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ABSTRACT

COMPARISON OF REDUCTIVE DEHALOGENATION BY MICROBIAL POPULATIONS ON ADSORPTIVE VERSUS NON-ADSORPTIVE BIOREACTOR SUPPORT MATERIALS

by
Linda S. Colella

The overall performance of two bioreactors was studied. The reactor with a wood-based activated carbon as a biosupport completely dehalogenated a higher feed concentration of trichlorophenol than that with Manville beads. The carbon reactor was further characterized by the development of adsorption isotherms for most of the class of chlorophenols. Competitive adsorption was investigated using an anaerobic medium, and a lignite-based carbon was studied for comparison. The order of adsorption strength on both carbons is trichloropenols> dichlorophenols> monochlorophenols, with the wood-based carbon having higher overall adsorption than the lignite-based carbon. The presence of the anaerobic medium decreased the extent of chlorophenol adsorption at lower liquid concentrations.

The investigation of the effect of a biofilm on the adsorption characteristics of the activated carbon showed that the biofilm decreased the rate at which adsorption equilibrium of 4-CP was obtained. However, the equilibrium itself was not effected. It was also determined that the organisms serve as adsorptive material.

COMPARISON OF REDUCTIVE DEHALOGENATION BY MICROBIAL POPULATIONS ON ADSORPTIVE VERSUS NON-ADSORPTIVE BIOREACTOR SUPPORT MATERIALS

by
Linda S. Colella

A Thesis
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COMPARISON OF REDUCTIVE DEHALOGENATION BY MICROBIAL POPULATIONS ON ADSORPTIVE VERSUS NON-ADSORPTIVE BIOREACTOR SUPPORT MATERIALS

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CHAPTER 1

INTRODUCTION

Phenolic compounds are classified by the Environmental Protection Agency as priority pollutants. Chlorophenols are one class of these hazardous chemicals which are used as fungicides and pesticides, and are produced during chemical processing and from wood preservation operations and pulp and paper mills. They are often found in industrial wastewaters as well as in the soil and groundwater near these types of industrial sites. Due to the toxicity and recalcitrance of this class of chlorophenols, investigators have been focusing their efforts on various methods of biodegradation.

Some investigators have shown that highly halogenated compounds can be more easily degraded via anaerobic reductive dehalogenation [Quensen *et al.* (1988); Neilson *et al.* (1988); Sahm *et al.* (1986); Goulding *et al.* (1986); Bollag (1974)], and others have shown that compounds that are less substituted can be mineralized by an aerobic microbial system [Chaudhry and Chapalamadagu (1991); Knackmuss (1982); DiGeronimo *et al.* (1979)]. It has even been shown that a combined anaerobic-aerobic system can be used for complete mineralization of chlorinated aromatics [Armenante *et al.* (1992)].

While investigating methods of biodegradation, researchers have shown that immobilized microorganisms have many advantages over a suspended microbial population [Ehrhardt and Rehm (1985),(1989); Westmeier and Rehm (1987); Keweloh *et al.* (1989)]. Washout of the biomass can be prevented using immobilized cells. An

increase in resistance to high concentrations of toxic chemicals, and a greater cell density resulting in higher degradation rates can be found when using an immobilized population.

Many researchers have investigated immobilized cells as mentioned above. However, few have compared the effect of different support materials. Maloney *et al.* (1984) compared bacterial TOC removal on GAC and sand finding similar bacterial removal on both. Morsen and Rehm (1990) compared phenol degradation by organisms immobilized on activated carbon and sintered glass. They found that the activated carbon system degraded about four times that of the sintered glass system in batch studies, and in continuous systems, the carbon system had a phenol degradation that reached 9.2 g/l-day while that of the sintered glass system was 6.4 g/l-day. The investigations previously mentioned involving immobilized cells utilized aerobic organisms.

The objective of this study was not only to compare two biosupport materials, but also to further characterize the complex bioreactor system using activated carbon as immobilization material. The investigation described in this work used an anaerobic microbial consortium to reductively dehalogenate 2,4,6-trichlorophenol (2,4,6-TCP) to 2,4-dichlorophenol (2,4-DCP) and eventually to 4-chlorophenol (4-CP)

CHAPTER 2

COMPARISON OF ACTIVATED CARBON AND MANVILLE BEADS AS THE MICROORGANISM SUPPORT IN BIOREACTORS

2.1 Introduction

In order to compare the support materials used for the immobilization of microorganisms, it was necessary to first investigate the overall performance of such systems. Therefore, to begin this study, two identical bioreactors were set up, the only difference being the biosupport material. One bioreactor used an activated carbon as the support material, which exhibited adsorptive properties. The second bioreactor was packed with Manville beads, a non-adsorptive silica material.

2.2 Reactor Set-up

2.2.1 Material List

A list of the materials used to assemble the reactor systems is given in Table 1.

Table 1: Reactor Material List

Company	Catalogue #	Description
ACE Glass (Vineland NJ)	5821-24	Jacketed chromatography column, ID:25 mm, L=300 mm, custom made with 4 #5037 sampling ports
	5838-78	Teflon Adapter with 1/4" NPT fitting
	5838-12	Connecting Adapter
SWAGELOK (Mountainside, NJ)	400-3-4TMT	Male Run Tee, 1/4" NPT, 1/4" OD tubing, Stainless Steel
	400-3	Union Tee, 1/4" tubing
Cole Parmer (Niles, IL)	L-07519-00	Masterflex pump head
	L-07519-50	Pump cartridges
	L-07520-35	Pump drive
	G-06485-13	Pharmed tubing, size 13
	G-06485-25	Pharmed tubing, size 25
	G-06392-21	Teflon Plug Valves
Fisher (Springfield, NJ)	14-176-179	Nalgene 890 Teflon FEP tubing, 1/4" OD, 3/16" ID
	SG-031778	Luer Lock Syringes, 50µl to 2 ml
Brinkmann Instruments (Westbury, NY)	Lauda K-2/R	Water Bath

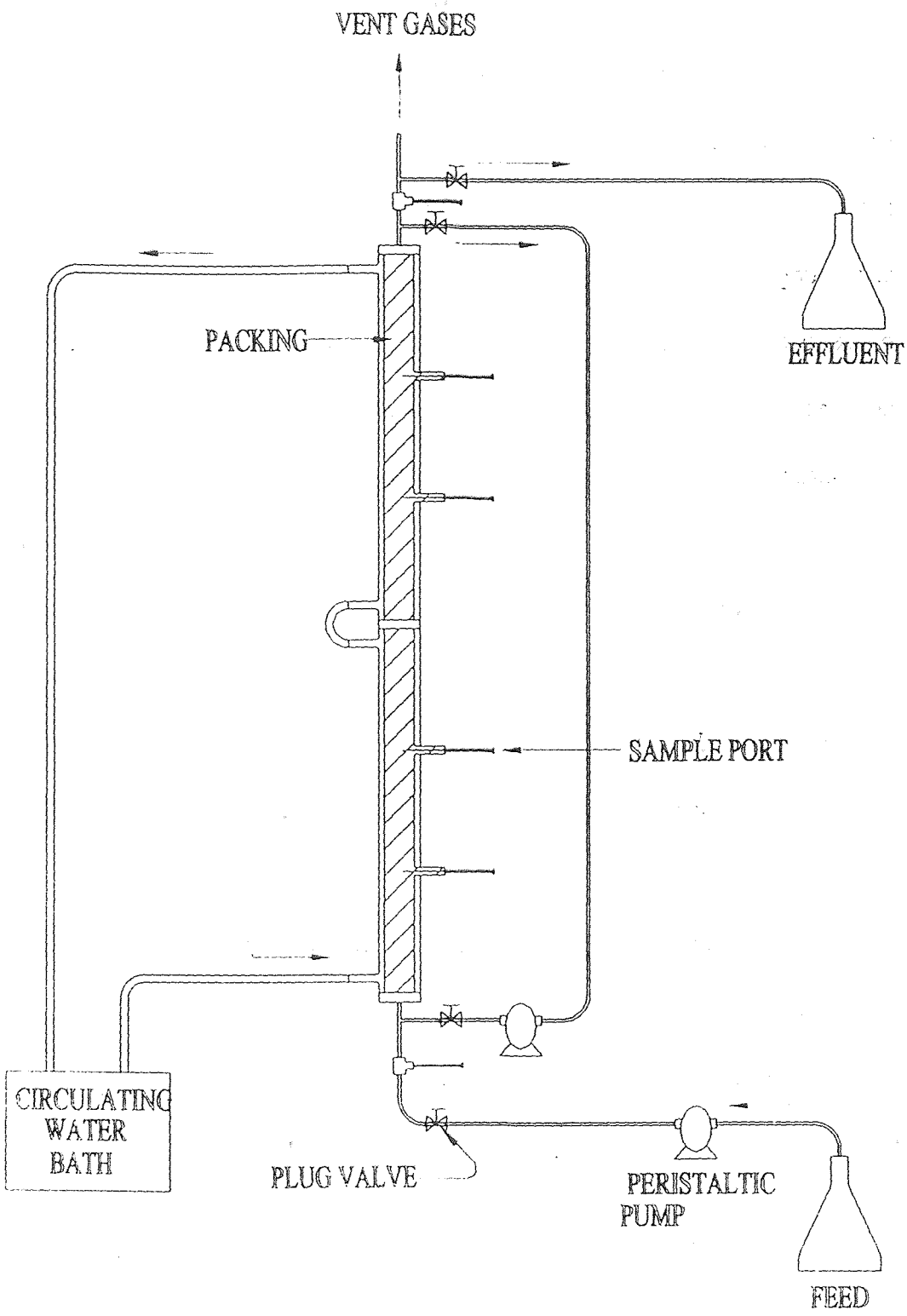

Figure 1: Reactor Set-Up

2.2.2 Reactor Description

2.2.2.1 Reactor Assembly The reactor set-up is shown in Figure 1. The set-up for the Manville packed reactor and the activated carbon packed reactor was identical. The total length of the effective reactor was 60 cm, consisting of two 30 cm jacketed chromatography columns connected by an open threaded connector. The top and bottom of the reactor were fitted with Teflon NPT adapters. Swagelok NPT fittings were attached to these in order to attach flexible Teflon tubing. A section of Pharmed tubing (approximately 6 inches in length) was attached to the Teflon tubing via tubing connectors, for use through the peristaltic pump on both the input feed line and the recycle line. A tube, separate from the effluent tube, led out of the top of the reactor in order to vent any gases produced. Teflon plug valves were place at the top and bottom of the reactor, outside the recycle loop, and at the beginning and end of the recycle loop to control the flow and allow for easy replacement of tubing sections. The columns were kept at 30°C using a circulating water bath to pump water through the column jackets at a flow rate of approximately 8 l/hr. The temperature was monitored by a thermometer on the water bath.

There were four sample ports on each column to sample within the reactor via syringe. Additional sample ports were located at the top and bottom of the reactor, outside the recycle loop, to sample the influent and effluent.

2.2.2.2 Reactor Operation The reactors were operated in an upflow mode with an initial flow rate of 2-3 ml/hr. The recycle flow rate was always kept much higher than the feed

flow rate in order to keep the system well-mixed (at an approximate 5:1 ratio), simulating a CSTR (continuous stirred tank reactor).

Samples (1 ml) were taken at the effluent sample port approximately daily, for chlorophenol determination. Samples (1 ml) were taken from the ports along the columns every few weeks to ensure the reactor operation was indeed CSTR mode. The variation of the liquid chlorophenol concentrations among the sampling ports was always less than 4.8%, confirming that these are well-mixed systems.

Effluent samples (50 ml) were collected approximately daily from the reactor tubing output for chloride ion determination. The time span of these sample collections were flow rate dependent.

2.2.3 Packing

The carbon reactor was packed with a commercially available wood-based activated carbon (WS 45; Chemviron Carbon, Bruxelles, Belgium). The carbon was in the form of cylindrical pellets approximately 2.5-5 mm in length (average diameter: 3.7 mm), and had a bulk density of 450 g/L (void fraction: 38-42%), a BET surface area of 1,100 m^2/g, and a total pore volume of 0.75 ml/g. The total dry weight of the carbon used was 95.38 g, with an inter-particle porosity of 21.3%. The working liquid volume of this column was calculated to be 382 ml by measuring the volume of liquid needed to fill the reactor after being packed with wetted carbon pellets.

The Manville reactor was packed silica-based cylindrical beads (Celite Bio-Catalyst Carrier R-635; Manville, Denver, CO). The beads had a diameter of 1/4" and a length of 1/4 - 1/2". The beads had a BET surface area of 1-2 m^2/g and a total pore

volume of 1.08 ml/g [Pak (1988)]. The total dry weight of these beads was 92.87 g with an inter-particle porosity of 47.2%. The working liquid volume of this column was calculated to be 300 ml by measuring the volume of liquid needed to fill the reactor after being packed with wetted Manville beads.

After the columns were packed, distilled water was pumped through the length of the reactors until the effluent pH was around neutral.

2.3 Media Preparation

The media composition used in both bioreactors is given in Table 2.

Table 2: Media Components

Chemical Compound	**Concentration (g/l)**
K_2HPO_4	0.225
KH_2PO_4	0.450
$(NH_4)_2SO4$	0.20
$MgSO_4 \cdot 7H_2O$	0.09
$FeSO_4 \cdot 7H_2O$	0.002
Bicine	5.0
Sodium Formate	2.0
$NaHCO_3$	2.5
Sodium Acetate	2.5

The medium, including the 2,4,6-TCP, were mixed in water and boiled for 15 minutes. The solution was then purged, while still boiling, with a N_2/CO_2 gas mixture for 15 minutes. The solution was then allowed to cool for 15 minutes, while still purging. The cooled solution pH was tested to insure that it was in the range of 8.0 to 8.8. If adjustment was necessary, the solution was either boiled to drive off the CO_2 to raise the pH, or purged with the gas mixture to replace the CO_2 and lower the pH, however,

adjustment was usually not needed. The solution was then autoclaved (AMSCO 2023) at 121°C and 2 atm to sterilize.

After the medium was completely cooled, the feed was sampled to determine the 2,4,6-TCP concentration. The initial feed concentration of 2,4,6-TCP was approximately 0.13 mM (the exact concentration was noted) for both reactors. The concentration in the carbon reactor was later increased to 1.0 mM, while that in the Manville reactor remained at 0.13 mM for the duration of the experiments.

2.4 Microorganisms

The microorganisms were obtained from the effluent of a 2,4,6-TCP dehalogenating bioreactor used by a previous student [Jou (1993)]. That reactor was operated anaerobically in a down flow fashion. Originally the microbial population was obtained from the Joint-Meeting Treatment Plant in Elizabeth, NJ and has been used in experiments for many years prior to those described in this thesis.

Several liters of effluent were collected over several days from the above mentioned reactor while it was still in operation. The effluent was not kept anaerobic. The effluent was pumped through the carbon and Manville reactors, which only contained distilled water (not sterilized), at a flow rate of approximately 15 ml/min and a recycle rate of approximately 45 ml/min. For a period of 16 hours, effluent samples (10 ml samples) were collected hourly from the reactor output until the optical density of the output was equal to that of the input, indicating that the packing was saturated with

biomass. The reactors were then allowed to operate on 100% recycle for 24 hours to ensure good biofilm adhesion.

2.5 Analytical Methods

2.5.1 Optical Density

The samples were analyzed using a spectrophotometer (Spectronic 20, wavelength: 440 nm) to determine the biomass concentration. The instrument absorbance was zeroed using a test tube of distilled water. The effluents samples collected from the reactors during microorganism loading were transferred to a glass test tube for analysis. The test tube was marked to ensure it was facing the same direction for each sample analysis. Concentration of the samples was needed to ensure that the absorbance readings were within the range where absorbance is linearly related to cell density (0.1 - 0.8). The effluent samples were initially concentrated eight times, by centrifuging the samples to settle the cells, removing the required amount of supernatant, and resuspending the cells using a vortex. After 7 hours of reactor biomass loading, the samples were concentrated only four times.

2.5.2 Chlorophenol Concentration

The concentrations of 4-CP, 2,4-DCP and 2,4,6-TCP in the liquid samples were determined by using an HPLC unit (Waters, Inc.), provided with a Waters 715 Ultrawisp Sample Processor, a Waters 600E System Controller, a Waters 484 Tunable Absorbance Detector set at 280 nm, and an Alltech Econosphere C8 50 Column (length=150 mm, ID=4.6 mm). The mobile phase used was a 50:50 mixture of two solutions, the first

containing 1% (v/v) acetic acid in methanol, and the second 1% (v/v) acetic acid in Milli-Q water. The flow rate was 1 ml/min and the mode of operation was isocratic. The concentration of the samples was determined using a calibration curve prepared by plotting the peak area versus concentration of four standards of known concentration.

2.5.3 Chloride Ion Concentration

The chloride concentration in the effluent was determined using a chloride electrode (Combination Chloride Electrode: Model 96-17B, Orion Research Inc. Laboratory Products Group, Boston, MA). A fresh low-level calibration curve was prepared by adding a known volume of chloride standard to 100 ml of Milli-RO water plus 1 ml of low-level ionic strength adjustor (ISA: Orion Catalogue No. 940011). The low-level ISA solution was prepared by diluting 20 ml ISA in 100 ml distilled water. The millivolt reading was recorded after two minutes. The millivolt readings were plotted versus the $\log_{10}$ of the chloride concentration. The samples were prepared by adding 1 ml of the low-level ISA per 100 ml of sample. The sample millivolt reading was taken after two minutes, as with the standard. The sample concentration was obtained utilizing the calibration curve. Since the calibration curve was not linear at this low level, 11 points were used ranging from 0.001 to 2 mM.

The chloride concentration of the samples was also analyzed using a method of known-addition in order to verify the results from the calibration curve. Sampling time was again two minutes. The agreement was found to be good.

2.5.4 Flow Rate

The flow rate was obtained by measuring a collected amount of effluent over a measured time period. Initially, at feed flow rates of 2-3 ml/hr, the samples were collected overnight. This time period was reduced to a few hours as the flow rates were increased.

2.6 Results and Discussion

2.6.1 Biomass Loading

During biomass loading, the biomass in the concentrated effluent samples prior to seven hours was outside of the linear range and therefore considered to be not useful. After this time the biomass in the output began to slowly increase. As seen in Figure 2, the Manville reactor increased fairly steadily, while the carbon reactor seemed to increase sharply after 11 hours, and slowly after that time. This may indicate that slightly more biomass was loaded in the carbon reactor between eight and eleven hours.

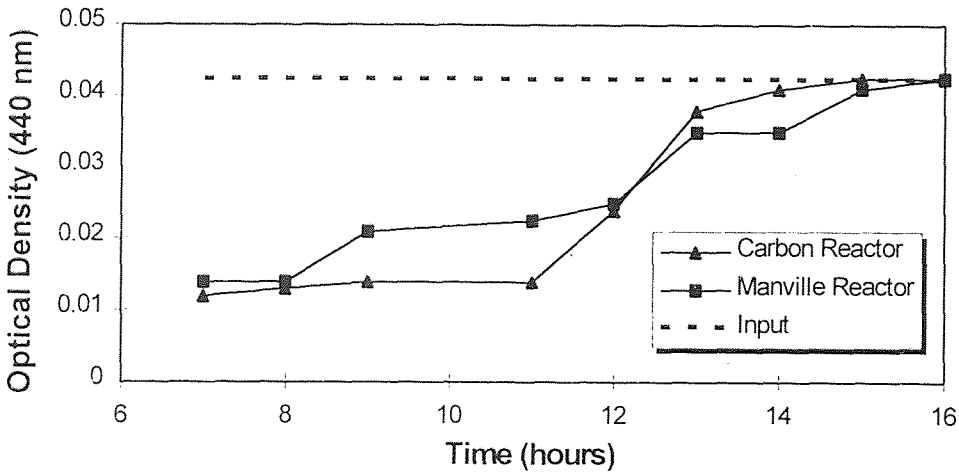

Figure 2: Optical Density During Reactor Loading

Both reactors reached biomass saturation at approximately the same time (Manville reactor: 16 hours, Carbon reactor: 15 hours). It would seem, from Figure 2, that there was only a small difference in the initial biomass loading of the two bioreactors.

2.6.2 Mass Balance

The following reaction occurred in both reactors is [Jou (1993)]:

$$2,4,6-TCP \xrightarrow{biomass} 2,4-DCP + Cl^- \xrightarrow{biomass} 4-CP + 2Cl^- \qquad (1)$$

The dehalogenation of 2,4,6-TCP, according to Equation 1, produces stoichiometric amounts of 2,4-DCP and 4-CP. Figure 3 shows that the sum of these three chlorophenol species in the effluent of the Manville reactor is in good agreement with the input 2,4,6-TCP to the reactor.

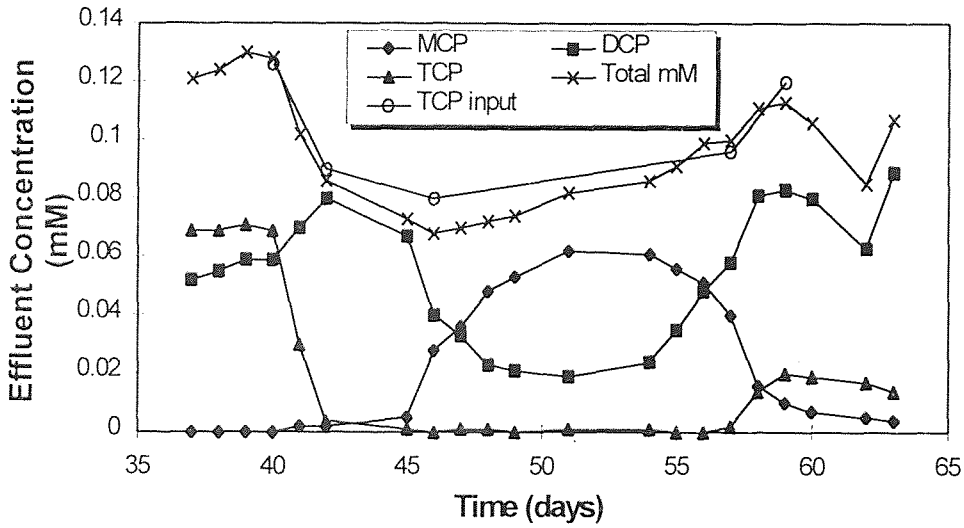

Figure 3: Effluent Chlorophenol Concentrations - Manville Reactor

In order to close the mass balance, the concentration of each of these species in Equation 1 needed to be measured. Based on the chlorophenol concentrations obtained by HPLC

analysis, the theoretical chloride concentration was calculated using the following equation:

$$[Cl-] = 3[2,4,6-TCP]_{(input)} - \{3[2,4,6-TCP] + 2[2,4-DCP] + [4-CP]\}_{(output)} \quad (2)$$

The values obtained from this equation were then compared to the chloride concentrations measured by the chloride ion electrode. The agreement between the calculated values and those obtained from the above equation was found to be good for the effluent samples taken from the Manville reactor, as seen in Figure 4.

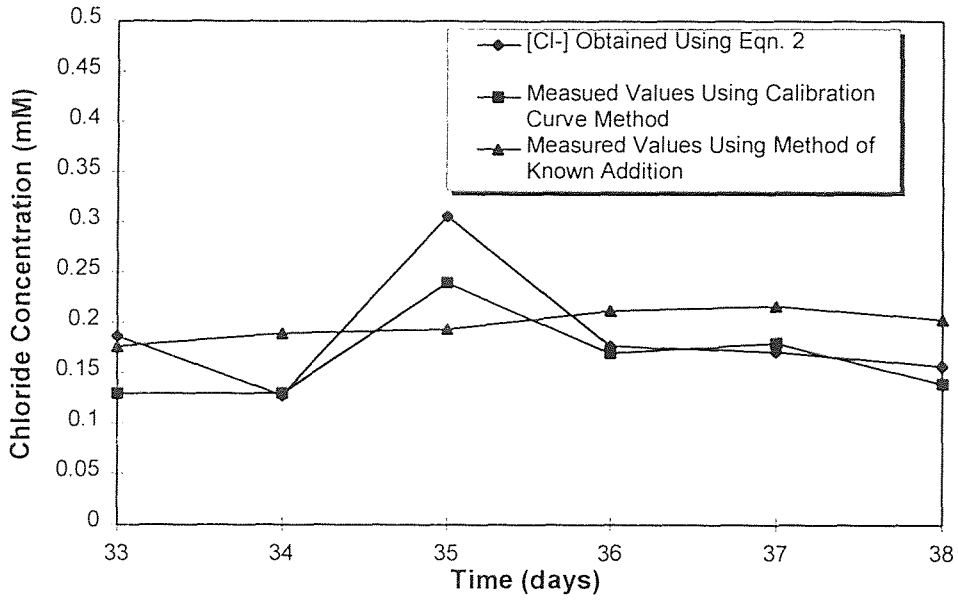

Figure 4: Chloride Analysis - Manville Reactor

However, the theoretical values of the chloride produced in the carbon reactor were always higher than the measured values, as seen in Figure 5. This indicates that adsorption was occurring at a faster rate than dehalogenation. Based on this conclusion, a breakthrough of TCP should eventually occur. In these samples, the agreement between

the values obtained from the calibration curve and those obtained from the method of know addition was good.

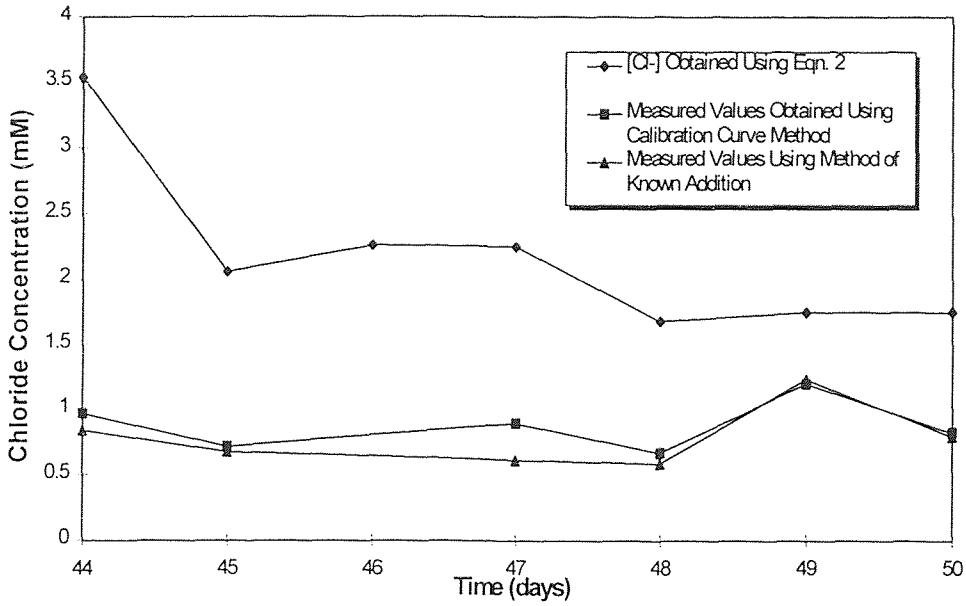

Figure 5: Chloride Analysis - Carbon Reactor

2.6.3 2,4,6-TCP Degradation Versus Flow Rate

At an initial 2,4,6-TCP feed concentration of 0.13 mM and a flow rate of approximately 2 ml/hr, no chlorophenol concentration was seen in the output of the carbon reactor based on HPLC analysis of the samples taken from the effluent sample port after sampling for a period of two weeks. The feed input was increased to 0.26 mM, to 0.39 mM and then to 0.75 mM, each for one week, with the same initial flow rate, and in all cases, no chlorophenols were detected in the effluent samples. An increase in the feed concentration to 1 mM began to show 2,4,6-TCP in the effluent samples. Once this feed concentration was established, the reactor was allowed to operate for two weeks to ensure a steady state biofilm at this feed condition.

It was assumed that degradation was occurring during these feed increases. Based on breakthrough curves found in the literature for 4-CP [Sorial *et al.* (1993)] and the adsorption isotherm data for 4-CP and 2,4,6-TCP found by Shirgaonkar *et al.* (1992) an estimate of the maximum 2,4,6-TCP carbon adsorption capacity was calculated. Initially, this calculation was based on the feed concentration of 2,4,6-TCP. Once the reactor output showed chlorophenols, the calculation was based on the output concentration. The maximum carbon adsorption capacity of 2,4,6-TCP was greatly exceeded in either case (i.e. over a factor of 10), and therefore breakthrough should have occurred if no degradation were occurring in addition to the adsorption. Chloride production also verified that degradation was occurring.

The Manville reactor continued to show degradation at the feed input of 0.13 mM and flow rate of approximately 2 ml/hr, therefore the feed concentration was not changed.

Once the flow rate experiments began, the reactors were allowed to operate for 24 hours after any change in flow rate before effluent samples were taken, to allow the system to reach a steady state. The residence time in the Manville reactor ranged from 150 hours at a flow rate of 2 ml/hr, to 11.8 hours at the maximum measured flow rate of 25.5 ml/hr. At a flow rate of 2 ml/hr, the residence time in the carbon reactor was 191.8 hours and at the maximum flow rate of 17.3 ml/hr, the residence times was 17.4 hours.

The percent 2,4,6-TCP degradation and flow rate data are presented in Tables 3 and 4.

Table 3: Manville Reactor - Flow Rate, 2,4,6-TCP Effluent Concentration and Percent Degradation

Manville Reactor: 2,4,6-TCP input 0.13 mM		
Flow Rate (ml/hr)	Percent 2,4,6-TCP Degradation	Effluent 2,4,6-TCP (mM)
1.6	99.2	0.001
2.2	100	0
2.7	99.2	0.001
5	100	0
6.9	100	0
8.2	100	0
9.6	100	0
12.4	100	0
13.1	100	0
14.4	100	0
19	96.2	0.005
20	80.8	0.025
24.9	71.5	0.037
25.5	67.7	0.042

Table 4: Carbon Reactor - Flow Rate, 2,4,6-TCP Effluent Concentration and Percent Degradation

Carbon Reactor: 2,4,6-TCP input 1 mM		
Flow Rate (ml/hr)	Percent 2,4,6-TCP Degradation	Effluent 2,4,6-TCP (mM)
2.2	100	0
4.4	100	0
5.1	100	0
9.4	100	0
11.7	100	0
11.9	99.4	0.006
16.5	99.1	0.009
17.3	95.6	0.044

A 95% conversion rate in the Manville reactor was achieved, with a TCP input of 0.13 mM, at flow rates up to 19 ml/hr. Above 19 ml/hr, the conversion rate dropped rapidly, as seen in Figure 6.

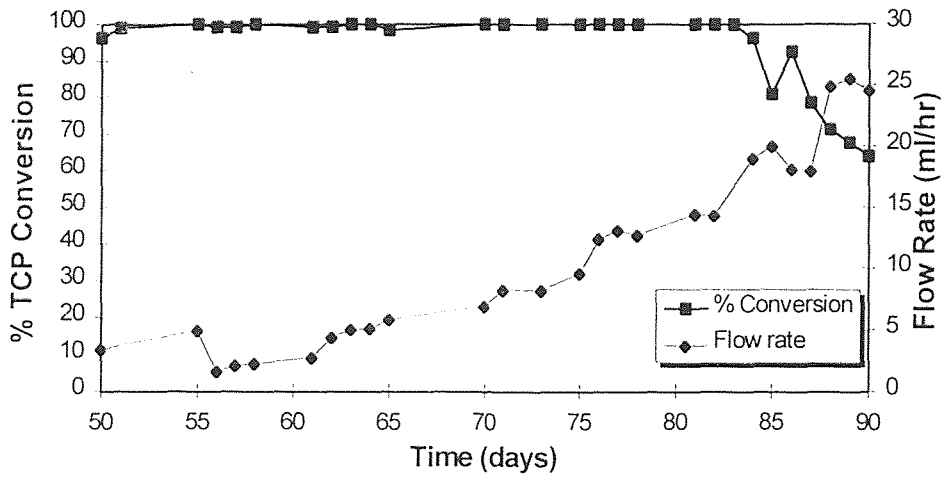

Figure 6: Flow Rate vs. TCP Conversion - Manville Reactor with a 2,4,6-TCP input of 0.13 mM

The carbon packed reactor can maintain above 95% TCP conversion up to 17.3 ml/hr, with a TCP input of 1 mM, which can be seen in Figure 7. At higher flow rates, air bubbles appeared in the carbon reactor and it was not possible to obtained valuable flow rate data. However, from the data that was obtained it is obvious that the carbon and Manville reactors can achieve approximately the same degradation at similar flow rates with the TCP molar input of the carbon reactor over 7 times that of the Manville reactor.

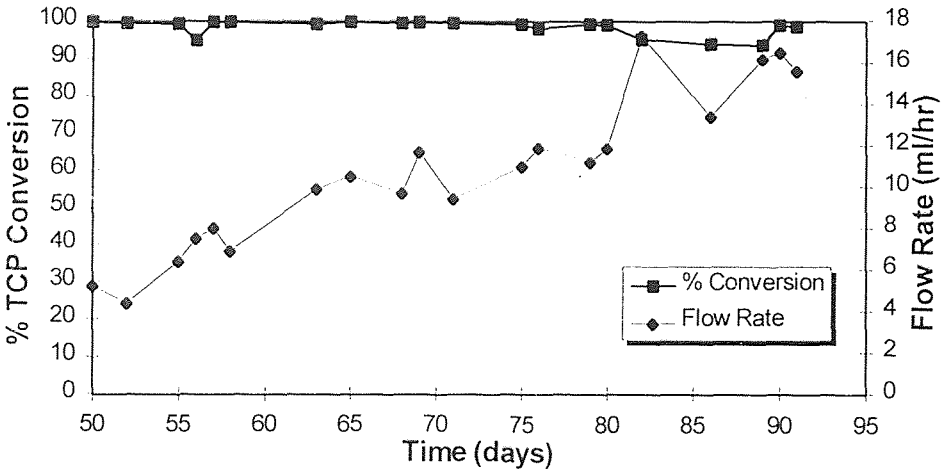

Figure 7: Flow Rate vs. TCP Conversion - Carbon Reactor with a 2,4,6-TCP input of 1 mM

It is important to note that "100% degradation" was not equivalent in these reactors. In the case of the carbon reactor, "100% degradation" also meant that the degradation products, 2,4-DCP and 4-CP, were not seen in the effluent. Since at higher flow rates, bubbles appeared in the carbon reactor, the rate of appearance of degradation products could not be determined. Less than 100% degradation in the carbon reactor meant a breakthrough of 2,4,6-TCP. This "breakthrough" could indicate carbon saturation since under these aerobic conditions (i.e. bubbles in the reactor), no dehalogenation could occur, and therefore the bioregeneration of the carbon would not be taking place. When the quantity of bubbles was reduced by changing the reactor mode to 100% recycle for several days, 2,4,6-TCP was no longer present in the output.

The Manville reactor always had these degradation products present, as seen in Figure 3. The maximum conversion of 2,4,6-TCP to 4-CP occurred at 2.7 ml/hr.

It may be helpful to compare the two reactors at similar conditions. Table 5 shows the effluent concentrations of each reactor at similar residence times and at similar flow rates. The residence times are based on the volume of the reactor, exclusive of the volume within the pores of the support materials, and the flow rates.

Table 5: Comparison of Reactors at Similar Conditions

	Effluent 4-CP (mM)	Effluent 2,4-DCP (mM)	Effluent 2,4,6-TCP (mM)	Feed 2,4,6-TCP (mM)	Residence Time (Flow Rate)
Manville Reactor	0.042	0.069	0	0.092	43.5 hrs (6.9 ml/hr)
Carbon Reactor	0	0	0	0.485	40.6 hrs (9.4 ml/hr)
Manville Reactor	0.056	0.035	0	0.118	60 hrs (5 ml/hr)
Carbon Reactor	0	0	0	1.182	73.5 hrs (5.2 ml/hr)

2.7 Bioreactor Conclusions

A bioreactor using activated carbon as the support material for the immobilization of microorganism could degrade a higher 2,4,6-TCP input than an identical reactor with Manville beads as the microbial support. Although no biomass balance was calculated, it appears from Figure 2 that the initial organism loading was approximately the same, yet at similar flow rates, a similar 2,4,6-TCP conversion was achieved in both reactors with the 2,4,6-TCP molar input of the carbon reactor over seven times that of the Manville reactor.

A measurement of the chloride ion concentration in the effluent of the Manville reactor served to close the mass balance. The theoretical chloride ion concentration in the carbon effluent was greater than the actual concentration, indicating that adsorption was occurring at a higher rate than dehalogenation, and breakthrough should eventually occur. Based on maximum adsorption capacity calculations, with no biodegradation, a breakthrough of 2,4,6-TCP should already have occurred.

The surface area of the activated carbon was over 1000 times that of the Manville beads. This increased surface area could result in an increased biomass per unit mass of packing. This could be one explanation for the increased activity of the carbon-packed reactor. However, the observed higher activity of the carbon reactor was not proportional to the higher surface area, possibly due to the lack of microbial access to the micropores of the carbon pellets.

Further characterization of the carbon packed bioreactor was desired in order to provide a basis for modeling, and eventually for scale-up purposes, some of which is presented in the following chapters.

CHAPTER 3

ADSORPTION ISOTHERMS FOR CHLORINATED PHENOLS ON ACTIVATED CARBONS

3.1 Introduction and Literature Survey

Activated carbon has many important uses. As an adsorbent for organics from the aqueous phase, it provides a useful and effective technique for the purification of industrial waste water. Activated carbon can also be used as the primary support material for microorganisms in bioreactors, which is its use in this research. In order to develop mathematical models for such reactors, it is necessary to have adsorption data for the specific activated carbon support being used.

Many of the isotherms found in the literature are for Filtrasorb-400, a coal-based activated carbon [Peel, *et al.* (1981); Vidic, *et al.* (1990); Shirgaonkar, *et al.* (1992); Abuzald and Nakhla (1994)]. Very few studies have covered more than one or two chlorophenols. The study by Shirgaonkar, *et al.* (1992) is a thorough study of the family of chlorophenols on Filtrasorb-400. Sorial, *et al.* (1993) compare the difference in adsorption capacity of various GACs, finding that adsorption capacity is in the order: coal-based > lignite-based > wood-based. Presented here are data for the class of chlorophenols on a wood-based carbon that is a commercially available alternative to Filtrasorb, as well as for an experimental type of activated carbon that is lignite-based. The anaerobic culture medium was used to investigate the effect of multiple solutes on the adsorption of the chlorophenols, and more accurately represents conditions within the bioreactor described in Chapter 2.

3.2 Materials and Methods

3.2.1 Activated Carbons

Carbon A1. This is a commercially available wood-based activated carbon (WS 45; Chemviron Carbon, Bruxelles, Belgium). The carbon was in the form of cylindrical pellets approximately 2.5-5 mm in length (average diameter: 3.7 mm), and had a bulk density of 450 g/L (void fraction: 38-42%), a BET surface area of 1,100 m^2/g, and a total pore volume of 0.75 ml/g. Before use the pellets were repeatedly washed with distilled water and dried overnight at 100 °C.

Carbon A2. This carbon was obtained by grinding the washed and dried Carbon A1 in a mortar and sieving the resulting powder. The fraction used in this work passed through the 140 mesh sieve but was retained on the 200 mesh sieve (approximate particle size: 75-106 mm).

Carbon B. This carbon is a lignite-based carbon produced at Queen's University. Lignite obtained from an open cast mine in Crumlin, Co. Antrim, UK, was heated to 800 °C for 40 minutes in a negligibly vented atmosphere in order to drive off volatiles and produce a porous carbon structure. After cooling the carbon was added to an iron nitrate solution to form a slurry (800 ml water, 80 g iron nitrate, 80 g carbon), mixed for one hour, vacuum filtered, and dried for 24 hours at 105 °C. The resulting particles (1-1.4 mm in size) were further activated at 850 °C by reaction with a measured amount of steam in a constant nitrogen atmosphere (partial steam pressure: 0.926 bar; total pressure: 1 atm). Additional information is provided elsewhere. [Allen *et al.* (1995)] The carbon particles were washed with distilled water and dried overnight at 100 °C prior to their use.

3.2.2 Adsorbates

The adsorbates used in this study are phenol, 2-chlorophenol (2-CP), 3- chlorophenol (3-CP), 4-chlorophenol (4-CP), 2,3-dichlorophenol (2,3-DCP), 2,4-dichlorophenol (2,4-DCP), 2,5-dichlorophenol (2,5-DCP), 2,6-dichlorophenol (2,6-DCP), 3,4-dichlorophenol (3,4-DCP), 3,5-dichlorophenol (3,5-DCP), 2,3,5 trichlorophenol (2,3,5-TCP), 2,3,6-trichlorophenol (2,3,6-TCP), 2,4,5-trichlorophenol (2,4,5-TCP), and 2,4,6-trichlorophenol (2,4,6-TCP). 2-CP, 4-CP, 2,4-DCP, 2,6-DCP, 2,4,5-TCP and 2,4,6-TCP (98% to 99% purity) were purchased from Sigma Chemical Company (St. Louis, MO). 3-CP, 2,3-DCP, 2,5-DCP, and 3,5-DCP, 2,3,5-TCP and 2,3,6-TCP (97% to 99% purity) were purchased from Aldrich Chemical Company, (Milwaukee, WI).

3.2.3 Solutions

Solutions of the adsorbates were prepared using either Milli-RO water or the anaerobic medium defined in Table 2.

3.2.4 Adsorption Procedure

High-concentration (250-10,000 mg/l) stock solutions were prepared for each adsorbate and used to prepare the adsorbing solutions by dilution with Milli-RO water or medium. The resulting concentrations used in the isotherm determination were typically in the range 100-4,000 mg/l for most adsorbates. Selected solubility values for chlorophenols are given in Table 6.

Table 6: Solubilities

Compound	Solubility
Phenol	82 g/l (15°C)
2-CP(ortho)	28,500 mg/l (20°C)
3-CP(meta)	26,000 mg/l (20°C)
4-CP(para)	27,100 mg/l (20°C)
2,4-DCP	4,500 mg/l (25°C)
2,4,5-TCP	1,190 mg/kg (25°C)
2,4,6-TCP	800 mg/l (25°C)

Source: *Patty's Industrial Hygiene and Toxicology* (1991), Clayton G.D., Clayton F.E., editors Wiley Pub., New York.

Adsorption experiments were conducted by adding about 0.1 g of one of the carbons (the exact weight was noted in each case) to 160 ml serum bottles filled with the appropriately diluted solution up to 130 ml. A nominal weight of 0.05 g of carbon was used in the experiments with 2,6-DCP, 2,3,5-TCP and 2,3,6-TCP due to the low solubility of these compounds. For each compound, 3 to 14 bottles (typically 10) were prepared, each one having a different initial chlorophenol concentration. Controls consisted of sample bottles containing the same concentration of the chemical, but without carbon pellets. All bottles were crimp sealed with butyl rubber stoppers, immediately autoclaved to prevent microbial degradation of the compounds, and incubated at 25 °C.

For all the experiments with Carbon A1 except that with 4-CP the bottles were initially allowed to stand for approximately thirty days to allow sufficient time to reach equilibrium. Samples (2 ml) were then taken and analyzed. Additional samples were taken again after 30 days and then after 7 more days to insure that equilibrium had been attained. All other bottles were sampled at 3 to 4 day intervals over a 30-day period. The

sample volumes for these ranged from 0.1 to 0.5 ml, depending on the dilution required for analysis.

Although chlorophenols are not very volatile under the conditions of these experiments, the head space of the bottles was occasionally analyzed by gas chromatography to verify that the disappearance of the compound in the liquid was due to adsorption and not volatilization.

3.2.5 Analytical Methods

All liquid samples were analyzed by HPLC (Waters, Inc.) provided with a Waters 715 Ultrawisp Sample Processor, a Waters 600E System Controller, a Waters 484 Tunable Absorbance Detector set at 280 nm, and an Alltech Econosphere C8 50 Column (length=150 mm, ID=4.6 mm). The mobile phase used was a 50:50 mixture of two solutions, the first containing 1% (v/v) acetic acid in methanol, and the second 1% (v/v) acetic acid in Milli-Q water. The flow rate was 1 ml/min and the mode of operation was isocratic. The samples were discarded once sampling was completed.

3.3 Interpretation of Experimental Data

Analysis of the gas samples from the headspace indicated that there were no appreciable amounts of the chlorophenol under exam in the headspace. In addition, the effect of multiple sampling of the liquid phase was found to have a negligible impact on the chlorophenol.

The concentration of chlorophenol in the samples changed as a function of time. However, at least 70% of chlorophenol uptake took place prior to the first sample

(7 days), in most cases, and minimal or no change was detected among the samples collected after two weeks.

The Freundlich isotherm is suitable for highly heterogeneous surfaces [Fritz and Schlunder (1981)]. The experimental equilibrium data, expressed as q_e (amount of chlorophenol adsorbed per mass of carbon, in mg/g) vs. C_e (final chlorophenol concentration in solution, in mg/L) were therefore interpreted using the Freundlich equation:

$$q_e = k \cdot C_e^{1/n} \tag{3}$$

In order to determine the constants k and $1/n$, the data were linearly regressed using the equation:

$$\log_{10}(q_e) = \log_{10}(k) + \frac{1}{n}\log_{10}(C_e) \tag{4}$$

An attempt was also made to interpret the data using the Langmuir equation. However, the fit was typically found to be rather poor.

3.4 Results and Discussion

3.4.1 Adsorption Results for Carbons A1 and A2 in Water

Based on the HPLC analysis of the liquid samples, chlorophenol uptake was still occurring up to six months with Carbon A1. Peel, *et al.* (1991) found that with phenols, sorbate-micropore interactions are likely to play an important role in the adsorption process within the internal micropores of the activated carbon, which could be responsible for the slow approach to equilibrium.

The Freundlich isotherm parameters for Carbon A1 in water are presented in Table 7.

Table 7: Freundlich Isotherm Parameters for Carbon A1 in Water

Compound	No. of Observations	a $(mg/g)(mg/l)^{1/b}$	b	Correlation Coefficient
2-CP	4	99.76	0.44	0.97
4-CP	14	47.86	0.34	0.85
2,3-DCP	5	270.64	0.12	0.94
2,4-DCP	5	242.18	0.14	0.87
2,5-DCP	6	253.51	0.18	0.89
3,5-DCP	4	299.66	0.087	0.78
2,4,5-TCP	3	354.83	0.044	0.57
2,4,6-TCP	3	396.28	0.044	0.98

Although most of these isotherms consist of only a few points, the data overall indicate that the strength of adsorption is trichlorophenols> dichlorophenols > monochlorophenols, as shown by the decreasing values of the parameter "a". Similar results were found by Shirgaonkar, *et al.* (1992) on Filtrasorb activated carbon (a coal-based carbon).

Equilibrium was reached more quickly using Carbon A2. This is probably due to greater exposed surface area, as well as more accessible micropores. The Freundlich isotherm parameters for this case are presented in Table 8.

Table 8: Freundlich Isotherm Parameters for Carbon A2 in Water

Compound	No. of Observations	a $(mg/g)(mg/l)^{1/b}$	b	Correlation Coefficient
Phenol	13	38.90	0.52	0.96
2-CP	11	194.84	0.15	0.93
3-CP	12	186.21	0.21	0.93
4-CP	13	177.83	0.13	0.92
2,3-DCP	9	398.11	0.15	0.94
2,4-DCP	11	407.4	0.19	0.85
2,5-DCP	8	213.8	0.18	0.76
2,6-DCP	9	177.83	0.21	0.99
3,4-DCP	11	281.83	0.16	0.85
3,5-DCP	12	239.88	0.12	0.94
2,3,5-TCP	8	316.2	0.20	0.89
2,3,6-TCP	9	416.9	0.16	0.95
2,4,5-TCP	12	245.47	0.16	0.91
2,4,6-TCP	13	85.11	0.11	0.87

A general decrease with a decrease in ring constituents is observed on Carbon A2 as with Carbon A1. For most of the compounds in Table 8, the values of the parameters "a" and "b" are higher for Carbon A2 than for Carbon A1, indicating greater adsorption as would be expected due to the increase in surface area and greater accessibility to the micropores. A good example of this is illustrated in Figure 8.

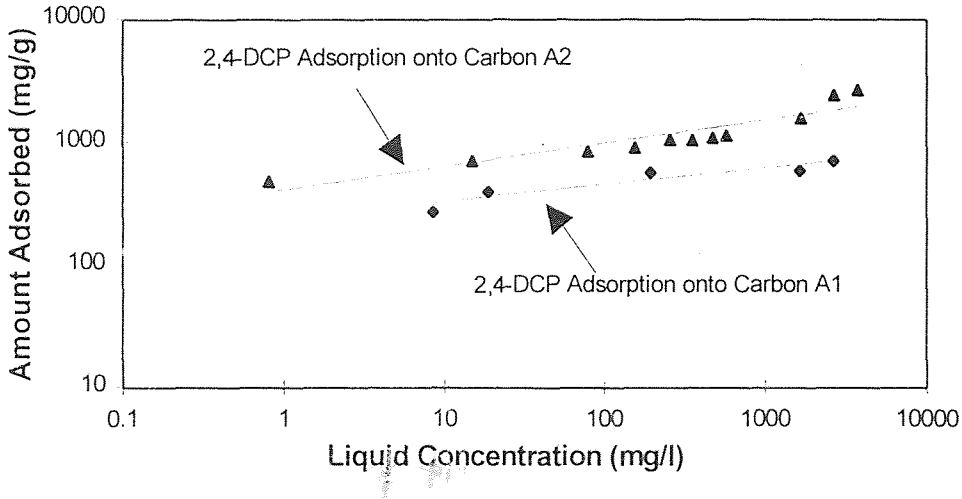

Figure 8: Adsorption of 2,4-DCP in Water on Carbons A1 and A2

3.4.2 Results for Carbon B in Water

The Freundlich isotherm parameters for the adsorption onto Carbon B are presented in Tables 9.

Table 9: Freundlich Isotherm Parameters for Carbon B in Water

Compound	No. of Observations	a $(mg/g)(mg/l)^{1/b}$	b	Correlation Coefficient
4-CP	14	3.39	0.72	0.88
2,4-DCP	14	61.66	0.16	0.92
3,5-DCP	11	112.26	0.097	0.52
2,3,5-TCP	9	112.2	0.15	0.95
2,4,6-TCP	9	77.62	0.15	0.91

On this activated carbon, a general decrease in the adsorption capacity with a decrease in constituents is again observed. The adsorption capacity of this experimental type of carbon was found to be much less than that of Carbon A1 or A2, which contradicts the findings by Sorial *et al.* (1993). A representative example is shown in Figure 9.

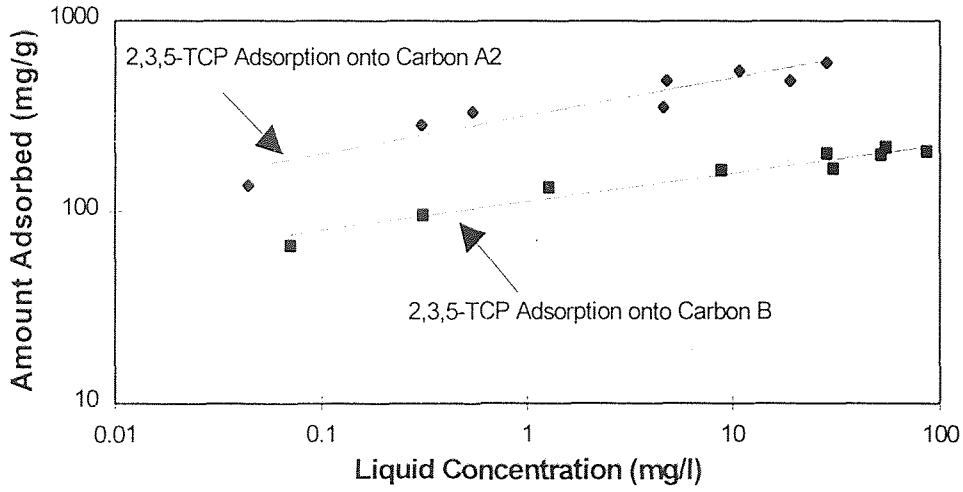

Figure 9: Adsorption of 2,3,5-TCP in Water on Carbons A2 and B

3.4.3 Effect of Media on Adsorption Capacity

The Freundlich isotherm parameters for adsorption in medium are presented in Table 10.

Table 10: Freundlich Isotherm Parameters for 4-CP in Medium

Adsorbate	No. of Observations	a $(mg/g)(mg/l)^{1/b}$	b	Correlation Coefficient
Carbon A1	13	51.29	0.34	0.90
Carbon A2	10	107.15	0.16	0.94
Carbon B	11	0.32	1.02	0.89

The Freundlich parameters for 4-CP adsorption onto Carbon A1 in medium and in water are very close, indicating a negligible difference in the adsorption capacity due to the competitive adsorption of the media components. This is seen as overlap in the scatter of the data, as shown in Figure 10.

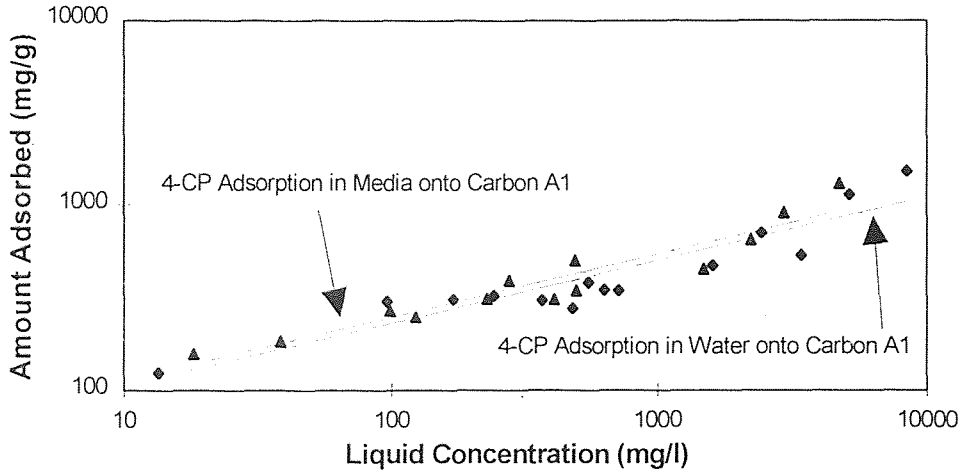

Figure 10: Adsorption of 4-CP on Carbon A1 in Water and in Media

For Carbon A2 and B, the parameter "a" is lower in medium than in water, indicating a lower extent of adsorption, which is the expected outcome due to competitive

adsorption. The value of the parameter "b", however, is higher for these carbons, which indicates that the effect of competitive adsorption decreases as the concentration of the chlorophenol increases (i.e. the adsorption capacity approaches that found in distilled water at very high concentrations). This phenomenon is shown in Figures 11 and 12.

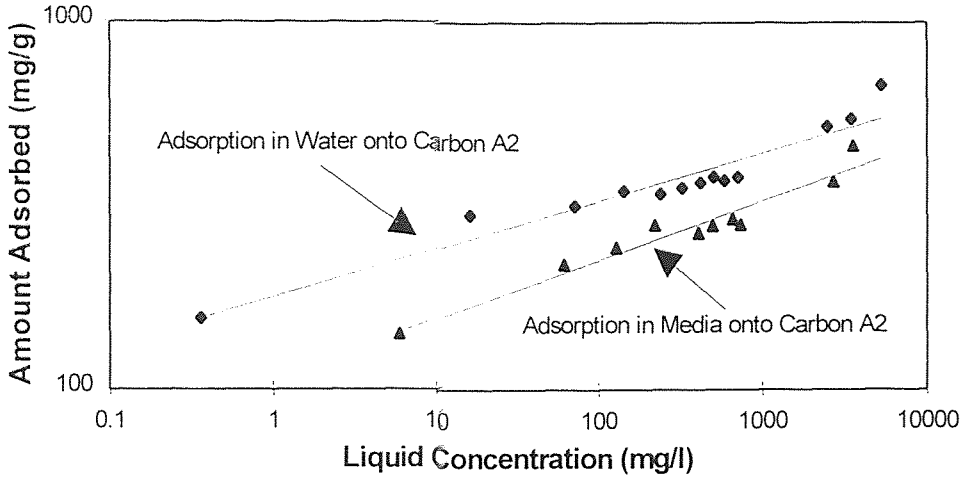

Figure 11: Adsorption of 4-CP on Carbon A2 in Water and in Media

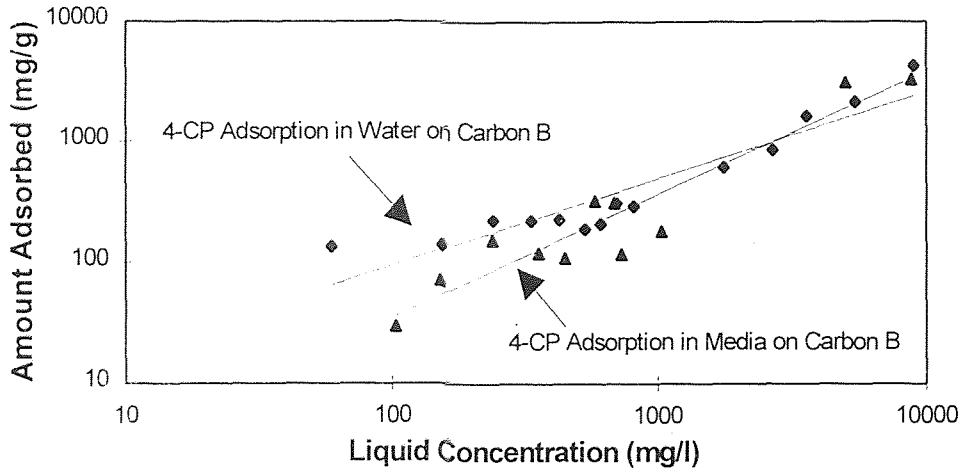

Figure 12: Adsorption of 4-CP on Carbon B in Water and in Media

3.4.4 Discussion

A study done by Sorial, *et al.* (1993) found that for the organic pollutants used, coal-based carbons had the highest adsorption capacity of the adsorbents tested. Lignite-based carbons had a somewhat reduced capacity, and wood-based carbons had a substantially decreased adsorption capacity. When comparing the results from this study with some of the those in the literature, a variety of results were found. For example, the "a" parameter for 4-CP on Carbon A2 was found to be in the range of the coal-based carbons in the study by Sorial, *et al.* (1993). Most of the results obtained here on Carbon A2 were very similar to those obtained by Shirgaonkar, *et al.* (1992) on Filtrasorb-400, another coal-based carbon.

Vidic, *et al.* (1990) have also shown that the presence of molecular oxygen has an effect on the adsorption capacity of activated carbons. In their study, no effect was noted on a wood-based carbon, but on those that were lignite-based, the adsorption capacity was increased in the presence of oxygen due to polymerization on the carbon surface. For phenol, our results on Carbon A2 were approximately the same as those found under anaerobic conditions and lower than those found under aerobic conditions in the study by Vidic, *et al.* (1990). This study was done under atmospheric conditions: therefore, the results found here should be between Vidic's anaerobic and aerobic results. However, this was not the case. For phenol, our results were found to be between the oxic and anoxic results obtained by Abuzald and Nakhla (1994), which would be expected.

It was also suggested in the study by Sorial, *et al.* (1993) that the manganese content of the activated carbon can effect adsorption capacity as well. The manganese

content of the carbons used in this study are not known and therefore a comparison would be inconclusive.

Hence, caution must be exercised when comparing the data obtained in this study to data in the literature, since slight differences in carbon characteristics and variations in the test conditions seem to have a substantial effect on the adsorption behavior of the carbon. Due to the variety of available types of activated carbon, and actual operating conditions, it would deem necessary to develop isotherms for the specific carbon and operating conditions to be used.

3.5 Adsorption Isotherm Conclusions

The wood-based carbon had a higher adsorption capacity in the ground form (Carbon A2) than in the pellet form (Carbon A1). Although only a few experimental points were obtained on Carbon A1, this finding is expected due to the increase in surface area of the ground form. The experimental lignite-based carbon had a lower adsorption capacity than either form of the wood-based carbon.

The competitive adsorption of the anaerobic medium had an effect only on Carbons A2 and B. The effect of competitive adsorption, a decrease in adsorption capacity, was predominant only at lower chlorophenol concentrations and decreasing to approach the single-solute adsorption capacity at higher chlorophenol concentrations.

CHAPTER 4

BIOSORPTION

4.1 Introduction and Literature Survey

Biosorption is defined as the uptake and accumulation of chemicals by microorganisms. The mechanisms involved may be surface adsorption or absorption in the components of the cell. The significance of this removal mechanism has been reviewed only within approximately the last ten years. Most researchers have used aerobic organisms, live and dead [Bell and Tsezos (1988); Tsezos and Bell (1988); Wang and Grady (1994); Muraleedharan and Venkobachar (1990)]. Kennedy, *et al.* (1992) used an anaerobic granular sludge and determined that biosorption is "considerable". There have been no studies to determine the effect of a biofilm on the adsorptive characteristics of activated carbon.

The primary goal of this set of experiments was to investigate the effect of the presence of a biofilm on the adsorption properties of the wood-based activated carbon in order to further characterize the carbon-packed reactor described in Chapter 2. The adsorption capacity of the microorganisms alone was also determined. In both of these cases, the main target compound was 4-CP, a metabolite of the bioreactor feed compound, 2,4,6-TCP. The compound 2,4,6-TCP was used in a small time-course experiment with the same objectives described above.

4.2 Materials and Methods

4.2.1 Stock Solution Preparation

Stock solutions of 4-CP and 2,4,6-TCP were made by dissolving a precisely weighed amount of the compound in Milli-RO water containing 50 μg/ml of the antibiotic chloramphenicol (Sigma Chemical Company; St. Louis, MO). The resulting stock solution concentrations were 9141 ppm (wt/vol) of 4-CP and 436.4 ppm (wt/vol) of 2,4,6-TCP.

4.2.2 Adsorbates

The first adsorbate material was activated carbon with a biofilm attached to its surface. These pellets were taken from the activated carbon packed reactor described in Chapter 2, after flow rate experiments were concluded and the reactor had been shut down for three days. The top of the reactor was removed, and the top six inches of packing material were removed along with enough reactor liquid to keep the removed pellets submerged. It was assumed that any additional adsorption from this liquid overnight would be negligible. The removed pellets were placed in a screw-top plastic contained and kept in a 30°C incubator overnight.

Activated carbon without a biofilm was also used. A sterile reactor, similar to those described in Chapter 2 with a packed bed length of 8 cm, was used to prepare virgin carbon pellets, with the objective of obtaining sterile carbon pellets with a similar background adsorption to those obtained from the bioreactor. The virgin carbon pellets were washed successively with distilled water until the rinse water was clear prior to

placement in the sterile reactor. Once packed, the reactor was fed with diluted anaerobic medium (dilution was 1:10), with no recycle present. The 2,4,6-TCP concentration in this feed medium was 0.75 mM, higher than the effluent concentration of the bioreactor. The sterile reactor was operated at approximately 10 ml/min (i.e. a residence time of around 3 minutes) until the effluent 2,4,6-TCP concentration was within 5% of the effluent 2,4,6-TCP concentration of the bioreactor. Once this level was obtained, the carbon pellets were removed from the sterile reactor and removed from the liquid to prevent further adsorption. These pellets were placed in a screw-top plastic container, identical to that described above, and kept in the same incubator overnight. The amount of 2,4,6-TCP adsorbed by the carbon during this process is on the order of 10 mg/g.

The third adsorbate was Manville beads with an attached biofilm. As with the carbon bioreactor, this reactor was shut down for three days prior to the removal of any packing. The top six inches of packing were removed from this reactor along with enough liquid to keep the pellets wet. Again, these beads were placed in a screw-top plastic container and kept in the incubator overnight.

The fourth material was Manville beads without a biofilm, which were prepared by repeated rinsing of virgin beads with distilled water until the rinse water pH was around neutral.

4.2.3 Experimental Procedure

4.2.3.1 4-CP Adsorption Isotherms In the case of 4-CP, five sets of serum bottles were set up. They are as follows:

A) Adsorptive support with biomass (i.e. activated carbon with biofilm).

B) Adsorptive support without biomass (i.e. activated carbon without biofilm).

C) Controls (i.e. no support, no biofilm).

D) Non-adsorptive support with biomass (i.e. Manville beads with biofilm).

E) Non-adsorptive support without biomass (i.e. Manville beads without biofilm).

Set A was used to obtain an adsorption isotherm in the presence of an anaerobic biofilm. Set B, carbon with no biofilm, was prepared to provide a background adsorption level of the carbon. Manville beads with a biofilm, set D, was used to determine the adsorption of the organisms alone. To ensure that no other mechanisms of loss occurred during the experiment, Set C, controls with no biomass and no support material, was used. Set E, Manville beads with no biofilm, was used to verify that this support material is indeed non-adsorptive.

Adsorbing solutions of 4-CP were prepared by diluting the stock solution with Milli-RO water containing 50 μg/ml of chloramphenicol. The total volume used in these experiments was 65 ml. In the case of sets A, B and D, 15 different concentrations between 96 ppm and 9141 ppm were used, each set done in triplicate. In the case of sets C and E, a single set of three different concentrations was used (91 ppm, 914 ppm and 9141 ppm).

Adsorption experiments were conducted by adding one pellet of support material was used in each bottle. The average weight of support material added was 0.067g, 0.063g, 0.34g and 0.36g for set A, B, D and E, respectively. All bottles were sealed with butyl rubber stoppers.

This experiment was carried out over a 27 hour period, with liquid phase sampling at Time 0, 2.8, 5 and 27 hours. All sets were done on the same day. The short duration of the experiment was to ensure that the biofilm remained attached to the carbon surface after inhibition, and to minimize the possibility of biodegradation.

4.2.3.2 2,4,6-TCP Time-Course Experiment The compound 2,4,6-TCP was used to perform a time-course experiment. The sets were the same as for 4-CP. However, only a single concentration, 436.4 ppm (wt/vol), was used and duplicates were set up for each set.

The liquid concentration was determined at 1, 2 and 3 hours, and then every 3 hours up to 30 hours.

4.2.4 Biomass Inhibition

The biomass was inhibited using the antibiotic chloramphenicol. The effectiveness of this antibiotic was previously tested on an actively dehalogenating suspended population. Two samples, 5 ml each, were taken from the lowest two column sample ports of each reactor described in Chapter 1. Two ml of a reactor sample was added to a test tube containing fresh, sterile media. A total of 10 test tubes were inoculated, five with samples from the Manville reactor and five with samples from the carbon reactor. Chloramphenicol was added to four test tubes, two inoculated with Manville reactor samples and two with carbon reactor samples, so that the resulting inhibitor concentration was 50 μg/ml. The tubes were sampled every 2-3 days for two weeks, and the samples

were analyzed for chlorophenol concentration. The antibiotic was found to inhibit dehalogenation activity in all four test tubes over the entire time period, while those tubes without any added antibiotic continued to be active. It is assumed that the antibiotic had the same effect on the immobilized population.

4.2.5 Analytical Methods

Samples volume ranged from 0.05 ml to 1.0 ml. The analytical methods for the determination of the liquid phase chlorophenol concentration are identical to those described in Section 3.2.5.

4.2.6 Data Interpretation

The liquid concentration of 4-CP, as determined by HPLC analysis, and the solid concentration of 4-CP per unit weight of support material, were plotted on a log-log scale to fit determine the Freundlich constants (see equation 4, Section 2.3).

The liquid concentration of 2,4,6-TCP was plotted versus time for each set of the time-course experiment.

4.3 Results and Discussion

4.3.1 Adsorption Isotherms of 4-CP

Isotherms for 4-CP were determined for Sets A, B and D. Other researchers have determined that biosorption occurs in less than one day [Bell and Tsezos (1987); Tsezos and Bell (1989); Kennedy, *et al.* (1992)], however, since here the objective was to

determine the effect of the presence of a biofilm on the adsorptive characteristics of activated carbon as well, samples were taken at 2.83 and 5 hours.

It can be seen from Figure 13, that there is only a small difference between the isotherms at 5 hours and 27 hours on the sterile carbon.

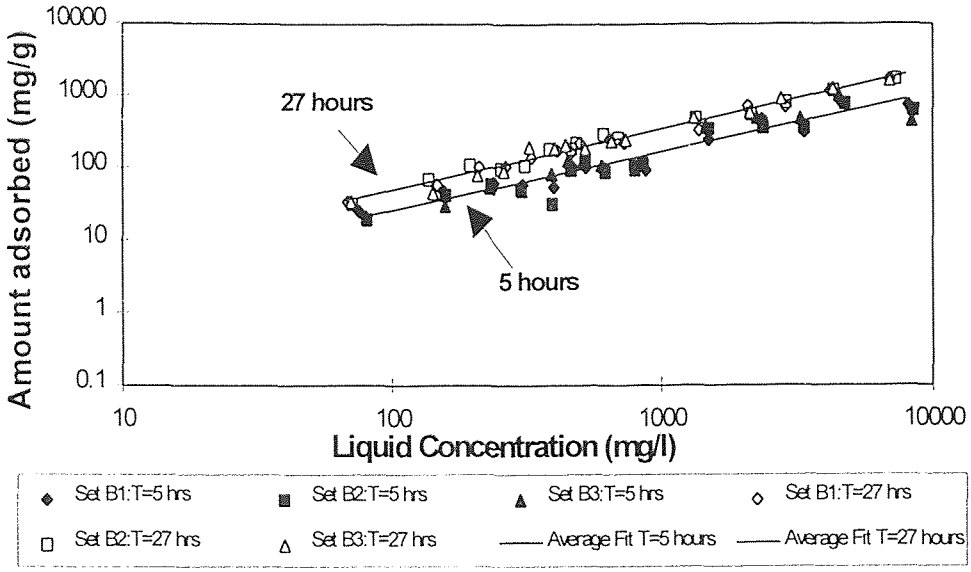

Figure 13: Adsorption of 4-CP - Sterile Carbon at 5 hours and 27 hours

In the presence of a biofilm, the uptake of 4-CP was much slower as seen in Figure 14. The 27 hour values of the activated carbon with and without a biofilm are very similar, indicating that the final equilibrium of the carbon is not greatly effected by the presence of a biofilm. It should be noted that at 2.8 hours, there was no significant uptake of 4-CP by either set of activated carbon. Although carbon usually has a rapid initial uptake [Peel *et al.* (1991)], the delay here can be attributed to the fact that virgin carbon was not used. Both sets of activated carbon used in this experiment already had a background level of adsorbed material. The amount of background material was not quantified, however,

qualitatively, the sterile pellets had adsorbed media components as well as 2,4,6-TCP. The carbon pellets taken from the bioreactor could have unused media components, 2,4,6-TCP, 2.4-DCP, 4-CP and/or any metabolic products.

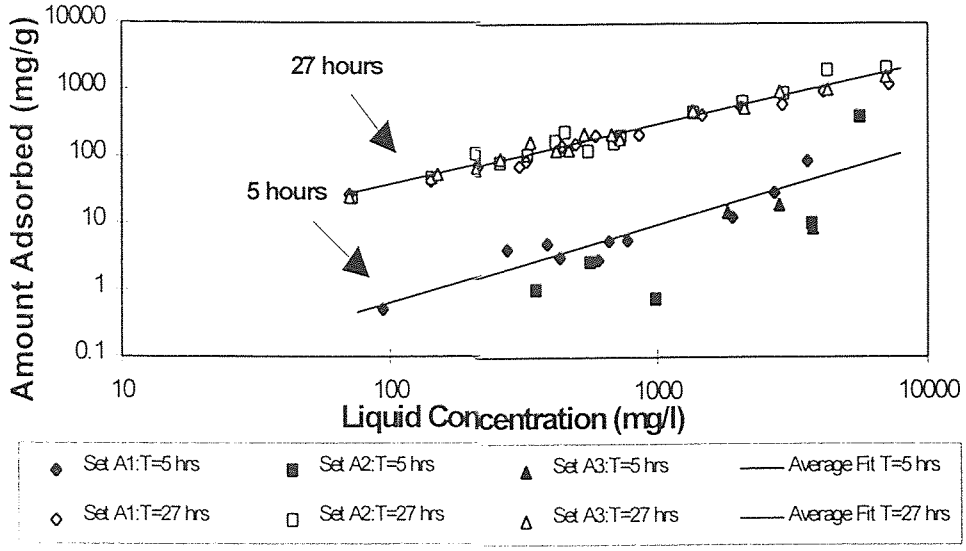

Figure 14: Adsorption of 4-CP - Carbon with Biofilm at 5 hours and 27 hours

There was some uptake of 4-CP by the organisms themselves, as seen in Figure 15, although initially (2.8 hours) there is much scatter in the data. The data from 5 hours and 27 hours are identical and with little scatter, indicating that biosorption equilibrium was reached between 2.8 and 5 hours. The average decrease in liquid 4-CP concentration from the initial liquid concentration, due to biosorption, was 21.6% over the entire concentration range.

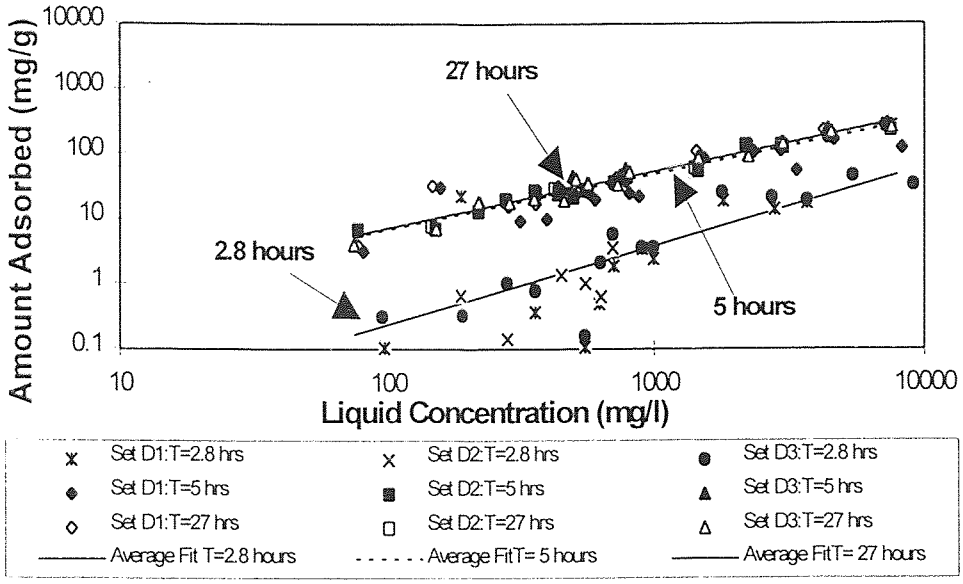

Figure 15: Adsorption of 4-CP - Manville with Biofilm at 2.8, 5 and 27 hours

The average Freundlich parameters, obtained from experimental data regression, for the sets described above are given in Table 11.

The liquid concentration of 4-CP in sets C and E remained constant +/- 4.8%, confirming that there are no other losses in the system and that the Manville beads are non-adsorptive.

Table 11: Average Biosorption Freundlich Parameters

Set/Time	a $(mg/g)(mg/l)^{1/b}$	b
A (carbon with biofilm)		
5 hours	0.0028[1]	1.18[1]
27 hours	0.56[2]	0.91[2]
B (sterile carbon)		
5 hours	0.64[2]	0.81[2]
27 hours	1.01[2]	0.85[2]
D (Manville with biofilm)		
2.8 hours	0.00091[3]	1.21[3]
5 hours	0.14[2]	0.85[2]
27 hours	0.13[2]	0.87[2]

1: Set A1 data only
2: Average of all 3 sets
3: Average of first and third sets only

The parameters of sets A and B cannot be directly compared with those for set D since it cannot be assumed that a biofilm on an activated carbon pellet would be the same as that on a Manville bead.

4.3.2 Time-Course Experiment

During the first three hours, the liquid concentration of 2,4,6-TCP actually increased in all sets, indicating that initially, desorption was occurring, after which the concentration began to decrease as was expected. This phenomenon was not detected in the case of the 4-CP isotherm experiment, possibly due to the first sample being at almost 3 hours.

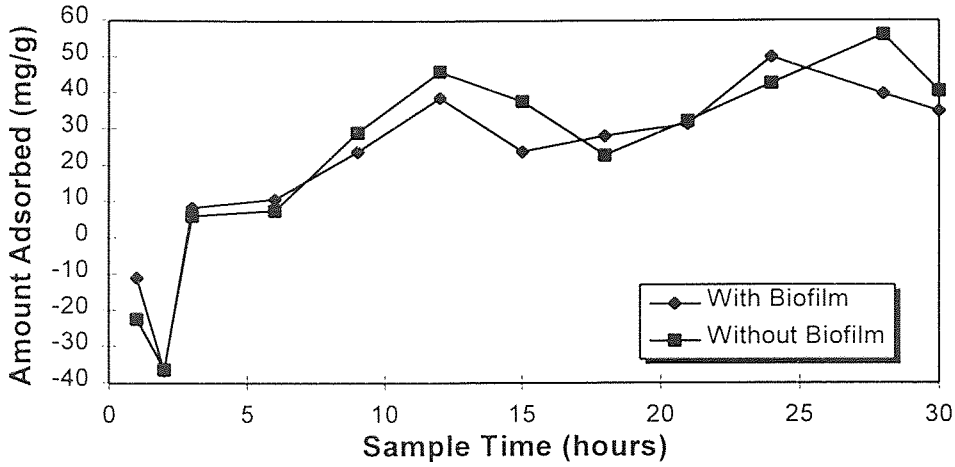

Figure 16: Adsorption of 2,4,6-TCP on Carbon with and without a Biofilm

The slowed adsorption rate due to the presence of a biofilm was not seen in this case. The adsorption on the carbon with and without the biofilm proceed at a similar rate and to a similar end, as seen in Figure 16. A possible explanation could be that there was a higher background adsorption of 2,4,6-TCP already present prior to the start of the experiment. This could also explain the slower initial uptake. In the case of the sterile

carbon, about 12 hours was needed to reach the equilibrium concentration, whereas only approximately 5 hours was needed for 4-CP.

Again an uptake of chlorophenol by the biomass was seen in the time-course experiment. Only approximately a 5% decrease in concentration was observed and a longer time was required to obtain equilibrium (10 hours), again possibly due to a higher initial 2,4,6-TCP concentration already adsorbed by the biofilm. This data is presented in Figure 17.

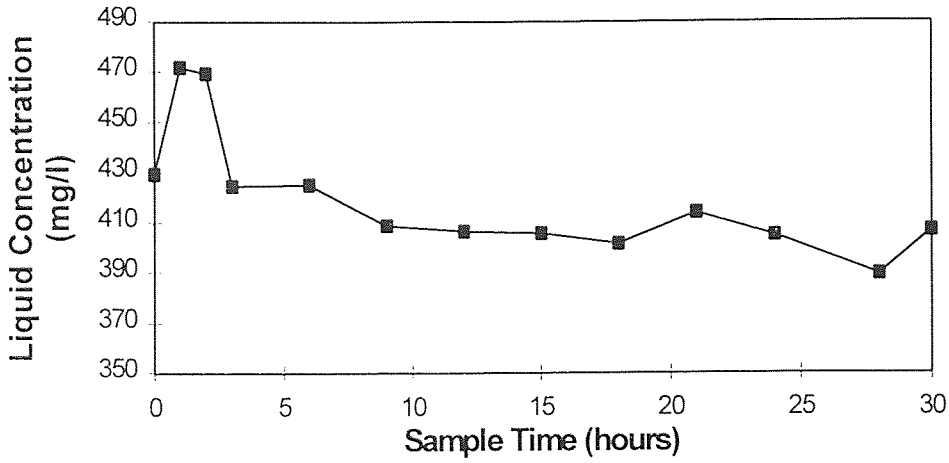

Figure 17: Adsorption of 2,4,6-TCP by a Biofilm

4.4 Biosorption Conclusions

The 4-CP adsorption isotherms of activated carbon both with and without a biofilm could be described using the Freundlich model. The initial uptake of the sterile carbon was slower than that of virgin carbon, due to the background adsorption that had already occurred. The presence of a biofilm served to slow the adsorption rate of 4-CP on activated carbon, however the equilibrium adsorption was not greatly effected.

The biosorption of 4-CP by the anaerobic organisms can also be described by the Freundlich equation. The initial uptake began very quickly (by 2.8 hours) and equilibrium was obtained shortly thereafter (by 5 hours). Approximately 21.6% of the initial 4-CP was adsorbed over the entire concentration range.

The difference in rate of the carbon uptake with and without biofilm was not seen in the 2,4,6-TCP biosorption time-course experiment, a longer time period was need in all cases for equilibrium to be obtained and only a 5% decrease in concentration was observed due to the biofilm alone. This could be due to a higher initial adsorbed concentration of 2,4,6-TCP prior to the start of the experiment.

CHAPTER 5

CONCLUSIONS

The bioreactor utilizing activated carbon as the microorganism support material has an overall superior performance than the reactor with Manville beads as the support material. The carbon reactor degraded over seven times the 2,4,6-TCP input of the Manville reactor, with similar flow rates and 2,4,6-TCP conversion rates.

When evaluating the adsorption characteristics of the wood-based activated carbon, it was found to have a higher adsorption capacity than the lignite-based carbon. It was also found that the effects of competitive adsorption due to the anaerobic medium are seen primarily at lower chlorophenol concentrations. At high concentrations, the effects become negligible.

The effect of the presence of a biofilm on the adsorption of 4-CP on the wood-based activated carbon was a decrease in the rate of adsorption. The equilibrium was not effected. This effect was not seen in the 2,4,6-TCP biosorption time-course experiment, possibly due to the higher initial adsorption level of 2,4,6-TCP.

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