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EVALUATION OF HIGH PERFORMANCE LIQUID CHROMATOGRAPHY PACKINGS FOR THE CONCENTRATION AND SEPARATION OF ORGANIC MATERIALS IN WATER

Progress Report from June 1, 1976 through September 30, 1977

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November 28, 1977

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Amberlite XAD-4 non-ionic adsorbent has been evaluated as a reverse phase concentrating agent for some aromatic and aliphatic compounds present in oily wastes dissolved in water Retention efficiences of 86 - 88% were found by extracting water samples before and after passing through XAD-4, aromatic concentrations being determined by liquid chromatography and

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aliphatic concentrations by an infrared technique. Effects of flow rate, sample size, and the weight of XAD-4 on retention efficiency are discussed.

XAD-4 has proven to be an excellent trapping agent for trace amounts of organic compounds in water.

Saturated solutions of naphthalene and β-naphthylamine in natural sea water were studied for biodegradation by pseudomonas. Both were found to be biodegradable using a qualitative comparative gas chromatographic technique. Both compounds showed end products having the same relative retention times, suggesting similar mechanisms. The low biodegradation rate shown by β-naphthylamine was attributed to the formation of an intermediate which accumulated in solution--the rate determining step.

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INTRODUCTION

The packing selected for evaluation was Rohm and Haas' polymeric adsorbent amberlite XAD-4, which is a copolymer of styrene and divinyl benzene, uniquely suited for the removal of nonpolar solutes from polar solvents. Because of its small average pore diameter (50 A) and high surface area (750 sq. meters/gram) it is well suited for the adsorption of solutes having a molecular weight less than 200. It is also unique for removing substances whose structures are only partially non-polar, such as surfactants or synthetic detergents. Many weak electrolytes, organic acids and amines are also adsorbed effectively if they are adsorbed under conditions in which they are not ionized (low pH's for acids and high pH's for amines).

The amberlites have been found to be highly effective for removing such substances as detergents, dyes, phenols, and a host of noxious and toxic hydrocarbons from waste streams. Amberlite XAD-4 has been found to be unusually effective for the adsorption of chlorinated phenols.

This study was concerned with determining the efficiency of XAD-4 adsorbent for extracting the dissolved oily wastes from sea water.

The XAD-4 material as received required purification, and, although the method of Junk (1) was satisfactory for aromatic compounds, for the aliphatic compounds, it was found necessary to extend the Soxhlet extractions first for an additional eight hours with methanol, and then for an additional eight hours with methylene chloride.

The standards were prepared in distilled water, synthetic sea water, and natural sea water, according to the method of Sniegoski (2). The efficiency of adsorption of the standards from the aqueous solutions was determined by analyzing the standard solutions before and after passage through the XAD-4 material. Retention efficiencies of 86 - 88% were obtained.

CHAPTER 1

EXPERIMENTAL

The following hydrocarbons were used without further purification: benzene, toluene, xylene (J. T. Baker), ethylbenzene, anthracene, n-hexadecane, n-octadecane (Eastman), n-tetradecane Chemical Samples Co.), and naphthalene (Allied). The liquid chromatograph consisted of the Milton-Roy Minipump (instrument model) and the Waters Associates U6K Universal Injector connected to the LDC Model 1205 UV Monitor (254 nm wavelength) through a glass column, 5 x 150 mm i.d., packed with Waters Associates Porasil B. The system operated at 1.8 ml min^{-1} (60% full stroke), and absorbance was recorded on the Hewlett-Packard 680 strip chart recorder.

The Beckman IR-8 Infrared Spectrophotometer was used to determine aliphatic hydrocarbon concentrations using the C-H stretch region, and was used in the single beam mode.

A. Procedure for Aromatics

Approximately 30 - 50 ml of distilled water was run through 0.5 g of purified XAD-4 resin occupying a 5.5 x 22 mm volume (3) above a small plug of fine glass wool in one end of a 180° glass stopcock fitted with a 50 ml reservoir at the top via a 2 cm length of 1/4 in. i.d. Tygon tubing. The flow rate was adjusted as nearly as possible to 10 ml min^{-1}. A 10 ml aliquot of the desired standard was added to the reservoir allowing it to mix with only the last 0.5 ml of distilled water present. After the sample had drained into a 30 ml separatory funnel fitted with a Teflon stopcock plug, the

resin was washed with ca. 5 ml of distilled water. The combined effluent was shaken vigorously with 2 ml of methylene chloride (Burdick and Jackson) for 2 min. and allowed to stand for 5 min. An additional 10 ml aliquot of the standard was likewise extracted to serve as a reference in determining the retention efficiency of the resin. Equal samples (typically 75 µl for benzene to 200 µl for anthracene) were drawn from the bottom layers for liquid chromatograph injection. The effluent extract was injected following the reference extract and this was repeated three times using the average calculated efficiency as the result of one trial.

B. Procedure for Aliphatics

Approximately 300 ml of distilled water was run through 10 g of purified and dried XAD-4 resin, ca. 1.5 x 10 cm in volume (3) in a chromatographic column similar to the one used by Junk et al., and fitted with glass wood plugs at both ends. The flow rate was adjusted to 10 ml $min.^{-1}$ and a 200 ml aliquot of the desired standard added in the same manner as with the aromatics (washing with ca. 20 ml water and collecting in a 500 ml separatory funnel). Extraction was performed using 5 ml of carbon tetrachloride (Mallinckrodt) as was also the case with a 200 ml reference aliquot of the standard. The lower layer was drained directly into a 1 cm quartz cell and placed in the sample beam of the ir. The 3000 - 2800 cm^{-1} region was scanned and the scan repeated superimposing a solvent blank to determine the exact peak height due to the extracted aliphatic. Efficiencies were calculated using a calibration curve to determine the concentrations represented by the peak heights. The scans were repeated and the results

averaged together.

C. Results and Discussion

Retention efficiencies for the aromatic compounds dissolved in water are reported in Table 1. Those for n-tetradecane, n-hexadecane, and n-octadecane were each found to average 86% (4) as the result of three trials with an overall standard deviation of 6.3% (5).

Three major parameters were found to have marked effects on the retention efficiency of XAD-4: the volume of the standard per weight of adsorbent, the flow rate, and the weight of adsorbent used. The effects of each were investigated and relationships were found graphically.

The retention efficiency of XAD-4 vs. flow rate at a constant volume of standard and weight of adsorbent was found to have a linear relationship with a slope of about -0.8 (6). The calculated regression line resulting from 14 trials is shown in Figure 1.

The efficiency of XAD-4 vs. the volume of standard at a given flow rate and weight of adsorbent was likewise found to be linear with the regression line as a result of 19 trials, shown in Figure 2, slope approximately -0.5. The effect on the retention efficiency of the weight of XAD-4 at a constant sample volume, flow rate, and bed diamter, is shown in Figure 3. At a flow rate of 10 ml min^{-1} the curve is a hyperbolic one.

The significance of this method of evaluation over other methods lies in the fact that no error can be introduced by the incomplete elution of compounds from the adsorbent in determining retention efficiencies. The method of purification required prior

Table 1. Retention Efficiencies[a] of One-Half Gram Quantities of Amberlite XAD-4 Porous Polymeric Adsorbent[b] Using 10 ml Samples of Selected Aromatic Standards[c] at a Flow Rate of 10 ml/min.

Compound	Solvent		
	Water	Synthetic Sea Water	Sea Water
	%	%	%
Benzene	88	86	86
Toluene	88	85	86
Xylene	90	89	89
Ethyl Benzene[d]	74	74	74
Naphthalene	87	88	87
Anthracene	89	90	88

Average retention efficiency excluding ethyl benzene = 88%

Standard deviation excluding ethyl benzene = 1.5%

[a]each percentage is an average of 3 or more trials.
[b]bed dimensions = 5.5 x 33 mm.
[c]saturated at 25°C.
[d]lower relative efficiency due to high water solubility.

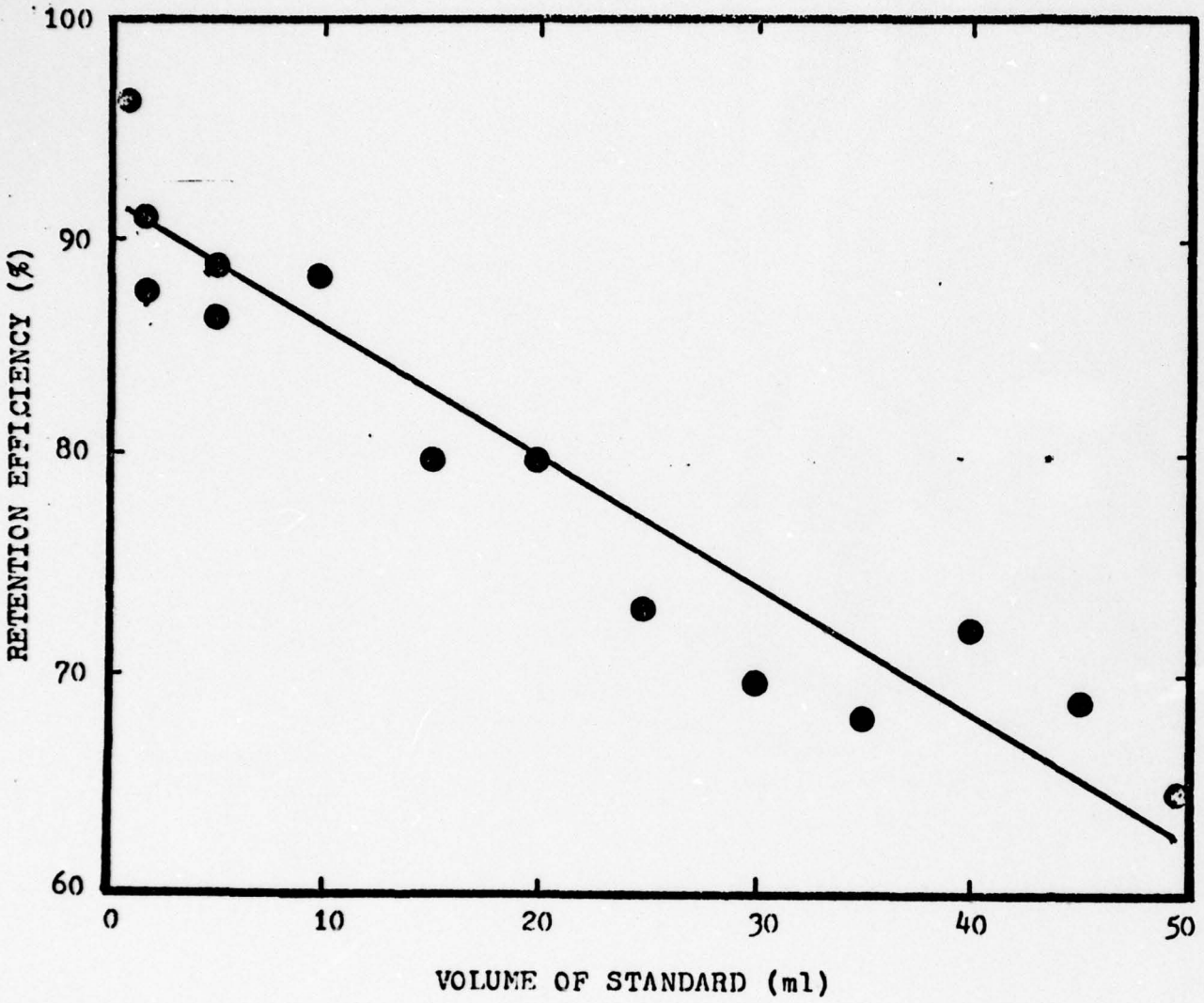


Figure 1: The effect of varying volumes of benzene-saturated water on the retention efficiency of XAD-4, the weight of resin (0.5 g) and flow rate (10 ml min^{-1}) remaining constant.

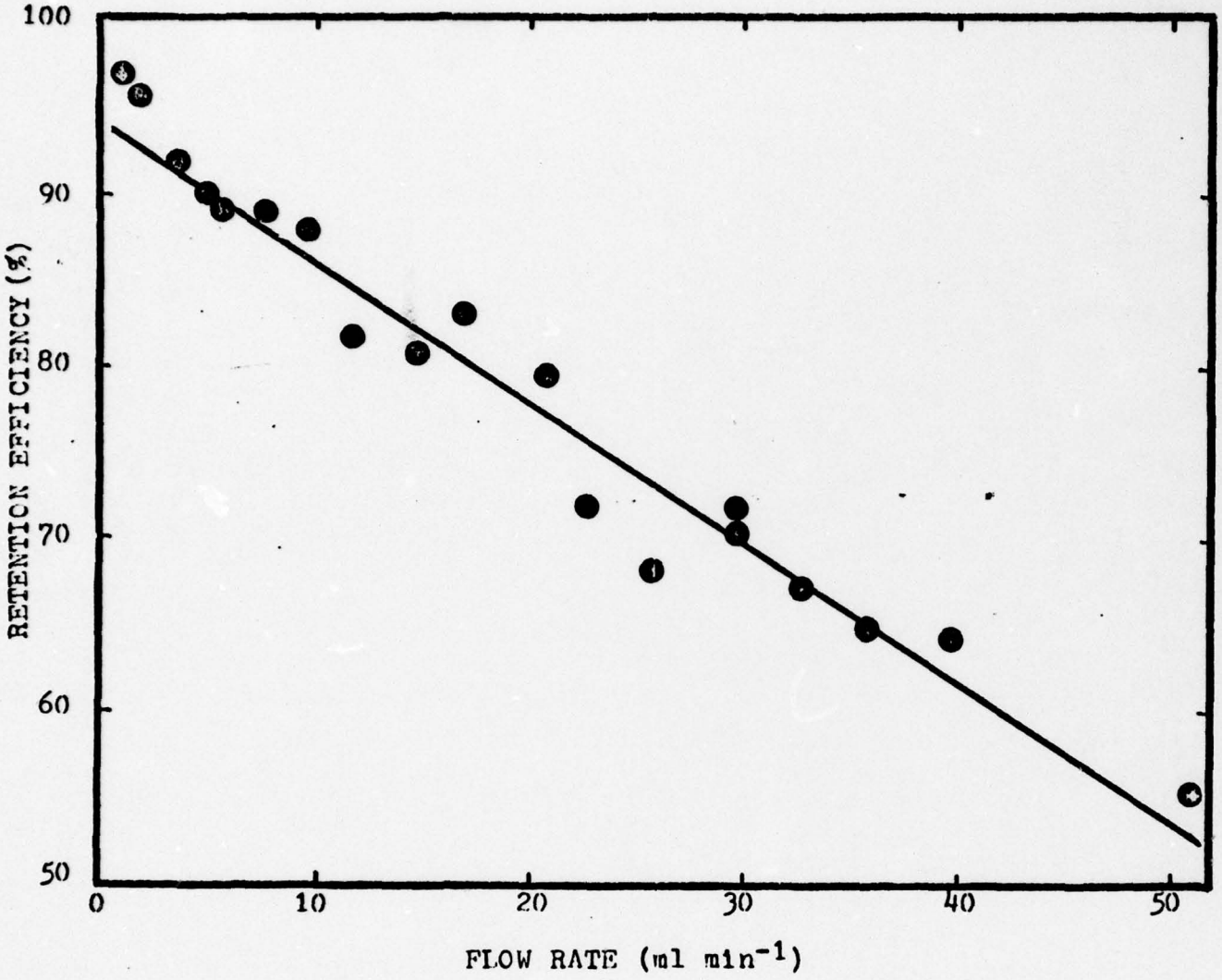


Figure 2: The effect of flow rate on the retention efficiency of XAD-4 with the weight of resin (0.5 g) and the volume of standard (10 ml) remaining constant.

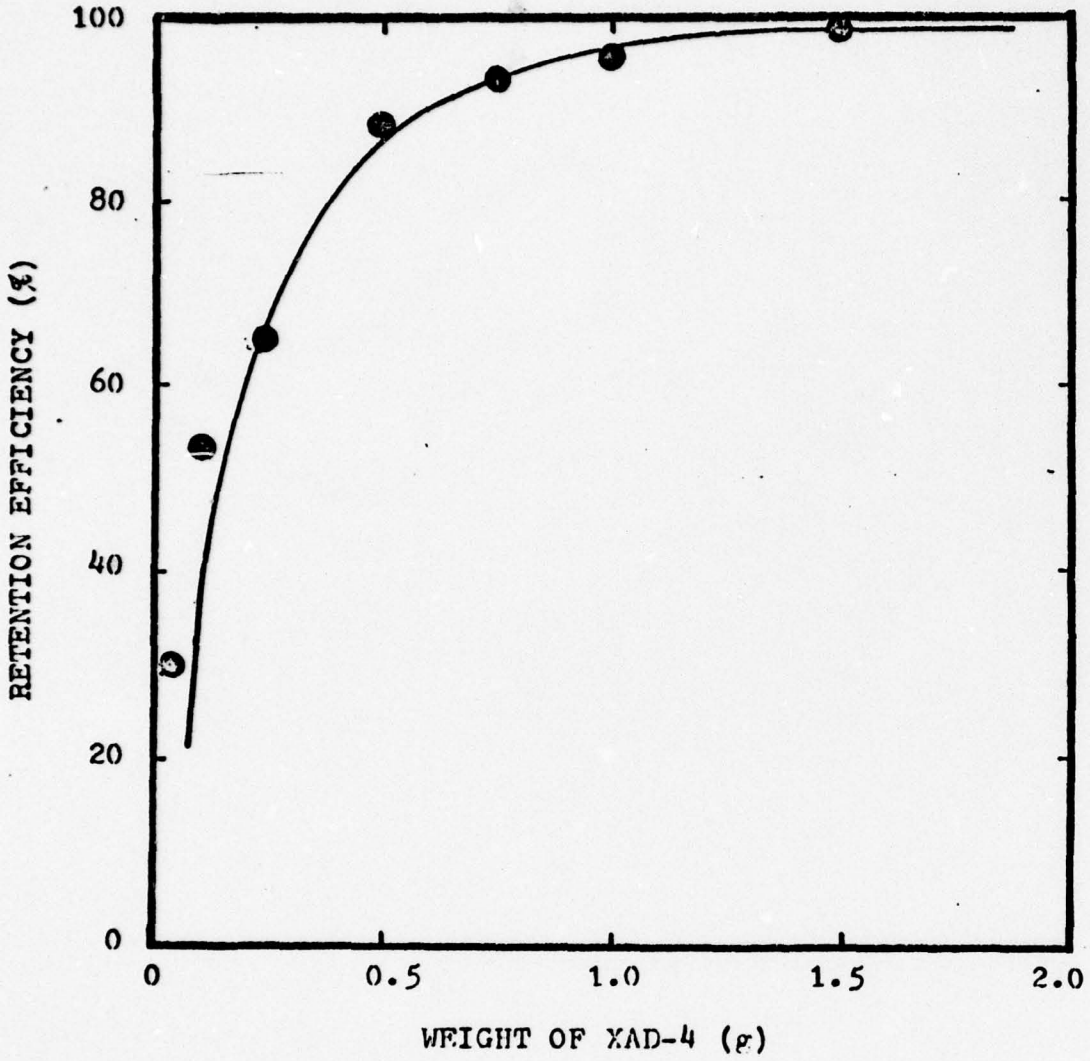


Figure 3: The effect of the weight of the XAD-4 resin bed on its retention efficiency. Flow rate (10 ml min^{-1}), the volume of standard (benzene-saturated water), and bed diameter are held constant.

to the aliphatic determinations denotes the difficulties which may be encountered in elution techniques. Without this purification, it was found that aliphatic-saturated water samples, after having been passed through the XAD-4 column, had high values of concentration, and, in some cases, higher concentrations than the standards. After extensive washing with methylene chloride, however, values were close to those obtained with the aromatic standards.

It is therefore concluded that these determinations have a lower probability of error than those previously published for retention efficiencies, a criterion necessary if quantitation of results is to be accurate.

CHAPTER 2

FURTHER STUDIES

A. Elution of Compounds from XAD-4

A 2 liter sample of water saturated with Naval Distillate (sample # MIL-F-24397) was prepared using the method of Sniegoski. A liter aliquot was passed through 50 g of XAD-4 at a flow rate of 10 ml $min.^{-1}$, corresponding to 10 ml per 0.5 g of resin. The retention efficiency was better than 90% and recovery was attempted by elution with sufficient methanol to wet the adsorbent, followed by a small amount of methylene chloride (total volume of solvents, 50 ml). Evaporation via the Kuderna-Danish technique yielded 2 ml of a cloudy solution which was devoid of any of the volatile organics found to be present in the original sample by extraction and gas chromatographic analysis.

An attempt was then made to determine the effects of a very large sample size in relation to the weight of resin used. A 500 ml aliquot of the same sample mentioned above was passed through 2.5 g of adsorbent at a flow rate of 10 ml $min.^{-1}$ The retention efficiency was found to be about 93% using the ir technique described in Chapter 1. This increased efficiency was attributed to the increasing affinity of the adsorbent for dissolved organics as more material was concentrated on the resin, i.e., the dissolved organics showed a high affinity for themselves. (Like dissolves like.)

Elution was accomplished with 4 ml of solvents. Concentration to half this volume using the Kuderna-Danish technique showed no change

in the components of the sample during the evaporation compared to an extracted sample analyzed via gc (although loss of volatile components was again extensive). Analysis by gc was accomplished using a 3% SE-30 on h.p. Chromosorb W column, 6 ft. x 1/4", a flow rate of 60 ml min^{-1} (He), and a temperature program of 50 - 215°C at 3° min^{-1}. Major components of the sample ranged from normal hexane through normal docosane as the major constituents with traces of aromatics and alkenes. Smaller, unidentified peaks were considered to be isomers, since no functional groups could be detected using ir. Aromatics were found to consist of benzene and lower substituted benzenes. Analysis was by comparison with standards.

Elution of benzene (from benzene-saturated water) from XAD-4 was studied with results and conditions reported in Table 2.

Since it was determined via the infrared spectra that hydrocarbons only were present in the samples, the use of gas chromatography was used for the most part for identification of compounds by comparison against standards, but the separation into classes had to be accomplished by other means.

Separation of aromatics from aliphatics was accomplished by using the Waters Associates Porasil B column (silica, 30 - 75 μ), 3/8" x 4', with methylene chloride as the solvent. Separation of aromatics via gc was then practical as are lc techniques. Polynuclear aromatics (PNA) were separated using the Waters Associates C_{18} μBondpak column, 6 x 300 mm, with acetonitrile:water, 60:40 ratio, as solvent. Separating standards of more than 4 or 5 known compounds was not possible using this method, however. It was

Table 2. Elution from XAD-4 with Small, Repetitive Amounts of Solvent.[a]

Solvent	Volume ml	Volume Recovered[d] ml	Concentration[e] ppm
MeOH[b]	0.5	0.35	2,600
CH_2Cl_2[c]	0.5	0.35	750
"	0.5	0.30	620
"	0.5	0.32	440
"	0.5	0.35	330
"	0.5	0.33	460
"	0.5	0.37	330
"	0.5	0.32	150
"	0.5	0.30	190

[a]attempted recovery of benzene adsorbed from benzene-saturated water, 10 ml sample passed through the column described for aromatic determinations in Chapter 1, flow rate 10 ml $min.^{-1}$

[b]used as the first solvent to remove traces of water remaining on the column.

[c]used for subsequent elution.

[d]lower volume due to unavoidable evaporation. Some error introduced by solvent remaining on the column; solvent was blown through using a small amount of compressed air.

[e]determined using the lc technique described in Chapter 1, and comparing peak areas to those of standards of known concentration.

determined that sample #MIL-F-24397 contained no PNAs using the known retention times of 9 compounds, i.e., anthracene, pyrene, biphenyl, acenaphthylene, naphthacene, benzo(α)pyrene, pyrilene, chrysene, and phenanthrene.

Problems encountered using the Porasil B column are mainly due to the fact that the column must be washed periodically--a procedure taking considerable time.

B. XAD-4 vs. XAD-2

XAD-2 was evaluated in the same manner as XAD-4 and was found to be approximately 10% less efficient, presumably due to the lower surface area per unit weight (330 m^2g^{-1} vs. 750 m^2g^{-1}).

C. Amberlite XAD versus Tenax GC as a Precolumn in Gas Chromatography

1. Introduction

Recently, many techniques involving the concentration, elution, and characterization of trace organic compounds have been developed. The majority of these methods utilize the Amberlite XAD (1, 7-16) and the Tenax-GC (17-21) nonionic polymeric adsorbents as the concentrating agents, due to their high capacities and retention efficiencies.

While solvent elution has proved a useful means to remove adsorbed materials from the resins (16), heat-stripping has become the method of choice, especially for the characterization of volatile organic compounds (12). Solvent requirements are minimal or nonexistent, and as a result, impurities introduced are minimized, and the concentration of the compounds remains relatively high in the eluted sample. Compounds eluted using this technique (usually employing the

gc injection port as an oven) have undergone gc analysis directly (16), or have been allowed to condense in cold traps for subsequent injection (22-24).

This study explores the elution of hydrocarbons from these adsorbents using solvent extraction, heat elution and direct gc analysis, and heat elution followed by condensation and injection. Among those parameters which influence the effectiveness of the methods are: the length of the gc column, the volatilities of the compounds, solvents used for elution, the length of the precolumn, and the shape of the cold trap used in the heat-stripping techniques.

2. Experimental section

Standard Preparation - A mixture of pentane, hexanes, heptane, octane, benzene, toluene, and xylene (25 ml each) was shaken and allowed to stand over 3 liter of distilled water. Aliquots of water were drawn from the bottom of the container (after allowing to stand for 48 hours) by means of a length of glass tubing (2).

Instrumentation - The gas chromatograph was the Hewlett-Packard #5750 Research Chromatograph, connected to the 7127A strip chart recorder. The flow rate was set at 70 ml min^{-1} (He). The detector was maintained at 250° and the injection port at 300° for use with the XAD-4 precolumn, and 320° for use with the Tenax-GC precolumn. The precolumns consisted of copper injection port inserts 7" x 1/4" in length (25), and packed for a 4" length with the resin. The analytical columns were 10% OV-1 on h.p. Chromosorb W, 6" x 1/4", and Tenax-GC, 60/80 mesh, 6" x 1/8". Both were packed in stainless steel tubing.

Cold Traps - Cold traps used to trap heat-eluted compounds were of seven varieties:

1) 8" x 1/4" copper tubing coiled one turn and connected permanently to the precolumn (26)

2) 6" x 1/8" copper tubing coiled one turn and detachable from the precolumn being used

3) 10" x 1/16" stainless steel tubing coiled three times and detachable from the precolumn (27)

4) 5' x 1/16" stainless steel tubing bent to the shape of a "U" having parallel sides 1/2" apart

5) 1/4" brass Swagelok union (brass) fitted with a small quantity of glass wool to trap the hydrocarbons when cooled

6) 9" x 1/4" copper insert through which a 1/8" copper tube was silver-soldered to act as a "micro cold trap"

7) a 4 mm o.d. glass tube, 4' in length, coiled to a helix 4" high and 1 1/2" in diameter.

Procedure: Heat-stripping - An aliquot of the standard was passed through a precolumn at a flow rate of ca. 10 ml min^{-1}. Excess water was blown through briefly using nitrogen (28), and the column centrifuged for 1 hour to remove residual water.

The precolumn was placed in the gc injection port with the flow rate turned to zero. The cold trap was connected to the precolumn before the flow rate was increased. After a period of time (5 to 10 minutes) the flow rate was set to zero and the trap removed. The trapped organic compounds, removed from the trap by addition of (29) a solvent, were then injected into the gas chromatograph.

Procedure: Solvent elution - After removal of residual traces of water by centrifugation, the resin was removed and washed with 2 ml methanol followed by 2 ml methylene chloride for 8 hours each in a Soxhlet extractor.

Results and Discussion - Table 3 summarizes the observations made using the heat-stripping technique.

3. Conclusions

Tenax-GC seems superior to XAD-4 as a precolumn packing material since volatile organics seem to be more easily desorbed by heat. Heat-stripping using the last technique listed in Table 3 proved to be the best method for analyzing the compounds adsorbed on the resin.

Further improvement of the method necessitates finding a solvent compatible with the analytical column, or vice versa.

Table 3. A Summarization of Observations Made Using the Heat-stripping Technique.

Trap	GC Column	Sample Size	Precolumn	Condition	Results
1	OV-1	250 ml	XAD-4	Temperature program 50-250° at 10°/min, trap eluted directly into column.	No resolution obtained.
2	OV-1	250 ml	XAD-4	Same as above, but trapping was accomplished without the trap being connected to the analytical column.	Some resolution achieved when trap was not connected to the column.
3	OV-1	10 ml 25 ml 100 ml	XAD-4	Same as above (Trap 2).	Improved resolution, 10 ml, since the larger samples plugged the trap.
4	OV-1	10 ml	XAD-4	Same as with Trap 3.	Resolution similar to Trap 3.
5	OV-1	250 ml	XAD-4	Trap cooled in crushed dry ice and packed in crushed dry ice during trapping. Other conditions the same as above.	Poor to fair resolution, reproducibility poor.
6	OV-1	250 ml	XAD-4	Dry ice-cooled acetone poured through 1/8" copper tube to trap compounds. Other conditions the same as above.	Poor resolution, poor reproducibility.
7	OV-1	1 liter	XAD-4	Trapped and eluted from trap with 0.2 ml MeOH.	Good resolution, reproducible, C_5 and C_6 obscured by the solvent peaks.
7	OV-1	1 liter	XAD-4	Same as above using CCl_3CF_3 as a solvent.	CCl_3CF_3 gave a large solvent peak and effectively stripped the OV-1 column.

Table 3. (continued)

Trap	GC Column	Sample Size	Precolumn	Condition	Results
7	OV-1	1 liter	XAD-4	As above, using CCl_4.	Good resolution, retention time of solvent between C_6 and beryene, some stripping of OV-1 column.
7	Tenax-GC	1 liter	Tenax-GC	Same as above.	Tenax-GC analytical column did not separate aromatics from aliphatics well, but resolution was very good for those peaks observed.
7	OV-1	1 liter	Tenax-GC	Same as above.	Resolution very good, reproducibility good, some column stripping by solvent.

Solvent elution from XAD-4 showed loss of volatiles and low concentration of less volatile compounds in the extract. Heat-stripping proved superior in all respects.

D. Concentration and Heat Elution of Samples Collected by NRL

Two identical one-liter samples of water containing trace amounts of dissolved oily wastes were passed through XAD-4 (1, 7-16) and Tenax-GC precolumns (17-21). The excess water was removed by spinning the columns in a centrifuge for two hours. This was considered to be a better technique than blowing dry with nitrogen since volatile compounds could be lost. Each column was then eluted in the heating block "B" (see Figure 4) by insertion first into a Pyrex glass tube adapted to fit the dry-ice acetone trap, which was then inserted into the heating block. Helium was used to elute the compounds. The temperature was programmed from ambient to the upper limit used to condition the column (approximately 0.5 hour). After eluting at the upper limit for 2 hours the trap was removed, washed out with 0.5 ml methanol and the solution analyzed by gas chromatography. The chromatograms resulting from the analysis of both columns were then compared to determine the differences between the two adsorbents. Tenax-GC revealed a slightly higher retention for very volatile compounds due, undoubtedly, to its smaller particle size, 60/80 mesh, compared to 20/50 mesh for XAD-4. In general, however, both functioned well and since Tenax-GC is 15 times more costly, we recommend XAD-4 for routine monitoring of the water.

E. The Biodegradability of Naphthalene in Sea Water

Naphthalene was found to be completely biodegradable in natural sea water (obtained from Ocean City, Maryland) during the evaluation in Chapter 1. An attempt to measure the rate of biodegradability showed that, after a four-month period, the sea water was no longer capable of degradation. With fresh water, it was found

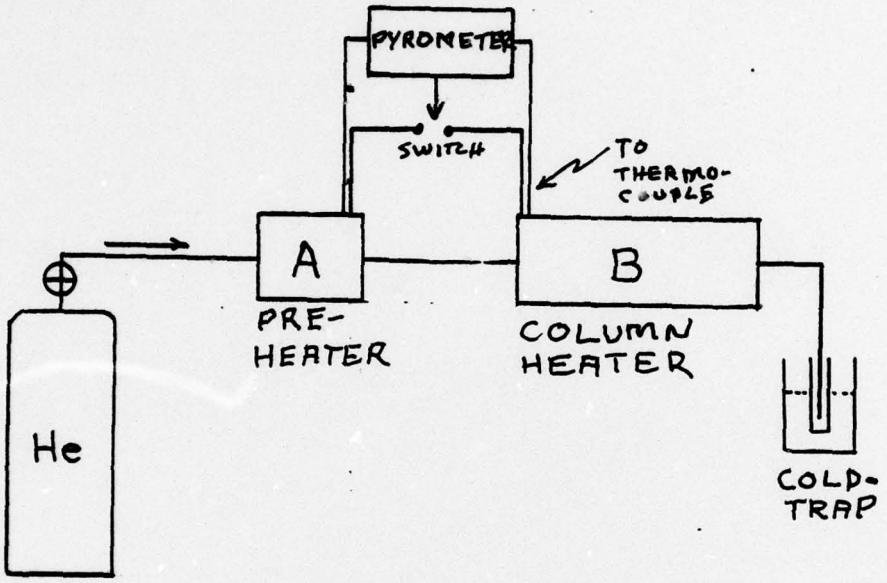


FIGURE 4

Apparatus for Heat-Stripping

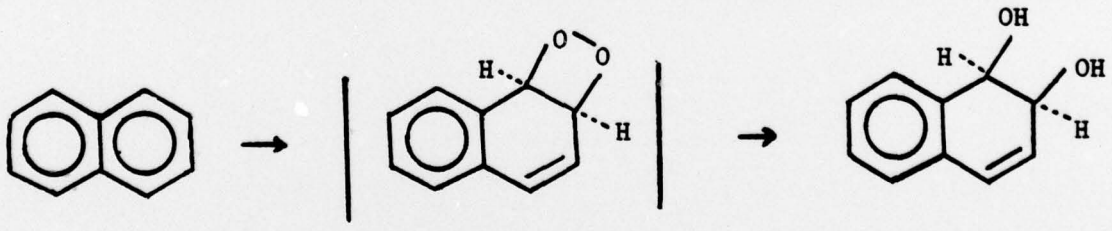
in the evaluative study that naphthalene was totally biodegradable in about two days.

1. Introduction

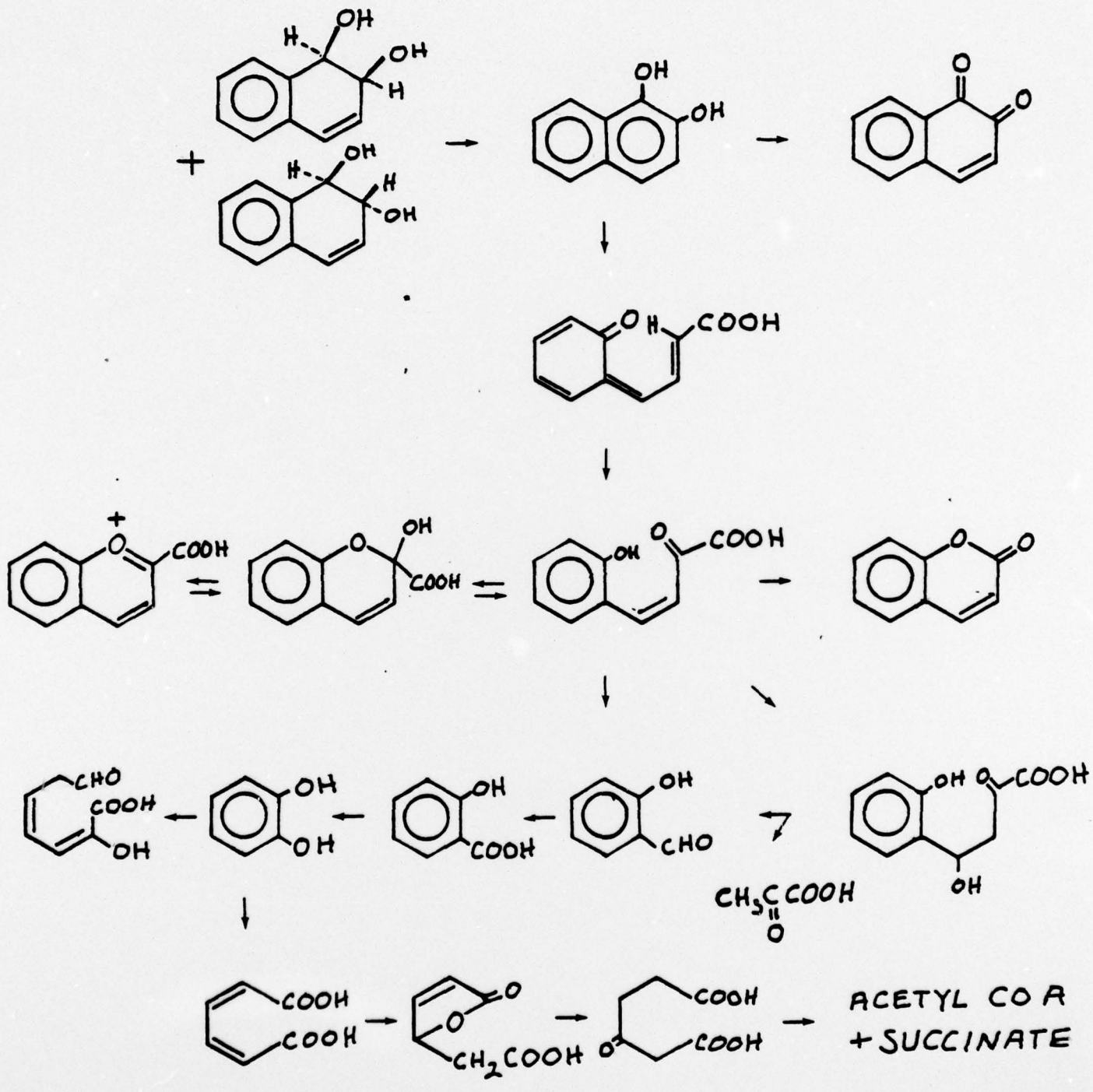
In recent years, the biodegradation of naphthalene and related products by pseudomonas NCIB 9816 (30-35) and pseudomonas putida PpG7 and ATCC 17483 (30,36) has become of interest. While most of the published work has been done with naphthalene (31-37, 39) other aromatics, such as phenanthrene (31,38,40), anthracene (31,38), dibenzyl (31), and methyl naphthalenes (40) have also been studied.

Pseudomonas, virtually omnipresent, are Gram-negative rods (39) which metabolize these hydrocarbons through an aerobic process. They can be grown readily on a number of carbon sources, such as naphthalene (31, 34) and salicylate (35,42). Generation time is independent of the carbon-source concentration above the amount needed for maximum growth.

The initiating step in the bioconversion of naphthalene to eventual non-aromatic products is the fixation of one molecule of oxygen by pseudomonas NCIB 9816 (33,38,39).



The product, cis-o-hydroxybenzalpyruvic acid, easily undergoes further reactions as outlined in Scheme I. Some trans-o-hydroxy-benzalpyruvic acid is also formed, but this undergoes isomerization to the more reactive cis form readily (32 , 33). The reaction is catalyzed



SCHEME 1
(40, 41)

by naphthalene dexoygenase (33, 35, 43) a Fe(II)-dependent enzyme requiring NADH for its action.

Three-membered rings are degraded by a similar pathway, outlined in Scheme II. No case of hydrocarbons bearing functional groups, e.g., β-naphthalamine, has been reported.

2. Purpose of Investigation

The purpose of this investigation was to qualitatively determine the extent of biodegradation of naphthalene and β-naphthylamine by psuedomonas present in natural sea water.

The significance of such a study could eventually lead to nonconcern over some polynuclear aromatic and other potentially carcinogenic compounds in certain mediums due to their natural decomposition.

3. Biodegradation: Qualitative Study of Naphthalene and β-Naphthylamine.

Rate Study - Studies of the rates at which naphthalene and β-naphthylamine were degraded were made using a liquid chromatographic technique monitoring the disappearance of aromatic compounds present in solution. Comparison of the sample containing psuedomonas and naphthalene in sea water against a saturated solution of naphthalene in synthetic sea water resulted in a curve shown in Figure 5. This curve, however, only represents biodegradation to products not absorbing at 254 nm, the wavelength monitored by the uv detector employed.

β-Naphthylamine, on the other hand, showed very little degradation as described above, and no curve was obtained in any reasonable length of time (10 days).

Qualitative Analysis of Degradation Products - The degradation

Figure 5

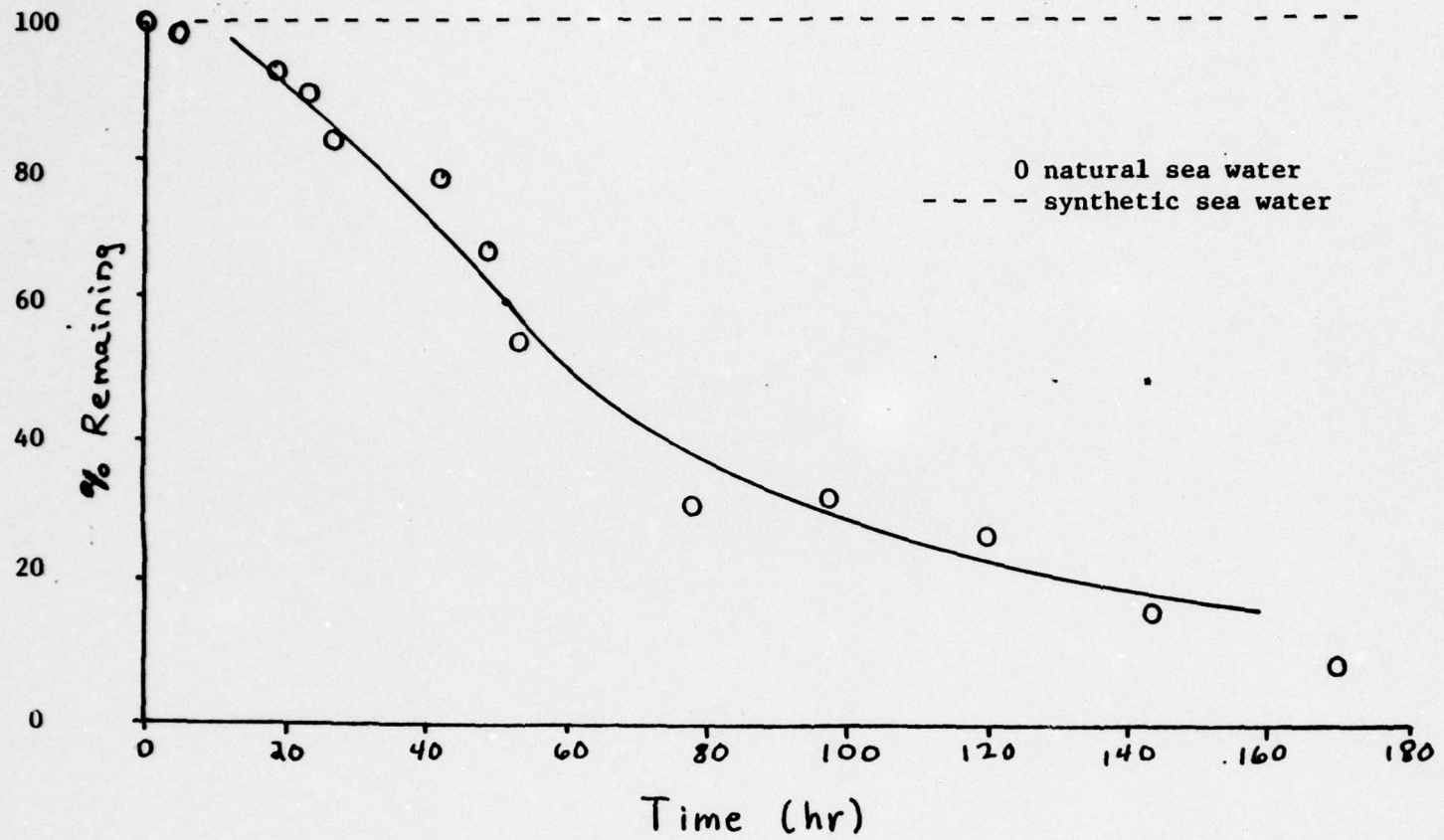
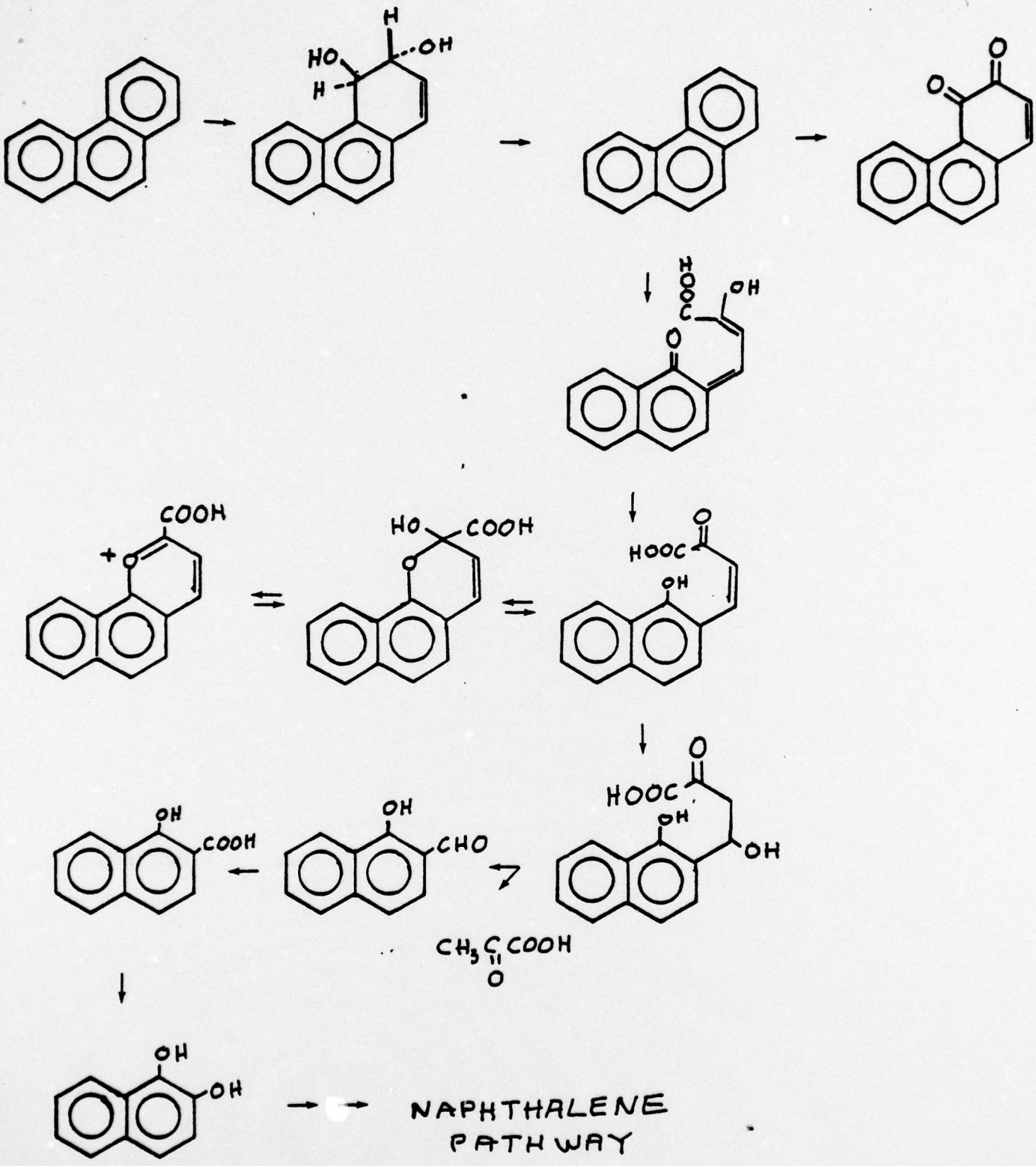


Figure 5. Percentage of naphthalene and aromatic degradation products remaining in natural sea water containing psuedomonas and naphthalene compared to saturated solution of naphthalene in synthetic sea water as a function of time.


HO
H
OH
O
O
HOOC
OH
COOH
HO
COOH
HOOC
OH
OH
COOH
OH
CHO
HOOC
OH
OH
CH3C
O
COOH
OH
OH
NAPHTHALENE
PATHWAY

SCHEME 2 (38)

products of naphthalene and β-naphthyamine may readily be analyzed in a qualitative sense through comparative gas chromatographic methods, as outlined in the experimental section. Solutions containing pseudomonas and the aromatics were extracted from solution with diethyl ether after acidification or other treatment and compared to similar samples which contained the compounds without metabolites. All were compared against standard solutions without the aromatics and against solvent blanks. Repeated runs were performed to confirm the production of a reproducible chromatogram in each case. Various samples prepared are outlined in Table 4.

Alkaline extractions were made using the β-naphthylamine samples due to the polar nature of the amine salts which may form in acidic solution. Acidic extractions were made in all cases to determine the presence of acidic products, which were in turn derivatized using diazomethane to enhance their volatility in the gas chromatograph.

4. Experimental Section

Naphthalene Degradation Curve - Data points for the naphthalene degradation curve were obtained as follows:

One liter samples of sea water and synthetic sea water were saturated with naphthalene (Eastman) by allowing to stand in closed containers at room temperature for a seven-day period. At the end of this time, 250 ml aliquots were withdrawn and filtered, and the degradation time measured from this point using liquid chromatography.

At time intervals, 10 ml aliquots of each sample were pipetted into 30 ml separatory funnels and shaken with 2 ml of methylene chloride for 2 minutes. After allowing to stand for an additional five minutes, 100 µl aliquots were drawn from the bottom layers and

TABLE 4

Preparation of Seven Samples Used to Determine the Presence of Naphthalene and β-Naphthylamine Metabolites Due to Pseudomonas in Natural Sea Water

Sample	Treatment before Extraction	Treatment after Extraction
#1 Naphthalene	Allowed to stand containing excess naphthalene for 4 mos.; acidified with HCl to pH 2	Derivatized with diazomethane
#2 Naphthalene	Sea water allowed to stand without naphthalene for 4 mos.; acidified to pH 2	Naphthalene added to extract; derivatized with diazomethane
#3 β-Naphthylamine	Allowed to stand containing excess β-naphthylamine for 4 mos.; acidified with HCl to pH 2	Derivatized with diazomethane
#4 β-Naphthylamine	Sea water allowed to stand without β-naphthylamine; acidified with HCl to pH 2	β-naphthylamine added to extract; derivatized with diazomethane
#5 β-Naphthylamine	Allowed to stand with excess β-naphthylamine for 4 mos.; NH_4OH added to achieve pH 12	None
#6 Sea Water	Allowed to stand for 4 mos.; NH_4OH added to achieve pH 12	None
#7 Sea Water	Allowed to stand for 4 mos.; acidified with HCl to pH 2	None

injected into the liquid chromatograph (43). Peak areas representing amounts of naphthalene in the samples were used to determine the percent degradation in the samples. The following formula was used to calculate the percent of naphthalene remaining in the sample:

$$\% \text{ remaining} = \frac{SW}{1.102 \times SSW} \times 100$$

where,

SW = concentration of naphthalene in the natural sea water sample

SSW = concentration of naphthalene in the synthetic sea water sample

1.102 = a constant correcting the peak area, SSW, to one equal to SW.

Data points are plotted in Figure 5. No appreciable degradation was found in 10 days in a similar sample containing β-naphthylamine (Fisher).

Degradation Products - Degradation products in both samples were qualitatively examined using the extracts outlined in Table 4. Those peaks present only in the aromatic samples containing pseudomonas, and not in the sea water samples, solvent blank, or individual compounds, were interpreted as being metabolites (44).

5. Results and Discussion

Upon examination of the chromatograms, differences were found when comparing samples containing degradation products to the sea water standards and the solvent blank. These differences are summarized in Table 5. It should be noted that, due to the large amount of organic matter present in sea water, at high levels of sensitivity some smaller peaks representing degradation products may be obscured. Therefore, only decisively different peaks are reported here.

Table 5. Summary of Metabolite Content of Samples Observed by Comparative Gas Chromatography.

NAPHTHALENE (acid)		β-NAPHTHYLAMINE (acid)	
Relative Retention Time[a]	Intensity[b]	Relative Retention Time	Intensity
32.5[c]	w - m	37.5[c]	s
38.1[c]	s	48.0[d]	vs

β-NAPHTHYLAMINE (alk.)		STANDARDS	
Relative Retention Time	Intensity	Compound	Retention Time
40.5[c]	w	Naphthalene	9.6
		β-Naphthylamine	21.5

[a]Retention times relative to diethyl ether.

[b]Intensities relative to average-sized peaks (signals):

w - weak
m - medium (average)
s - strong
vs - very strong

[c]Increase in levels of existing compounds.

[d]Peak not observed in other samples.

It is evident from these data that naphthalene and β-naphthylamine may undergo biodegradation through similar mechanisms, culminating in a product already present from such degradations in sea water. The acidic extracts of both compounds showed a large increase in intensity of the peak occurring at a relative retention time of ca. 38, and this was interpreted as being the end product.

A very large peak was observed at retention time = 48 in the β-naphthylamine sample after having undergone degradation. This may represent an intermediate product containing an amino group which takes more time to be transformed to lower products. This would then be the rate determining step in the process.

It is therefore logical to conclude that β-naphthylamine is biodegradable by pseudomonas, and that the end product is an acid (Scheme 1). Further study is therefore warranted since many other amine-group containing aromatics may also be biodegradable along with other potential carcinogens.

An example of the usefulness of pseudomonas may be found in water purification as the initial treatment, thus removing a possible large number of carcinogenic agents. Further studies should include research using ^{14}C-labelled compounds and gc-ms analysis of degradation products.

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3. This is in agreement with the recommended height to diameter ratio of Junk, et al., (reference 1).

4. This is contrary to the result reported in the recent EPA Report, National Environmental Research Center, Office of Research and Development, "Isolating Organic Water Pollutants: XAD Resins, Urethane Foams, Solvent Extractions," EPA-660/4-75-003, Corvallis, Oregon, June 1975.

5. This equals the value given in the work by Junk, et al., reference 1, for 110 individual determinations.

6. Flow rates above 22 ml min^{-1} were obtained using a source of compressed air introduced at the head of the column.

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25. This is a standard insert which must be used with this particular instrument.

26. Centrifugation not employed due to the irregular shape of the trap-precolumn combination.

27. Flow rate used for condensing material in the trap was 20 ml min^{-1}.

28. It was found that nitrogen effectively stripped the Tenax-GC precolumn of volatiles, and therefore, only centrifugation was employed to remove water.

29. Carbon tetrachloride was used for this extraction. It was further found that this solvent slowly strips the OV-1 analytical column used.

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43. The liquid chromatograph used consisted of a Milton-Roy mini-pump connected to an LCD 1205 uv detector through a Waters Associates U6K injection and a 1/4" x 6" column consisting of Waters Associates Porasil B.

44. The gas chromatograph was a Hewlett Packard 5750 research instrument, and was connected to a 7127 A strip chart recorder. The column consisted of 10% OV-1 on h.p. Chromosorb W, 1/4" x 72".