THE REMOVAL OF CARCINOGENIC POLYCYCLIC AROMATIC HYDROCARBONS BY ACTIVATED SLUDGE

by

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I. INTRODUCTION

The current rapid expansion of the American chemical industry into new and diverse fields has led to production of sophisticated new products such as pesticides, herbicides, and other synthetic compounds. While these products have proved to have wide benefit for our society, a significant waste treatment problem has also evolved with the development of highly refractory exotic compounds as by-products in the manufacturing scheme. These compounds become part of the plant waste effluent and enter waste treatment plants in trace amounts. Many of these compounds are forms of polycyclic aromatic hydrocarbons, some of which have proven to be carcinogenic to mammals by contact or upon ingestion, Hartwell (1) and Shubik and Hartwell (2).

In recent years numerous carcinogenic compounds have been isolated from natural waters. Wedgewood and Cooper (3) recovered various polycyclic hydrocarbons such as 3, 4-benzpyrene and 1, 2-benzanthracene from sewage solids and industrial plant effluents. Other investigators, Shimkin, Koe, and Zechmeister (4), Hueper and Ruchoft (5), and Ruchoft et. al. (6) have also identified similar carcinogenic compounds in both marine and fresh water environments. A more exacting series of studies by Borneff and co-workers (7), (8), (9), demonstrated the presence of a wide range of polycyclic aromatic hydrocarbon carcinogens in surface waters.

Popular attention has been concentrated primarily on nutrients and other compounds which have caused a "visible" change in the environment. No tolerance limits for carcinogens exist, and the high cost of equipment required for analysis of these specific compounds is prohibitive for most treatment laboratories. Also, knowledge of transport mechanisms is virtually nonexistent.

While the immediate effect of trace organics upon humans is unknown, Heuper (10) has warned that a potential danger does exist if these compounds are not removed from drinking and bathing waters. This is especially pertinent if continual ingestion of trace amounts leads to critical tissue concentrations by accumulative storage of compounds of this type through repeated exposure. Also, the increasing productive capacity of industrial sources of these pollutants can only result in greater concentration entering and accumulating in the potable waters of our country, serving to magnify the dangers of exposure.

Much of these industrial discharges enter conventional biological waste treatment plants either via municipal sewer systems or to privately operated plant units. The aeration tank of the widely used activated sludge treatment system is probably the most efficient obstacle to the passage of dissolved and colloidal organics contained in the plant effluents and hence to down-stream drinking water supplies.

Malaney, et. al. (11) investigated these compounds as to amenability of removal from wastewaters by oxidation in the activated sludge process. Results indicated that removal of these potentially harmful compounds by oxidation in the aeration tank is insignificant. The sludge also showed no appreciable ability to acclimate to the oxidation of these compounds. However, since an estimated 70% of the BOD removal in an aeration tank is affected by adsorption on the floc, it is important to investigate potential removal of the carcinogenic organics by the natural floc adsorptive mechanism. If these organics are thus removed and sent to the digester and ultimately to drying beds, an effective mechanism for eliminating these dangerous organics is thus operative. However, if this is not the case, an additional treatment unit such as activated carbon beds may have to be employed ultimately.

II. REVIEW OF LITERATURE

A carcinogen is an agent or process which significantly increases the yield of malignant neoplasms in a given cell population. Yamagiwa and Ichikawa (12) in 1918 induced cutaneous or skin tumors in experimental animals by painting their ears with coal-tar. In 1932 Kenneway et al (12) were the first to demonstrate that a pure chemical was capable of inducing cancer in experimental animals. Introducing the chemical by both oral and subcutaneous routes, Hueper, Wiley and Wolfe (12) showed that 2-naphthylamine induced benign and malignant tumors of the bladder using dogs as experimental animals.

Carcinogenic compounds have been detected in many phases of the environment. Ruchhoft et al (6) found that refinery gravity oil separator effluents, usually discharged without treatment, contained aromatic hydrocarbons which were determined to be carcinogenic. Wedgewood and Cooper (3) have stated that effluents discharged from gas works, washings from macadam roads and the atmosphere are sources of aromatic hydrocarbons such as 3,4 Benzpyrene. They detected the presence of 3,4 Benzpyrene at a concentration of 3 ppm in sewage "humus" which separated from the effluent after treatment of the crude sewage in a plant receiving gas works ammoniacal liquor and de-oiled carburetted water gas effluent. Careful analysis of human urine and feces has failed to

reveal the presence of these complex hydrocarbons and therefore sources other than domestic are indicated.

Shimkin et al (4) showed the presence of polycyclic carcinogenic hydrocarbons, including 3,4 Benzpyrene, in chromatographic fractionation of extracts obtained from a sample of the thatched barnacle. The possibility of tarry materials, from ships or submarine oil wells, being carried to the filter feeding intertidal sedentary animals constituted the potential external source for aromatic polycyclic hydrocarbons.

Incidental food additives such as petroleum waxes were analyzed by William Lijinsky et al (13) for the content of Benzo-a-pyrene and similar carcinogenic polycyclic hydrocarbons. Gas chromatography was the method utilized for quantitative and qualitative determination of these compounds at levels of 0.1 ppm in the original wax. Harold J. Dawson Jr. (14) employed a gas chromatograph equipped with an electron capture detector and a hydrogen flame detector arranged in series for analysis of polynuclear aromatic hydrocarbons contained in heavy oils. The electron capture detector exhibited a high selectivity and sensitivity for selected carcinogenic hydrocarbons such as Benz-a-anthracene, 3-Methylcholanthrene, Benzo-a-pyrene and Dibenz-a,h-anthracene.

B.B. Chakraborty and R. Long (15) investigated the formation of polycyclic aromatic hydrocarbons during incomplete

combustion of hydrocarbons in diffusion flames. Individual hydrocarbons present in the soot samples were determined by programmed temperature gas chromatography using dual flame ionization detectors. By comparing the relative retention times of the peaks obtained from soot extracts with those of pure compounds and by ultraviolet absorption spectrophotometry of the fractions separated by gas chromatography, peaks in the gas chromatogram were identified.

Disadvantages relating to gas chromatographic analysis of tars were encountered by W. Lijinsky and G. Mason (16). They determined that many compounds of great interest because of their carcinogenicity have retention times in this system identical with noncarcinogenic hydrocarbons and therefore they cannot be positively identified by this means.

Warburg studies were performed by Malaney et al (11) to determine the oxidative activity of several activated sludges when subjected to high (500 mg/l) concentrations of polycyclic aromatic hydrocarbons. The results indicated that for practically all cases, after 144 hours, the compounds were not metabolized by the sludge and in some cases were toxic to the microorganisms. Further experimentation is necessary to define the oxidative behavior of organisms of activated sludge in the presence of polycyclic aromatic hydrocarbons before it can be concluded that activated sludge cannot metabolize these compounds.

Engineers and Sanitarians must decide whether the presence of carcinogenic chemicals in the environment is to be prohibited absolutely or whether maximum limits should be established. Little work has been carried out on the quantitative aspects of the induction of cancer by chemicals resulting in the lack of experimental bases to define permissible limits for carcinogenic hazards.

III. MATERIALS AND METHODS

A. Specialized Materials

1. Benzo-a-pyrene, Practical Grade. Sigma Chemical Co. Used in substrate.
2. 20-Methylcholanthrene, Practical Grade. Sigma Chemical Co. Used in batch study substrate.
3. 1,2-Benzanthracene, Sigma Chemical Co. Used in batch study.
4. 1,2,5,6-Dibenzanthracene, Sigma Chemical Co. Used in batch study substrate.
5. B-Naphthylamine, Recrystallized. Sigma Chemical Co. Used in batch study substrate.
6. 9,10-Dimethyl-1,2-benzanthracene, Sigma Chemical Co. Used in batch study substrate.
7. Acetone, (CH_3COCH_3), Fisher Scientific Co. Certified ACS. Used in substrate.
8. Peristaltic Pump, Model 1201, Harvard Apparatus Co., Inc. Used to supply substrate to pilot plant.
9. Magnetic Stirrer, Wilkens-Anderson Co. Used to mix liquor in pilot plant.
10. Flexa-Mix Stirrer, Fisher Scientific Co. Used to continually mix substrate.
11. Benzene, (C_6H_6), Fisher Scientific Co. Certified ACS. Used to extract carcinogens from liquids and solids.

12. Peristaltic Pump, Sigma Motor. Used to recycle sludge.
13. Solution Shaker, Eberbach Corp. Used to agitate batch study samples.
14. Extraction Apparatus, Pyrex, Soxhlet. Used in extraction of carcinogens.
15. Filter Paper, Whatman, No. 40. Used to separate liquid from sludge.
16. Alkyl Benzene Sulfonate, Soap and Detergent Assoc. Used in substrate.
17. Ammonium Chloride, (NH_4Cl). Allied Chemical Co. Certified ACS. Used in buffer for substrate.
18. Bacto-Dextrose, Difco Laboratories. Used in substrate.
19. Bacto-Peptone, Difco Laboratories. Used in substrate.
20. Bacto-Yeast Extract, Difco Laboratories. Dehydrated. Used in substrate.
21. Dipotassium Hydrogen Phosphate, (K_2HPO_4), Fisher, Certified ACS. Used in buffer for substrate.
22. Potassium Dihydrogen Phosphate, (KH_2PO_4), Fisher, Certified ACS. Used in buffer for substrate.
23. Sodium Phosphate, ($Na_2HPO_4 \cdot 7\ H_2O$), Baker, Reagent Grade. Used in buffer for substrate.
24. Gas Chromatograph, Bechman GC-5. Used in analysis for carcinogens.

25. Gas Chromatograph, Varian Aerograph, Model 1700. Used in analysis for carcinogens.

26. Strip Chart Recorder, Bausch and Lomb, Model VOM-7. Used with Bechman GC-5 to record results.

27. Strip Chart Recorder, Sargent, Model SR. Used with Varian Aerograph Model 1700 to record results.

28. 10 ul Syringe, The Hamilton Company. Used to inject samples into Bechman GC-5.

29. 5 ul Syringe, Scientific Glass Engineering PTY, Ltd. Used to inject samples into Varian Aerograph Model 1700.

30. Chemicals and other materials for tests according to Standard Methods (17).

B. Methods

1. Preparation of Standards for Chromatographic Analysis.

Standard solutions of polycyclic aromatic hydrocarbons were maintained for quantitative determination of carcinogens found in a given sample. Each standard was prepared by dissolving a selected weight of each compound in 10 milliliters of benzene. The standards were stored in screw-cap test tubes under refrigeration. A complete list of the concentrations used is given in Table I.

TABLE I

CONCENTRATION OF STANDARDS

Constituent	Concentration
20-Methylcholanthrene	1, 5, 10, 20, 30, 40, 50, 60, 70, 80, 90 and 100 ng/ul
1,2-Benzanthracene	1, 5, 10, 20, 30, 40, 50, 60, 70, 80, 90 and 100 ng/ul
Benzo-a-pyrene	1, 5, 10, 20, 30, 40, 50, 60, 70, 80, 90 and 100 ng/ul
1,2,5,6-Dibenzanthracene	1, 5, 10, 20, 30, 40, 50, 60, 70, 80, 90 and 100 ng/ul
B-Naphthylamine	1, 5, 10, 20, 30, 40, 50, 60, 70, 80, 90 and 100 ng/ul
9,10-Dimethyl-1,2-benzanthracene	1, 5, 10, 20, 30, 40, 50, 60, 70, 80, 90 and 100 ng/ul

2. Laboratory Activated Sludge Plant.

As illustrated in Figure 1, the lab activated sludge plant consisted of an aeration tank, settling tank, sludge return pump and a substrate feed pump. The primary substrate (Table II) was fed at a rate of 250 ml per hour. The aeration tank had a working volume of 4 liters providing a detention time of 8 hours at the normal substrate feed rate. Diffused air released through an airstone near the bottom provided aeration and some mixing. A magnetic stirrer was used to insure thorough mixing of the mixed liquor. The mixed liquor passed by gravity to a 750 ml separatory funnel which acted as a settling tank. The settling tank had a working volume of 500 ml which provided a detention time of one hour at normal flow rates. Supernatant was continuously removed from the settling tank to a constant-head tank via a siphon. Effluent was discharged to a 24 liter container. Settled sludge was recycled to the aeration tank at a rate of 250 ml per hour, which provided a recirculation ratio of 1 to 1. The plant was originally seeded with sludge obtained from the aeration tank at the Corning Glass Works sewage treatment plant, Blacksburg, Virginia.

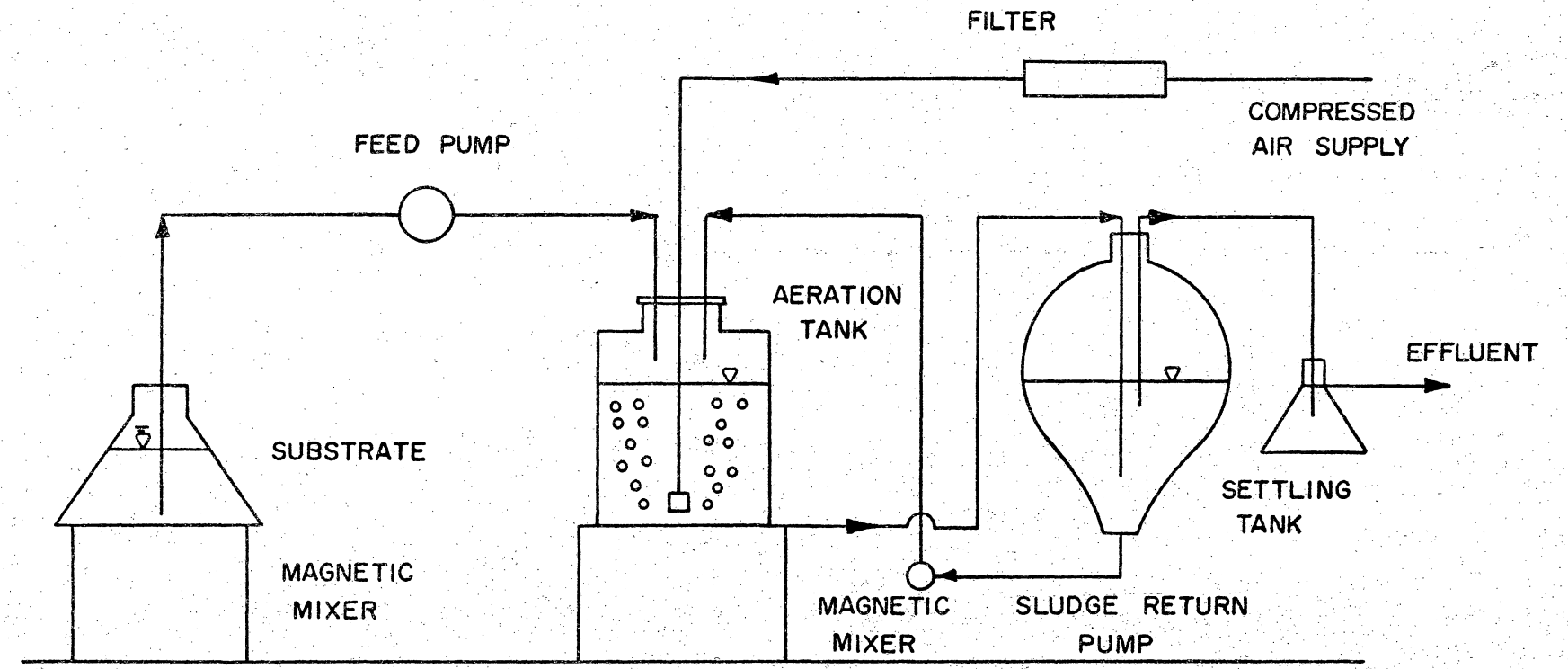

FIGURE 1. LABORATORY SCALE ACTIVATED SLUDGE PLANT

3. Substrate.

The primary substrate was initially prepared every 48 hours in a 12 liter pyrex carboy and autoclaved to prevent decomposition before being pumped to the aeration tank. The composition of the primary substrate is shown in Table II. For details pertaining to additions made to the primary substrate see Experiment 3.

4. Addition of Carcinogens to Substrate.

The polycyclic aromatic hydrocarbons used in this investigation are highly insoluble in water. Small amounts of these compounds introduced into a beaker of water remained on the surface, apparently strongly hydrophobic in nature.

These compounds are soluble in organic solvents, including benzene and acetone. Acetone was considered less toxic to the activated sludge organisms and was utilized as the solutioning and introductory agent. For detailed procedure see Experiment 2 and 3.

5. Extraction of Carcinogens.

The carcinogenic polycyclic aromatic hydrocarbons were extracted by a modified Soxhlet technique (18). For detailed procedure see Appendix A.

TABLE II

COMPOSITION OF PRIMARY SUBSTRATE

Constituent	Quantity
Peptone	200 mg/l
Dextrose	50 mg/l
Yeast Extract	40 mg/l
ABS	1 mg/l
Phosphate Buffer	25 ml/l
Tap Water	200 ml/l
Distilled Water	To Volume

6. Analysis of Carcinogens.

The carcinogenic polycyclic aromatic hydrocarbons were analysed qualitatively and quantitatively by gas chromatography. For detailed procedure see Experiment I.

7. Total Suspended Solids (Nonfilterable Residue).

The method for determining suspended solids was that contained in Standard Methods (17).

8. Volatile Suspended Matter.

The method for determining volatile suspended solids was that outlined in Standard Methods (17).

9. Chemical Oxygen Demand.

Determination of the chemical oxygen demand of the primary substrate was performed by the reflux method as contained in Standard Methods (17).

10. Sludge Volume Index.

The method for determining the sludge volume index of the aeration tank mixed liquor was that contained in Standard Methods (17).

11. pH Determination.

A Beckman glass calomel electrode pH meter was used to measure the pH of the aeration tank liquor.

IV. EXPERIMENTAL RESULTS

Experiment I. Determination of Optimum Methodology for Detection of Selected Polycyclic Aromatic Hydrocarbons.

The object of this experiment was to establish a satisfactory routine analysis for the quantitative evaluation of polycyclic aromatic hydrocarbons. Several researchers have devoted vast amounts of time and patience in pursuit of this goal (13, 14, 15, 16).

The literature (13, 14, 15, 16) indicated that analysis by gas chromatography was the method of choice for the hydrocarbons under study.

A one ul sample (Figure 2) was introduced by a 10 ul syringe into the injection chamber which was maintained at 250 C. The stream of helium carrier gas carried the sample into the column packed with 100/120 mesh Chromsorb WHP coated with 4% SE-52. Sample partition occurred in the column and the separated components entered the detector developing signals which activated the recorder which made a permanent record of the sample components as a traced chromatograph. Qualitative and quantitative evaluations are based upon the time of elution and the peak areas and/or height, respectively. (Figure 3 and 4).

Standard Solutions

Standard benzene solutions of selected polycyclic aromatic hydrocarbons were prepared as described in the previous chapter. These solutions were then subjected to

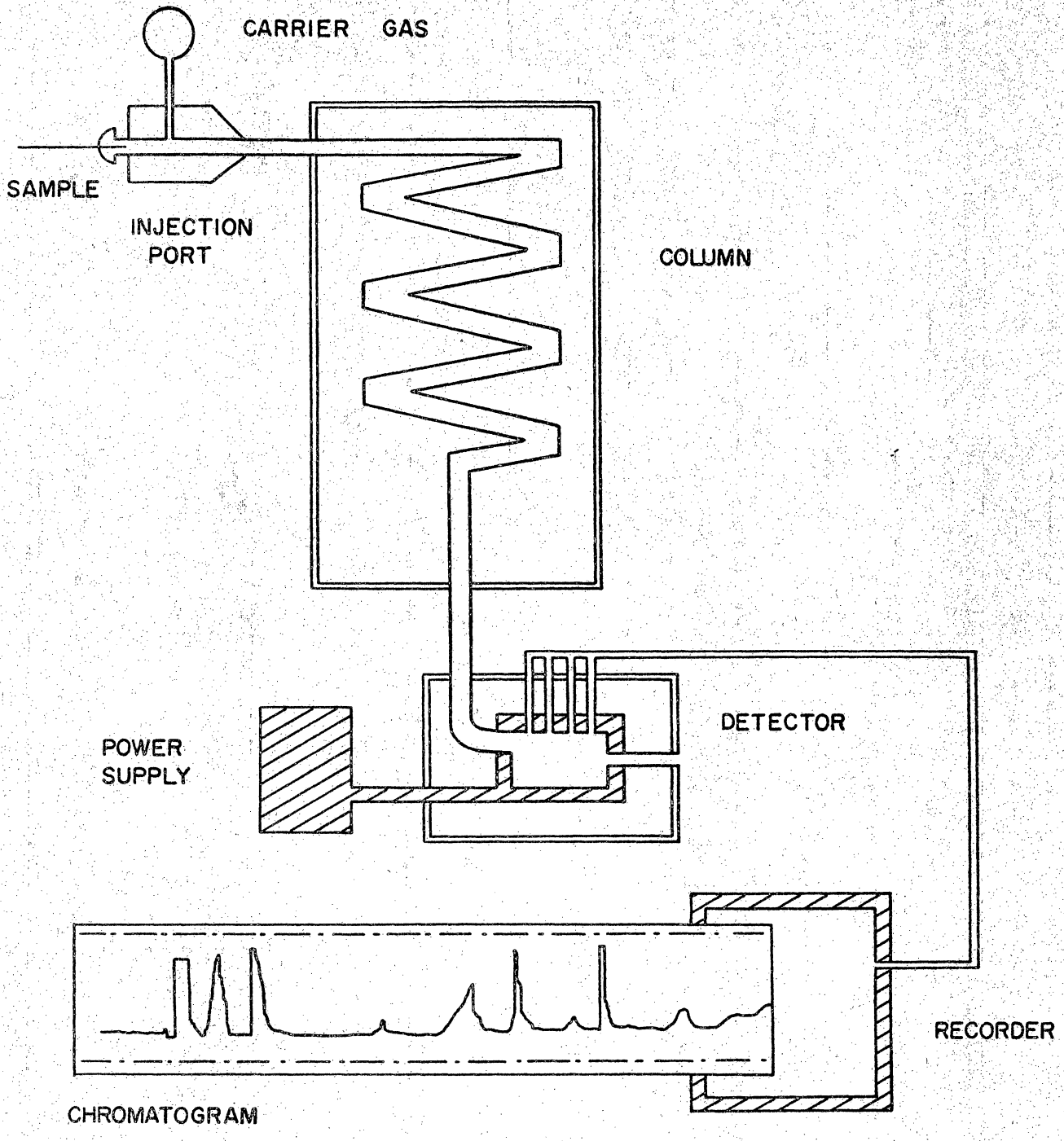

FIGURE 2. BASIC DIAGRAM OF GAS CHROMATOGRAPH

gas chromatographic analysis.

Analysis

A Beckman GC-5 and a Varian Aerograph Model 1700 were employed in analyzing the standard solutions. The detailed accessories and parameters which produced the most accurate and reliable results are listed for each instrument, respectively, in Tables III and IV.

Results

The Beckman GC-5 instrument did not yield a satisfactory chromatogram. Figure 3 is an illustration of the best results quantitatively and qualitatively obtainable. The elution time for Benzo-a-pyrene at the stated conditions (Table III) was 3 minutes and 10 seconds. An extreme degree of "tailing" was observed, with the last trace of Benzo-a-pyrene being emitted at a time delay greater than ten minutes. This period was contingent upon the amount of Benzo-a-pyrene injected. Greater concentrations and repeated use of the compound resulted in more prolonged "tailing". An increase in the concentration of the sample did not produce any significant increase in peak height, but did increase the area under the peak.

Through experimentation a Varian Aerograph Model 1700 Gas Chromatograph equipped with a hydrogen flame detector was determined the best available equipment for quantitative and qualitative analysis of polycyclic aromatic hydrocarbons.

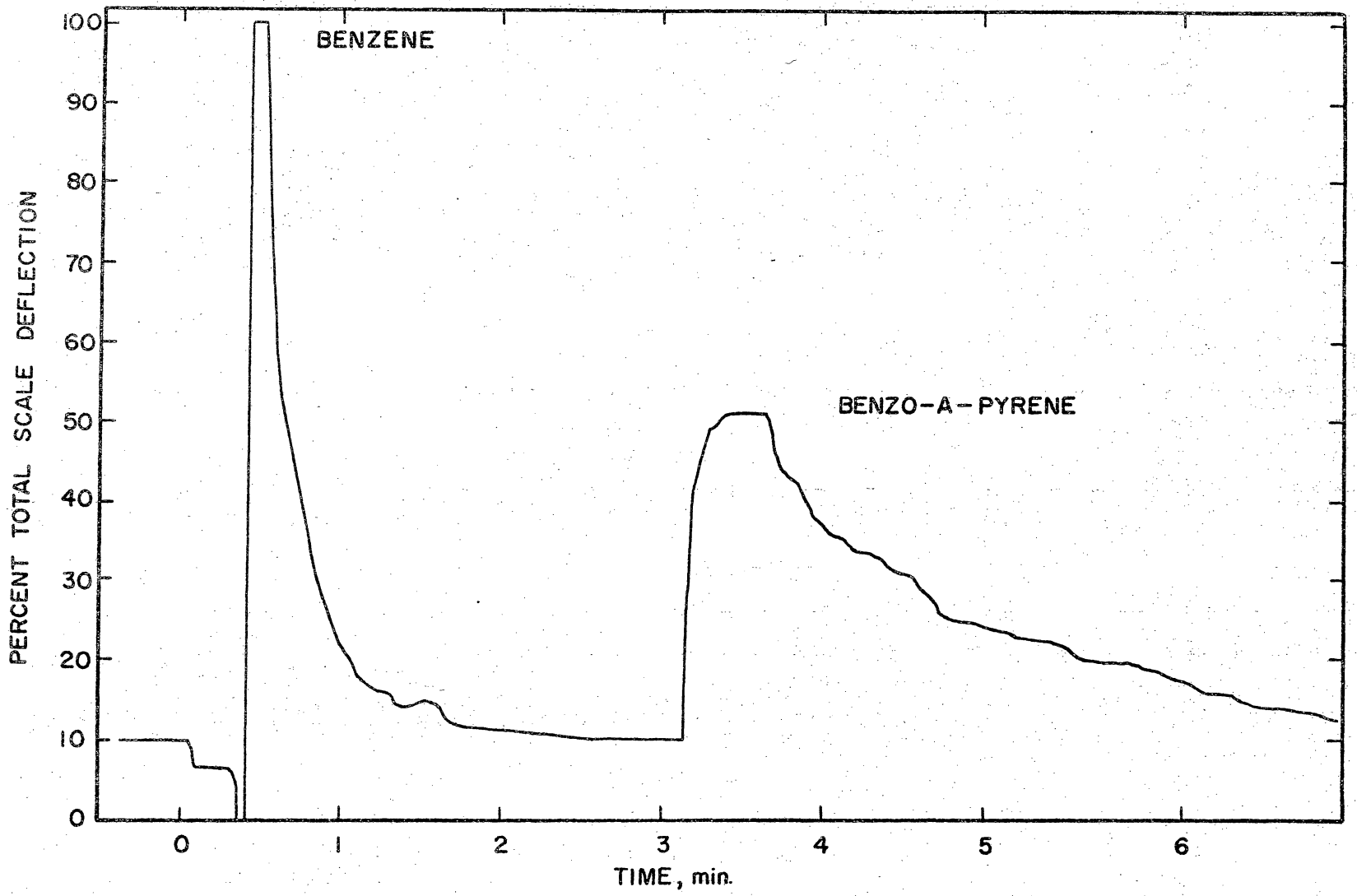

FIGURE 3. CHROMATOGRAM OF BENZO-A-PYRENE
(BECKMAN GC-5)

TABLE III

GAS CHROMATOGRAPH EQUIPMENT AND OPERATING PARAMETERS

Instrument	Beckman GC-5
Column	¼" x 6' x 2mm (glass)
Solid Phase	100/120 Chromsorb WHP
Liquid Phase	4% SE-52
Detection	Electron Capture
Discharge Voltage	215 volts
Carrier Gas	Helium 30 cc/min
Discharge Gas	Helium 65 cc/min
Standardization Gas	CO_2 3 cc/min
Operating Temperatures	
Column	235° C.
Detector	250° C.
Inlet	250° C.
Line	260° C.
Polarizing Voltage	669
Source Current	7 ma
Bias Voltage	0
Range and Attenuator	Set to yield maximum peak with minimum baseline distortion.

TABLE IV

GAS CHROMATOGRAPH EQUIPMENT AND OPERATING PARAMETERS

Instrument	Varian Aerograph Model 1700
Column	1/8" x 5' (s. s.)
Solid Phase	100/120 Chromsorb WHP
Liquid Phase	3% SE-30
Detection	Hydrogen Flame
Carrier Gas	Helium 30 cc/min
Operating Temperatures	
Column	285° C.
Detector	265° C.
Inlet	260° C.
Range and Attenuator	Set to yield maximum peak with minimum acceptable base-line distortion

The elution time at specified conditions (Table IV) was 4 minutes and 25 seconds for Benzo-a-pyrene. The peak (Figure 4) given by this compound was very well defined with a sharp rise and a short base. As shown in Figure 5, the relation of peak height to Benzo-a-pyrene concentration (ng/ul) was a straight line. This factor made for high quantitative accuracy, and the Varian Aerograph was chosen for analysis of samples in Experiment II and III.

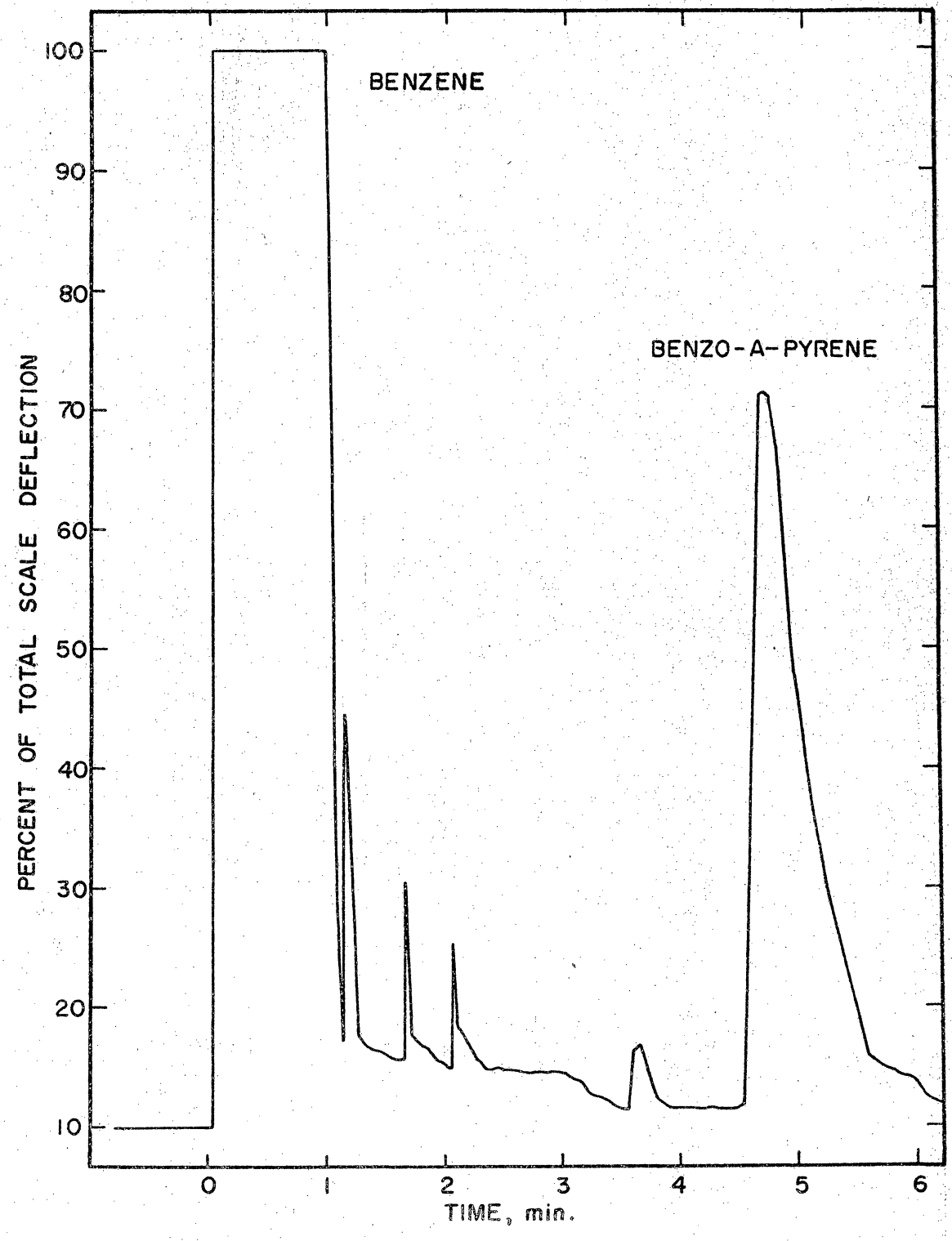

FIGURE 4. CHROMATOGRAM OF BENZO-A-PYRENE
(VARIAN AEROGRAPH MODEL 1700)

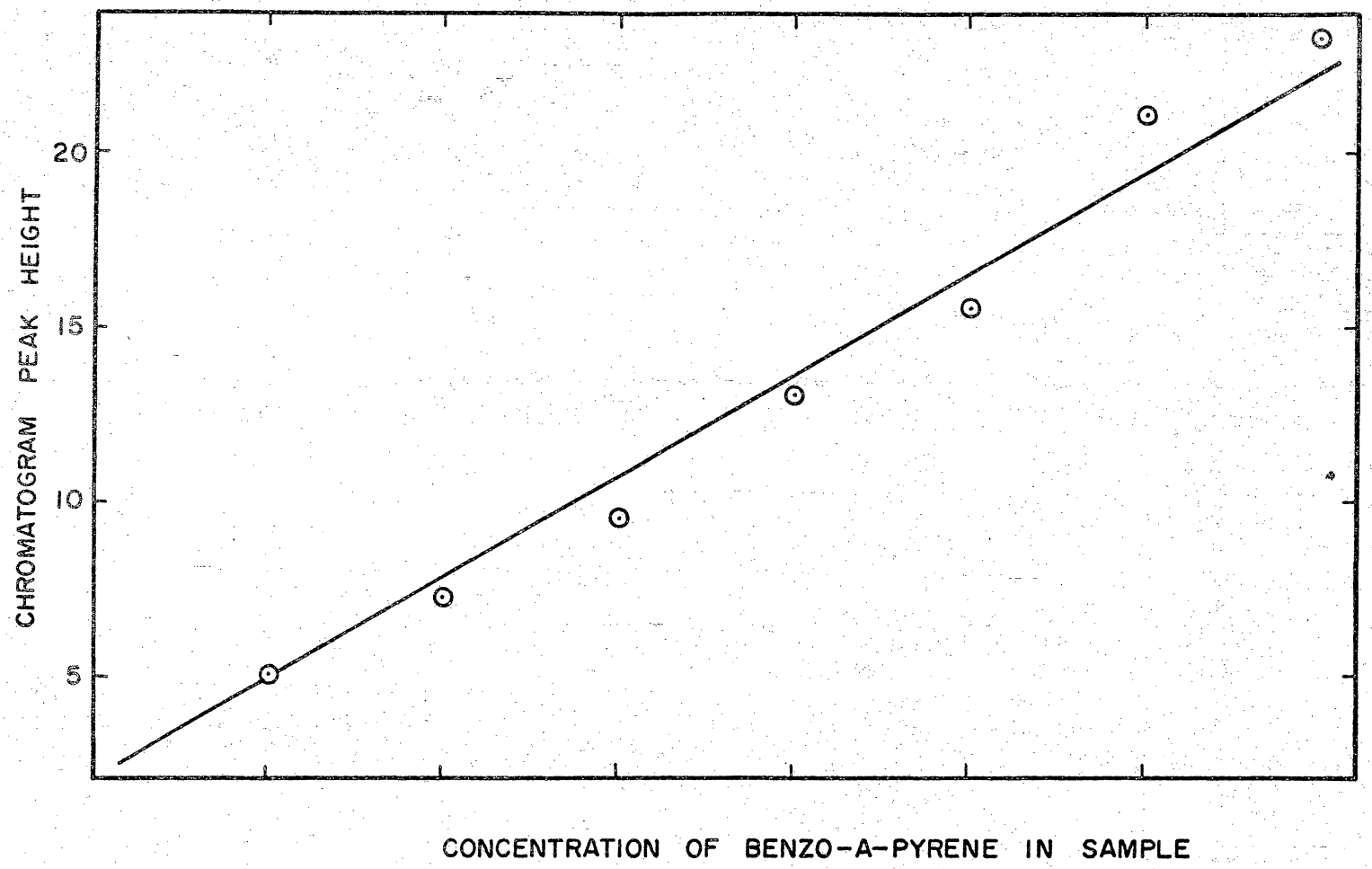

FIGURE 5. CONCENTRATION VS. PEAK HEIGHT

Experiment II. The Removal of Benzo-a-pyrene by Activated Sludge - Batch Unit.

The object of this experiment was to quantitatively determine the extent of Benzo-a-pyrene removal from activated sludge mixed liquors by adsorption on the sludge floc. Measured amounts of Benzo-a-pyrene were added to a flask containing 250 ml of fresh activated sludge mixed liquor. The flasks were stoppered and agitated for eight hours, in order to coincide with the detention time in the laboratory activated sludge plant. The sludge solids were allowed to settle for one hour prior to drawing off the supernatant.

The sludge slurry was removed from the flask and filtered through a Buchner funnel. The organics contained in the sludge cake, filtrate and supernatant were then individually extracted with benzene in a Soxhlet apparatus. The extracts were subsequently analyzed for the content of Benzo-a-pyrene by gas chromatography.

Activated Sludge

The activated sludge used in this experiment may be considered typical, having the following characteristics; total suspended solids, 2230 mg/l; volatile suspended solids, 1920 mg/l; per cent volatile solids in suspended solids, 86; sludge volume index, 110; pH, 7.5; color, light brown; large numbers of stalked ciliates and rotifers. The sludge had been acclimated to the primary substrate for approximately

four months and had reached a steady state condition.

Benzo-a-pyrene Admixture

Two stock solutions of Benzo-a-pyrene dissolved in acetone were prepared. Solution A and B contained 2.1 mg and 8.0 mg, respectively, with each dissolved in 100 ml of organic solvent. This provided stock solutions containing 21,000 ng/ml and 80,000 ng/ml. Quantitative additions of Benzo-a-pyrene were made to the flasks of activated sludge mixed liquor as shown in Table V.

Results

The extracts from the sludge were dark green in color indicating that a wide variety of organics were present. The chromatograms exhibited a high continuous background which supported this hypothesis. The peak for Benzo-a-pyrene projected above the organic background which served as a baseline. Background peaks exhibiting up to 60% scale deflection were recorded.

The control sample containing no Benzo-a-pyrene yielded no peaks above the organic baseline. As illustrated in Table V, when endogenous activated sludge was subjected to low concentrations (0.08 mg/l and 0.17 mg/l) of Benzo-a-pyrene, only a trace of the compound was detected in the sludge extract and none was detected in the supernatant or filtrate extracts. The Benzo-a-pyrene was undetectable in this case due to mechanical losses, including adsorption on glass surfaces.

At concentrations of 0.32 mg/l and 0.64 mg/l, Benzo-a-pyrene was recovered in the sludge extract to the extent of 38% and 13% of the amount injected, respectively. Benzo-a-pyrene was not found on the filtrate extracts. Erratic losses were evident again indicating mechanical interferences.

At the higher concentrations of 1.28 mg/l and 2.56 mg/l, 31% and 53% of the Benzo-a-pyrene, respectively, was recovered from the sludge extract. The supernatant extracts contained 9% and 11% of the total amount of Benzo-a-pyrene injected, indicating that the sludge floc had adsorbed the material up to its capacity. The filtrate extracts contained no Benzo-a-pyrene.

TABLE V

THE REMOVAL OF BENZO-A-PYRENE BY ACTIVATED SLUDGE (BATCH STUDY)

Sample	Benzo-a-pyrene Added	Benzo-a-pyrene Detected		
		Supernatant	Filtrate	Sludge
Control	0	0	0	0
1	21,000 ng	0	0	trace
2	42,000 ng	0	0	trace
3	80,000 ng	0	0	30,000 ng
4	160,000 ng	0	0	20,000 ng
5	320,000 ng	30,000 ng	0	100,000 ng
6	640,000 ng	70,000 ng	0	340,000 ng

Experiment III. The Removal Of Benzo-a-pyrene By Activated Sludge - Continuous Flow Unit.

The object of this experiment was to determine to what extent Benzo-a-pyrene was removed when fed continuously to the laboratory activated sludge plant. Benzo-a-pyrene was added to the primary substrate which was pumped to the aeration tank for twenty-four hours where it underwent eight hours of contact with the sludge floc. The effluent was collected, extracted and analyzed for Benzo-a-pyrene. The mixed liquor in the aeration tank was combined with the contents of the settling tank and allowed to stand for one hour. The supernatant was drawn off and analyzed for Benzo-a-pyrene. The remaining sludge, simulating waste activated sludge, was filtered through a Buchner funnel and the dried sludge and filtrate were analyzed separately for Benzo-a-pyrene by gas chromatography.

Activated Sludge

The activated sludge used in this experiment had the following characteristics: total suspended solids, 2010 mg/l; volatile suspended solids, 1630 mg/l; per cent volatile solids in suspended solids, 82; sludge volume index, 87; pH, 7.0; color, light brown; large numbers of stalked ciliates and rotifers. Due to the addition of acetone, small numbers of filamentous organisms were found. This sludge was the same as that used in Experiment II, after acclimation for two additional months.

Substrate

The substrate was modified for this experiment by adding to the primary substrate a solution of acetone containing Benzo-a-pyrene during the final twenty-four hours of feeding time. A stock solution was prepared by dissolving thirty five milligrams of Benzo-a-pyrene in 100 milliliters of acetone. 2.85 milliliters of stock solution was added to two liters of primary substrate resulting in a concentration of 1.5 mg/l acetone and 0.5 mg/l Benzo-a-pyrene.

Results

The extracts from various portions of the mixed liquor and effluent exhibited characteristics similar to those of Experiment II; the sludge extract was dark green in color and the respective chromatogram displayed a high background of organic contamination. The gas chromatograph was unable to detect Benzo-a-pyrene in either the effluent, supernatant, or sludge filtrate. 0.5 milligram of the compound was detected on the sludge cake, indicating the possibility of compound modifications due to enzymatic activity. Table VI lists the above determinations.

TABLE VI

THE REMOVAL OF BENZO-A-PYRENE BY A CONTINUOUSLY FED LABORATORY ACTIVATED SLUDGE PLANT

Detention Volume	Benzo-a-pyrene Added	Benzo-a-pyrene Recovered			
		Effluent	Supernatant	Filtrate	Sludge
4 Liters	3.0×10^6 ng	0	0	0	0.5×10^6 ng

V. DISCUSSION OF RESULTS

The results of Experiment III indicate that Benzo-a-pyrene was effectively removed from a synthetic sewage mixture when subjected to biological treatment in the aeration tank of a conventional activated sludge system in the laboratory. The mechanisms involved appeared to be adsorption followed by compound modification. As shown in Table VI, the concentration of Benzo-a-pyrene in effluent from the plant was below detection through gas chromatographic analysis. Seventeen per cent (17%) of the Benzo-a-pyrene injected was recovered from the sludge.

It should be noted that the addition of acetone containing Benzo-a-pyrene to distilled water produced a suspension of small Benzo-a-pyrene particles in the water. This also occurred when the stock solution was added to the primary substrate. As the suspension was pumped to the aeration tank, the particles adhered to any available surface. Most of these were dislodged, but it is possible that some remained in the hose resulting in a loss which was not accounted for in Table VI.

Melaney et al (11) determined that most polycyclic aromatic hydrocarbons were not amenable to biological oxidation, and in some instances were toxic to activated sludge organisms. However, the abnormally high concentrations employed (i.e. 500 mg/l) render these results ques-

tionable when applied to a functional activated sludge unit receiving trace amounts of the carcinogen. The concentration of Benzo-a-pyrene introduced into the aeration tank, in this investigation, was 0.75 mg/l in twenty-four hours assuming all of the compound entered and remained in the reactor. At this low concentration, acclimation of the sludge is feasible, resulting in oxidation of the Benzo-a-pyrene. This hypothesis was partially supported by results observed following the addition of low concentrations of acetone to the aeration tank; a filamentous organism developed after acclimation to the acetone, indicating that the organic solvent was serving as a secondary food source. Acetone in greater concentrations was toxic to the activated sludge microorganisms.

When Benzo-a-pyrene was injected into a batch activated sludge unit undergoing endogenous respiration due to the lack of an appropriate food source, Benzo-a-pyrene was detected in the supernatant (eventually the effluent) in generally increasing concentrations as the amount of Benzo-a-pyrene added was increased. Table V shows that at concentrations of 0.08, 0.17, 0.32, and 0.64 mg/l Benzo-a-pyrene in the mixed liquor, the carcinogen was below the level of detection in the effluent. When added at concentrations of 1.28 and 2.56 mg/l, Benzo-a-pyrene was detected in the effluent at 9% and 11% of the total amount injected, respectively. At these higher concentrations active adsorption sites were occupied by the suspended particles of

Benzo-a-pyrene and due to the inactive state of the sludge there was no increase in cell metabolism. As the active adsorption sites were filled, excess Benzo-a-pyrene was detected in the effluent.

This investigation provided useful information pertinant to the development of a reliable technique for the analysis of polycyclic aromatic hydrocarbons. The study was limited to the use of Benzo-a-pyrene because of difficulty encountered in the gas chromatographic analysis presented in Experiment I. The basic structural similarity of carcinogenic polycyclic aromatic hydrocarbons leads logically to the postulate that these other compounds would react in a manner similar to that of Benzo-a-pyrene.

This investigation involved the study of polycyclic aromatic hydrocarbons in a suspended form and at relatively low concentrations (i.e. 0.5 mg/l). Under these conditions, experimental results indicate that the aeration chamber of a typical activated sludge treatment system provides an effective barrier to these insidious carcinogenic compounds.

VI. CONCLUSIONS

1. A Varian Aerograph Gas Chromatograph equipped with hydrogen flame detector was found to be effective in detecting carcinogenic polycyclic aromatic hydrocarbons such as Benzo-a-pyrene.
2. The polycyclic aromatic hydrocarbon under study, Benzo-a-pyrene, was not detected in the effluent when introduced at a concentration of 0.5 mg/l into the aeration tank of an activated sludge plant.
3. The actively metabolizing activated sludge microorganisms are more effective in removing Benzo-a-pyrene than the endogenous organisms contained in the batch study.
4. The mechanism exhibited by the activated sludge in removing Benzo-a-pyrene was a combination of adsorption followed by compound modification.
5. Warburg studies utilizing concentrations below 500 mg/l of polycyclic aromatic hydrocarbons are needed to further define the metabolic uptake of these compounds.
6. On the basis of this study the aeration tank of a conventional activated sludge treatment system proved an effective treatment process for wastewaters containing low concentrations of carcinogenic polycyclic aromatic hydrocarbons. (i.e. 0.5 mg/l)

VII. SUMMARY

Experimental data obtained in the study of the removal of Benzo-a-pyrene in the aeration chamber of an activated sludge system indicated that carcinogenic polycyclic aromatic hydrocarbons of this type may be eliminated from the plant effluent through conventional biological treatment processes.

Qualitative and quantitative analysis for Benzo-a-pyrene was performed using a Varian Aerograph Model 1700 Gas Chromatograph equipped with a hydrogen flame detector.

Benzo-a-pyrene was added at various concentrations to a number of flasks containing activated sludge mixed liquor during the batch study. The continuous study involved the sustained introduction of Benzo-a-pyrene at a concentration of 0.5 mg/l into the aeration chamber of a laboratory activated sludge unit. The batch activated sludge unit produced a considerably lower removal of the compound when compared to the continuously fed activated sludge aeration tank. The mechanism of removal appeared to be adsorption of the Benzo-a-pyrene followed by compound modification. This evidence indicates that metabolic studies should be continued.

Experimental results of this study indicate that the activated sludge treatment process could be effectively used to treat wastwaters containing low concentrations (i.e. 0.5 mg/l) of carcinogenic polycyclic aromatic hydrocarbons.

VIII. BIBLIOGRAPHY

1. Hartwell, J. L., "Survey of Compounds Which Have Been Tested for Carcinogenic Activity." 2nd Ed., Pub. Health Service Publ. No. 149, U.S. Govt. Printing Office, Washington, D.C. (1951).

2. Shubik, P., and Hartwell, J. L., "Survey of Compounds Which have been Tested for Carcinogenic Activity." Pub. Health Service Publ. No. 149, Supp. I, U.S. Govt. Printing Office, Washington, D.C. (1957).

3. Wedgewood, P., and Cooper, R. L., "The Detection and Determination of Traces of Polynuclear Hydrocarbons in Industrial Effluents and Sewage." Analyst (Brit.). 79, 163 (1954).

4. Shimkin, M. B., Koe, B. K., and Zechmeister, L., "Instance of Occurrence of Carcinogenic Substances in Certain Barnacles." Science, 113, 650 (1959).

5. Hueper, W. C., and Ruchhoft, C. C., "Carcinogenic Studies on Adsorbates of Industrially Polluted Raw and Finished Water Supplies." Arch. Ind. Hyg. and Occupational Med., 9, 488 (1954).

6. Ruchhoft, C. C., Middleton, F. M., Braus, H., and Rosen, A. A., "Taste and Odor Producing Components in Refinery Gravity Oil Separator Effluents." Ind. and Eng. Chem., 46, 284 (1954).

7. Borneff, J., and Knerr, R., "Carcinogenic Substances in Water and Soil," Arch. Hyg. u. Bakteriol. (Germany), 143, 890 (1959); 143, 405 (1959); 144, 81 (1960).

8. Borneff, J., and Fischer, R., "Carcinogenic Substances in Water and Soil." Arch. Hyg. u. Bakteriol. (Germany), 145, 241 (1961); 146, 183 (1962).

9. Borneff, J., "Carcinogenic Substances in Water and Soil." Arch Hyg. u. Bakteriol, (Germany), 144, 249 (1960).

10. Hueper, W. C., "Cancer Hazards from Natural and Artificial Water Pollutants." Proc. Conf. on Physiological Aspects of Water Quality, Washington, D. C. (1960).

11. Malaney, G. W., Lutin, P. A., Cibulka, J. J., and Hickerson, L. H. "Resistance of Carcinogenic Organic Compounds to Oxidation by Activated Sludge," J. Water Pollution Control Federation, Vol. 39, p. 7020, (1967).

12. Clayson, D. B., Chemical Carcinogenesis, Little, Brown and Company, Boston, (1962).

13. Lijinsky, W., Domsky, I. I., Ward, J., "A Procedure for the detection of Carcinogenic Polynuclear Hydrocarbons in Petroleum Waxes Using Electron Capture." Journal of Gas Chromatography, May, (1964), p. 152.

14. Dawson, H. J., "Detection of Traces of Polynuclear Aromatics in Hydrocarbons by Gas Chromatography," Analytical Chemistry, Vol. 36, p. 1852, (1964).

15. Chakraborty, B. B., Long, Ronald, "Gas Chromatographic Analysis of Polycyclic Aromatic Hydrocarbons in Soot Samples," Environmental Science and Technology, Vol. 1, p. 828, (1967).

16. Lijinsky, W., Mason, G., "Programmed Temperature Gas Chromatography of Tars and Pyrolysates of Naphthalenes at Moderate Temperatures." Journal of Gas Chromatography, September, (1963), p. 12.

17. American Public Health Association, Standard Methods for the Examination of Water and Wastewater, New York, American Public Health Association, 12th Edition, (1965).

18. Stanley, T. W., Meeker, J. E., Morgan, M. J., "Extraction of Organics from Airborne Particulates," Environmental Science and Technology, Vol. 1, p. 927, (1967).

19. McNair, H. M., Bornelli, E. J., Basic Gas Chromatography. Varian Aerograph: Walnut Creek: Calif., (1968).

IX. APPENDICES

APPENDIX A

Benzo-a-pyrene Extraction

When extracting from the liquid samples (effluent, supernatant and filtrate) add 100 ml of the sample to a flask containing 100 ml of benzene. Attach the flask to a medium size Soxhlet apparatus. Heat the mixture, containing four glass beads, until the water begins to boil. Extract the Benzo-a-pyrene for six hours at the rate of 12 cycles per hour. Transfer the two-phase mixture to a separatory funnel and decant the benzene. Evaporate the extract to 10 ml. Inject a one microliter sample into the gas chromatograph. Use the following formula to determine the total weight of Benzo-a-pyrene present in the extract.

$$\text{Total Weight BaP} = \frac{\text{ng detected}}{\text{ul injected}} \text{ X volume of extract (ul)}$$

To extract Benzo-a-pyrene from activated sludge, obtain a sample of mixed liquor from the aeration tank or batch study and allow it to settle for one hour. Decant the supernatant and filter the settled sludge through a Buchner funnel with Whatman No. 40 filter paper. Peel the filter cake from the funnel and cut with scissors into four equal pieces. Place these slices in an extraction flask and add 100 ml benzene. Extract the Benzo-a-pyrene for six hours at the rate of 12 cycles per hour. Remove the extract and evaporate to 10 ml. Inject a one microliter sample into the gas chromatograph.

Use the above equation to determine the total weight of Benzo-a-pyrene present in the extract.

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THE REMOVAL OF CARCINOGENIC POLYCYCLIC AROMATIC HYDROCARBONS BY ACTIVATED SLUDGE

by

William Jewett McCaw III

Abstract

Benzo-a-pyrene, a carcinogenic polycyclic aromatic hydrocarbon, was injected into the aeration tank of an activated sludge pilot plant in an investigation to determine the ability of activated sludge to effectively treat this compound.

The method employed to segregate Benzo-a-pyrene from other organics was a modified Soxhlet technique followed by gas chromatographic analysis. The amount of Benzo-a-pyrene detected in the effluent, supernatant, sludge filtrate and sludge cake was tabulated to determine the mechanism of removal.

The experimental results indicated that adsorption appeared to be the primary mechanism of removal. The concentration of Benzo-a-pyrene in the effluent collected from the pilot plant was below the level of detection by gas chromatography.

On the basis of the experimental evidence activated sludge provides satisfactory treatment for wastes containing low concentrations (i.e. 0.5 mg/l) of Benzo-a-pyrene, a carcinogenic polycyclic aromatic hydrocarbon.