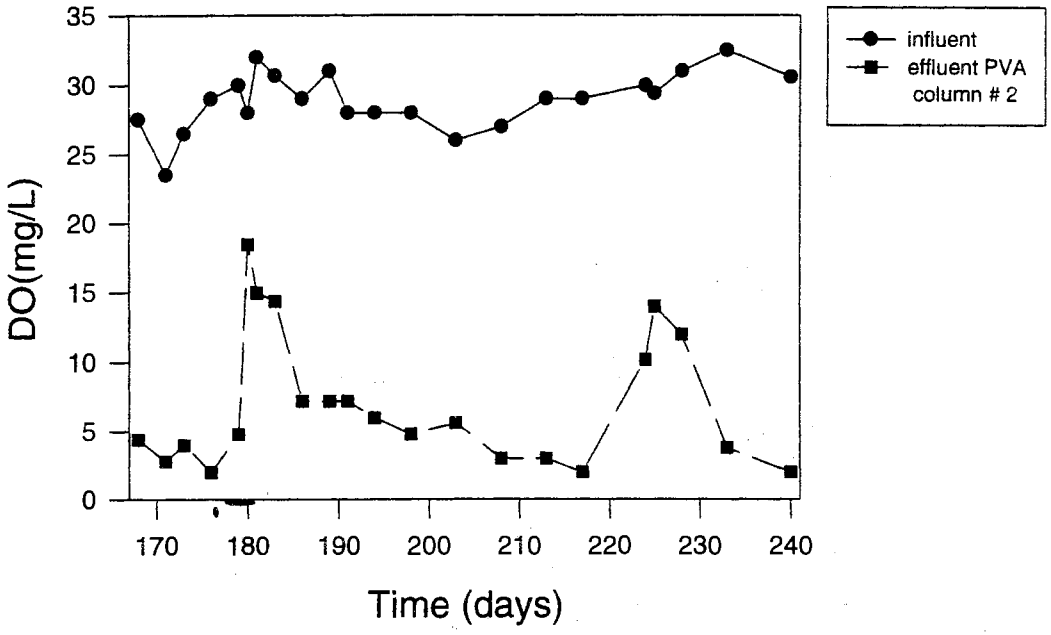

Figure 106. PVA column #2 DO changes during high shock loads.

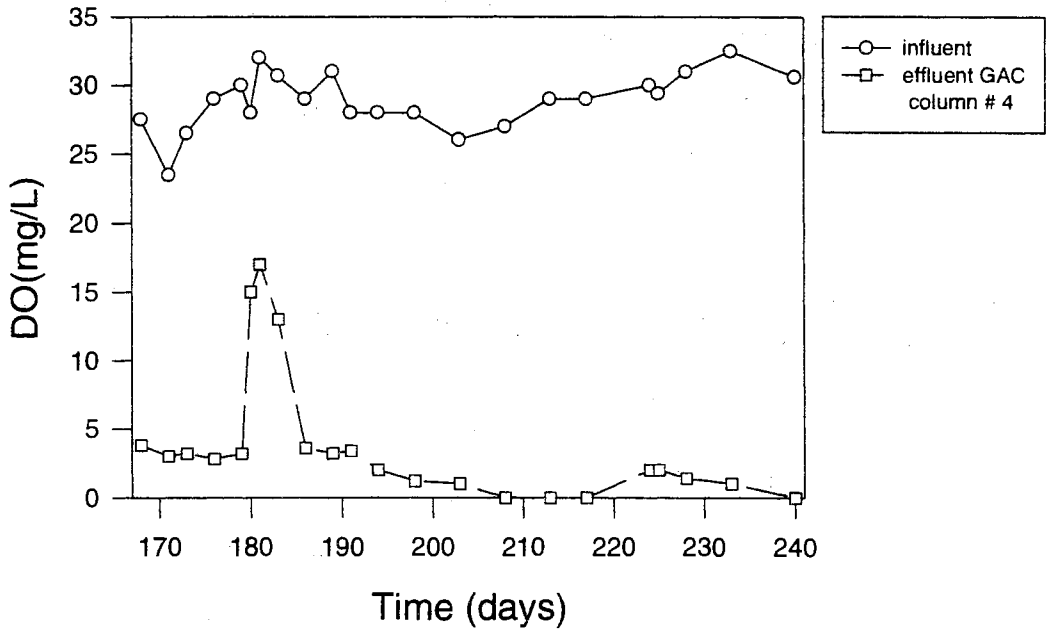

Figure 107. GAC column #4 DO changes of GAC column #4 during high shock loads.

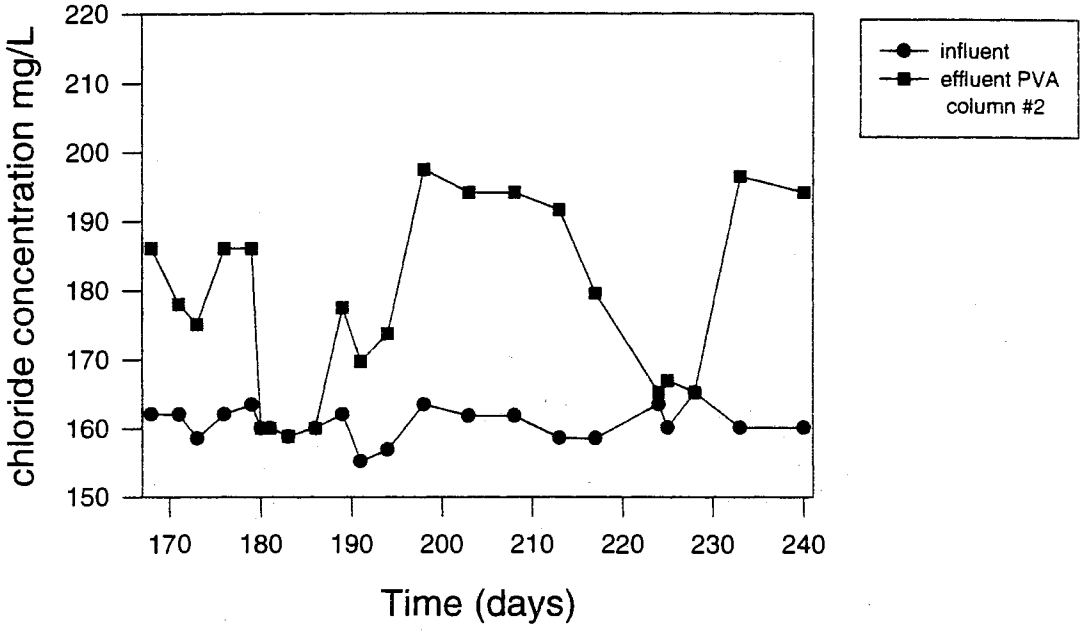

Figure 108. PVA column #2 chloride release changes during high shock loads.

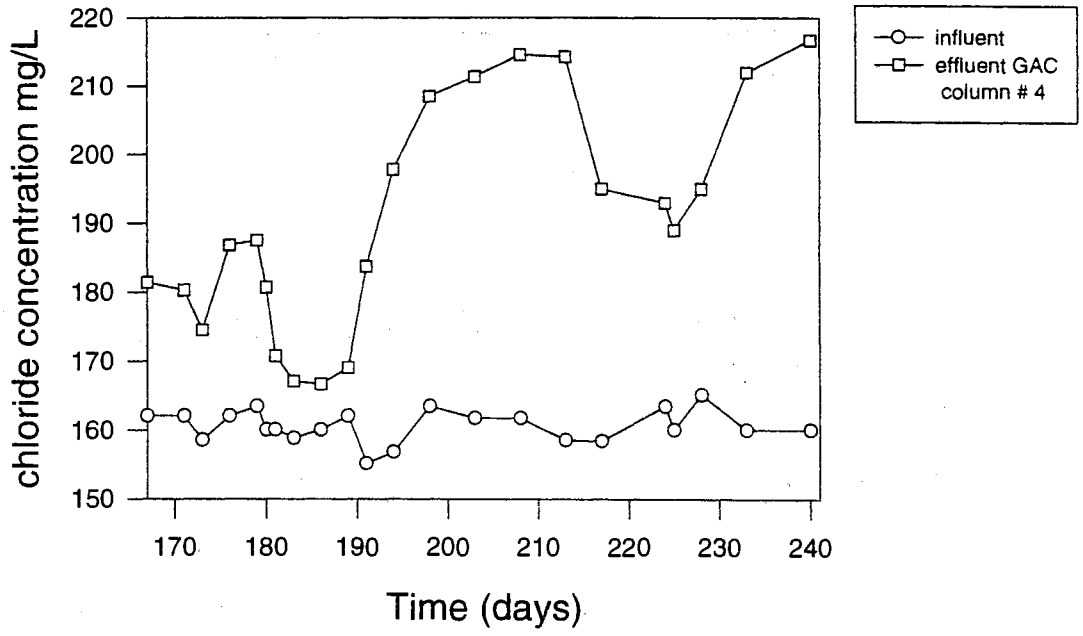

Figure 109. GAC column #4 chloride release changes during high shock loads.

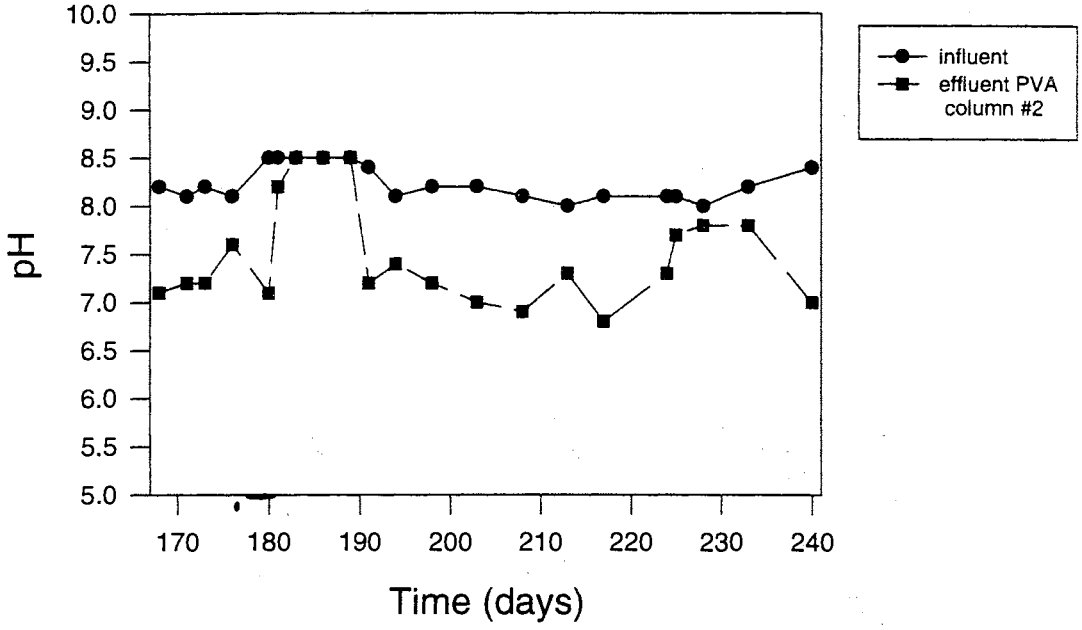

Figure 110. PVA column #2 pH changes during high shock loads.

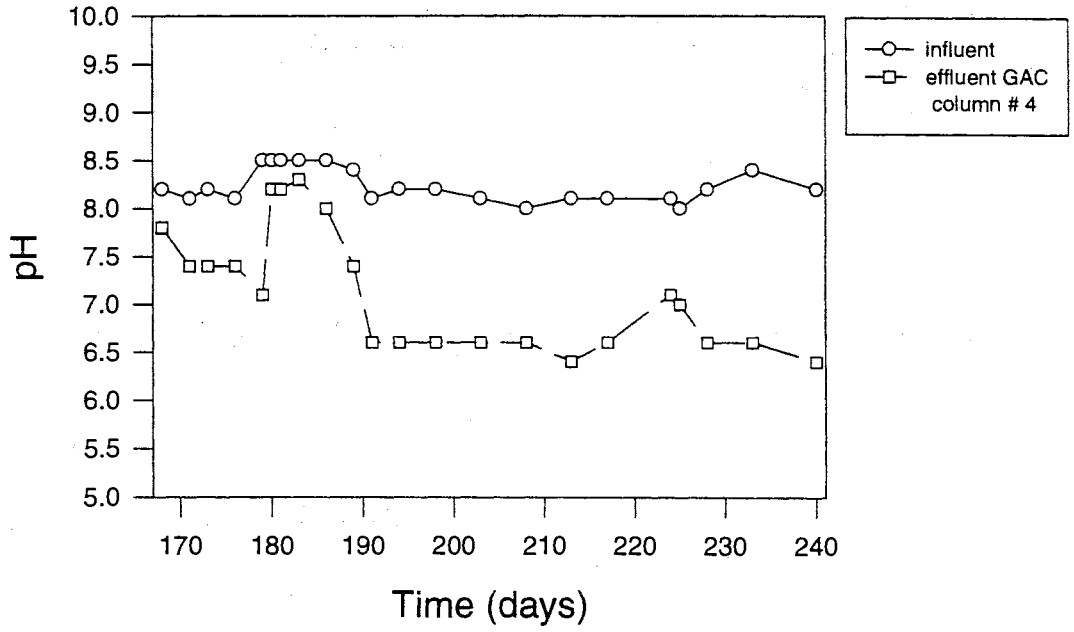

Figure 111. GAC column pH changes during the shock loads.

To study the effects of low DO on TCP degradation performances and recovery, the oxygen supply to the PVA column #1 and GAC column #3 was discontinued twice during 70 days for 50 hours each time. Figures 112-119 show the columns responses to these upset. During the steady state operation, TCP concentration in the influent was 40.0 mg/L and the flow rate was 2 ml/min. The DO during the steady state operation (days 167-179, 182-224, and 228-240) was maintained above 27.0 mg/L. PVA column # 1 reacted quickly to the low DO during both interruptions of DO. In both cases, increases and recoveries of effluent TCP concentrations followed the same pattern. The recovery time was shorter after the second interruption of DO. After the first interruption of DO, PVA column #1 took about 21 days to reduce the influent TCP concentration down to 4.0 mg/L as seen in Figure 112. This is 90% removal of the 40.0 mg/L influent TCP. The percent removal increased from 90% to 95% between day 203-217. The results shown in Figures 114, 116, and 118 are consistent with the effluent TCP concentration removal by PVA column #1. During the first interruption, effluent shown zero TCP removal supported with corresponding results of pH drop, DO change or chloride release.

After the second interruption, immobilized cells in PVA column #1 recovered within 11 days and reduced TCP concentration by 90% (Figure 112), with corresponding DO consumption (Figure 114), chloride release (Figure 116) and pH drop (Figure 118) in the effluent. These results demonstrated the sensitivity of immobilized cells in PVA column and, at same time, the tolerance of these cells toward the low DO influent.

The GAC column also reacted to the interruption of DO. The influent TCP continued to be biodegraded despite the deficiency of dissolved oxygen as indicated by

chloride release, effluent pH drop, and 100% TCP removed (Figures 113, 117, 119). Both anaerobic activity and adsorption were responsible for the removal of influent TCP during the deficiency of DO as indicated by chloride release and pH drop of the effluent. Results shown in Figures 117 and 119 support the partial removal of TCP by anaerobic bacteria. The amount of chloride release and pH drop in the effluent correspond with only 40% of influent TCP dehalogenated by anaerobic bacteria (insufficient DO). Once the oxygen was restarted after each interruption of DO, the aerobic bacteria began to recover and start to consume influent TCP. The activity of aerobic bacteria was evident from oxygen uptake by the GAC immobilized cells. Theoretically, dehalogenation of 40.0 mg/LTCP releases about 21.6 mg/L Cl^-. During day 189-217, the average chloride release was 33.0 mg/L chloride. A possible explanation for extra 45% chloride release is the result of biodegradation of TCP already adsorbed on GAC by anaerobic bacteria. The average GAC column effluent pH was 6.8. According to the pH curve (Figure 15), the effluent pH drop from 8.1 to 6.8 show that 10.5 mL volume of 0.1 N HCl would be required for this drop. This is 37.3 mg/L Cl^- concentration which was close to the chloride release (measured). The pH drop in the effluent along with chloride release support dehalogenation of TCP. It is clear that the DO provided was insufficient for aerobic bacteria to biodegrade TCP already adsorbed by carbon and release an extra 45 % chloride. Therefore, the dehalogenation of TCP already adsorbed is believed to be the result of anaerobic biodegradation. During the second interruption of DO (day 224, 225), the aerobic immobilized cells in the GAC column #3 unlike anaerobic bacteria were inactive. Adsorption and anaerobic dehalogenation were responsible for 100% removal of TCP on days 224 and 225. It is theorized that anaerobic dehalogenation of

some TCP resulted in the effluent chloride release and pH drop during the second DO interruption as shown in Figures 117 and 119.

These results demonstrated the sensitivity of aerobic immobilized cells and , at the same time, the tolerance of these cells toward low DO. The PVA-immobilized cells were unable to degrade TCP during oxygen upset. In both cases of DO interruption, increases and recoveries of effluent TCP concentrations followed the same pattern. The second time recovery times were shorter. The GAC column #3 offered both adsorption and anaerobic biodegradation during the interruptions of DO. The adsorption capacity of GAC offered 100% removal of TCP. The TCP adsorbed onto carbon subsequently was released and consumed by bacteria (bioregeneration) (Kim *et al.*, 1989). Insufficient DO promoted the activity of anaerobic bacteria which resulted to biodegradation of TCP, release of chloride, and drop of pH. Since anaerobic bacteria are slower growers they could not grow to a significant enough number in 50 hrs to do any good for TCP removal so then either they are present in the column all the time or some of the degraders may be facultative. Once the oxygen supply restarted, the GAC immobilized cells resumed their activity and continued to biodegrade TCP.

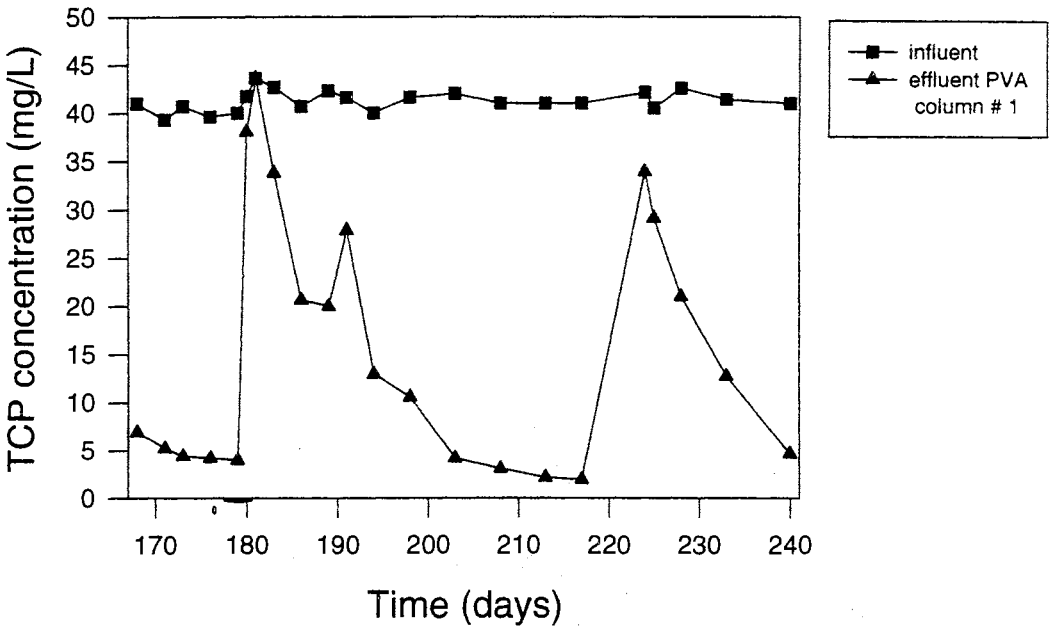

Figure 112. PVA column # 1 effluent TCP concentrations response to the low DO.

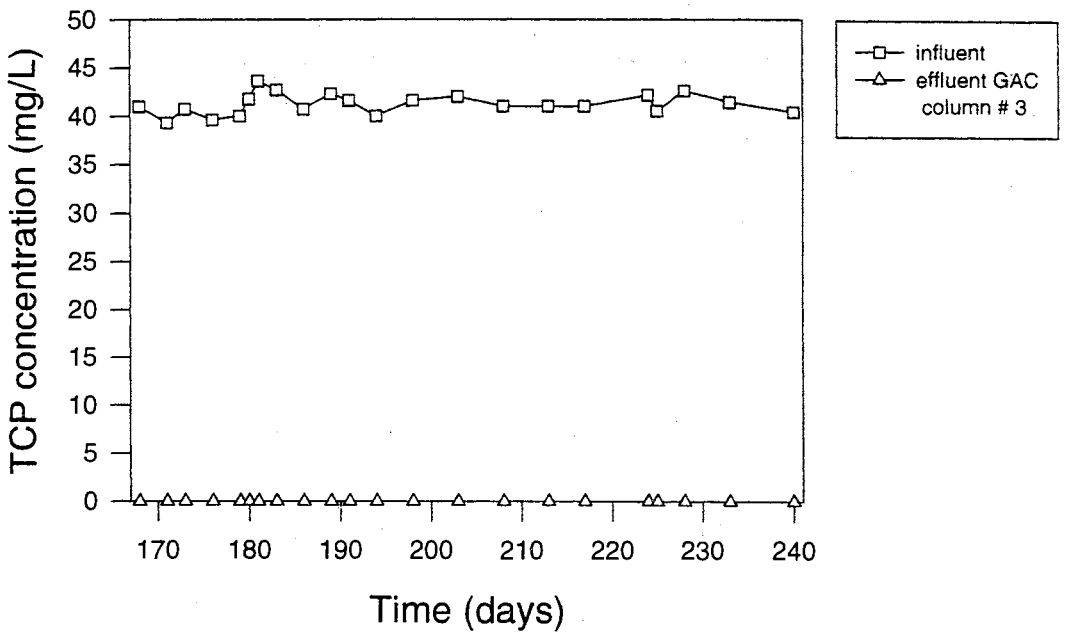

Figure 113. GAC column #3 TCP concentrations response to the low DO.

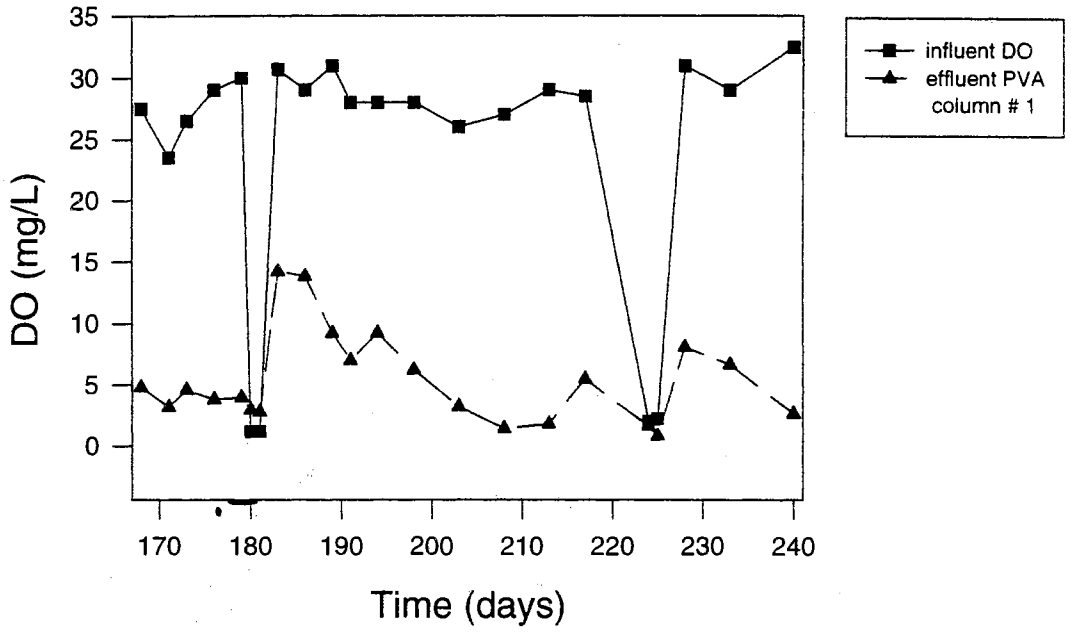

Figure 114. PVA column #1 DO uptake responses to the influent DO interruptions.

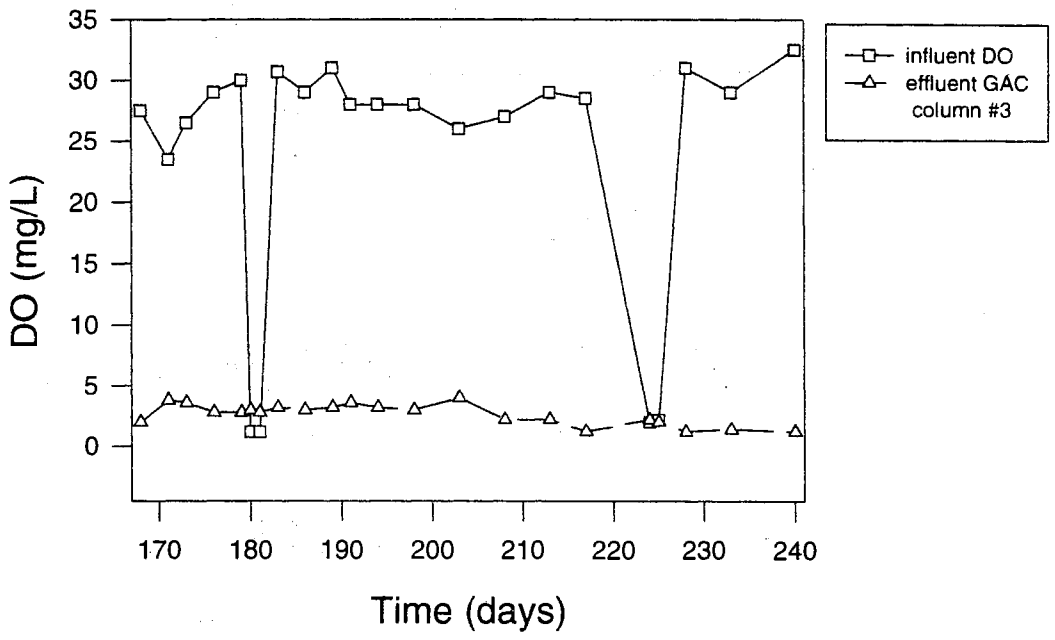

Figure 115. GAC column #3 DO uptake responses to the influent DO interruptions.

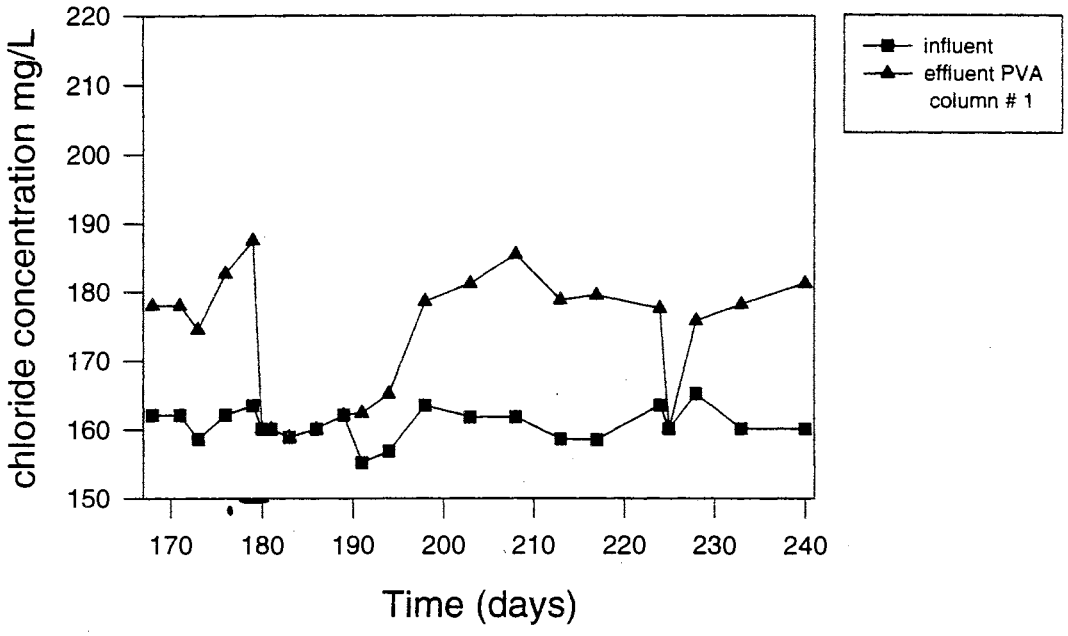

Figure 116. PVA column #1 chloride releases during and after influent DO upsets.

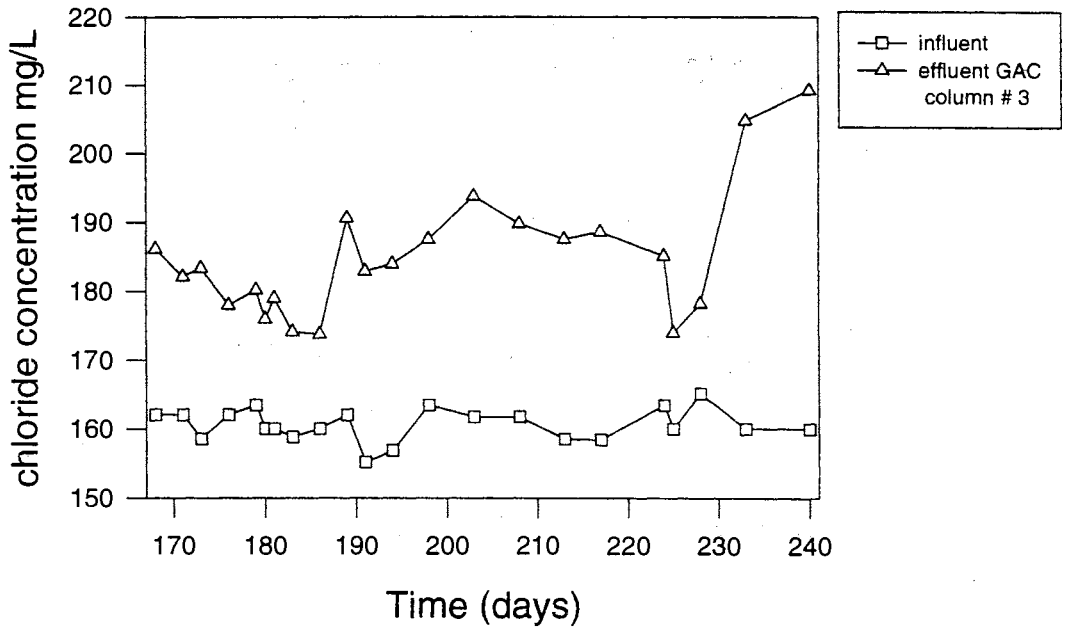

Figure 117. GAC column #3 chloride releases during and after influent DO upsets.

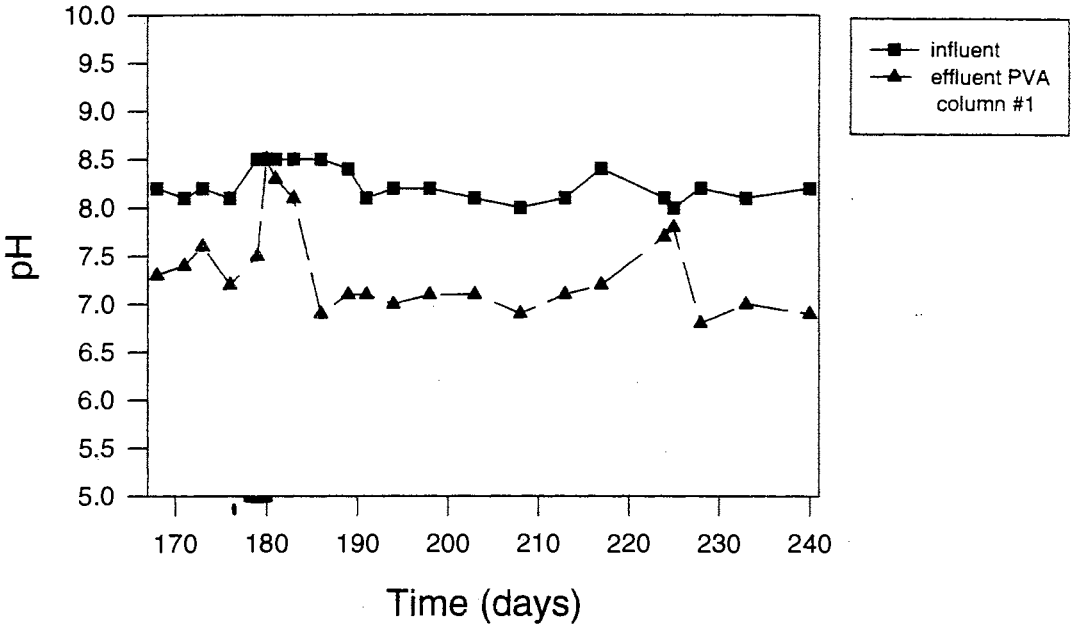

Figure 118. PVA column #1 effluent pH changes during and after influent DO upsets.

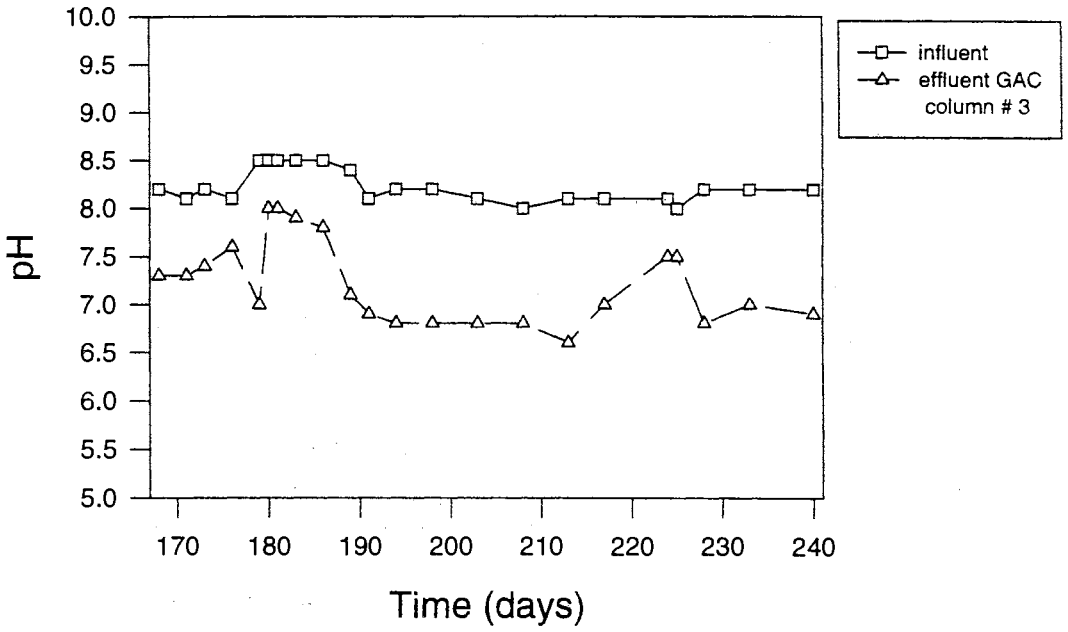

Figure 119. GAC column #3 effluent pH changes during and after influent DO upsets.

GC/MS

Intermediates Formation

Samples taken on day 122 from all four columns effluent were analyzed by GC/MS. The results are presented in Figures 120-124. The results presented here demonstrate the degradation of TCP by both PVA and GAC columns with no phenol or intermediate (ring compound) production.

Gas Production

Both GAC columns were shown to release more chloride than expected from dehalogenation of 40.0 mg/L TCP (column study No.9) during steady state operation (days 168-179, 183-217, 228-240). The production of extra chloride was believed to come from dehalogenation of TCP already adsorbed on the GAC columns by anaerobic bacteria. Dehalogenation of TCP by an anaerobic bacteria should release methane gas and chloride (Haggblom *et al.*, 1988). An attempt was made to detect the production of any gases in GAC column #3 and #4 after the 70 day column experiment No. 9. Therefore, effluent samples were collected very carefully in a closed system on day 241 from both GAC columns and analyzed by GC/MS. The results are shown in Figures 126 and 127. These samples were compared to an ambient air sample analyzed by GC/MS (Figure 125). The depletion of oxygen (indicating aerobic activity), production of methane (anaerobic activity), and production of CO_2 are demonstrated in both GAC column effluent. The CO_2 and methane production in the column effluents were not

quantified. Both CO_2 and methane were present in amounts greater than in the ambient air.

Another attempt was made to collect gas samples from the headspace of all four columns. The influent feed pump was stopped on day 248. The gas samples were collected from the head space about 12.0 hours later that day. The gas samples were analyzed by GC/MS. The GC/MS results of gas samples from the columns headspace were compared to a GC/MS result of air sample. The results are presented in Figures 128-132. The depletion of oxygen in all four columns gas samples indicates aerobic activity. The methane gas and CO_2 were present in both the GAC and PVA columns. Methane gas with MW of 16 was present in all four columns gas sample. The methane gas was not present in the ambient air sample. The CO_2 gas detected in all four columns showed (in quantitative terms) more CO_2 was present in the columns than air. The quantities of oxygen, methane, and carbon dioxide were not determined.

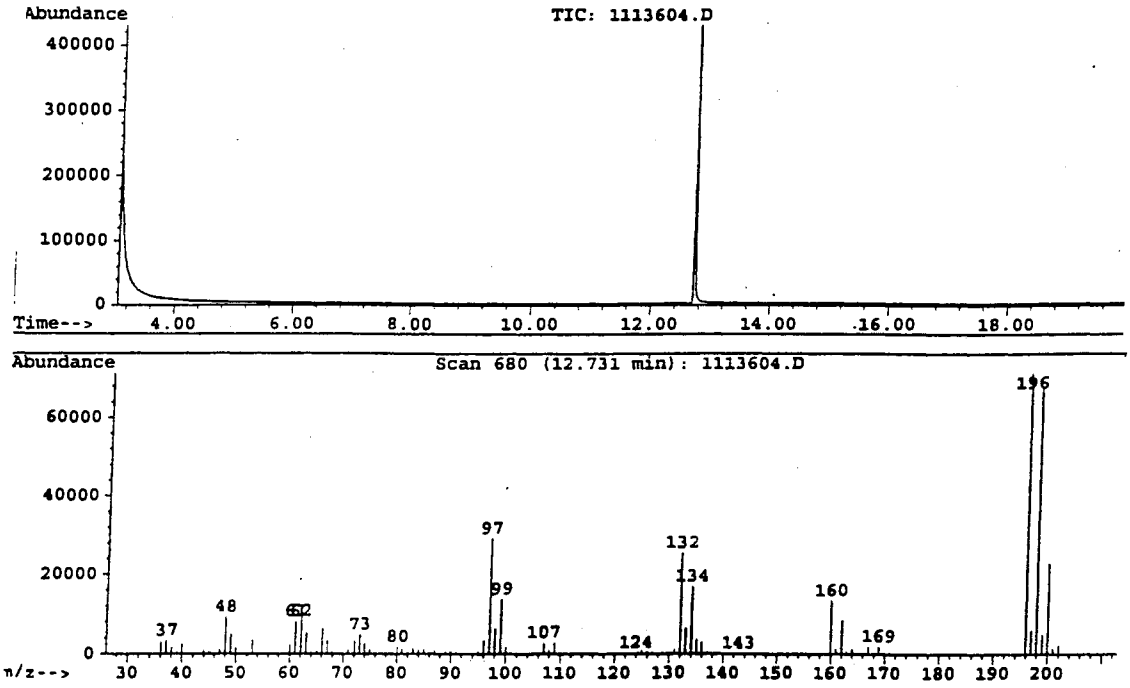

Figure 120. Influent, GC/MS

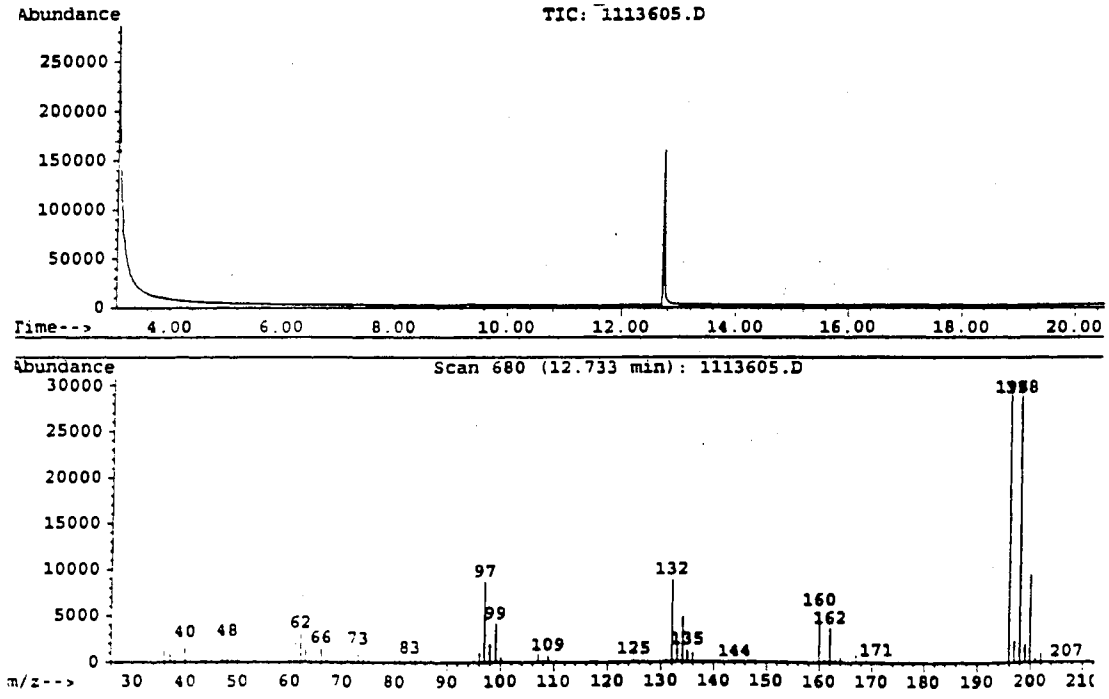

Figure 121. effluent PVA column #1, GC/MS.

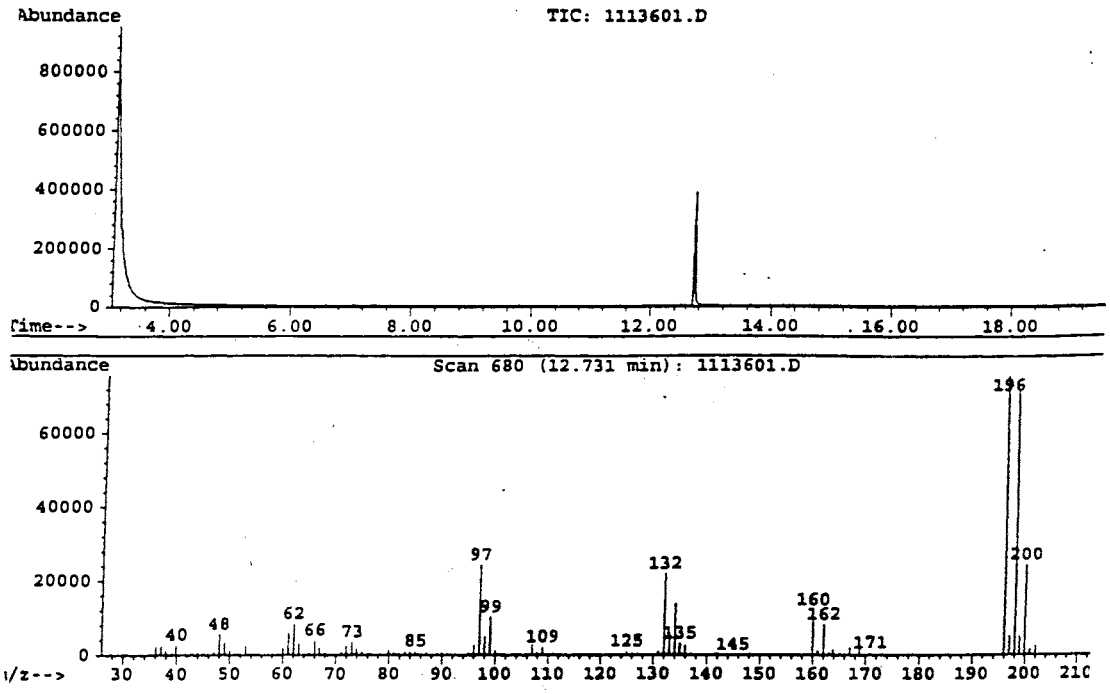

Figure 122. Effluent PVA column #2, GC/MS

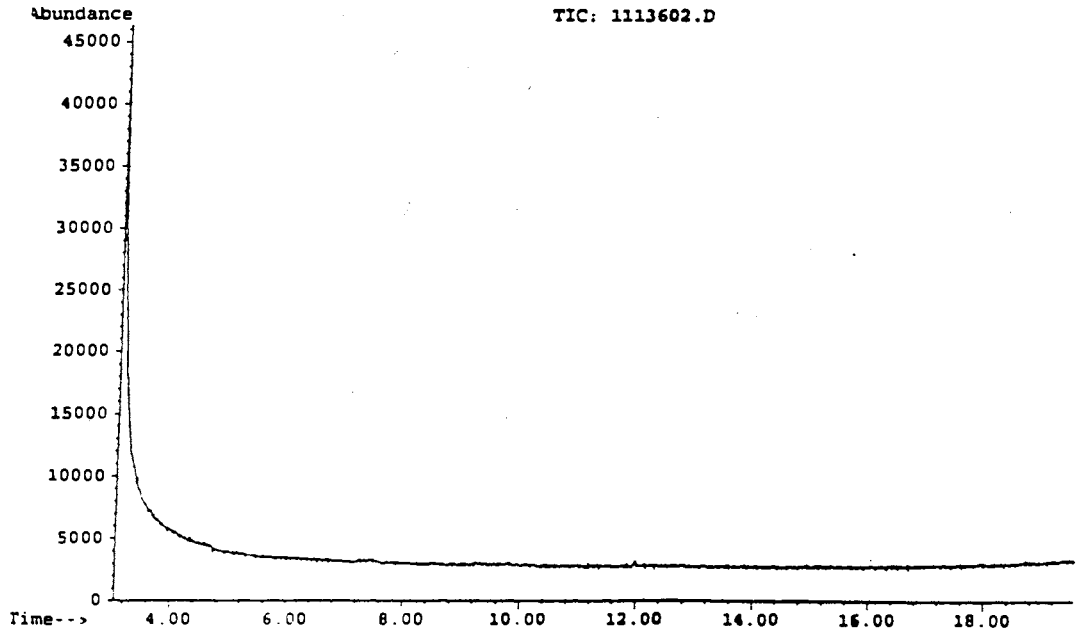

Figure 123. Effluent GAC column #3, GC/MS.

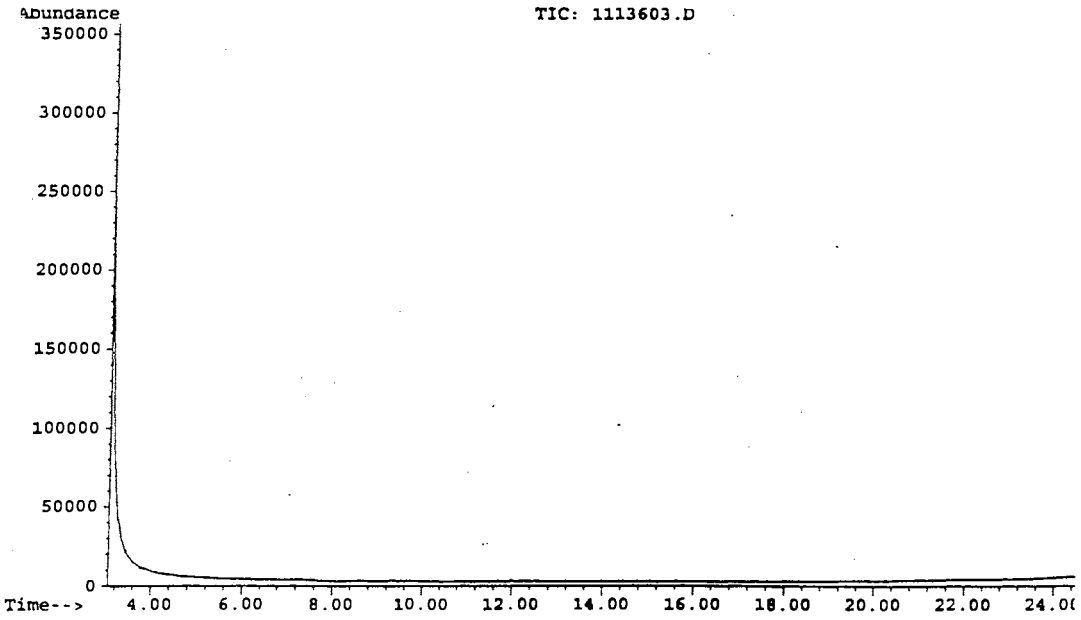

Figure 124. Effluent GAC column #4, GC/MS.

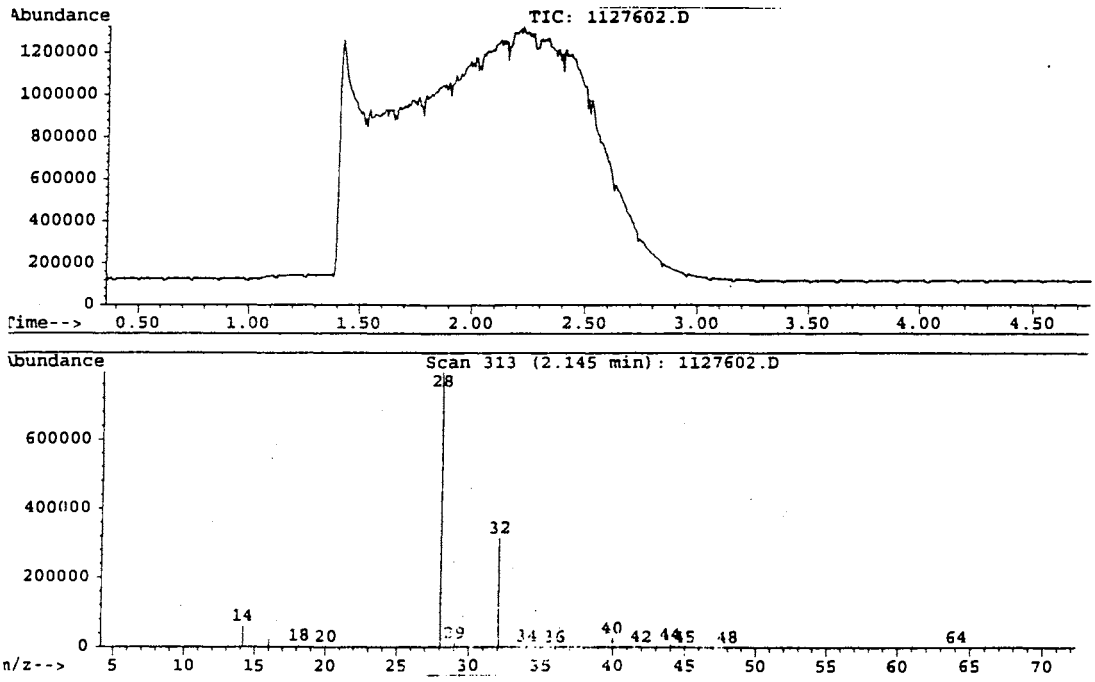

Figure 125. Air sample, GC/MS.

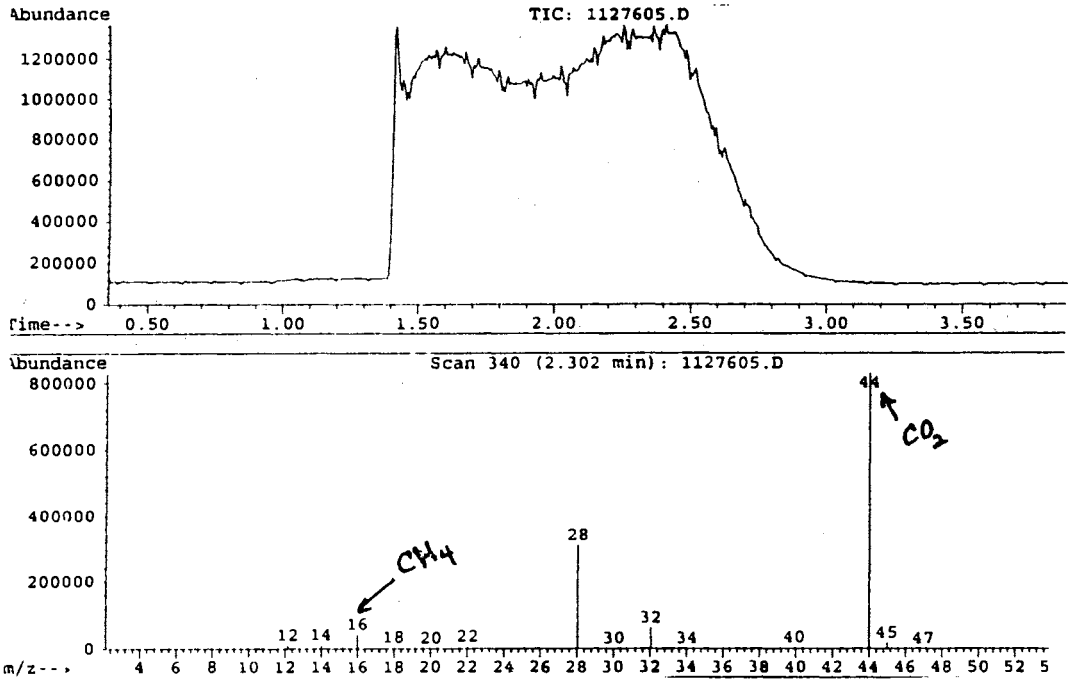

Figure 126. Effluent GAC column #3, GC/MS.

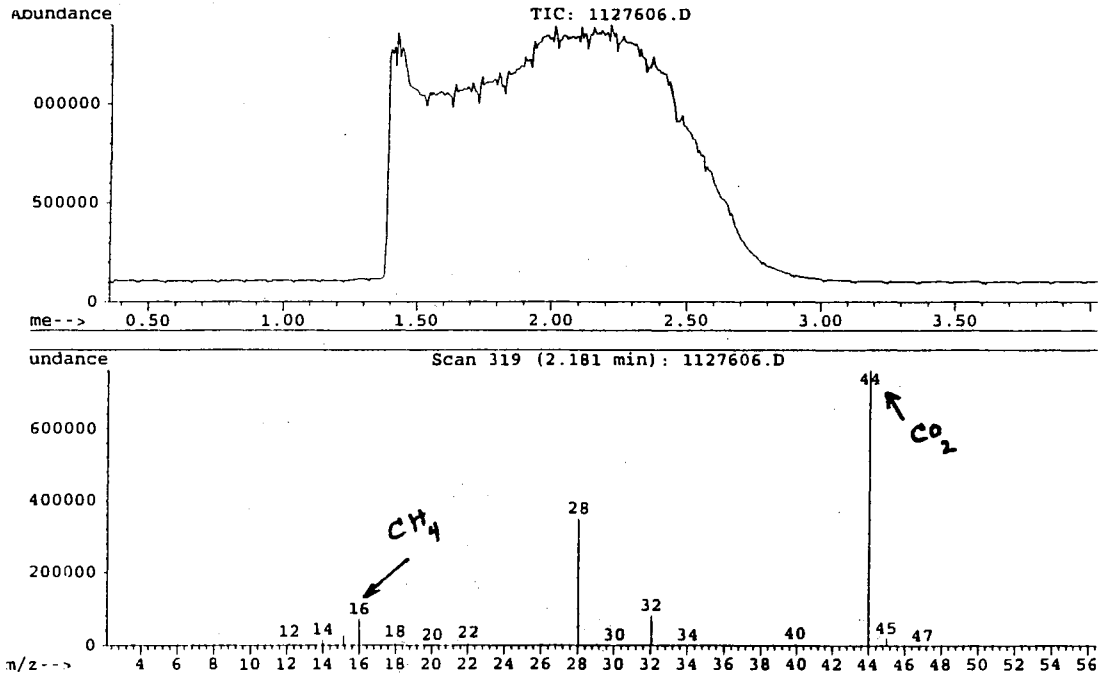

Figure 127. Effluent GAC column #4, GC/MS.

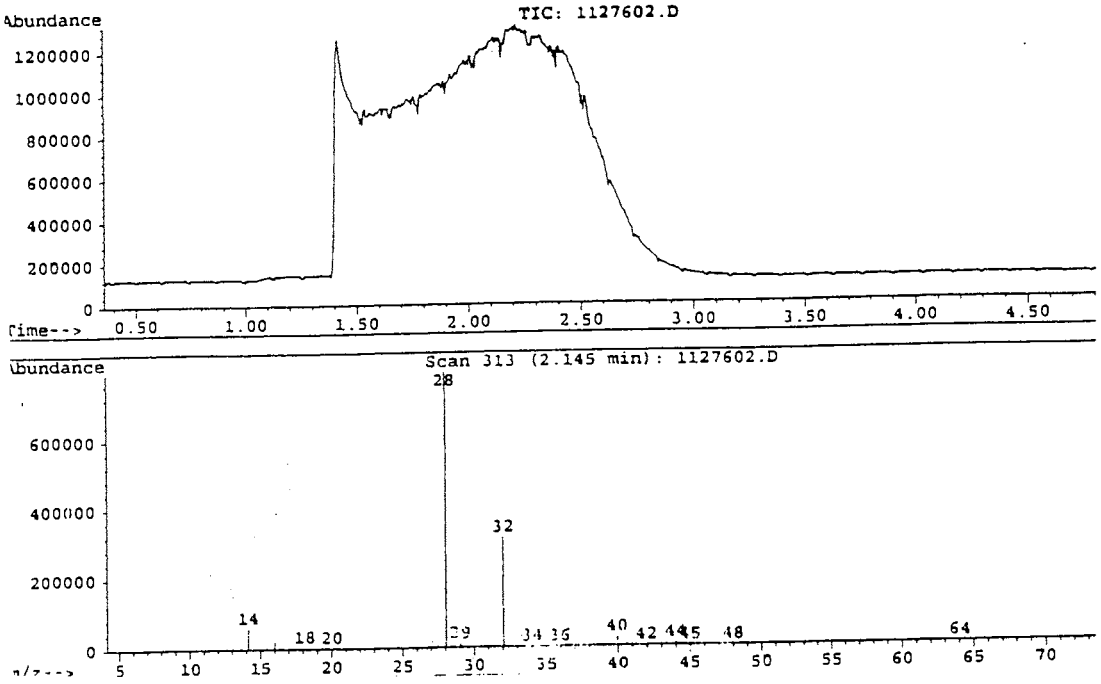

Figure 128. Air sample, GC/MS.

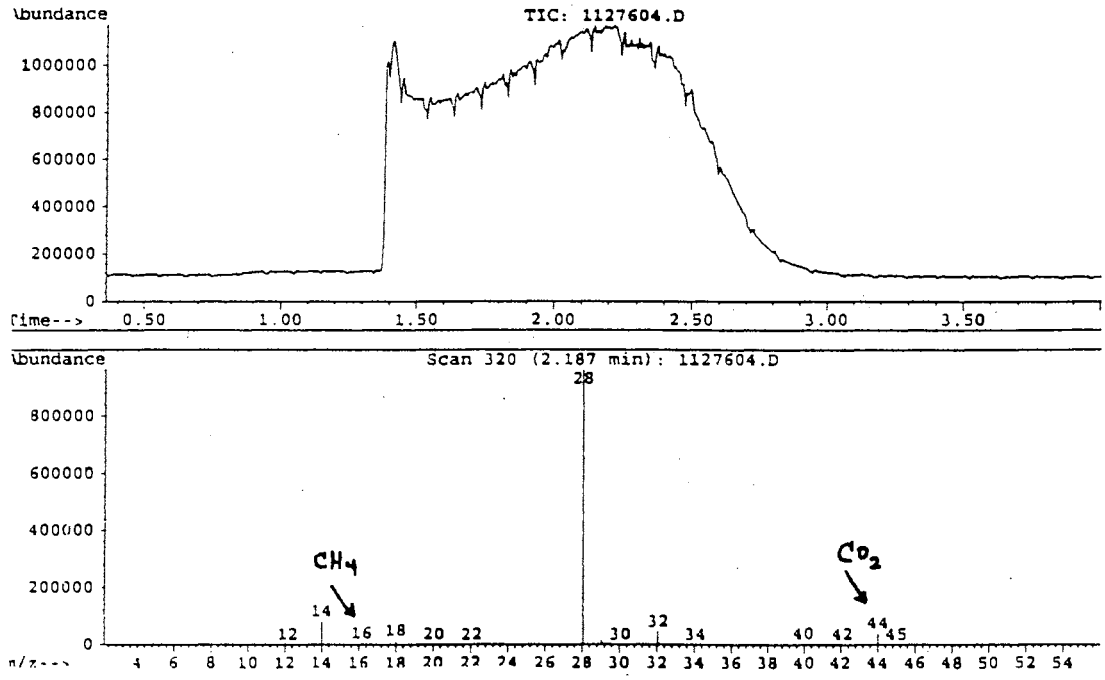

Figure 129. Effluent PVA column #1, GC/MS.

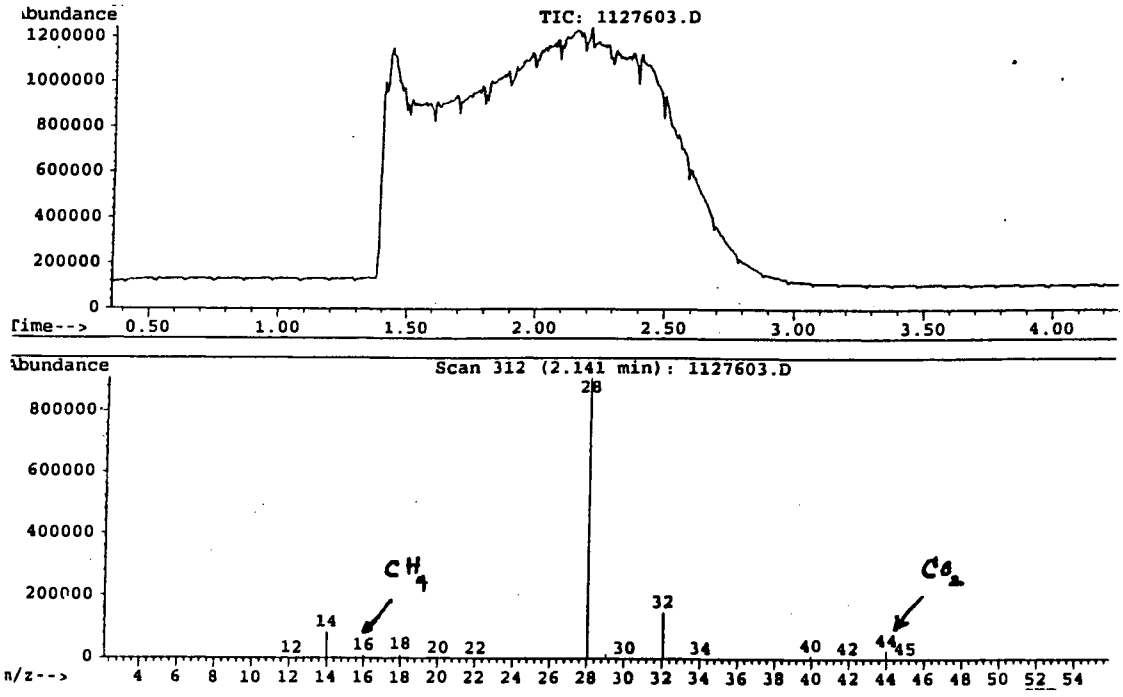

Figure 130. Effluent PVA column #2, GC/MS.

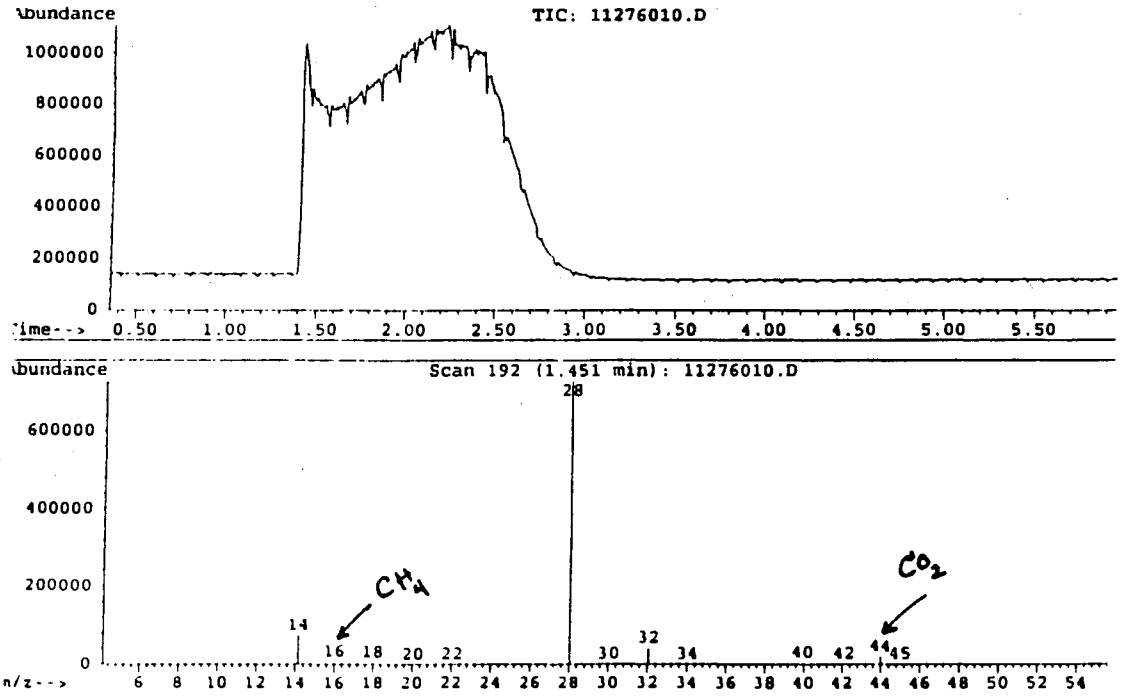

Figure 131. Effluent GAC column #4, GC/MS.

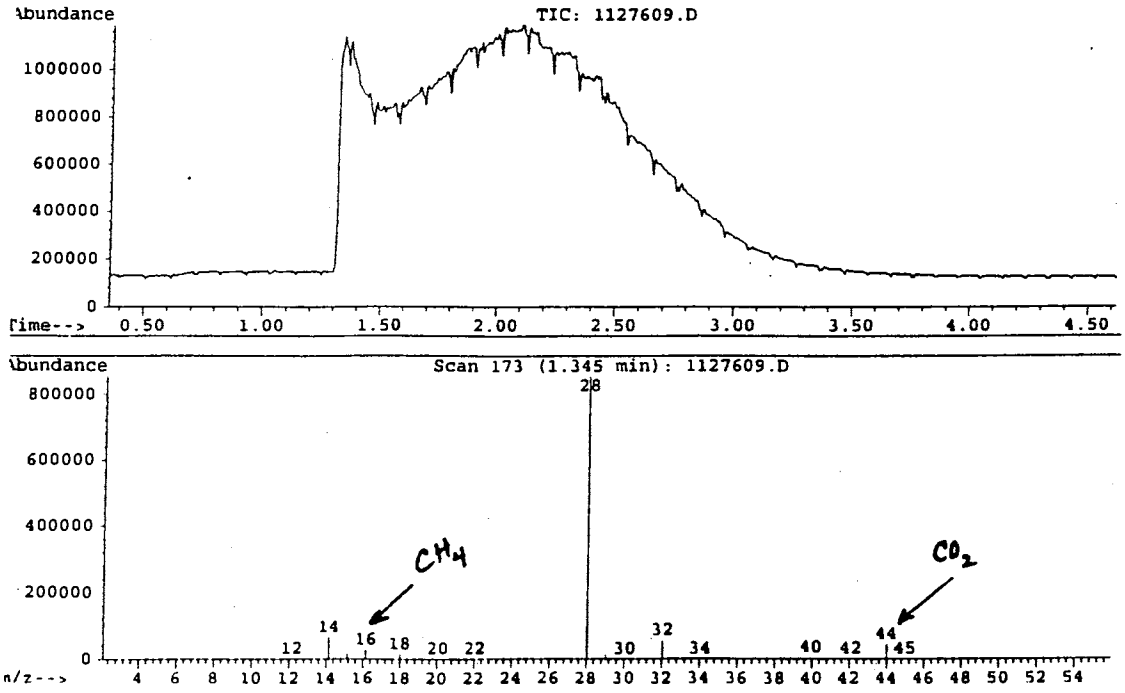

Figure 132. Effluent GAC column #4, GC/MS.

Scanning Electron Micrographs

Activated Sludge

Figure 133 shows the mixed culture of microorganisms that were immobilized in both PVA and GAC. The organisms are approximately 1-2 μ long and about 0.5 - 1 μ wide. Figure 134 shows the presence of protozoa in the mixed culture. Figures 133 and 134 were magnified 6000 X and 400 X, respectively.

PVA Beads

Figure 135 shows the surface of a freshly made PVA bead without microorganisms. The bead in Figure 135 has a smooth outer surface and has been magnified 72 X. Figure 136 shows the surface of a freshly made PVA bead with immobilized microorganisms. The bead in Figure 136 shows the outer surface is smooth and was magnified 72 X. Figure 137 shows the outer surface of a PVA bead with microorganisms, which was 9 months old, magnified 66X, from the column study. Figure 138, is an enlargement of the outer surface of the 9 months old PVA bead from the column study (300 X). Figure 138, shows the network of pores on the outer surface. Figure 139, shows the deterioration of the outer surface in a bead from the column study after 9 months which was magnified 30X. Figure 140 show the severely deteriorated bead after 9 months of column study and was magnified 24 X. Figures 141-143 show the inside of a PVA bead with microorganisms which were magnified 11000 X, 3600 X, and 780 X, respectively. The beads in Figures 141-143 are 2 days, 45 days, and 9 months old, respectively. The star-shape feature in Figure 133, was speculated to be the

bacteria that aggregated together. Figures 142 and 143 show the spherical shaped cells inside the PVA beads. These cells are approximately 1.4 X 10^{-3} mm to 4.0 X 10^{-3} mm in diameter. The inner surface of the 9 month old PVA bead appears to be more porous than the 45 days old bead. Figures 144-146, show the microcolonies of the beads fromPVA column #2 after the shock load of TCP concentration. The beads in Figures 144-146 were magnified 1800 X, 1500 X, and 11000 X, respectively.

GAC

Figures 147 and 148 show the outer surface of GAC before and after immobilization. The GAC was magnified 72 X in both Figures 147 and 148. The outer surface of the GAC in Figure 148 appears to be smooth. The presence of the immobilized cells on the GAC after immobilization was verified in Figure 149 where the cells were magnified 20000 X. The immobilized cells on the GAC of columns #3 and #4 after 9 months of column studies are still present and shown in Figures 150 and 151. The immobilized cells were magnified in Figures 150 and 151 16000 X. Figure 152 show the immobilized cells on GAC from column #4 after the high shock load. The bacteria colonizing the surface seem to be producing slime, as seen in Figures 150-152. The slime matrix allows attachment to the substrate as well as other cells (Weber *et al.*, 1978). The appearance of protozoa shown in Figure 153 after 9 months of continuous column operation indicates a more advanced microbial colonization of the surface (Weber *et al.*, 1978). The establishment of an ecosystem including bacteria and bacteriovorous protozoans is evidence of improved mineralization of organics, as well as nutrient regeneration. (Weber *et al.*, 1978).

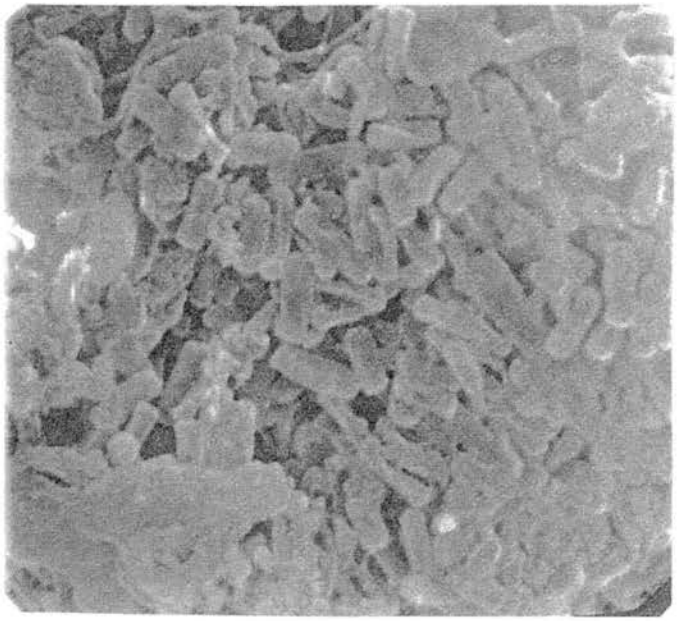

Figure 133. Centrifuged biomass prior to immobilizing into PVA beads or GAC (6000X)

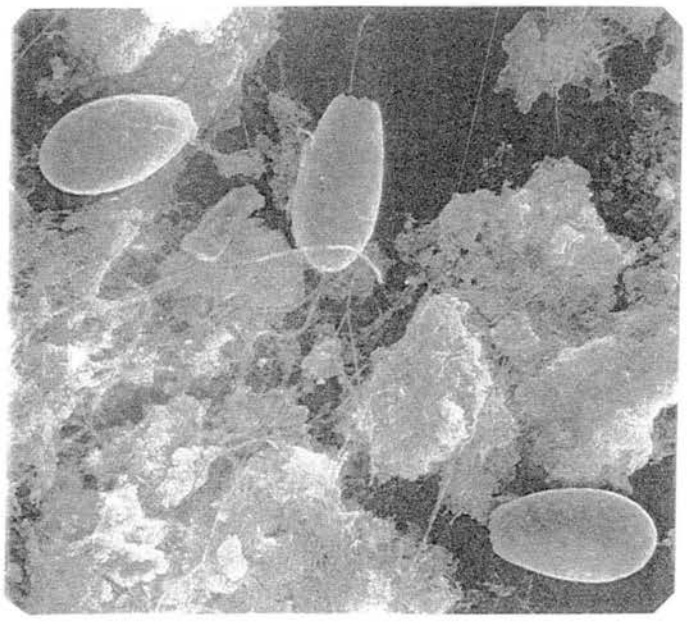

Figure 134. The presence of protozoa in the centrifuged biomass prior to immobilization (400 X)

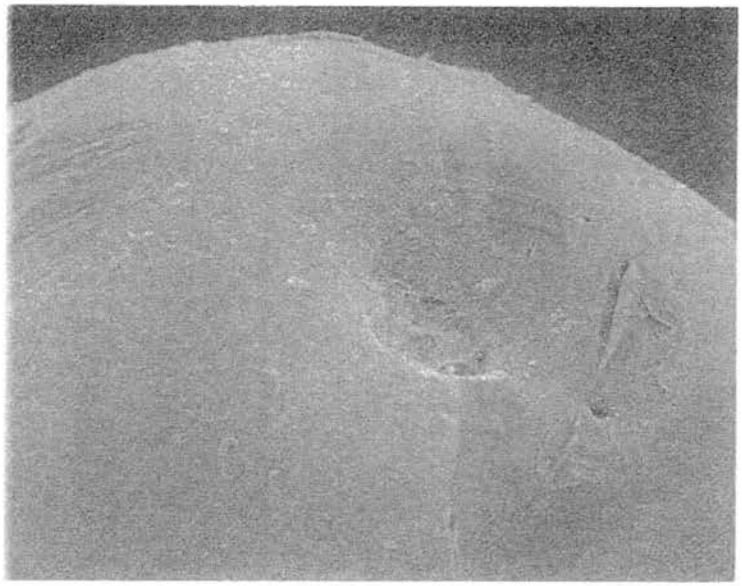

Figure 135. The outer surface of 2 days old PVA bead without microorganisms (72 X).

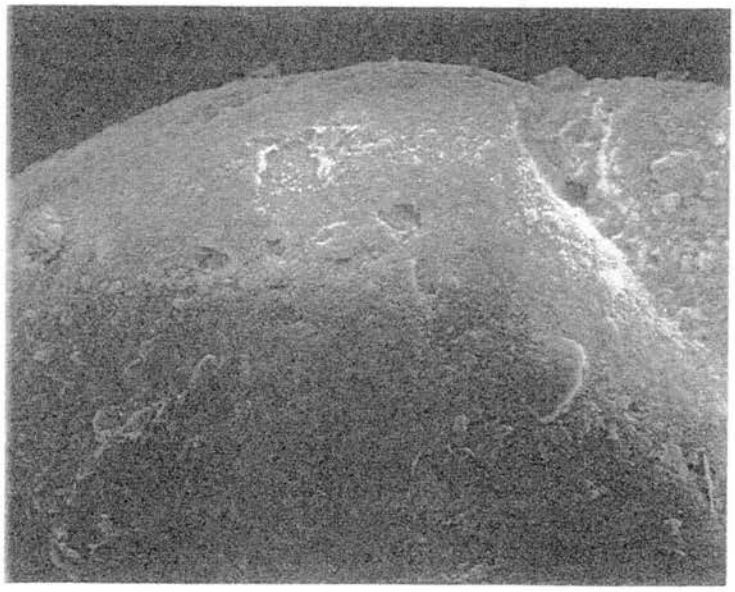

Figure 136. The outer surface of the PVA bead with immobilized bacteria 2 days old (72 X).

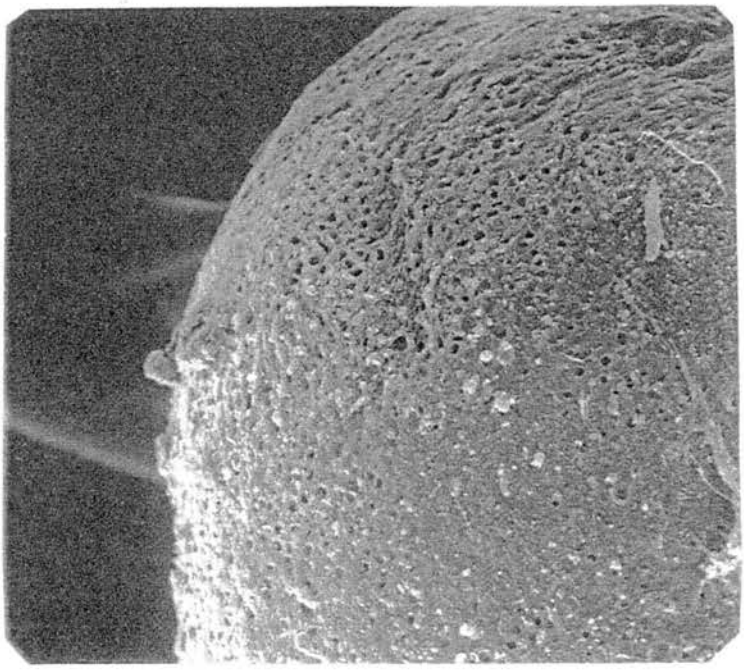

Figure 137. The outer surface of PVA bead with microorganisms, 9 months old (66 X).

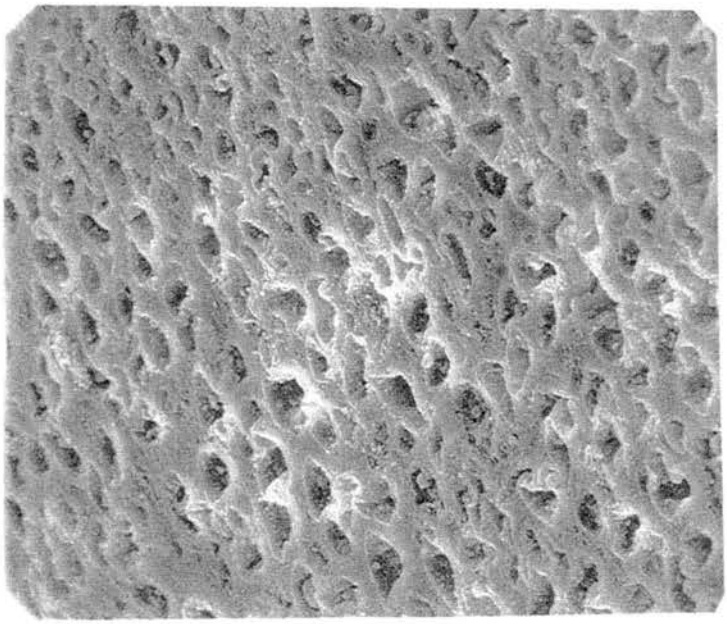

Figure 138. An enlargement of the outer surface of 9 months old PVA bead from column study (300 X).

Figure 139. The deterioration of the outer surface of a 9 months old PVA bead (930X).

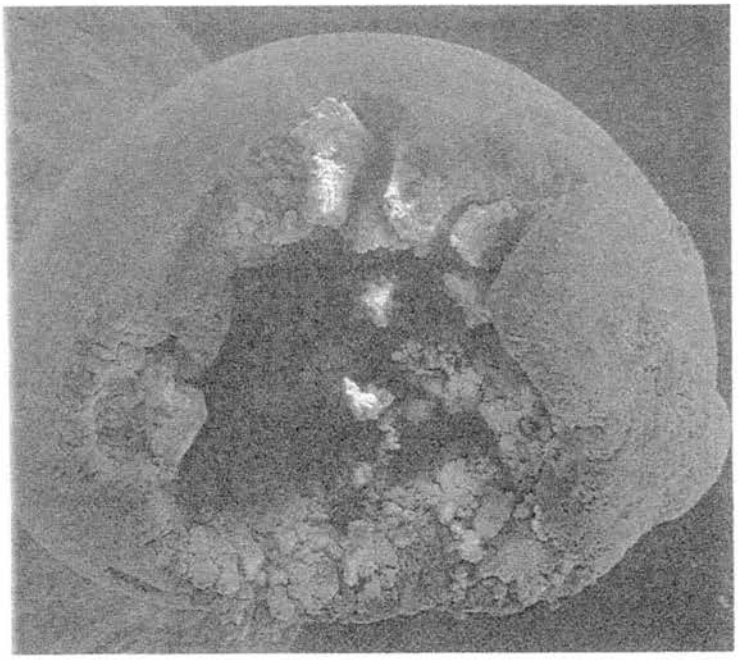

Figure 140. A severely deteriorated 9 months PVA bead (24 X).

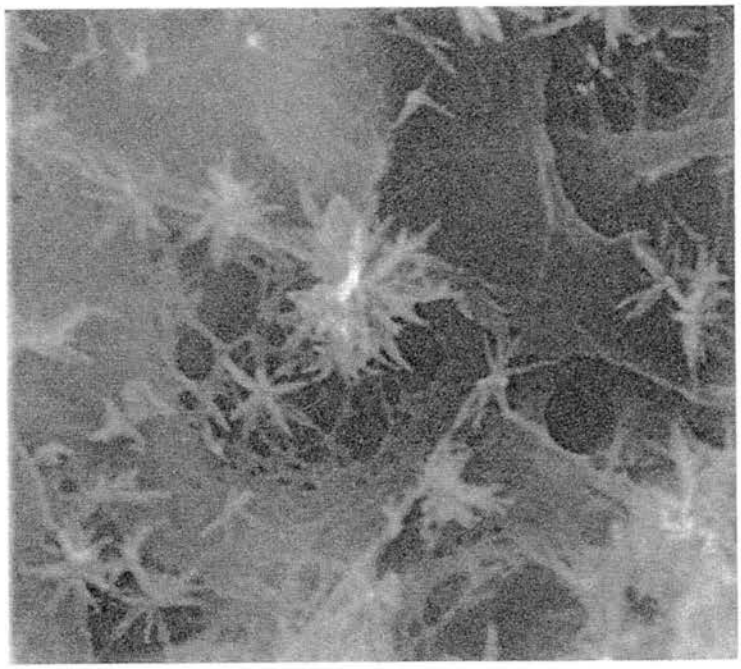

Figure 141. Inside of PVA bead with microorganisms, 2 days old (11000 X).

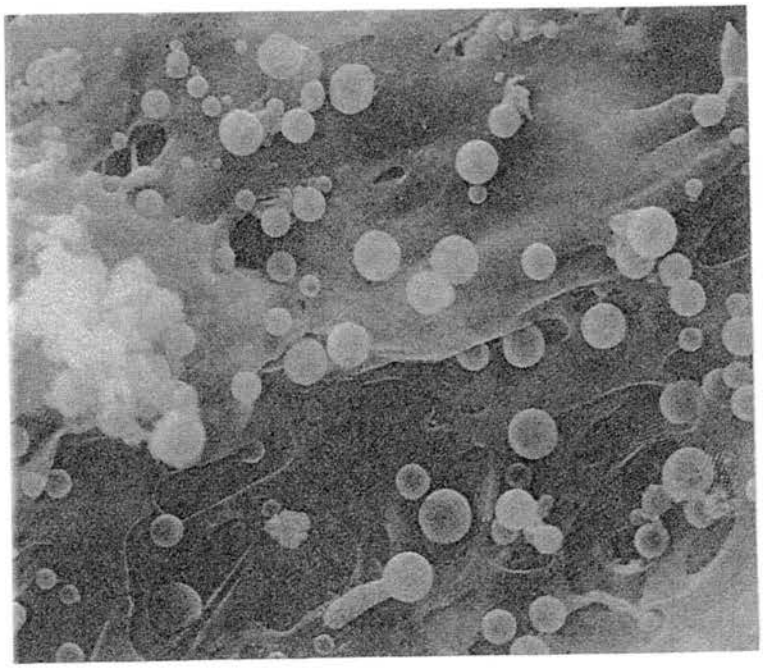

Figure 142. Population of the cells inside the PVA 45 days old (3600 X).

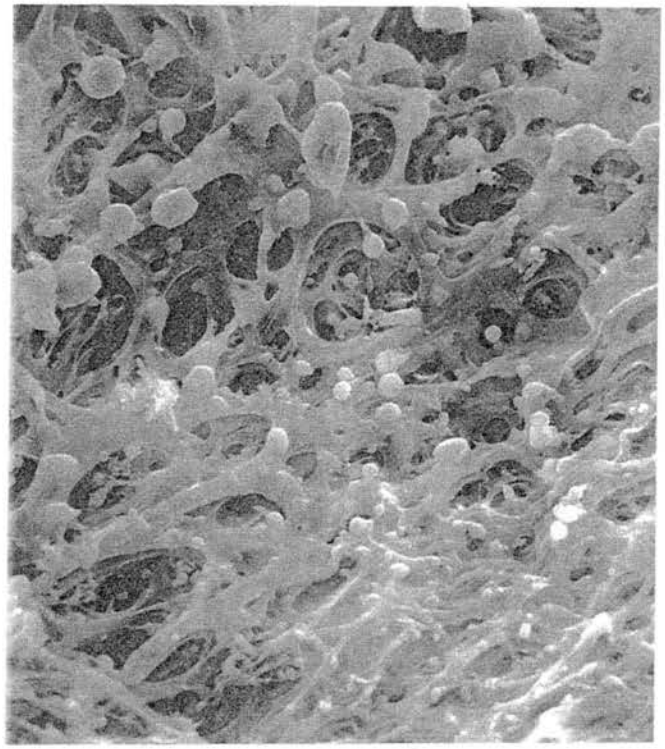

Figure 143. The PVA bead inside, 9 months old (780 X).

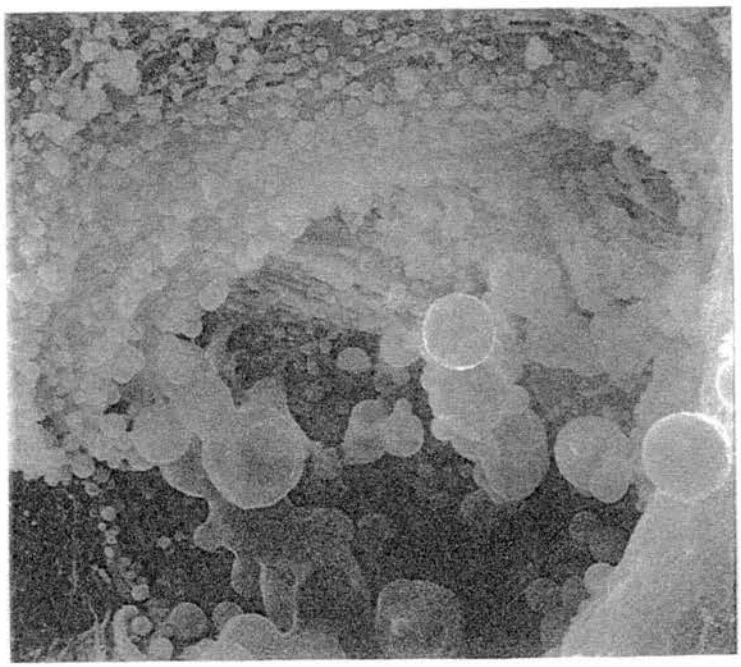

Figure 144. Population of cells inside the PVA beads after second shock load (1800 X).

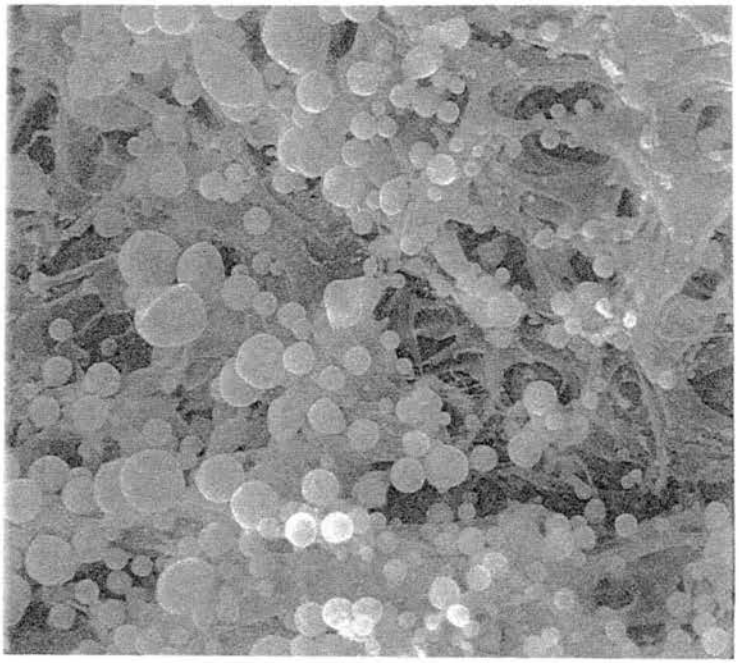

Figure 145. The microcolonies inside PVA beads, 9 months old (1500 X).

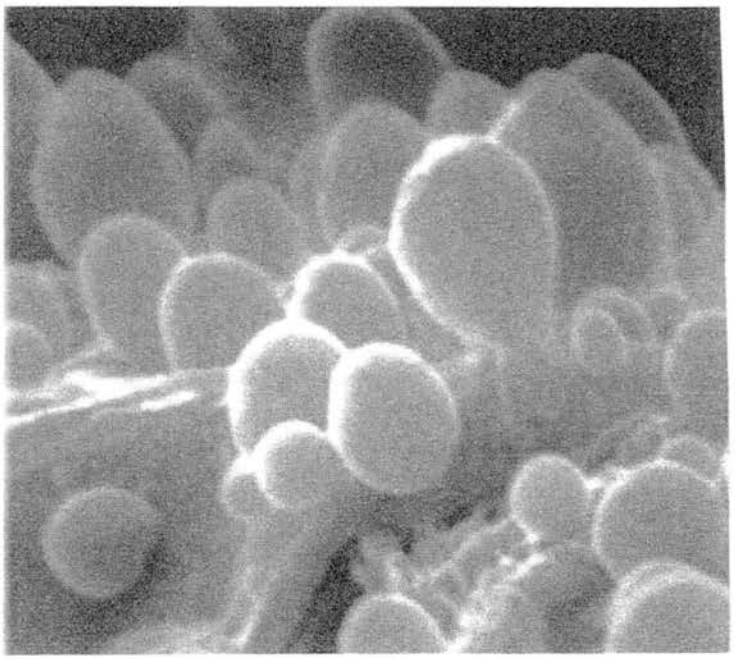

Figure 146. Biofilm formation inside PVA beads, 9 months old (11000 X).

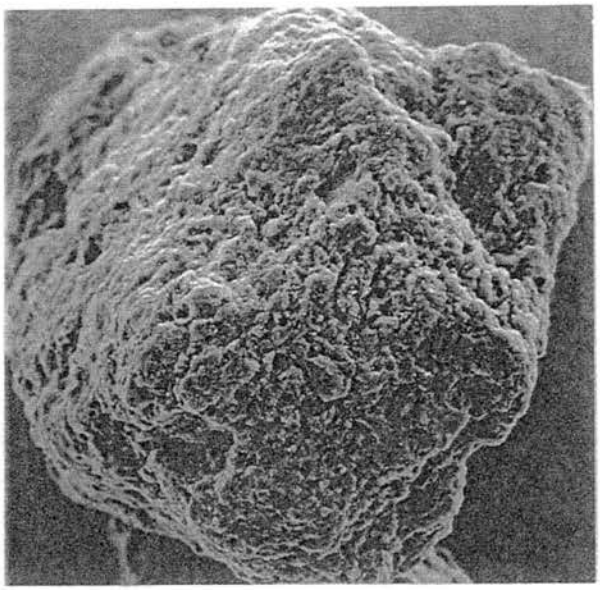

Figure 147. The outer surface of GAC before immobilization, (72 X).

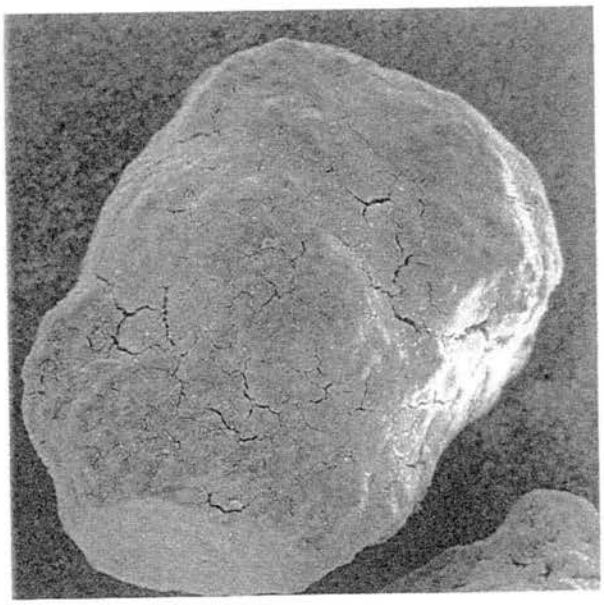

Figure 148. The outer surface of GAC after immobilization (42 X).

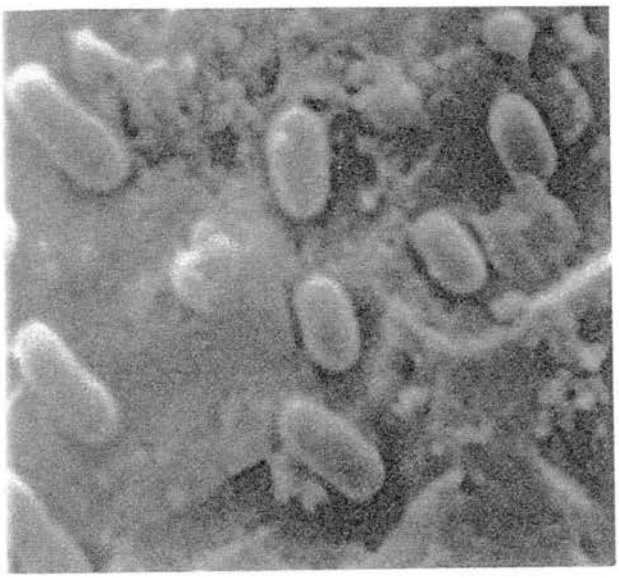

Figure 149. The immobilized cells on GAC, 14 days old (20,000 X).

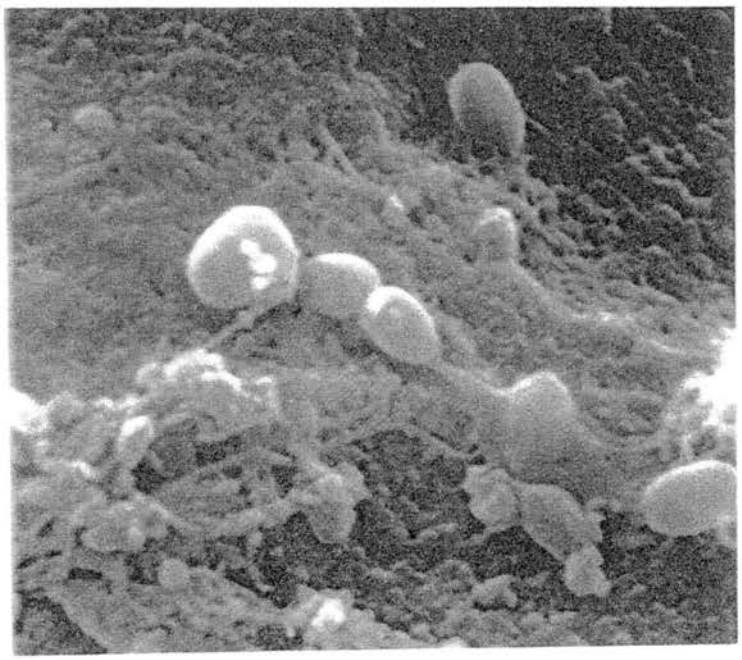

Figure 150. The bacteria colonization shown by slime production, GAC column #3 (16000 X).

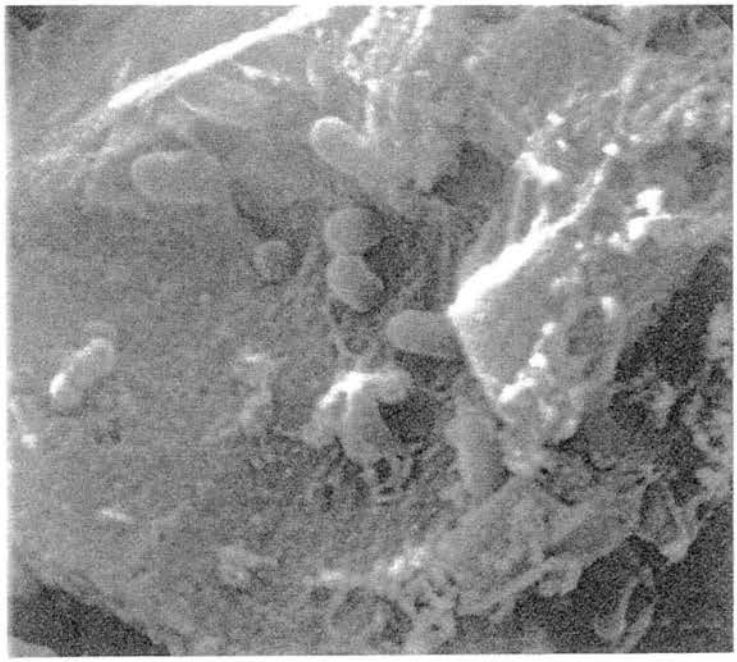

Figure 151. The bacteria colonization shown by slime production, GAC column #4 (16000 X).

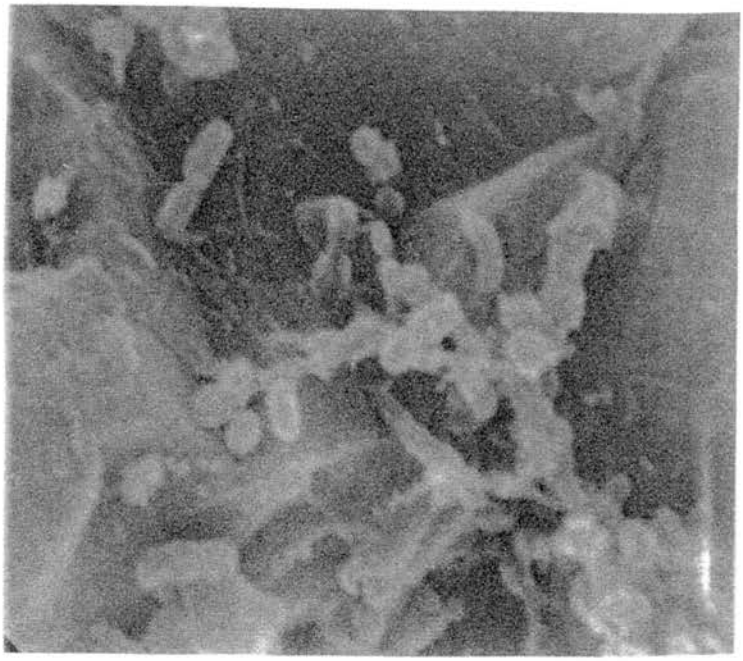

Figure 152. The population of the GAC immobilized cells shown in GAC column #4 after shock load (16,000 X).

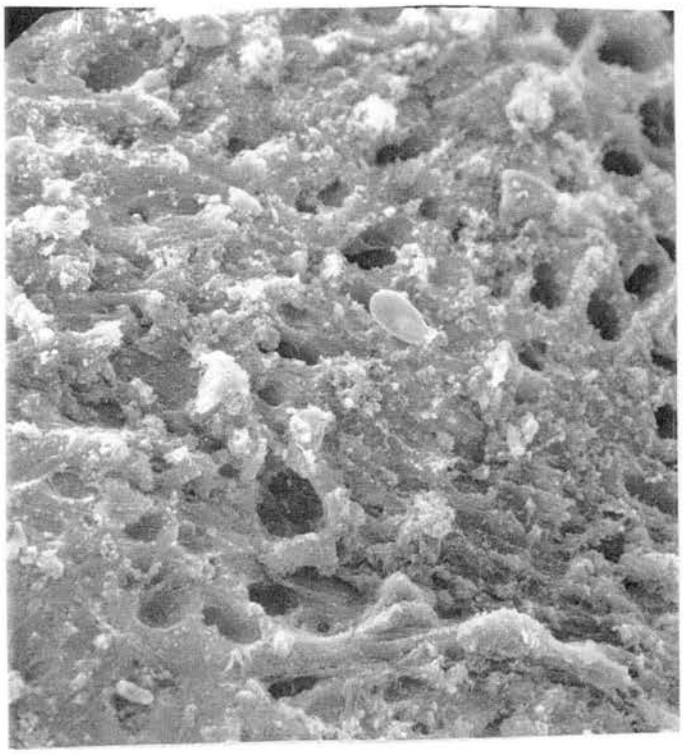

Figure 153. The appearance of protozoa in both PVA and GAC (not shown), after 9 months (240 X).

Summary of Discussion

The main objectives of this study were to demonstrate the capabilities of PVA-immobilized cells and 3% GAC-immobilized cells/sand as two novel permeable barrier media to biodegrade TCP in groundwater under a variety of operating conditions. In this study, the effects of loading rate, HRT, shock load, and low DO on the removal efficiency of PVA-immobilized cells and 3% GAC-immobilized cells were investigated.

Few studies have been done on the biodegradation of chlorophenols by immobilized bacteria. Immobilized cells in alginate were able to biodegrade phenol and tolerate the toxicity of high loads (Bettmann and Rehm 1984). The immobilized microorganisms on GAC could utilize most of the adsorbed phenol (Ehrhardt and Rehm 1985). The GAC in this case operated like a "buffer and depot"; it protected the immobilized microorganisms by adsorbing toxic phenol concentrations and set low quantities of the adsorbed phenol free for biodegradation. Polyurethane-immobilized *Flavobacterium* cells degraded pentachlorophenol (PCP) at initial concentrations as high as 300 mg/L (O'Reilly and Crawford 1989). The use of immobilized cells on PVA and GAC as biological permeable barrier media has never been investigated.

In order to investigate GAC-immobilized cells and PVA-immobilized cells as biological permeable barrier media, it is necessary to design and test these barriers under different operating conditions such as different loading rates, HRT's, deficiency of DO, and high shock loading for extended period of time. PVA-immobilized cells and 3% GAC-immobilized cells/sand were characterized and tested during 166 days of continuous operation under different loading rates, HRT's, and nutrient (C:N:P) ratios.

Both PVA and GAC immobilized cells were subsequently tested under high shock load and low DO conditions. The discussion of the various experimental results conducted during the study are presented as follows.

The results from the 166 days of continuous column experiments on PVA-immobilized cells (Table 9) proved that an elimination capacity of 100% TCP is feasible for loads up to 0.3 g L^{-1} d^{-1} (HRT= 24.5 minutes). At the loading rate of 0.6 g L^{-1} d^{-1} (HRT=12.3 minute), the TCP removal efficiency of PVA-immobilized cells was reduced to 91%. At the highest loading rate of 1.2 g L^{-1} d^{-1} (HRT= 12.3 minutes), the total TCP removal was 67%. Valo *et al.* (1990) used a semi batch biofilter with immobilized *Rhodococcus* to remove TCP, TeCP, and PCP from synthetic groundwater in pilot scale plant. Partial (30-60%) degradation of chlorophenols was achieved at the average loading rate of 0.01-0.07 g L^{-1} d^{-1} (HRT= 80 h). Makinen *et al.* (1993) achieved 99.7% chlorophenols (TCP, TeCP PCP) removal in an aerobic fluidized-bed reactor at a maximum loading rate of 0.45 g L^{-1} d^{-1} and a hydraulic retention time of 5 h. As compared to earlier studies, PVA-immobilized cells in this project operated at higher TCP loading rates and lower HRTs and produced a better quality effluent. The PVA-immobilized cells survived high shock loads of 4.12 and 4.7 g L^{-1} d^{-1} of TCP and recovered within 16 and 5 days, respectively. Betmann and Rehm (1984) concluded that immobilized *Pseudomonas* in alginate were protected by immobilization and were able to degrade phenol up to 2 g/L in less than 2 days. The immobilization of cells into PVA in this research was n to protect the microorganisms against the toxicity of TCP. An adequate oxygen supply was crucial,as shown in column experiments No.2 and No.9.

PVA-immobilized cells removal efficiency of TCP was affected by low DO in the influent. PVA-immobilized cells recovered within 21 and 11 days from first and second interruption of DO, respectively, and continued to biodegrade TCP. Increases and recoveries of effluent TCP concentrations followed the same pattern as reported by Makinen *et al.* (1993). A significantly lower elimination capacity of PVA-immobilized cells columns could generally be to traced to an insufficient oxygen supply, and high loading rates with a corresponding decrease in HRT

The elimination capacity of GAC-immobilized cells of 100% TCP is feasible regardless of the organic load (up to 1.2 g L^{-1} d^{-1}). The results confirm that GAC, even with a substantial development of bacterial activity shown by biodegradation of TCP during 166 days of operation, maintains a substantial adsorption capacity. This confirms the earlier results by Kindzierski *et al.* (1992). They noted that GAC maintained about one quarter of its virgin adsorption rate for phenol even with a substantial development of bacterial growth within its micropores. In this research immobilized cells on GAC were surveyed high shock loads (50.0 hr each) and DO interruptions (twice 50.0 hr each). GAC protected immobilized cells from shock loading through rapid initial adsorption into pores and slow subsequent release by desorption. This desorption accompanied by biodegradation of the desorbed TCP (bioregeneration) was shown during column study No. 9 after the first and second shock load. The evidence of activated carbon bioregeneration has been seen by many researchers. Suidan (1980) noted that the carbon equivalent of the gaseous products exceeded the organic carbon removed by the process and indicated that the extra gaseous products were the result of

the bioregeneration. Andrews and Trapasso (1984) showed that several compounds normally thought of as non-biodegradable were adsorbed on activated carbon and subsequently metabolized by attached microorganisms. During the interruption of DO, the microorganisms were unable to biodegrade TCP influent TCP was removed by adsorption on GAC (as shown with no chloride released, or pH dropped in the effluent). During steady state operations extra chloride was released in the effluent as the result of dehalogenation of TCP already adsorbed on GAC by attached microorganisms (bioregeneration).

Biological degradation of chlorophenols under aerobic conditions is known to release chloride, decrease DO and pH in the effluent (Makinen *et al* 1993). The results obtained from running PVA-immobilized cells and GAC-immobilized cells systems for approximately 240 days, indicated that the contribution of chloride release, DO consumption, and pH drop in the effluent were all important in the evaluation of removal efficiency of TCP in this study. During this study, the measured chloride release from dehalogenation of TCP under aerobic conditions agreed well with those calculated from GC results.

Jarvinen *et al.* (1994) concluded that aerobic chlorophenol biodegradation does not result in partially dechlorinated metabolites. They claim mineralization of chlorophenols (CP) since all CP removals were confirmed by chloride release and no chlorinated intermediates were found. Makinin *et al.* (1993) concluded that the chloride release and H^+ generation (pH decrease) is an indication of chlorophenol mineralization. The results of this research can be directly compared to the above studies. The results of GC/MS confirm that no chlorinated intermediates or phenol was found in the PVA and

GAC columns effluent. Amounts of CO_2 and methane gas greater than found in ambient air were detected in the GAC columns as the results of anaerobic biodegradation of TCP already adsorbed on GAC. This provides a possible explanation for the extra chloride release with the corresponding pH drop in the GAC columns effluent. The presence of aerobic and anaerobic activities in both GAC and PVA columns were confirmed by GC/MS after at the end of this research.

Comparison of GAC-Immobilized Cells with PVA-Immobilized Cells

One of the main objectives of this project was to compare the performance of GAC-immobilized cells to PVA-immobilized cells as two novel permeable barrier matrices on the basis of elimination capacity, ease of operation, stability over extended period of time, tolerance under toxic shock loads and low DO, and capital cost.

The results from this comparison (Table 10) demonstrate that 3% GAC - immobilized cells/ sand is superior to PVA-immobilized cells for its adsorption capabilities. The 3% GAC-immobilized cells/sand recovered from the high shock loads faster than PVA-immobilized cells. GAC-immobilized cells were able biodegrade TCP already adsorbed on GAC which can extend the life of GAC (bioregeneration). Unlike 1% of PVA-immobilized cells beads, 3% GAC-immobilized cells shown no signs of deterioration.

Both PVA-immobilized cells and 3% GAC-immobilized cells as permeable barriers compare to conventional surface treatment process offer low cost and efficient process to remove TCP from groundwater in-situ.

Table 9. Elimination Capacity During 166 days of PVA Column Studies No. 1-8

COLUMN STUDY	PVA COLUMN #1 (10.0 cm bed)	PVA COLUMN #2 (20.0 cm bed)
No. 1 TCP=10 mg/L flow=1ml/min	Reached 100% after 17.0 days	Reached 100 % after 13.0 days
No. 2 TCP=20 mg/L flow=1ml/min	Reduced from 100% to 68%	Reduced from 100 % to 76%
No. 3 TCP=20 mg/L flow=1ml/min	Reached 100% since day 1	Reached 100 % since day 1
No. 4 TCP=20 mg/L flow=1ml/min	Reached 100% since day 1	Reached 100 % since day 1
No. 5 TCP=30 mg/L flow=1ml/min	Reached 98%	Reached 100 %
No. 6 TCP=20 mg/L flow=2ml/min	Reached 100% after 8-10 days	Reached 100 % after 6 days
No. 7 TCP=20 mg/L flow=4ml/min	Reached 78%(trans*) Reached to 91%(ss**)	Reached 94%(trans) Reached 100% (ss)
No. 8 TCP=40 mg/L flow=4ml/min	Reached 54%(trans) Reached 67%(ss)	Reached 76%(trans) Reached 81%(ss)

*tran=transition periods.

** ss= steady state periods.

Table 10. Comparison of PVA-immobilized cells and 3% GAC-immobilized cells/sand

Basis of Comparison	PVA-immobilized cells	3% GAC immobilized cells
Removal efficiency	100 % for load up to 300 mg $L^{-1}.d^{-1}$	100 %
Ease of operation	easy to handle	easy to handle
Stability	most of the beads remained firm and elastic. a few of the beads severely damaged during 240 days of continuous operation.	no physical damage was observed.
Tolerance	survived high shock load and deficiency of DO, recovered from high shock load and low DO within 11-21days.	survived high shock load and low DO, biodegradation of TCP was affected by low DO and high shock. maintained 100% efficient by adsorption and biodegradation during high shock load and low DO.
Capital Cost	chemicals needed (boric acid, PVA) = $\$55.0/ft^3$.	$\$ 8.0/ft^3$ (3% GAC/ Silica sand)

Significance of Research

This research demonstrated that PVA-immobilized cells and 3% GAC-immobilized cells were able to biodegrade TCP from groundwater under various operational conditions. The immobilized cells were protected against toxic shock loads by the PVA and GAC. This research demonstrated that PVA-immobilized cells would be a successful media for use in a trench-based permeable barrier to remove TCP up to 0.3 g L^{-1} d^{-1}. PVA-immobilized cells tolerated low DO and recovered (100% efficiency) within 11-21 days. After 240 days of operation, some of the PVA beads showed signs of deterioration as shown by SEM. The 3% GAC-immobilized cells columns were remained 100% efficient throughout this research (except during 50 hr shock load). This research demonstrated that bioregeneration occurred as adsorbed TCP was desorbed. The adsorption capacity and biodegradation activity of GAC provided a better permeable barrier matrix than the PVA-immobilized cells. The bioregeneration of GAC by immobilized cells extent the life of GAC and eliminate the need to excavate and replace the media.

CHAPTER V

CONCLUSIONS

For the first time, PVA-immobilized cells and 3% GAC immobilized cells/sand were evaluated as two novel permeable barrier media in a side by side study under various operational conditions during 240 days of operation. This laboratory investigation were designed to remove TCP in-situ from groundwater. The results of this research corroborated previous investigations as well as provided new basis for operation of biological permeable barrier using PVA-immobilized cells and 3% GAC-immobilized cells/sand. The conclusions that can be drawn from this research are:

1) PVA-immobilized cells and 3% GAC-immobilized cells would be two successful media for use in a trench-based permeable barrier to remove TCP in-situ from groundwater.

2) PVA-immobilized cells can provide 100-91% removal efficiency at an applied loadings of 0.3-0.6 g $L^{-1}d^{-1}$. GAC-immobilized cells can provide 100% removal efficiency at applied loadings of 1.2 g $L^{-1}d^{-1}$.

3) A comparison of removal efficiencies between two columns of varied sizes containing PVA-immobilized cells demonstrated the effect of HRTs. The TCP concentration in the effluent of PVA column #1 (10.0 cm bed) was higher than PVA column #2 (20.0 cm bed). The removal efficiency of the PVA columns #1 and #2 reduced to 67% and 81% at applied loadings of 1.2 g $L^{-1}.d^{-1}$ and 0.6 g $L^{-1}.d^{-1}$,

respectively. The HRTs for column #1 and #2 were 12.3 and 24.5 minutes, respectively, at those loading conditions.

4) Cells were protected against high shock loads by immobilization on PVA beads and recovered to steady state conversion within 11-21 days. PVA-immobilized cells tolerated deficiency of dissolved oxygen and regained their activity once they received adequate DO. GAC maintained substantial adsorption capacity even with development of bacterial growth. The survival of the immobilized cells in spite of the addition of a shock load was the result of rapid adsorption of TCP by GAC. Bioregeneration occurred as adsorbed TCP was desorbed and metabolized by immobilized cells. This was shown by the extra chloride release, with corresponding pH drop in the effluent, after adsorption capacity of GAC was exhausted by a high shock load of TCP. PVA-immobilized cells were unable to offer any of the TCP adsorption advantages provided by GAC-immobilized cells. Bioregeneration may eliminate the need to excavate and replace the media.

5) After 240 days of continuous operation, over 99% of PVA-immobilized cells appeared to be resilient, firm, and structurally sound. The micrographs of the beads showed them to be more porous than initial beads. The channels and pockets within the beads appeared larger than initial beads. The micrographs of the beads after 240 days showed mirocolonies formation inside the PVA beads. The micrographs of GAC-immobilized cells showed microcolonies of the cells on inner surfaces of GAC.

6) PVA-immobilized cells will remain permeable and structurally sound over time (240 days). PVA-immobilized cells can tolerated high shock loads, low DO and resumed its biological activity to a steady state in a matter of a few days. PVA-

immobilized cells remained 100-91% efficient at applied loadings of 300 mg $L^{-1}.d^{-1}$ and 600 $mgL^{-1}d^{-1}$, respectively. PVA-immobilized cells completely dehalogenated TCP without formation of chlorinated intermediates or phenol.

7) GAC-immobilized cells offered 100% removal of TCP by a combination of biological degradation and physical adsorption. The cells functioned as biological processors and the GAC functioned as a support and adsorbent barrier. The GAC may be biologically regenerated, eliminating the need to excavate and replace the media. GAC-immobilization protected cells from high shock loads by rapid TCP adsorption. Biodegradation of TCP by GAC-immobilized cells dehalogenated TCP without formation of chlorinated intermediates or phenol.

CHAPTER VI

RECOMMENDATIONS

The potential for the use of PVA-immobilized cells and 3% GAC-immobilized cells/sand as two novel permeable barrier media to remove TCP in-situ from groundwater has been demonstrated. Important operational factors likely to influence the removal efficiencies of these barrier were also investigated. Based on the findings of this research, the following recommendations for future studies are proposed:

1). Investigate the removal efficiency of these barriers to remove multiple substrates such as mixtures of PCP, TeCP and TCP.

2). Investigate the use of immobilization of anaerobic pure cultures on PVA and GAC to remove chlorophenol(s) in-situ from groundwater.

3). Investigate the use of other sources of oxygen such as hydrogen peroxide for aerobic biodegradation of chlorophenol(s) using PVA-immobilized cells and 3% GAC-immobilized cells/sand.

4). Investigate the effect of other factors, such as low temperature, low pH and interruption of nutrient addition to the influent groundwater. This would assist in design and operation of permeable barriers using PVA and GAC immobilized cells.

5). Investigate the capital cost and operating costs of these barriers and compare to other technologies.

6). Evaluate different methods to measure growth rate of bacteria within the PVA-immobilized beads.

7). Evaluate 3% ion-exchange resins immobilized cells/sand as a permeable barrier to remove TCP in-situ from groundwater.

8). Evaluate the removal efficiencies of 3% ion-exchange immobilized cells/sand, PVA-immobilized cells, and 3% GAC-immobilized cells to remove TCP from groundwater under variety of operating conditions.

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APPENDIXES

APPENDIX A

Table A.1. Physical and Chemical Properties of Chlorophenols

Physical/ chemical Properties	MCP	2,4 DCP	2, 4, 6 - TCP	2,.3,4,6- TeCP	PCP
Molecular Mass	128.56	163.0	197.5	231.98	266.35
Density	1.26	1.28	1.49	1.6	1.978
Boilimg Point (° C at 760 mm)	174.9	210	246	246.0	310
Melting Point (°C at 760 mm)	9.0	45	69.5	70	190
Flash point (°C)	63.9	62.0	113.9		
Vapor pressure	1(12.1 °C)	1(76.5 °C)		1(100° C)	0.12(100 °C)
Log n-octanol/water partition coefficient	2.15	3.06		4.10	5.01

Source: WHO, 1989.

Table A.2: Groundwater Analysis

Parameter	Concentration	EPA Method 40 CFR Part 136
Specific Conductance	1045.7 µmhos/cm	120.1
pH	7.9 std unit	150.1
Alkalinity (total)	237.3 mg/L	310.2
Solids (total dissolved)	515.1 mg/L	160.1
Nitrite-Nitrate as N	0.5 mg/L	353.2
Hardness (total)	106.9 mg/L	130.1
Chloride	143.9 mg/L	325.2
Sulfate	32.9 mg/L	375.2
TOC	1.5 mg/L	------

APPENDIX B

Table B.1. Characteristics of PVA Beads and Mixture of (3%) GAC/Silica Sand

Parameter	Packed Bed of PNA Beads	Packed Bed of (3%) GAC/Sand
pH	8.1	8.1
Specific Gravity	1.008	1.63
Density (ρ) (g/cm^3)	0.987	1.62
Porosity (%)	25	30
Permeability Coefficient (K) (cm/s)	0.1425	0.0162
Compressibility Index (C_c) (m^2/kN)	4.08×10^{-3}	2.87×10^{-5}
Particle Size (mm)	3.8	0.4
Soil Classification	uniform rounded fine gravel	uniform rounded medium sand

* Density of water at 22° C was 0.9793 g/cm^3.

APPENDIX C

Table C.1. Raw Data of Equilibrium Study

Time (hours)	TCP concentration (mg/L)
0.0	19.5
3.0	4.8
5.0	2.0
8.0	0.5
10.0	0.2
20.0	0.1
24.0	0.0

Table C.2 Isotherm Study (Freundlich Isotherm)*

m (g GAC/L)	C (mg TCP/L)	Log(q)	q = x/m	Log(C)
0.0	19.54	2.199	158.11	1.2909
0.001	10.7692	2.098	125.38	1.0322
0.01	5.1852	1.970	94.24	0.7148
0.05	4.1176	1.940	86.16	0.6146
0.1	3.0882	1.890	77.02	0.4897
0.5	3.0	1.882	76.13	0.4771

* $\log q = \log K + 1/n \log C$

Table C.3 Raw Data for Adsorption Study on Silica Sand

sand time (hr)	TCP (mg/L)
0	20.0
3	19.2
7	19.6
12	19.8
24	19.9

Table C.4 Raw Data For Adsorption Study on Copper Screen

TCP (mg/L)	screen time (hr)
10	0
10	3
10	7
10	12
10	24

Table C.5 Raw Data for Immobilization Time Study

immob. time (hr)	adsorbed cell/g (10^6)
0	0.0
1	1.0
3	2.1
7	3.7
10	4.2
13	4.3
16	4.4
20	4.4
24	4.4

Table C.6. Kinetic* Study of Free Cells

Time (hrs)	TCP (mg/L)	Cl^- Theoretical (mg/L)	CL^- Measured (mg/L)	VSS (mg/L)
0.0	10.0	658.0	659.0	1035
1.0	4.0	661.24	663.2	1035
3.5	2.9	661.9	664.6	1075
5.0	0.0	662.9	666.0	1110
12.0	0.0	663.35	666.5	1136
24.0	0.0	663.35	666.0	1147

*The rate of growth of bacterial cells for a batch culture can be defined by the following expression (Tchobanoglous and Burton, 1991):

$$r_g = dX/dt$$

Where

r_g = rate of bacterial growth, mg $L^{-1}hr^{-1}$

X = concentration of microorganisms, mg/l

t = time, hr

Table C.7. Kinetics of TCP Adsorption on GAC and GAC(cell)

Time (hr)	TCP concentration (mg/L) GAC/cell	TCP concentration (mg/L) GAC
0	500	500
5	300	450
10	190	350
20	110	300
30	60	250
40	50	249
50	50	250
60	50	250

APPENDIX D

Table D.1 Data of Tracer Study Column #3

Time (min)	C (mg/L)	C_t	θ	E	θ^2	$E\theta^2$	V (L)
12	0.00	0.00	0.0817	0.0000	0.00667	0.0000	0.0015
24	0.40	9.60	0.1634	0.0529	0.0267	0.0014	0.0035
36	0.40	14.40	0.2451	0.0529	0.0601	0.0032	0.0045
48	1.80	86.40	0.3268	0.2380	0.1068	0.0254	0.0075
60	1.60	96.00	0.4084	0.2115	0.1668	0.0353	0.0090
72	2.60	187.20	0.4901	0.3437	0.2402	0.0826	0.0100
84	2.60	218.40	0.5718	0.3437	0.3270	0.1124	0.0110
96	2.10	201.60	0.6535	0.2776	0.4271	0.1186	0.0130
108	3.10	334.80	0.7352	0.4098	0.5405	0.2215	0.0150
120	8.33	999.60	0.8169	1.1012	0.6673	0.7348	0.0170
132	12.20	1610.40	0.8986	1.6128	0.8074	1.3023	0.0180
144	13.75	1980.00	0.9803	1.8178	0.9609	1.7467	0.0190
156	14.00	2184.00	1.0619	1.8508	1.1277	2.0872	0.0222
168	8.63	1449.84	1.1436	1.1409	1.3079	1.4922	0.0252
180	6.53	1175.40	1.2253	0.8633	1.5014	1.2961	0.0277
192	4.75	912.00	1.3070	0.6280	1.7083	1.0727	0.0317
204	2.90	591.60	1.3887	0.3834	1.9285	0.7393	0.0357
216	2.60	561.60	1.4704	0.3437	2.1620	0.7431	0.0387
228	1.90	433.20	1.5521	0.2512	2.4089	0.6051	0.0427
240	1.70	408.00	1.6338	0.2247	2.6692	0.5999	0.0457
252	0.70	176.40	1.7155	0.0925	2.9428	0.2723	0.0487
264	0.00	0.00	1.7971	0.0000	3.2297	0.0000	0.0511
276	0.00	0.00	1.8788	0.0000	3.5300	0.0000	0.0526
288	0.00	0.00	1.9605	0.0000	3.8436	0.0000	0.0551
300	0.00	0.00	2.0422	0.0000	4.1706	0.0000	0.0570
312	0.00	0.00	2.1239	0.0000	4.5109	0.0000	0.0600
324	0.00	0.00	2.2056	0.0000	4.8646	0.0000	0.0630
336	0.00	0.00	2.2873	0.0000	5.2316	0.0000	0.0690
348	0.00	0.00	2.3690	0.0000	5.6120	0.0000	0.0730
360	0.00	0.00	2.4506	0.0000	6.0057	0.0000	0.0770
Total	92.59	13600				13.2921	

Table D.2. Data of Tracer Study for Column #4

Time (min)	C (mg/L)	C_t	θ	E	θ^2	$E\theta^2$	V (L)
12	0.0	0.0	0.0653	0.0000	.0043	0.0000	.0020
24	0.4	9.6	0.1306	0.0944	.0171	0.0016	.0040
36	0.5	18.0	0.1960	0.1180	0.0384	0.0045	.0065
48	1.9	91.2	0.2613	0.4482	0.0683	0.0306	.0090
60	0.5	30.0	0.3266	0.1180	0.1067	0.0126	.0115
72	0.3	21.6	0.3919	0.0708	0.1536	0.0109	0.0133
84	0.4	33.6	0.4573	0.0944	0.2091	0.0197	0.0158
96	0.5	48.0	0.5226	0.1180	0.2731	0.0322	0.0183
108	0.6	64.8	0.5879	0.1415	0.3456	0.0489	0.0208
120	1.2	144.0	0.6532	0.2831	0.4267	0.1208	0.0233
132	1.1	145.2	0.7186	0.2595	0.5163	0.1340	0.0258
144	3.6	518.4	0.7839	0.8492	0.6145	0.5218	0.0283
156	4.4	686.4	0.8492	1.0380	0.7212	0.7485	0.0308
168	4.8	806.4	0.9145	1.1323	0.8364	0.9470	0.0330
180	9.7	1746.0	0.9799	2.2882	0.9601	2.1970	0.0358
192	11.0	2112.0	1.0452	2.5949	1.0924	2.8347	0.0388
204	7.9	1611.6	1.1105	1.8636	1.2332	2.2982	0.0413
216	5.3	1144.8	1.1758	1.2503	1.3826	1.7286	0.0433
228	3.2	729.6	1.2412	0.7549	1.5405	1.1629	0.0463
240	2.6	624.0	1.3065	0.6133	1.7069	1.0469	0.0488
252	1.5	378.0	1.3718	0.3539	1.8818	0.6659	0.0513
264	1.8	475.2	1.4371	0.4246	2.0653	0.8770	0.0535
276	0.9	248.4	1.5024	0.2123	2.2574	0.4793	0.0563
288	0.4	115.2	1.5678	0.0944	2.4579	0.2319	0.0588
300	0.4	120.0	1.6331	0.0944	2.6670	0.2517	0.0613
312	0.0	0.0	1.6984	0.0000	2.8846	0.0000	0.0641
324	0.0	0.0	1.7637	0.0000	3.1108	0.0000	0.0669
336	0.0	0.0	1.8291	0.0000	3.3455	0.0000	0.0699
348	0.0	0.0	1.8944	0.0000	3.5887	0.0000	0.0727
360	0.0	0.0	1.9597	0.0000	3.8405	0.0000	0.0752
Total	64.9	11920.0		15.3099		16.4072	

APPENDIX E

Table E.1. pH curve

APPENDIX F

Table F.1 pH Raw Data for Column Study No.1

Day	INF (1,2)	Col. #1	Col.#2	Col. #3	Col. #4	INF (3,4)
1	8.1	7.8	7.8	7.9	7.4	8.3
4	8.1	7.8	7.8	8.1	8.1	8.3
7	8.1	7.9	8.0	7.9	7.9	8.3
13	8.1	7.6	7.7	7.8	7.5	8.3
17	8.2	7.7	7.8	7.9	7.5	8.3
19	8.1	7.5	7.6	7.9	7.5	8.1
21	8.3	7.5	7.5	8.0	7.5	8.1
23	8.3	7.5	7.5	7.8	7.6	8.1
26	8.3	7.6	7.6	8.0	7.5	8.3
29	8.2	7.6	7.6	7.9	7.5	8.2

Table F.2 DO Raw Data for Column Study No.1

Day	INF (1,2)	Col. #1	Col. #2	Col.#3	Col. #4	INF (3,4)
1	8.6	7.7	7.8	8.2	7.6	8.5
4	8.6	7.5	7.8	8.2	7.4	8.5
7	8.4	7.3	7.7	8.4	7.4	8.5
13	8.4	7.0	7.5	7.8	7.6	8.5
17	8.6	7.2	7.7	7.9	7.7	8.4
19	8.6	7.1	7.7	7.9	7.3	8.6
21	8.4	6.9	7.6	7.8	7.4	8.5
23	8.4	6.6	7.5	7.7	7.4	8.3
26	8.4	6.6	7.4	7.6	7.1	8.4
29	8.4	6.5	7.5	7.6	7.2	8.4

Table F.3 Cl^- Raw Data for Column Study No.1

Day	INF (1,2)	Col. #1	Col. #2	Col. #3	Col. #4	INF (3,4)
1	161	162	162	154	159	152
4	158	159	160	154	159	152
7	151	154	156	150	154	150
13	155	157	160	160	162	153
17	156	161	162	164	164	160
19	155	162	161	168	170	164
21	155	162	164	168	169	164
23	158	162	163	164	164	159
26	158	164	164	167	164	159
29	158	164	162	167	164	159

Table F.4 TCP^- Raw Data for Column Study No.1

Day	INF (1,2)	Col. #1	Col. #2	Col. #3	Col. #4	INF (3,4)
1	11.0	10.2	10.0	0	0	9.5
4	11.0	9.25	7.6	0	0	11.5
7	11.0	4.5	2.5	0	0	11.3
13	10.75	1.75	0.1	0	0	9.5
17	10.0	0.5	0	0	0	11.5
19	10.0	0	0	0	0	9.5
21	10.0	0	0	0	0	9.4
23	10.2	0	0	0	0	9.4
26	12.0	0	0	0	0	12.0
29	9.5	0	0	0	0	9.5

APPENDIX G

Table G.1 pH Raw Data for Column Study No.2

Day	INF	Col. #1	Col. #2	Col. #3	Col. #4
33	8.3	7.5	7.7	8.0	7.8
35	8.4	7.4	7.4	7.8	7.8
38	8.3	7.2	7.3	7.4	7.4
40	8.4	7.4	7.4	7.6	7.6
42	8.3	7.4	7.4	7.4	7.7
44	8.1	7.4	7.4	7.9	7.7
47	8.2	7.9	7.9	8.0	8.0
49	8.3	8.1	8.1	8.1	8.1
50	8.4	8.2	8.2	8.2	8.1
54	8.3	7.8	8.0	8.1	8.1
55	8.3	8.1	8.2	8.2	8.2
58	8.0	7.9	7.8	7.9	7.9

Table G.2. DO Raw Data for Column Study No.2

Day	INF	Col. #1	Col. #2	Col. #3	Col. #4
33	9.2	2.2	3.2	3.2	3.8
35	9.0	2.8	3.0	3.0	3.8
38	9.0	3.2	3.6	2.4	3.8
40	8.6	2.9	3.4	3.2	2.0
42	9.0	3.4	3.6	2.9	3.5
44	8.8	3.2	3.0	2.4	3.0
47	9.0	2.2	3.2	3.0	2.5
49	8.4	3.6	3.2	4.0	3.2
50	8.6	3.6	3.0	3.8	3.4
54	8.2	2.6	2.8	2.3	3.0
55	8.4	2.6	2.6	2.3	2.8
58	8.6	2.4	2.2	2.4	2.2

Table G.3. Cl Raw Data for Column Study No.2

Day	INF	Col. #1	Col. #2	Col. #3	Col. #4
33	158.5	170.5	163.3	163.3	170.5
35	163.3	177.9	170.5	170.5	170.5
38	158.5	170.5	172.4	170.5	172.4
40	158.5	170.5	163.3	163.3	170.5
42	158.5	170.5	173.5	173.5	170.5
44	158.5	170.5	173.5	173.5	170.5
47	160.8	167.7	167.7	170.5	170.5
49	161.0	168.0	171.8	168.0	168.0
50	161.0	171.8	168.0	168.0	168.0
54	159.8	167.7	167.7	167.7	170.5
55	159.8	168.0	167.7	167.7	169.0
58	161.8	168.7	170.4	168.7	170.4

Table G.4. TCP Raw Data for Column Study No.2

Day	INF	Col. #1	Col. #2	Col. #3	Col. #4
33	19.9	0.0	0.0	0	0
35	19.8	0.0	0.0	0	0
38	20.0	0.0	0.0	0	0
40	22.0	1.2	0.8	0	0
42	20.2	1.6	1.2	0	0
44	20.0	1.0	1.0	0	0
47	19.7	2.2	2.4	0	0
49	20.5	2.8	3.0	0	0
50	23.0	3.2	2.5	0	0
54	19.0	4.2	3.0	0	0
55	19.0	5.9	4.6	0	0
58	21.0	6.5	4.9	0	0

APPENDIX H

Table H.1. pH Raw Data for Column Study No.3

Day	INF	Col. #1	Col. #2	Col. #3	Col. #4
64	8.4	8.2	8.3	8.3	8.2
66	8.1	7.6	7.6	7.8	7.8
69	8.0	7.3	7.3	7.4	7.4
71	8.0	7.3	7.4	7.4	7.4
74	8.0	7.3	7.4	7.4	7.3
75	8.1	7.3	7.4	7.3	7.2
78	7.8	7.3	7.4	7.6	7.6
79	7.8	7.3	7.3	7.5	7.4
81	7.9	7.4	7.3	7.4	7.4
82	8.1	7.6	7.5	7.6	7.5

Table H.2. DO Raw Data for Column Study No.3

Day	INF	Col. #1	Col. #2	Col. #3	Col. #4
64	22.0	2.0	2.0	3.0	2.8
66	29.0	5.2	4.8	5.2	4.6
69	21.0	3.2	2.1	2.8	2.2
71	20.0	1.8	2.1	2.0	2.0
74	29.0	4.6	4.4	4.2	4.8
75	26.0	4.2	4.0	4.2	4.0
78	21.0	2.0	2.0	2.0	2.2
79	22.0	2.2	2.0	2.0	1.8
81	20.0	1.0	1.8	2.2	1.8
82	25.0	3.2	3.2	2.4	3.2

Table H.3. Cl Raw Data for Column Study No.3

Day	INF	Col. #1	Col. #2	Col. #3	Col. #4
64	156.0	162.9	162.9	166.4	166.4
66	161.8	172.2	174.0	172.2	174.0
69	162.9	172.4	172.4	170.0	170.0
71	158.5	168.7	168.7	170.4	170.4
74	158.5	165.2	168.7	168.7	168.7
75	158.5	170.4	170.4	172.2	172.2
78	161.0	171.7	171.7	171.7	171.7
79	161.0	168.0	168.0	172.0	172.0
81	160.0	171.7	171.7	175.0	171.7
82	161.8	168.7	174.0	168.7	174.0

Table H.4. TCP Raw Data for Column Study No.3

Day	INF	Col. #1	Col. #2	Col. #3	Col. #4
64	21.0	0	0	0	0
66	20.0	0	0	0	0
69	19.8	0	0	0	0
71	17.9	0	0	0	0
74	19.2	0	0	0	0
75	20.0	0	0	0	0
78	19.8	0	0	0	0
79	21.5	0	0	0	0
81	20.0	0	0	0	0
82	20.0	0	0	0	0

APPENDIX I

Table I.1. pH Raw Data for Column Study No.4

Day	INF	Col. #1	Col. #2	Col. #3	Col. #4
83	7.8	7.6	7.7	7.6	7.6
84	7.8	7.4	7.4	7.4	7.4
86	7.8	7.3	7.4	7.5	7.5
88	8.0	7.5	7.5	7.5	7.5
89	8.1	7.7	7.7	7.7	7.7
90	8.0	7.6	7.6	7.6	7.6
91	7.8	7.6	7.6	7.6	7.6
92	7.9	7.3	7.4	7.6	7.7
94	8.0	7.5	7.3	7.5	7.5
96	8.0	7.5	7.5	7.6	7.5

Table I.2. DO Raw Data for Column Study No.4

Day	INF	Col. #1	Col. #2	Col. #3	Col. #4
83	30.0	4.8	4.4	4.2	4.8
84	27.0	3.8	4.2	5.2	5.2
86	23.0	4.0	4.6	3.8	4.0
88	23.0	2.8	3.0	3.8	2.8
89	22.0	4.8	3.8	4.6	3.8
90	29.0	4.4	4.0	3.2	4.0
91	26.0	3.8	3.8	3.6	3.4
92	30.0	3.6	3.6	3.4	3.6
94	32.0	4.8	4.6	4.2	4.0
96	26.0	3.6	3.6	4.0	3.8

Table I.3 Cl Raw Data for Column Study No.4

Day	INF	Col. #1	Col. #2	Col. #3	Col. #4
83	158.5	168.7	170.4	170.4	168.7
84	165.2	175.8	175.8	172.2	175.8
86	165.2	175.8	177.6	172.2	177.6
88	166.9	177.6	175.8	175.8	177.6
89	168.7	181.3	183.2	181.3	183.3
90	165.2	177.5	175.8	175.8	175.8
91	163.3	170.5	174.2	174.2	170.5
92	162.9	177.4	177.4	177.4	177.4
94	163.3	174.2	174.2	174.2	174.2
96					

Table I.4 TCP Raw Data for Column Study No.4

Day	INF	Col. #1	Col. #2	Col. #3	Col. #4
83	21.5	0	0	0	0
84	19.8	0	0	0	0
86	20.0	0	0	0	0
88	19.0	0	0	0	0
89	19.4	0	0	0	0
90	19.4	0	0	0	0
91	19.8	0	0	0	0
92	22.0	0	0	0	0
94	21.0	0	0	0	0
96	20.0	0	0	0	0

APPENDIX J

Table J.1 pH Raw Data for Column Study No.5

Day	INF	Col. #1	Col. #2	Col. #3	Col. #4
97	7.9	7.3	7.3	7.4	7.3
99	8.1	7.4	7.6	7.5	7.5
101	8.0	7.5	7.4	7.5	7.6
103	7.9	7.2	7.1	7.3	7.3
105	8.1	7.2	7.2	7.4	7.4
107	7.9	7.1	7.0	7.1	7.2
109	7.9	7.0	6.9	7.1	7.2
111	7.9	6.9	7.0	7.3	7.3

Table J.2 DO. Raw Data for Column Study No.5

Day	INF	Col. #1	Col. #2	Col. #3	Col. #4
97	28.0	3.0	3.0	2.8	3.0
99	33.0	4.4	4.1	3.0	3.0
101	29.0	2.4	1.8	2.2	1.8
103	31.0	2.8	2.9	3.4	2.9
105	29.0	2.8	3.0	2.9	1.8
107	31.0	3.2	2.8	3.3	1.8
109	30.0	2.9	2.4	3.0	2.2
111	31.0	2.8	2.9	3.3	2.4

Table J.3. Cl Raw Data for Column Study No.5

Day	INF	Col. #1	Col. #2	Col. #3	Col. #4
97	164.5	175.4	175.4	183.1	175.4
99	168.0	183.1	183.1	183.1	191.1
101	168.0	191.1	191.1	191.1	191.1
103	163.3	177.9	185.7	177.9	185.7
105	163.3	181.8	181.8	181.8	181.8
107	163.3	177.9	185.7	185.7	185.7
109	170.0	193.3	193.3	193.3	193.3
111	163.3	170.5	170.5	185.7	177.9

Table J.4. TCP Raw Data for Column Study No.5

Day	INF	Col. #1	Col. #2	Col. #3	Col. #4
97	30.36	0	0	0	0
99	29.54	0	0	0	0
101	31.0	0	0	0	0
103	29.3	0	0	0	0
105	31.57	0.56	0.73	0	0
107	31.0	0	0	0	0
109	30.5	0	0	0	0
111	29.5	0	0	0	0

APPENDIX K

Table K.1. pH Raw Data for Column Study No.6

Day	INF	Col. #1	Col. #2	Col. #3	Col. #4
113	7.9	7.4	7.3	7.4	7.2
115	7.8	7.6	7.2	7.3	7.2
117	7.9	7.5	7.1	7.3	7.2
119	8.0	7.3	7.3	7.3	7.1
121	8.0	7.3	7.4	7.4	7.4
123	8.0	7.2	7.2	7.3	7.2
124	8.2	7.4	7.4	7.4	7.3
126	8.0	7.3	7.1	7.3	7.1
127	8.0	6.9	6.9	7.0	6.9

Table K.2. DO. Raw Data for Column Study No.6

Day	INF	Col. #1	Col. #2	Col. #3	Col. #4
113	24.0	5.8	5.8	3.8	3.4
115	24.0	7.8	5.2	3.6	3.2
117	22.0	7.9	4.8	3.0	3.2
119	30.0	6.2	6.8	3.8	4.4
121	26.0	3.4	3.2	3.0	3.0
123	30.0	6.0	6.0	4.8	6.0
124	25.5	4.2	4.2	3.2	4.1
126	23.0	2.6	2.8	2.8	2.8
127	28.0	4.8	5.4	5.0	4.2

Table K.3. Cl Raw Data for Column Study No.6

Day	INF	Col. #1	Col. #2	Col. #3	Col. #4
113	158.5	168.7	168.7	170.4	172.2
115	160.1	160.1	170.4	172.2	173.9
117	160.1	165.2	170.4	172.2	172.2
119	161.8	172.2	173.9	172.2	173.9
121	163.5	173.9	175.8	173.9	175.8
123	158.5	168.7	170.4	168.7	170.4
124	160.1	172.2	172.1	170.4	172.1
126	163.5	175.8	177.6	175.8	177.6
127	163.5	175.8	175.8	175.8	177.6

Table K.4. TCP Raw Data for Column Study No.6

Day	INF	Col. #1	Col. #2	Col. #3	Col. #4
113	22.8	8.20	4.2	0	0
115	22.6	18.23	1.82	0	0
117	21.8	18.38	4.79	0	0
119	23.6	2.15	N.D	0	0
121	21.2	1.2	0	0	0
123	20.5	0	0	0	0
124	21.0	0	0	0	0
126	23.4	0	0	0	0
127	22.2	0	0	0	0

APPENDIX L

Table L.1. pH Raw Data for Column Study No.7

Day	INF	Col. #1	Col. #2	Col. #3	Col. #4
128	8.0	7.3	7.1	7.1	7.1
130	8.0	7.3	7.1	7.1	7.1
132	8.0	7.3	7.1	7.1	7.1
134	8.0	7.3	7.1	7.1	7.1
136	7.9	7.4	7.0	7.0	7.0
138	8.0	7.2	7.1	7.1	7.1
140	7.8	7.1	7.1	6.9	6.9
142	7.9	7.2	7.2	7.0	7.0

Table L.2. DO. Raw Data for Column Study No.7

Day	INF	Col. #1	Col. #2	Col. #3	Col. #4
128	26	7.4	6.8	7.6	8.2
130	26	7.8	6.8	6.6	5.8
132	27	7.4	7.8	7.6	8.2
134	28	8.0	6.8	6.6	5.8
136	29	9.6	9.0	6.6	5.8
138	30	7.6	7.2	6.0	5.8
140	29	9.2	8.8	7.0	5.8
142	28	7.8	7.0	6.6	5.8

Table L.3. Cl Raw Data for Column Study No.7

Day	INF	Col. #1	Col. #2	Col. #3	Col. #4
128	159.0	163.5	165.2	168.7	170.4
130	160.1	165.2	170.4	170.4	172.2
132	161.8	168.7	172.2	170.4	172.2
134	159.0	166.9	172.2	168.7	170.4
136	163.5	170.4	175.8	172.2	175.8
138	160.1	170.4	170.4	170.4	172.2
140	160.1	168.7	168.7	168.7	170.4
142	156.9	166.9	168.7	166.9	168.7

Table L.4. TCP Raw Data for Column Study No.7

Day	INF	Col. #1	Col. #2	Col. #3	Col. #4
128	21.12	8.92	1.20	0	0
130	22.60	4.77	1.84	0	0
132	20.40	4.30	1.00	0	0
134	21.90	4.40	0	0	0
136	21.80	2.80	0	0	0
138	19.80	2.30	0	0	0
140	21.30	2.10	0	0	0
142	20.50	0	0	0	0

APPENDIX M

Table M.1. pH Raw Data for Column Study No.8

Day	INF	Col. #1	Col. #2	Col. #3	Col. #4
150	8.1	6.9	6.9	6.5	6.7
152	8.0	7.0	7.0	6.5	6.6
154	8.1	7.0	6.8	6.7	6.7
156	8.1	7.0	6.7	6.6	6.7
158	8.1	6.7	6.7	6.7	6.6
160	8.4	6.8	6.8	6.7	6.6
162	8.5	6.9	7.0	6.8	6.7
164	8.4	6.9	6.9	6.7	6.6

Table M.2. DO. Raw Data for Column Study No.8

Day	INF	Col. #1	Col. #2	Col. #3	Col. #4
150	27.5	12.0	7.6	3.2	3.6
152	27.0	12.2	7.4	2.4	3.6
154	26.5	11.8	8.6	5.0	3.2
156	26.5	8.8	4.8	7.6	4.0
158	27.5	8.8	6.2	4.2	3.2
160	30.0	11.2	6.2	6.8	4.0
162	28.6	7.2	3.0	4.0	7.8
164	27.2	8.6	5.8	4.2	3.8

Table M.3. Cl Raw Data for Column Study No.8

Day	INF	Col. #1	Col. #2	Col. #3	Col. #4
150	162.1	172.9	176.6	180.4	182.4
152	158.6	170.6	180.5	182.4	184.4
154	158.6	169.2	172.9	182.4	176.6
156	162.1	176.6	180.5	182.4	182.4
158	158.6	172.9	180.5	184.4	184.4
160	155.2	165.6	172.9	176.6	180.5
162	158.6	169.2	180.5	176.6	169.2
164	162.1	176.6	180.5	182.4	182.4

Table M.4. TCP Raw Data for Column Study No.8

Day	INF	Col. #1	Col. #2	Col. #3	Col. #4
150	41.0	19.49	10.80	0	0
152	40.2	18.30	10.20	0	0
154	41.74	19.15	9.50	0	0
156	39.4	16.94	7.72	0	0
158	40.0	13.60	8.00	0	0
160	40.5	18.10	7.90	0	0
162	40.3	10.10	9.60	0	0
164	41.2	12.30	7.60	0	0

APPENDIX N

Table N.1. pH Raw Data for Column Study No.9 (col. 1 & 3)

day	inf. (1,3)	col. 1	col. 3
168	8.2	7.3	7.3
171	8.1	7.4	7.3
173	8.2	7.6	7.4
176	8.1	7.2	7.6
179	8.5	7.5	7.0
180	8.5	8.0	8.0
181	8.5	8.5	8.0
183	8.5	8.3	7.9
186	8.5	8.1	7.8
189	8.4	6.9	7.1
191	8.1	7.1	6.9
194	8.2	7.1	6.8
198	8.2	7.0	6.8
203	8.1	7.1	6.8
208	8.0	6.9	6.8
213	8.1	7.1	6.6
217	8.1	7.2	7.0
224	8.1	7.7	7.5
225	8.0	7.8	7.5
226	8.2	6.8	6.8
233	8.4	7.0	7.0
240	8.2	6.9	6.9

Table N.2. pH Raw Data for Column Study No.9 (col. 2 & 4)

day	inf. (2,4)	col. 2	col. 4
168	8.2	7.1	7.8
171	8.1	7.2	7.4
173	8.2	7.2	7.4
176	8.1	7.6	7.4
179	8.5	7.1	7.1
180	8.5	8.2	8.2
181	8.5	8.5	8.2
183	8.5	8.5	8.3
186	8.5	8.5	8.0
189	8.4	7.2	7.4
191	8.1	7.4	6.6
194	8.2	7.2	6.6
198	8.2	7.0	6.6
203	8.1	6.9	6.6
208	8.0	7.3	6.6
213	8.1	6.8	6.4
217	8.1	7.3	6.6
224	8.1	7.7	7.1
225	8.0	7.8	7.0
226	8.2	7.8	6.6
233	8.4	7.0	6.6
240	8.2	6.9	6.4

Table N.3. DO Raw Data for Column Study No.9 (col. 1 & 3)

day	inf. (1,3)	col. 1	col. 3
168	27.5	4.8	2.0
171	23.5	3.2	3.8
173	26.5	4.6	3.6
176	29.0	3.8	2.8
179	30.0	4.0	2.8
180	1.2	3.0	3.0
181	1.2	2.8	2.8
183	30.7	14.2	3.2
186	29.0	13.8	3.0
189	31.0	9.2	3.2
191	28.0	7.0	3.6
194	28.0	9.2	3.2
198	28.0	6.2	3.0
203	26.0	3.2	4.0
208	27.0	1.4	2.2
213	29.0	1.8	2.2
217	28.5	5.4	1.2
224	2.0	1.6	2.2
225	2.2	0.8	2.0
226	31.0	8.0	1.2
233	29.0	6.6	1.4
240	32.5	2.6	1.2

Table N.4. DO Raw Data for Column Study No.9 (col. 2 & 4)

day	inf. (2,4)	col. 2	col. 4
168	27.5	4.4	3.8
171	23.5	2.8	3.0
173	26.5	4.0	3.2
176	29.0	2.0	2.8
179	30.0	6.8	3.2
180	28.0	18.5	3.6
181	32.0	15.0	15.0
183	30.7	14.4	17.0
186	29.0	7.2	13.8
189	31.0	7.2	3.2
191	28.0	7.2	3.4
194	28.0	6.0	2.0
198	28.0	4.8	1.2
203	26.0	5.6	1.0
208	27.0	3.0	0
213	29.0	3.0	0
217	28.5	2.0	0
224	30.0	10.2	2.0
225	29.4	14.0	2.0
226	31.0	12.0	1.4
233	29.0	3.8	1.0
240	32.5	2.0	0

Table N.5. Cl Raw Data for Column Study No.9 (col. 1 & 3)

day	inf. (1,3)	col. 1	col. 3
168	162.1	178.0	186.1
171	162.1	178.0	182.1
173	158.6	174.5	183.3
176	162.1	182.7	178.0
179	163.5	187.5	180.2
180	160.1	160.1	176.0
181	160.1	160.1	179.0
183	158.9	158.9	174.1
186	160.1	160.1	173.8
189	162.1	162.1	190.6
191	155.2	162.4	182.9
194	156.9	165.2	195.6
198	163.5	178.6	197.5
203	161.8	181.2	193.8
208	161.8	185.5	198.8
213	158.6	178.8	194.4
217	158.5	179.5	188.0
224	163.5	177.6	185.1
225	160.1	160.1	174.0
226	165.2	175.8	178.2
233	160.1	178.2	204.8
240	160.1	181.3	209.3

Table N.6. Cl Raw Data for Column Study No.9 (col. 2 & 4)

day	inf. (2,4)	col. 2	col. 4
168	162.1	186.1	181.4
171	162.1	178.0	180.3
173	158.6	175.1	174.5
176	162.1	186.1	186.8
179	163.5	186.1	187.5
180	160.1	160.1	180.7
181	160.1	160.1	170.8
183	158.9	158.9	167.2
186	160.1	160.1	166.7
189	162.1	177.5	169.1
191	155.2	169.7	183.7
194	156.9	173.7	197.8
198	163.5	197.5	208.5
203	161.8	194.1	211.4
208	161.8	194.1	214.6
213	158.6	191.6	214.3
217	158.5	179.5	195.0
224	163.5	165.2	193.0
225	160.1	166.9	189.0
226	165.2	165.2	195.0
233	160.1	196.5	212.0
240	160.1	194.2	216.7

Table N.7. TCP Raw Data for Column Study No.9 (col. 1 & 3)

day	inf. (1,3)	col. 1	col. 3
168	41.0	6.95	0
171	39.3	5.25	0
173	40.7	4.40	0
176	39.6	4.20	0
179	40.0	4.00	0
180	41.75	38.06	0
181	43.6	43.60	0
183	42.7	33.80	0
186	40.7	20.70	0
189	42.3	20.02	0
191	41.6	27.90	0
194	40.0	13.00	0
198	41.6	10.60	0
203	42.0	4.20	0
208	41.0	3.10	0
213	41.0	2.19	0
217	41.0	1.92	0
224	42.14	33.90	0
225	40.5	29.13	0
226	42.6	21.04	0
233	41.4	12.80	0
240	41.0	4.69	0

Table N.8. TCP Raw Data for Column Study No.9 (col. 2 & 4)

day	inf. (2,4)	col. 2	col. 4
168	41.0	6.1	0
171	39.3	5.0	0
173	40.7	4.9	0
176	39.6	4.7	0
179	40.0	4.0	0
180	566.0	432.2	182.3
181	566.0	710.4	565.0
183	42.7	57.6	59.0
186	40.7	21.4	0
189	42.3	13.7	0
191	41.6	7.8	0
194	40.0	7.2	0
198	41.6	5.3	0
203	42.0	3.2	0
208	41.0	3.4	0
213	41.0	0	0
217	41.0	0	0
224	563.0	450.4	0
225	591.0	487.0	48.1
226	42.6	90.9	5.8
233	41.5	0	0
240	40.0	0	0

VITA

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