FATE OF SELECTED PRIORITY POLLUTANTS IN BATCH BIOLOGICAL REACTORS

By

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CHAPTER I

INTRODUCTION

Consumer products containing poisonous ingredients are common household items in a majority of American homes. Safety procedures need to be improved, but the responsible adult is becoming more educated to the dangers of these toxic chemicals. As a result, directions are being followed more closely and safety precautions are noted on the labels which warn the user of potential health hazards.

Medical research data often suggests that certain chemicals may be responsible for a variety of cancers, birth defects, respiratory problems, nervous disorders, and other serious health problems. Toxicologists and environmentalists are producing scientific evidence that not only is aquatic life affected and killed by negligent chemical use and/or disposal, but the environment as a whole is endangered. The damage may be an esthetic problem, but the probability exists that serious health problems occur when people are exposed to polluted water and a chemically endangered environment. As a result, a growing awareness of toxic chemicals and their effects on their environment and personal health is developing.

The danger of toxic substances is being imprinted daily on the minds of the American people. Apprehension and anger occur when a toxic chemical is detected in recreational areas resulting in the loss of leisure activities such as swimming or fishing. Fear is experienced

when a toxic substance is detected in municipal water reservoirs or underground water reserves. Water treatment plants do not have the capability to remove certain toxic chemicals, and the damage to underground water reserves may be so extensive or expensive to treat that the water is no longer available for safe consumption.

The U.S. Congress passed the Basic Federal Water Pollution Control Act over 25 years ago, but chemical pollution continues to be a national problem. Waste chemicals are disposed directly into natural waterways or into municipal sewer systems without pre-treatment. The Public Owned Treatment Works (POTWs) receives the wastewater containing toxic substances and it is not known what effect, positive or negative, the plant's processes have on the specific toxic pollutant. The POTWs and the final receiving waters must be protected from toxic substances, and it is imperative that extensive research be conducted in order to determine the most successful treatment for specific toxic compounds. Stripping properties, chemical reactions, biodegradability, bioaccumulation, intermediate by-products, and synergistic effects should be thoroughly investigated before recommending treatment or design.

This research will explore the ultimate fate of selected toxic priority pollutants utilizing a "batch" system. The fate of selected pollutants will be analyzed in four areas:

1. Can a toxic priority pollutant be biologically oxidized?
2. Is the pollutant being stripped?
3. Are volatile by-products produced during biodegradation?
4. Are base/neutral and acid extractable by-products being produced by the microorganisms?

CHAPTER II

LITERATURE REVIEW

A. Legislation

Due to the demand of society on organic chemical products, there is an increasing level of these substances being introduced into the aquatic environment. While "it is not possible to guarantee a risk free society, nor is a risk free society necessarily the best society, it is often necessary to accept the risks of chemicals . . . when the benefits warrant their use" (1, p. 1864). The United States Congress has recognized that chemical risks must be monitored and have passed numerous laws to control the discharge of toxic substances into the environment.

The Basic Federal Water Pollution Control Act (PL 84-660) was approved July 9, 1956. It was later amended by the 1961 Federal Water Pollution Control Act Amendments (PL 87-88) and by the Water Quality Act of 1965 (PL 89-234). This amended act empowers the federal government to protect the rights of the states in preventing and controlling water pollution. The act provides support for technical research in regard to wastewater treatment along with financial aid to the state and municipalities in connection with the prevention and control of water pollution (2).

The Clean Water Restoration Act of 1966 (PL 89-753) called for a comprehensive study of the effects of pollution, including sedimentation, in the estuaries and a consideration of use trends which will influence

future pollution problems. The act ordered the assembly, coordination, and organization of all existing data, and the identification of problems in need of study (3).

In 1972, Public Law 92-500 was added to the Federal Water Pollution Control Act. This amendment required that by 1983, secondary treatment --the absolute minimum in wastewater treatment--is to be accomplished by the Best Practicable Waste Treatment Technology (BPWTT). The general approaches to the BPWTT are treatment and discharge to receiving waters, treatment and reuse, and land application. If the BPWTT does not meet existing water quality standards, then the act requires advanced waste treatment, temporary storage of treatment effluent, and facilities for abating pollution from combined sewer overflows (4).

The 1976 Toxic Substances Control Act (PL 94-469) was another attempt by Congress to protect human health and the environment by requiring testing and necessary use restriction on certain chemical substances. The act states that it is the responsibility of

> manufacturers to develop adequate data on the health and environmental effects of chemical substances, the chemical substances presenting health and environmental risks should be regulated, and that regulation should not create unnecessary barriers to technological innovation (5, p. 3).

It also gives the Environmental Protection Agency (EPA) administrator the authority to use other laws such as the Federal Water Pollution Control Act, the Clean Water Act, along with others which would protect against unreasonable risks (6).

The Federal Water Pollution Control Act of 1972 was amended again by the Clean Water Act of 1977 (PL 95-217) which gave the EPA the authority to establish and enforce pretreatment standards. At this point several environmental groups (Natural Resources Defense Council,

Environmental Defense Fund, National Audubon Society, etc.) sued the EPA for its unaggressive implementation of the Federal Water Pollution Control Act (7). On June 7, 1978, a court settlement was reached which established a regulatory mandate for the development and management of a control program. The 1978 court decision later became known as the "EPA Consent Decree" which required the EPA to develop a list of specific substances to be controlled. The discharge limitations of these substances, based on Best Available Technology (BAT), and methods for quantitating these pollutants is to be promulgated (8).

B. Priority Pollutants

1. Significance

The American Chemical Society has reported that over 4 million chemical substances are now in existence with the number increasing at the rate of 1000 per year. Because of industrial and technological demands, over sixty-three thousand of these compounds pose a potential threat to the natural environment (1). Of equal concern to environmentalists is the enormous production rate of specific compounds. For example, in 1965, approximately 778 million pounds of acrylonitrile was produced with a value of over $48 million. Acrylonitrile has been proved to be highly toxic to aquatic life; nevertheless, most of the industrial plants producing or using this toxic substance had only primary or no waste treatment (9).

As data have continued to accumulate regarding the environmental effects of toxic substances, the news media have educated and informed the public about the results of negligent chemical use. Fish have been

poisoned by phthalates, cattle by polybrominated biphenyls, horses by dioxins, forest seedlings by fluorides, oysters by chloramines, and wheat crops by zinc. Humans, on the other hand, have experienced deleterious effects on the nervous system from lead, cyanide, and kepone; on the liver from carbon tetrachloride; on the kidneys from ethylene glycol; on the lungs from asbestos and beryllium; suspected reproductive disorders from dioxins; skin, lung, and liver cancer from arsenic; leukemia and blood disorders from benzene; and bladder cancer from benzidine (1).

Halogenated hydrocarbons, which have been detected in many municipal drinking waters, are of particular interest because of their potential as a carcinogen. Phenols have been found toxic to fish and have an adverse effect on fish and water taste. Phenol can be detected in water by its odor and taste in concentrations as low as 0.01 to 0.1 mg/ℓ. Furthermore, the reaction of phenol and chlorine in finished drinking water results in chlorophenols which have a stronger odor and taste, as much as 100 times that of phenol (10).

A 1977 EPA study of surface waters involved 15 of the nation's major drainage basins. The investigation included 204 water samples and a wide variety of toxic chemicals was found to be present in 12 of the 15 basins (1). Several processes may occur when toxics enter natural waters: biodegradation, bioaccumulation, volatilization, chemical reactions, and/or synergistic action. Intermediates produced by these processes may increase or decrease the total toxic effect (1). Many of the substances are biologically oxidized very slowly or are biologically recalcitrant, persisting in the water and becoming adsorbed in the sediments (11).

With new developments in analytical techniques, there has been a growing awareness of toxics in the air and water. A GC/MS method has

been developed for the rapid and simultaneous determination of halogenated hydrocarbons otherwise obscured by more abundant hydrocarbons. Atomic absorption and a variety of chromatography techniques have enabled researchers to detect substances in water to parts per billion and in some cases to parts per trillion (12). It has been estimated that all 4 million compounds known to man could be detected in a sample of drinking water at concentrations of one part per trillion or higher (5).

Epidemiological studies have suggested that the 25 percent death rate due to cancer is caused by environmental factors. Dr. Phillip Handler, President of the National Academy of Sciences, has pointed out that if this atrocious death rate is due to an exposure of some environmental factor, it must exist at large concentrations and should be easily identifiable. This has evoked Handler to ask, "Why have we failed to detect a carcinogen on so vast a scale?" (1, p. 1863). He has proposed the following hypothesis. A chemical agent responsible for cancer may be a normal metabolic product of the body. A prime candidate could be the superoxide ion, O_2^-, which results from the one electron reduction of molecular oxygen. The superoxide radical degrades to a hydroxyl radical which has long been considered to be the intermediate that attacks DNA. Handler's research has determined that the superoxide formation is a function of oxygen tension; the higher the tension, the greater formation of the superoxide ion. It is possible that cancer may be the price animal life pays for living in an oxygen atmosphere (1).

2. Identification

Before 1972, all discharge permits were granted according to the level of BOD_5, pH, and TSS. In a few industrial cases COD was used and

sometimes a specific compound was included. In 1977, a Congressional commission reported that further reduction of BOD standards produced very little effect on receiving water quality. COD was significant only when it was reported in terms of the environmental impact of the chemical constituents that made up the residual COD (8).

Parker (13) has suggested that parameters other than BOD_5, COD, and TOC could be used to measure biological response. The monitoring of ATP and/or the enzyme dehydrogenase may be a more effective surrogate measurement. His research utilized the concept of oxygen uptake and ATP measurement in order to quantify the strength of toxics in wastewater. However, Parker has admitted that there are two problems associated with his research:

1. While the BOD does serve as an indication of biodegradability, it is not a sensitive measurement of the degradability of compounds that are not the major source of substrate.

2. Pure cultures are required.

The priority pollutant policy of the EPA was ushered into action because of the realization that toxic substances must be controlled and that surrogate measurements would not suffice. The courts, therefore, charged the EPA to accomplish the following:

1. Publish a list of toxic pollutants for which effluent limitations and guidelines would be required. Sixty-five compounds and compound classes were recommended as an initial starting list.

2. Establish effluent limitations of all compound classes which require BAT.

3. Establish new source performance standards and pretreatment standards for all 21 industrial categories (1) (see Table 1).

TABLE I

POINT-SOURCE CATEGORIES

Timber products processing
Steam electric power plants
Leather tanning and finishing
Iron and steel manufacturing
Petroleum refining
Inorganic chemicals manufacturing
Textile mills
Organic chemicals manufacturing
Nonferrous metals manufacturing
Paving and roofing materials
Paint and ink formulation and printing
Paint and ink
Printing and publishing
Soap and detergent manufacturing
Auto and other laundries
Plastic and synthetic materials
Manufacturing
Pulp and paperboard mills and converted
paper products
Rubber processing
Miscellaneous chemicals
Adhesives
Gum and wood chemicals
Pesticides
Pharmaceuticals
Explosives manufacturing
Machinery and mechanical products manufacturing
Aluminum forming
Battery manufacturing
Coil coating
Copper forming
Foundries
Plastics processing
Porcelain enamel
Mechanical products
Electrical and electronic components
Electroplating
Ore mining and dressing
Coal mining

The immediate problem was to establish sampling techniques, storage, and analysis methods for the priority pollutant list. Because of the thousands of potential compounds and the financial cost of analysis, the EPA established priorities to select a representative compound from each compound class. The following criteria were used:

1. All specifically named compounds on the original list were included.
2. Frequency of a compound detected in the natural waters.
3. Chemical production data were employed.

As a result, 129 toxic priority pollutants were named and now are recognized as the EPA's priority pollutants. The list includes 31 purgeable organics, 46 base/neutral extractable organics, 11 acid extractable organics, 26 pesticides, 13 metals, and 2 miscellaneous (14).

With the identification of 129 toxic substances, the EPA felt that it was making rapid progress toward control and enforcement. However, several problems were encountered before the program was initiated. GC/MS was the only analytical technique which could detect substances in the desired ppm or ppb range. This method created severe financial problems since it required expensive equipment and highly trained professionals to operate and interpret the data. Also, no data existed on the presence of priority pollutants in industrial wastewater; and there were no established guidelines to be used in determining effluent limitations for each pollutant. No one knew whether existing wastewater treatment plants had any positive or negative effects on the pollutants or if the toxic substance had a harmful effect on the plant itself. As a result, contractors were assigned the task of collecting data on the occurrence and treatability of the pollutants as well as producing cost estimates

(8). Much of the contractual data have been completed and the GC/MS analysis has been suggested for many of the organics, but gas and liquid chromatography equipped with special detectors have been determined to be acceptable procedures (15).

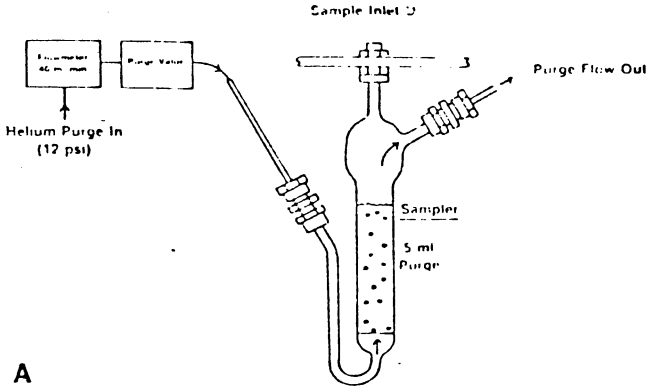
3. Analysis Procedures

Two major techniques have been employed to separate and concentrate toxic organics from the wastewater. Volatile compounds can be collected from the solution by bubbling an inert gas through the solution, while the other compounds undergo a liquid/liquid extraction process. After collection the substances are analyzed by gas-liquid chromatography or GC/MS (15).

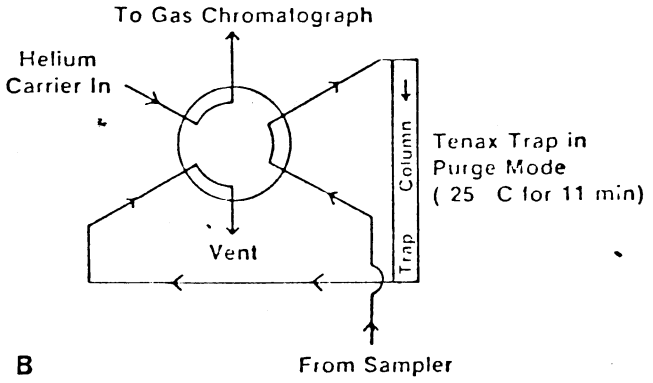
The established method for concentrating volatiles is called the "purge and trap" method (Figure 1) which is based on the work done by Bellar and Lichgenberg (16). Helium or nitrogen are bubbled through the solution, driving the dissolved volatiles into the gaseous phase. The resulting gas is passed through a trap containing tenax, silica gel, or charcoal, which adsorbs the compounds. Once adsorbed, the inert gas is backflushed through the trap as the trap is heated rapidly to 180°C. The volatiles are driven off the trap to the GC column where it is analyzed by an isothermal or programmed technique (15).

Liquid/liquid extraction (Figure 2) involves one to two liters of water sample extracted with three 30 mℓ aliquots of methylene chloride. The organic fraction is separated from the water by a separatory funnel and concentrated by evaporation using a Kuderna-Danish apparatus. The organic solution is evaporated to a specific volume and analyzed by chromatography (15). Complete analytical procedures along with sampling

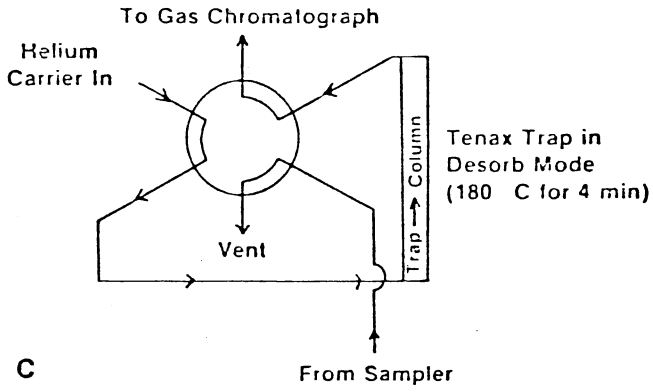
Figure 1. Purge-and-Trap Apparatus. A: Sampler Tube; B: Operation in Purge Mode; C: Operation in Desorb Mode (16)


Sample Inlet
Purge Flow Out
Helium Purge In
(12 psi)
Sampler
5 ml
Purge

A

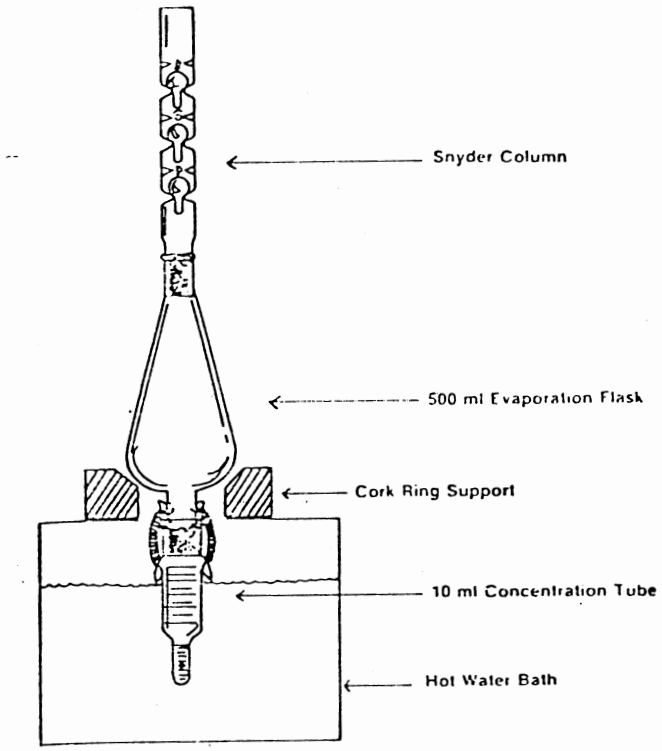

To Gas Chromatograph
Helium
Carrier In
Column
Trap
Tenax Trap in
Purge Mode
(25 C for 11 min)
Vent
From Sampler

B


To Gas Chromatograph
Helium
Carrier In
Trap
Column
Tenax Trap in
Desorb Mode
(180 C for 4 min)
Vent
From Sampler

C

Figure 2. Kuderna-Danish Concentrator (16)


Snyder Column
500 ml Evaporation Flask
Cork Ring Support
10 ml Concentration Tube
Hot Water Bath

storage, quality control, etc. are found in the December 3, 1979 Federal Register (17).

4. Industrial Treatment

There is growing evidence that the POTWs biological treatment process has removal capabilities for many of the priority pollutants. However, the most economical, effective, and common sense approach to treatment has been found to be at the industrial point source. Although good housekeeping, controlling leaks and spills, is not necessarily a treatment, it is absolutely essential in pollution control. As an example, one pound of an organic dissolved in one million gallons of water is 0.12 ppm, which is about 10 times the needed control level. Because the pollutants are in their most isolated and concentrated form, there is a variety of processes that can be applied for effective treatment (8).

Distillation can be utilized to separate volatile components from the waste stream. The volatile compounds are carried upward and out of the column, leaving the higher boiling-point materials behind. While evaporation is an expensive volatilization method, it can be used as a desalting process, but the remaining residue will need stabilization and final disposal. Solvent extraction is a popular process, particularly when a valuable substance is involved and when the substances has low solubility in water. Not only can the substance be separated and reused, but the solvent can be recovered and recycled (8).

Adsorption by activated carbon and resins is more applicable at the plant site rather than as a tertiary treatment at the POTWs. It is also possible to recover the absorbed materials and at the same time recondition the activated carbon for future use. Ion exchange resins are

usually designed for specific metals, but they can be used to polish some chemical coagulation effluents. Reverse osmosis is a membrane separation technique used for desalting and removing dissolved solids from specific industrial wastewaters. Chemical oxidation is more attractive for a specific waste and therefore is used on a more limited scale (8).

Biological oxidation is probably the most cost effective and therefore the most popular process in industrial treatment. Industry can utilize biooxidation as the only removal operation or may choose it as the last phase of its pollution management. Biological treatment is especially useful at the point source, because microorganisms can be acclimated to a specific toxic priority pollutant which otherwise may cause a toxic shock to the biological treatment process of a POTW (8).

Wet oxidation is a process using water, molecular oxygen, and suspended organics in a relatively high pressure reactor. This process oxidizes the dissolved organics to carbon dioxide and water. Any contaminants that remain tend to stay in the aqueous phase, thereby reducing air pollution to a minimum. Also, if the waste stream contains reusable chemicals, they can be recovered along with part of the thermal energy (18).

5. Research Methodology

Bunch and Chambers' (19) biodegradability test for organic compounds was conducted in a 250 mℓ erlenmeyer flask containing 100 mℓ of inoculated medium. Ten mℓ of settled sewage was used to seed 90 mℓ of BOD dilution water containing 5.0 mg of yeast extract and 2.0 mg of the specific test compound. The flasks were incubated at room temperature under static conditions. Subcultures were made with fresh medium for three consecutive

weeks. Biodegradability was measured by observing the extent of turbidity in each culture. The undegraded portion of each test compound was determined by different chemical analysis. The test also provided an indication of the time required for acclimation along with producing rapid biodegradable results. The method is a simple screeding procedure for comparing the degradability of organic compounds and should not be considered as the absolute test for biodegradability.

Tabak et al. (11) used the test of Bunch and Chambers to study the biodegradability of 114 toxic organics. The test was modified to include water insoluble and/or volatile compounds that were included on the EPA's toxic priority pollutant list. Biodegradability was observed by using TOC and DOC measurements rather than turbidity, and chemical analysis of remaining test compounds was accomplished using gas chromatography. It must be remembered that this test is still considered a screening procedure; and shaker flask techniques, aerated batch studies, and/or complete mix continuous flow activated systems should be used to obtain further data on the biodegradability of the tested organics.

Kincannon et al. (20) and Medley (21) conducted biodegradability studies using sealed complete mix continuous flow activated sludge reactors. The activated sludge systems were operated at mean cell residence times, θ_c, of two, four, and six days. Influent, effluent, mixed liquor suspended solids, and off-gas samples were collected and analyzed over a sixty-day period. Biodegradability was observed by BOD_5, TOC, and COD measurement, and specific toxic compound analysis was accomplished using GC methods as published in the Federal Register (17). Batch reactor studies were also completed using a three-day mean cell residence time. The top of the reactor was fitted with a ground glass stopper with inlet

and exit ports to analyze for any volatile compound or by-products produced during the experiment. Biodegradability was observed by measuring TOC and specific compounds by gas chromatography. Priority pollutants were also exposed to different concentrations of ozone to determine if chemical oxidation has an effect on their biodegradability. The initial concentration of the pollutants was 100 mg/ℓ and after treatment with ozone was fed to a complete mix continuous flow reactor. Biodegradability was measured in terms of ultimate BOD to COD ratios along with ultimate BOD to TOC ratios. The two ratios were found to be very similar (20).

Liu et al. (22) studied biodegradation using an enclosed cyclone fermentor with inlet and outlet ports to collect any volatile compounds. A basic growth medium was used along with the emulsion stabilizing agent, sodium ligninsulfonate. The test compound was added to the medium and seeded with samples from lake sediments, soil, and activated sludge from a POTW. With this variety of inoculants, metabolism and cometabolism studies were conducted under both aerobic and anerobic conditions. The microbial response was measured by estimating bacteria numbers from a surface plating technique. The biodegradation of the specific compound was followed by gas chromatography.

Pitter (23) used a batch-type reactor to study the biodegradation of 123 organic compounds. The specific compound being tested was added to 1000 to 1500 mℓ of a biological medium with the test compound being the sole source of carbon. The initial COD was adjusted to 200 mg/ℓ and then seeded with a volume of activated sludge that would equal 100 mg/ℓ. When a volatile substance was suspected, an additional test was run without inoculum to distinguish between volatilization and biodegradation of

the compound. The batch reactors were placed in a dark room and aerated with a magnetic stirrer. Biodegradation was then followed by collecting 50 mℓ samples and conducting COD analysis. The experiment for each compound was carried out until no decrease in COD was observed. At this point the rate of biodegradation could be calculated in terms of mg of COD per gram per hour.

Randall and Knopp (24) have conducted studies on the detoxification of priority pollutants by the wet oxidation process. Solutions of 5 to 12 g/ℓ were prepared and one liter of the solution was injected into the autoclave. Compressed air was then added to the autoclave and the contents were heated to the desired temperature. Experiments were conducted at 150°C, 275°C, 320°C, and 275°C with the addition of copper II ion used as a catalyst. After cooling, samples of the liquid were analyzed by gas chromatography. Toxicity experiments were also conducted with the liquid products on Daphnia magna.

C. Specific Compound Classes

1. Phenols

The ultimate fate of phenol in industrial wastewaters is controlled by biological oxidation. Photooxidation, metal-catalyzed oxidation, and possibly volatilization play a minor role in the compound's removal from wastewater. The biological oxidation of phenol was first observed by Happold and Key (25). Several types of microorganisms have been identified as phenol degrading organisms including soil microorganisms, pseudomonas, Bacillus, and several strains of yeast. The metabolic pathway of the microbial degradation was well established by Buswell (26). Baird et al. (27) reported that concentrations of 1 mg/ℓ to 10 mg/ℓ of phenol

was biodegraded beyond the limits of detection. However, at 10 mg/ℓ, phenol began inhibiting oxygen uptake and at 100 mg/ℓ, only 20 percent of the phenol was removed. McKinney et al. (28) reported that by using long acclimation periods, activated sludge could metabolize up to 500 mg/ℓ of phenol without toxic effects.

The volatilization of phenol must be considered since its vapor-liquid distribution ratio has been reported as 1.8 at atmospheric pressure by Hakuta (29). However, phenol is generally considered to be a nonvolatile substance since it has a low vapor pressure and a high solubility in water. If volatilization of phenol in wastewater does prove to be an effective removal technique, then it is generally felt that it would undergo rapid photooxidation in the atmosphere. Perelshtein and Kaplin (30) conducted experiments with natural sunlight to demonstrate the photooxidation of phenol in aqueous solutions.

The fate of the chlorophenols in wastewater is undetermined but biological oxidation may be the best approach to treatability. Aly and Faust (31) conducted degradation experiments with 2,4-dichlorophenol using natural lake water. An initial concentration of 100 µg/ℓ was completely biodegraded after 9 days, and concentrations of 500 and 1000 µg/ℓ were 97.5 percent eliminated in 30 days. Soil microorganisms also seemed to be very effective in degrading 2,4-dichlorophenol (32).

The treatability of the nitrophenols is also an area of concern, because oxidation, volatilization, hydrolysis, and biological oxidation all seem to be ineffective in treating nitrophenols in wastewater. Howard et al. (33) reported that biological oxidation is prevented in aquatic environments because of the nitrophenol's ability to uncouple the oxidative phosphorylation process.

Results from Tabak's (11) experiments indicated a 100 percent degradation with a two-week acclimation period for phenol, 2-chlorophenol, 2,4-dichlorophenol, 2,4,6-trichlorophenol, 2,4-dimethylphenol, p-chloro-m-cresol, 2-nitrophenol, 4-nitrophenol, and 2,4-dinitrophenol. Pentachlorophenol also was 100 percent degraded after the fourth week of acclimation, but 4,6-dinitro-o-cresol was not biodegraded to any extent after the fourth week.

Data from the complete mix continuous flow reactors of Kincannon et al. (20) and Medley (21) revealed a significant decrease in BOD_5, TOC, and COD for phenol, 2,4-dichlorophenol, and 2,4-dinitrophenol. GC results indicated a 95 percent removal for 2,4-dichlorophenol, while phenol and 2,4-dinitrophenol had a removal of 99.9 and 99.3 percent, respectively. Kincannon et al.'s research demonstrated that all removal was accomplished by biodegradation. Batch studies conducted by Kincannon et al. indicated a 100 percent removal of phenol and a 63 percent removal of 2,4-dinitrophenol. Their research also showed that ozone had a definite impact on the biodegradation of 2,4-dinitrophenol. The ultimate BOD/TOC ratios increased from 0.1 to 0.4 when the ozone concentration was increased from 10 to 50 mg/ℓ.

Randall and Knopp's (24) wet oxidation experiments also revealed a high removal efficiency with phenols. At 275°C, a removal efficiency greater than 95 percent for 2-chlorophenol, 2,4-dimethylphenol, 4-nitrophenol, and phenon was reported. Pentachlorophenol demonstrated an 82 percent removal, but was increased to 97.3 percent with the addition of the copper catalyst. All phenols exhibited approximately 100 percent removal when the temperature was increased to 320°C.

2. Aromatics

The biodegradation of benzene does not appear to be a successful removal process. However, Gibson et al. (34) showed that Pseudomonas putida could metabolize benzene as its sole carbon source. Walker and Colwell (35) demonstrated the degradation of benzene with the presence of cometabolites. Although data are limited, volatilization seems to be the best removal method. Mackay and Leinonen (36) showed that the volatilization of benzene was not significantly affected by changes in temperature. They also suggested that the evaporation rate would be greater in turbulent waters than in quiescent waters.

Alexander and Lustigman (37) demonstrated that the addition of chlorine to the benzene ring reduced the biological oxidation by microorganisms. Ware and West (38) reported that the more halogenated a compound becomes, the more resistant it is to biodegradation. Chlorobenzene and the dichlorobenzenes may undergo biological oxidation after long acclimation periods, but removal by volatilization appeared to be the best hope for removal (32). Thom and Agg (39) reported the biodegradation of chlorobenzene in a biological sewage treatment system, but no removal of 1,2-dichlorobenzene was noted.

Tabak (11) showed that the di-, tri-, and hexachlorobenzenes were subject to partial biodegradation after the second week of acclimation, but were then reduced to less than 30 percent after the fourth week. Chlorobenzene was removed after the second week at a rate of 77 percent and was 100 percent degraded after the fourth week. Benzene, nitrobenzene, ethylbenzene, and toluene were all 100 percent biologically oxidized after the second week of adaptation. Tabak suggested that the gradual loss in biological activity with the multiple chlorinated

benzenes was probably due to loss of synergistic activity or the accumulation of toxic metabolic by-products. Tabak's experiments also revealed that volatilization does not play a vital role in the removal of these aromatics from wastewater.

Kincannon et al. (20) also studied the treatability of benzene, nitrobenzene, 1,2-dichlorobenzene, and 1,3-dichlorobenzene in complete mix continuous flow reactors. The BOD_5, TOC, and COD were reduced significantly for all three values of mean cell residence times with the exception of the two-day θ_c for nitrobenzene. The COD effluent for nitrobenzene measured 105 mg/ℓ for the two-day θ_c. The removal percentages for all four aromatics were greater than 97.8 percent. Kincannon et al. demonstrated that the majority of the removal was accomplished by biological oxidation. Off-gas measurements indicated only 16 percent of the benzene and 22 percent of 1,2-dichlorobenzene were stripped from the wastewater. A removal process for 1,3-dichlorobenzene was not determined. The compound was not detected in the off-gas analysis, but two unknown volatiles were noted. It was suggested that 1,3-dichlorobenzene may have been converted to these intermediates and stripped from the reactor.

3. Halogenated Aliphatics

Pearson and McConnell (40) reported that the majority of the literature that is available regarding the biodegradation of halogenated aliphatics indicates that low molecular weight chlorinated hydrocarbons are not oxidized by microorganisms. Thom and Agg (39) suggested that several snythetic chemicals could be removed by biological sewage treatment if acclimation is achieved; however, no research data were provided. Their

list contained dichloromethane, 1,2-dichloroethane, 1,1,1-trichloroethane, 1,1,2,2-tetrachloroethane, 1,2-dichloropropane, along with other halogenated hydrocarbons. Altmann and Lawlor (41) reported that 1,2-dichloropropane may not be biodegraded by organisms in the wastewater but can be utilized by several soil microorganisms.

The volatilization process may be effective in removing halogenated hydrocarbons. Dilling et al. (42) discussed the experimental half-life of these compounds as they were being stirred at 200 rpm. The half-life for dichloroethane and 1,1,1-trichloroethane was demonstrated to be approximately 20 minutes, while the half-life for 1,1,2,2-tetrachloroethane, 1,2-dichloropropane, 1,2,3-trichloropropane, and 1,2,2,3-tetrachloroethane were all approximately 50 minutes. Gabel and Ponnamperuma (43) have suggested that hydrolysis may be the most important process in removing 1,2-dichloropropane.

Tabak's (11) biodegradability studies on chlorinated hydrocarbons indicated a low to moderate biodegradability with the exception of hexachloroethane, which was 100 percent degraded. This also seemed to be an exception to the general rule of Ware and West (38). Dichloromethane also was 100 percent degraded, but all other compounds were reported to be moderately biodegraded after the fourth week of acclimation. Tabak's volatile control experiments revealed that stripping would not play a major role in the removal of these compounds from wastewater. The stripping of most compounds was reported less than 10 percent, with the exception of the chloroethylenes which was approximately 30 percent.

Kincann et al. (20) and Medley's (21) research with continuous flow systems indicated treatability in terms of BOD_5, TOC, and COD for 1,1,2,2-tetrachloroethane, 1,2-dichloromethane, 1,2-dichloroethane, 1,1,1-tri-

chloroethane, and 1,2-dichloropropane. The removal of each specific compound was shown to be 99 percent with the exception of tetrachloroethane which was 94.5 percent. The removal process for dichloromethane was shown to be predominantly by biological oxidation at 93 percent. However, all other chlorinated compounds tested indicated a volatilization level of 100 percent. Batch studies with these compounds were incomplete, but it was clearly demonstrated that small amounts of ozone made 1,2-dichloropropane rapidly biodegradable. The ultimate BOD/TOC ratio increased from 0.1 to 0.6 when the ozone concentration was increased from 0.0 to 0.2 mg/ℓ.

4. Nitrogen Compounds

The treatability of acrylonitrile in wastewater has been demonstrated by Lank and Wallace (9) during the anaerobic digestion process of POTWs. Slave et al. (32) reported an acclimated sludge capable of biologically oxidizing up to 35 percent of the acrylonitrile. The relatively high vapor pressure suggests that this compound may be treated by volatilization. Broderius (44) reported that hydrogen cyanide followed first order stripping, and it has been suggested that since acrylonitrile has a similar structure, it may also undergo significant volatilization in wastewater (32).

Tabak (11) reported a 100 percent biodegradation of acrylonitrile with the first week of acclimation. Volatilization data were not reported.

Kincannon et al. (20) and Medley's (21) research with continuous flow reactors indicated good removal in terms of BOD_5, TOC, and COD when acrylonitrile was the test compound. The COD was reduced from 480 mg/ℓ

to 74 mg/ℓ and the BOD_5 and TOC were reduced to 4.0 mg/ℓ and 11 mg/ℓ, respectively. No stripping of the acrylonitrile was observed with the continuous flow units, but the data from the batch reactors indicated 25 percent stripping. Both systems produced 100 percent removal from the wastewater. The ozonation experiment revealed a very negative impact on the biodegradation of acrylonitrile. The ultimate BOD/TOC ratio was reduced from 2.0 to 0.8 with the increase in ozone concentration.

Randall and Knopp's (24) wet oxidation experiments also demonstrated removal. Using an initial concentration of 8.0 mg/ℓ, 99 percent of the acrylonitrile was destroyed at 275°C; 99.5 percent was destroyed at 275°C with copper catalyst; and 99.9 percent was destroyed when the temperature was increased to 320°C.

D. Kinetics and Design

Beltrame et al. (45) used a continuous flow reactor with cell recycle to study the kinetics of phenol biodegradation. He reported no substrate inhibition, and concluded that kinetics of phenol degradation with cell recycle was similar to other well known aerated waste treatment.

Shamat and Maier (46) used continuous flow units and batch reactors to study the degradation of chlorinated aromatics. Shamat's data from batch reactors demonstrated a linear substrate removal from which he concluded that existing mathematical models are adequate for analyzing test data. Also, the growth parameters u_m and K_s were shown to be similar in both the continuous flow and batch reactors, which indicated that the same kinetic relationships can be used for both systems.

Current kinetic design models are derived from the same substrate balance. Stover and Kincannon (47) suggested that stripping and adsorption factors should be included in the mass balance. Adsorption data were collected on several compounds, but there was not enough evidence to support adsorption as a major removal process. However, it was suggested that pesticides on the toxic priority list, which are known to have high adsorbing qualities, could be a contributing factor to overall design. Kincannon, Stover, and Chung (48) used a continuous flow system without microorganisms to demonstrate that stripping could be a major removal process. 1,2-Dichloropropane, dichloromethane, benzene, and 1,2-dichlorobenzene were reported to be 100 percent removed from their nonbiological system. Stover and Kincannon (47) used the identical compounds in the continuous flow reactors with the addition of acclimated microorganisms to show that stripping properties are not necessarily the same with biological treated systems. 1,2-Dichloropropane was the only compound of the four which demonstrated 100 percent volatilization. It was suggested that volatilization experiments must be done on biological reactors receiving priority pollutants and included in the mass balance when stripping plays a major role in the removal process.

E. Current Innovative Research

Envirokinetics of Harrison (49) have developed a treatment procedure using nuclear wastes to detoxify chemical wastes. Gamma rays from Cesium 137 break down the chemical bonds of the toxic chemicals and the ionized molecules reform into less harmful substances. Klibanov (50) stated that a combination of horseradish and hydrogen peroxide can be used to treat priority pollutants. The enzyme peroxidase, which is present in the

horseradish, acts as a catalyst between hydrogen peroxide and specific pollutants. The pollutants are converted to insoluble compounds which could be separated and burned. The EPA is funding a project which uses supercritical water, water at 706°F and at a pressure of 3000 pounds/inch, to oxidize hazardous wastes. It is hypothesized that supercritical water breaks down the organics into carbon dioxide and water (51).

Sawyer and Roberts (52) reported that superoxide, oxygen molecules containing an extra electron, will break down chloroorganics into carbonate and chloride ions. However, the waste must be water-free or the superoxide loses its stability. Kearney (53) submitted a technique in which oxygen is bubbled into a solution containing toxic chemical and irradiated with ultraviolet light at the same time. Chemical bonds are broken down which enables soil microorganisms to metabolize the simpler molecules.

CHAPTER III

MATERIALS AND METHODS

A. General

Initially, a large, heterogeneous group of microorganisms having the potential of metabolizing toxic organic chemicals were cultivated. The successful growth of these microbes required an appropriate inoculum source and synthetic wastewater containing carbon sources plus nutrients.

Biological reactors of a POTWs provided a logical choice for the microbial seed because of the number and variety of microorganisms growing in the reactor. The seed employed throughout the experiment was collected from the municipal sewage treatment plant at Ponca City, Oklahoma. This plant was chosen because of its successful activated sludge process and the presence of a large oil refinery located in that community. The microbes were collected, added to the complex wastewater, and aerated with compressed air.

The constituents of the complex wastewater included "Sego," ammonium sulfate, and phosphoric acid dissolved in two liters of tap water. The ingredients of "Sego" provided a carbon source, protein, vitamins, and a balance of chemical elements. The vitamin requirements for microorganisms vary greatly; therefore, the vitamin component of "Sego" was considered to increase the potential growth of microorganisms that might not have otherwise developed in the reactor. The addition of ammonium

sulfate and phosphoric acid insured that nitrogen and phosphorus were not growth limiting factors.

B. Batch System

1. Design

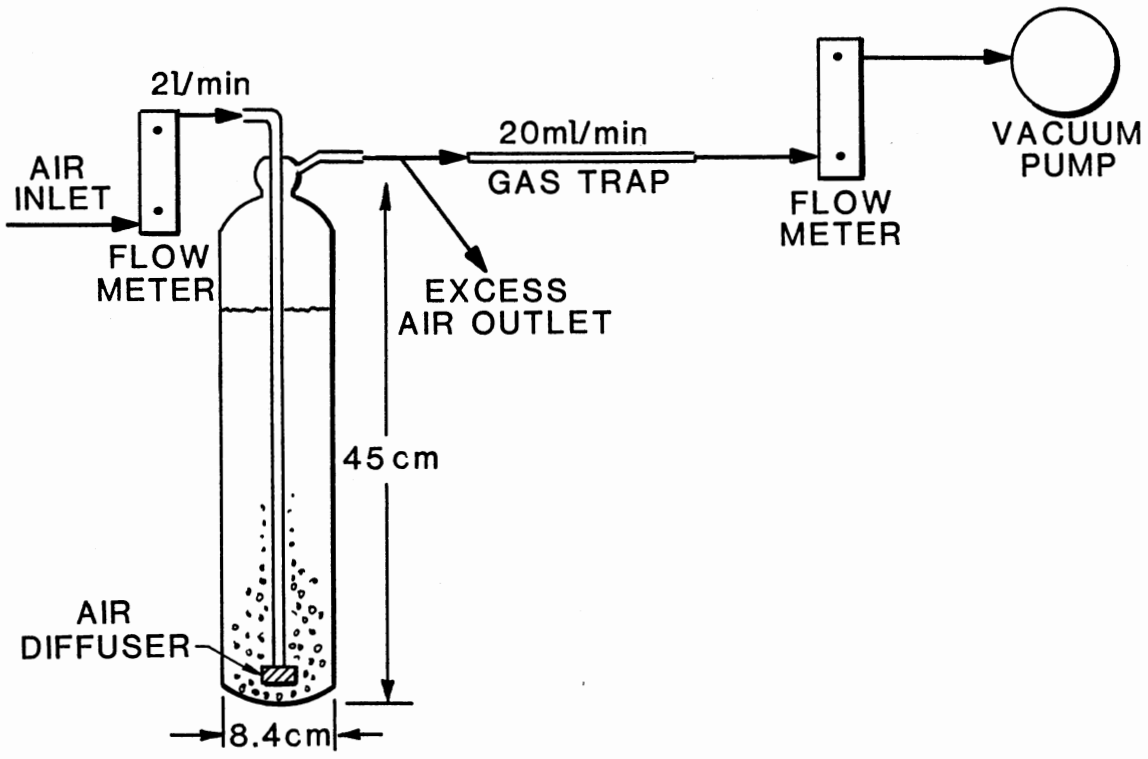
An enclosed batch reactor was designed to allow for the collection of liquid and gaseous samples (Figure 3). The volume of the reactor was designed to hold two liters of mixed liquor with one-third liter of free-board space above the liquid. The top of the reactor contained ground glass fittings with inlet and outlet exit ports. Compressed air entered through the inlet at a controlled rate of 2.0 ℓ/min, bubbled through the mixed liquor, and exited along with any volatiles through the exit ports. Gas samples were collected by connecting a gas trap, packed with six inches of tenax and four inches of silica gel, to the exit port and a small vacuum pump. A standardized flow meter was used to control the flow of air through the trap at 2.0 mℓ/min. Liquid samples were collected by removing the glass fitting at the top of the reactor.

2. Synthetic Wastewater

The complex wastewater contained 10 mg/ℓ of nitrogen and 2 mg/ℓ of phosphorus. The nitrogen and phosphorus source were ammonium sulfate and phosphoric acid, respectively. Two batch reactors containing two liters of liquid were run simultaneously, and the following calculations were made for the total volume of four liters:

$$\frac{0.01 \text{ g of N}}{\text{liter}} \times \frac{1 \text{ mole } (NH_4)_2\ SO_4}{28 \text{ g of N}} \times \frac{132 \text{ g of } (NH_4)_2\ SO_4}{\text{mole of } (NH_4)_2\ SO_4}$$

Figure 3. Batch Reactor and Volatile Collection System



BATCH REACTOR

$$\times 4.0 \text{ liters} = 0.188 \text{ g of } (NH_4)\ SO_4;$$

$$\frac{0.002 \text{ g of P}}{\text{liter}} \times \frac{\text{mole of } H_3PO_4}{31 \text{ g of P}} \times \frac{98 \text{ g of } H_3PO_4}{\text{mole of } H_3PO_4}$$

$$\times \frac{1.0 \text{ m}\ell \times 85\%}{1.8 \text{ g}} \times 4.0 \text{ liters}$$

$$= 0.01 \text{ m}\ell \text{ of } H_3PO_4.$$

The ingredients listed on the "Sego" label included concentrated skimmed milk, sugar, vegetable oils, edible cellulose, magnesium sulfate, artificial flavor, salt, cellulose gum, magnesium oxide, sodium ascorbate (Vitamin C), ferric orthophosphate, carrageenan, atocopherylacetate (Vitamin E), niacinamide, zinc oxide, copper gluconate, calcium pantothenate, Vitamin A palmitate, pyridoxine hydrochloride (Vitamin B_6), riboflavin phosphate (Vitamin B_2), thiamin hydrochloride (Vitamin B_1), folic acid, biotin, potassium iodide, Vitamin D_3, and Vitamin B_{12}.

The nutritional information recorded on a ten-ounce can of "Sego" included 11 grams of protein, 34 grams of carbohydrate, 5 grams of fat, and the U.S. recommended daily allowance of 25 percent for each of the following:

1. Vitamins A, D, E, C, B_6, B_{12}
2. Folic acid
3. Thiamin
4. Riboflavin
5. Niacin
6. Biotin
7. Pantothenic Acid
8. Calcium
9. Phosphorus
10. Iodine
11. Iron
12. Magnesium
13. Copper
14. Zinc

An initial volume of three milliliters of "Sego" was added to two liters of tap water to form the complex wastewater.

3. Experimental Procedure

Two liters of sewage from the activated sludge sample were added to the batch reactor. Three mℓ/ℓ of "Sego," ten mg/ℓ of nitrogen, and two mg/ℓ of phosphorus were added to the reactor. The resulting mixture was aerated with compressed air at 2.0 ℓ/min. After 24 hours, one-third of the mixed liquor, two-thirds of a liter, was wasted; the remaining suspension was allowed to settle; and one-third of the remaining liquid was decanted, leaving one-third of the mixed liquor remaining in the reactor. The two-liter volume was restored by adding tap water with the initial feed. A mean cell residence time, θ_c, of approximately three days was established using the above wasting procedure. The feeding and wasting procedure was continued until there was adequate biomass.

After the biomass had developed, a toxic priority pollutant was selected and 10 mℓ/ℓ of this test compound was added to the basic feed. With the addition of the pollutant to the reactor, the biomass was observed closely to determine if the pollutant was producing a negative effect on the microorganisms. The concentration of the pollutant was increased by 10 mg/ℓ each day until a concentration of 100 mg/ℓ was being added to the reactor. At this point the volume of "Sego" was decreased daily from 3.0 mℓ/2ℓ to 0.5 mℓ/2ℓ. The same acclimation technique was employed for each selected priority pollutant throughout the experiment.

An additional experiment was conducted to determine if the microorganisms could utilize the priority pollutant as its sole source of carbon. The addition of "Sego" was eliminated in the feed to one of the reactors containing an established biomass. A noticeable loss in biomass

was observed after two or three days, and the biomass continued to decrease until it became almost nonexistent. It was concluded that cometabolism would be essential throughout the experiment.

An experiment also was conducted to determine if the growth of microorganisms was affected by eliminating the nitrogen and phosphorus. Three mℓ of "Sego" only was added to the batch reactor during a five-day period and a definite drop in biomass was observed. It was concluded that excess nitrogen and phosphorus would be essential to insure constant growth.

Liquid and volatile sampling from the reactor was initiated after a biomass was well established with 100 mg/ℓ of the test pollutant, 0.5 mℓ/ℓ of "Sego," and the original concentrations of nitrogen and phosphorus. Three gas samples and three liquid samples for extraction were collected for each pollutant tested. The first collection occurred immediately after adding the basic feed; the second was taken during maximum substrate utilization; and the third at the end of substrate utilization. A preliminary growth study was completed over a 24-hour period, and a TSS and TOC versus time graph was prepared. The collection time for gas and liquid extraction was determined from this preliminary growth curve. After these times had been established a final experiment run was conducted.

During the final run two 25 mℓ mixed liquor samples were collected. One was used to determine TSS, and the other was stored in a refrigerator until TOC and GC analyses were completed. Two to three volatile samples were collected by inserting the gas trap between the exit port of the reactor and the small electric vacuum pump. The flow rate through the trap was adjusted to 20 mℓ/min. Volatiles were collected over a time period varying from 15 to 60 minutes depending upon the characteristics

of the toxic priority pollutant. After the collection period the gas trap was disconnected from the exit port and stored in a refrigerator until GC analysis could be completed. The biomass was allowed to settle briefly, and 600 mℓ of the liquid were decanted and also stored in a refrigerator until extraction could be achieved.

Total suspended solids and total organic carbon was measured in accordance with the procedures listed in Standard Methods for the Examination of Water and Wastewater (54). The sampling, storage, extraction procedures, and GC techniques were followed as detailed in the December 3, 1979, Federal Register (17).

Twenty-five mℓ of mixed liquor were collected and filtered through 0.45 μm filters. The filters were placed in pre-weighed aluminum cans and placed in a drying oven at 103°C for a minimum of two hours. After cooling the pans in a desiccator, the pans were weighed to determine the dry weight of the suspended solids. The concentration of the TSS was calculated by dividing the dry weight of the solids by the volume of the sample.

Total organic carbon was determined by using a Beckman TOC analyzer. Total carbon and inorganic carbon standards were carefully prepared and small aliquots were injected in the analyzer. A response versus concentration graph was prepared for both standards which allowed the determination of the concentration of the total carbon and inorganic carbon in the unknown sample. The total organic carbon concentration is the difference in total carbon and inorganic carbon concentrations.

The concentration of the priority pollutant in the mixed liquor was followed by using the purge and trap or direct-inject techniques of gas-liquid chromatography. Acrylonitrile, 1,2-dichloropropane, benzene, and

1,1,2,2-tetrachloroethane concentrations were determined by the purge and trap techniques. Phenol, 2,4-dinitrophenol, and 1,2-dichlorobenzene concentrations were determined by extracting liquid samples with methylene chloride, then analyzing on the GC by the direct-inject procedure.

Standard solutions were prepared for each procedure and small aliquots were subjected to GC analysis. The results allowed a standard curve for each pollutant to be developed. Small aliquots were taken from the stored 25 mℓ liquid sample or the extracted sample and analyzed with the correct procedure.

The stripping of the priority pollutant, along with possible gaseous by-products, was determined by replacing the gas trap on the GC unit with the volatile traps collected during the experimental run. The amount of the test pollutant was then determined from the previously prepared standard curve and its volatilization calculated. The presence of intermediates was noted but not identified. The off-gas analysis was determined by inserting a carbowax column in the GC. An isothermal procedure or a temperature program of 80°C to 150°C at 8°C/min was utilized. The attenuation was set at 1 and the range at 10.

Nonvolatile intermediates remaining in the liquid were either base/neutral or acid extractable compounds. The base/neutral organics were separated by adding three 30 mℓ volumes of methylene chloride. Hydrochloric acid was added to the remaining liquid sample, reducing the pH to less than two; the acidified solution was extracted with three 30 mℓ volumes of methylene chloride. The base neutral and acid extracts were concentrated by using the Kuderna-Danish procedure (Figure 2).

The base/neutral extract was analyzed by injecting small aliquots onto the SP 2250 column of the GC unit. A temperature program of 85°C

to 265°C at 8°C/min was used with the final temperature being held for an additional five minutes. The attenuation was set at 1 and the range at 10. The acid extractables were analyzed on the SP 1240 DA column in the GC unit. A temperature program of 80°C to 185°C at 8°C/min was used with the final temperature being held at the final temperature limit. The attenuation and range settings were identical to that of the base/neutral analysis.

CHAPTER IV

RESULTS

A. General

The seven toxic priority pollutants were chosen as representative compounds from four general compound groups. Acrylonitrile was selected from the nitrogen containing compounds, phenol and 2,4-dinitrophenol from the phenols, benzene and 1,2-dichlorobenzene from the aromatics, and 1,1,2,2-tetrachloroethane and 1,2-dichloropropane from the halogenated aliphatics. The research data collected over a two-year period will be summarized according to the compound group and will be presented by the following graphs.

1. A 24-hour growth curve which includes TSS, TOC, and the concentration of the test pollutant versus time.

2. A chromatograph for off-gas analysis which displays the presence of the specific pollutant plus volatile intermediates.

3. A bar graph for the volatiles which demonstrates the number of intermediates detected, the relative amounts, and the change of each specific intermediate over the collected times.

4. A chromatograph for the base/neutral and acid intermediates produced in the liquid wastewater which shows the presence of the acid or base/neutral test compound along with other organic by-products existing in the wastewater.

5. A bar graph for the liquid intermediates which presents the same information as the bar graphs for the volatiles.

B. Nitrogen Containing Compounds

1. Acrylonitrile

The growth curve for acrylonitrile is presented in Figure 4. A gradual increase in TSS is noted accompanied by a gradual decrease in TOC. The sludge yield was determined at the end of substrate utilization, t_8, and was found to be 2.41. Acrylonitrile, however, decreased rapidly from 110 mg/ℓ to 20.0 mg/ℓ in the first three hours, and then slowly declined to its final concentration of 2.0 mg/ℓ at the end of ten hours.

The standard curve resulting from the acrylonitrile standard solutions is shown in Figure 5. The quantity of acrylonitrile in the unknown samples was determined by reading the GC response of the sample and referring to the standard curve. The identical procedure was used for all seven toxic priority pollutants.

The chromatograph resulting from the off-gas analysis revealed only acrylonitrile; no volatile intermediates were detected. The volatilization of acrylonitrile as summarized in Table II indicates that acrylonitrile was being stripped from the batch reactor. The percentage of volatilization was calculated for the first hour of operation, t_0; the second hour, t_2; and the eighth hour, t_8. Approximately 25 percent stripping was observed for all three samples.

The chromatographs in Figures 6, 7, and 8 represent the acid extractable compounds detected at the times t_0, t_2, and t_{24}. Several intermediates are noted but only ten have an area response greater than 10^4. A bar graph (Figure 9) displaying these ten intermediates demonstrates the

Figure 4. Growth Curve for Acrylonitrile Demonstrating TOC and Acrylonitrile Removal and TSS Production

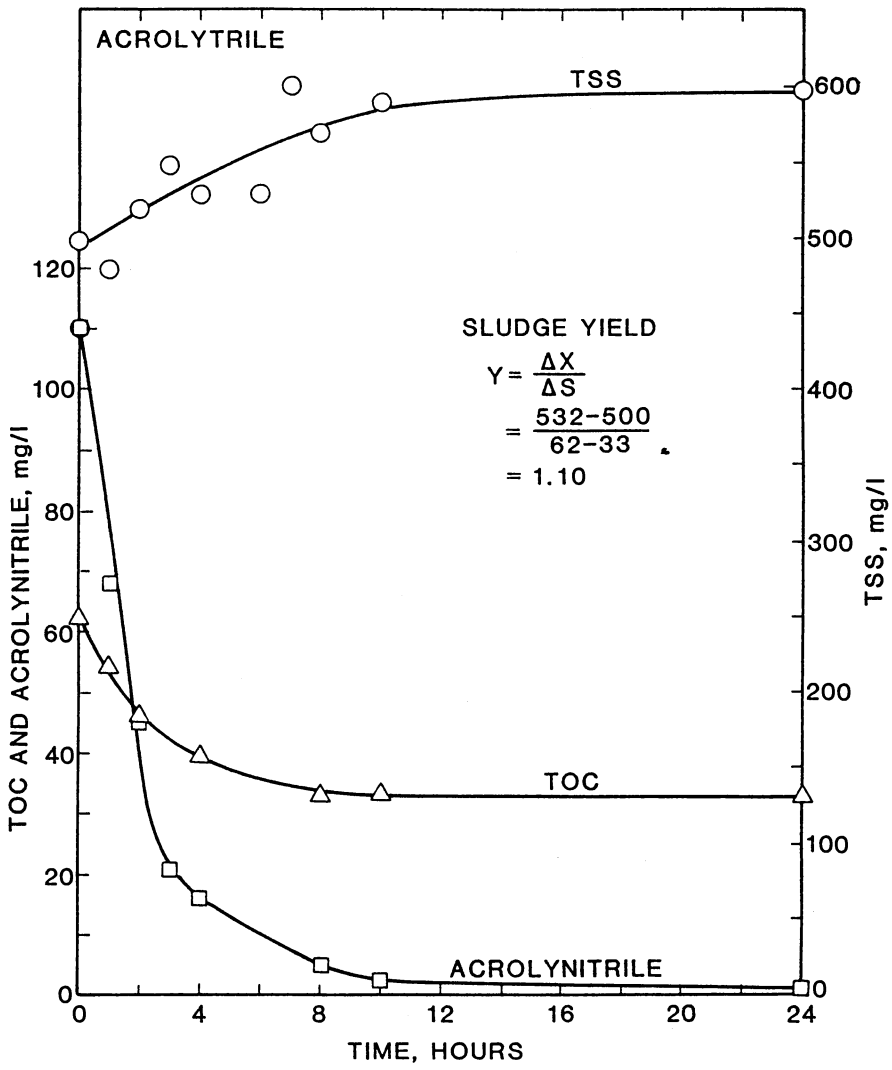

ACROLYTRILE
TSS
SLUDGE YIELD
Y = ΔX/ΔS
= 532-500/62-33
= 1.10
TOC
ACROLYNITRILE
TOC AND ACROLYNITRILE, mg/l
TSS, mg/l
TIME, HOURS
0
20
40
60
80
100
120
0
100
200
300
400
500
600
0
4
8
12
16
20
24

Figure 5. Standard Curve for Acrylonitrile

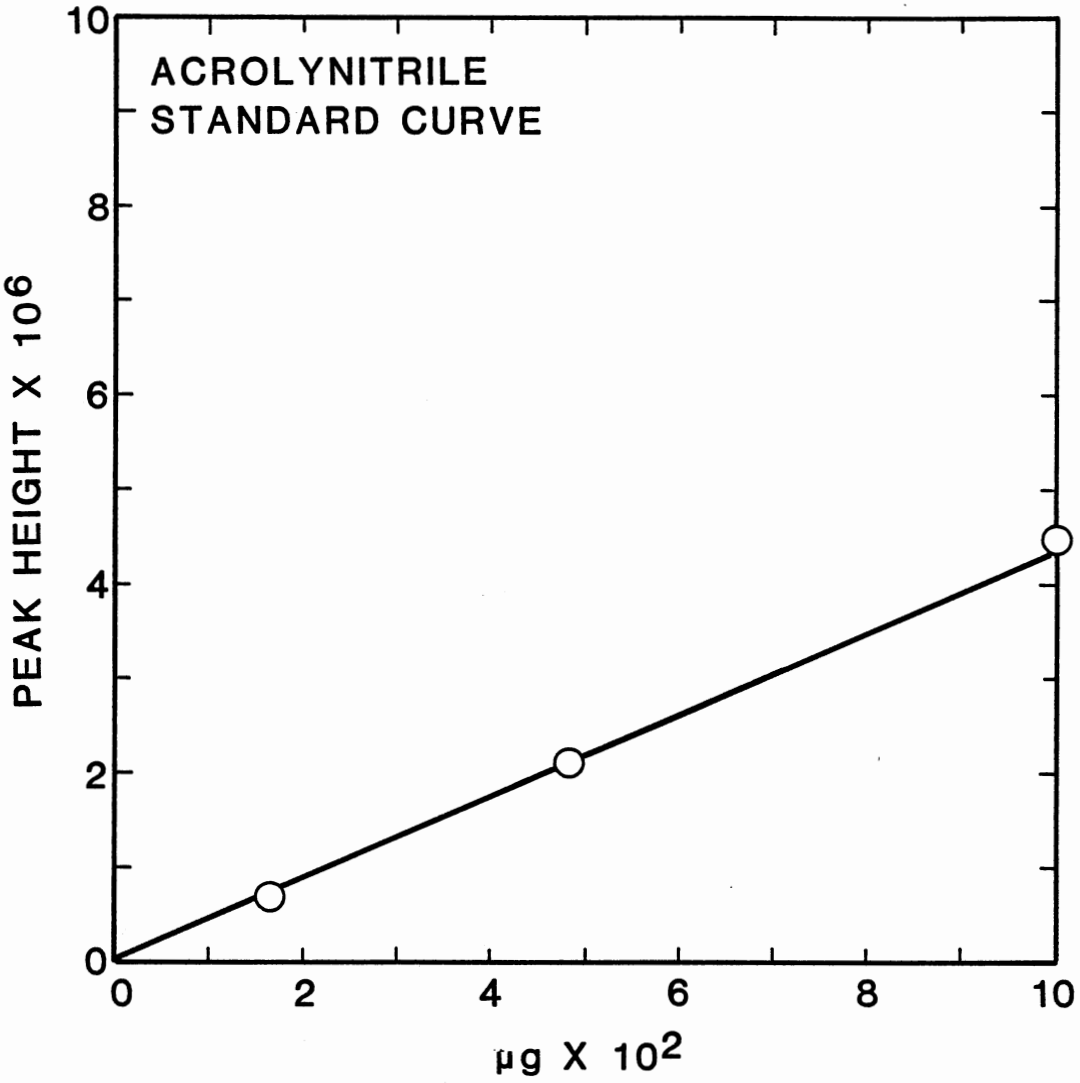

ACROLYNITRILE
STANDARD CURVE
PEAK HEIGHT X 10^6
µg X 10^2
0
2
4
6
8
10

TABLE II

VOLATILIZATION ANALYSIS FOR ACRYLONITRILE

Time	Area Response x 10^5	Mg	Dilution Factor x mg	Total Removal, mg	Volatilization, Percent
t_0	6.2	0.140	14.0	72.0	19.4
t_2	4.7	0.105	10.5	42.0	25.0
t_8	0.5	0.005	0.5	2.0	25.0

Air flow rates: Reactor rate = 2.0 l/min; trap rate = 20.0 ml/min.

Dilution factor: 20/2000 = 0.01.

Figure 6. Chromatograph for the Acrylonitrile Acid Fraction Collected at the Initial Time t_0

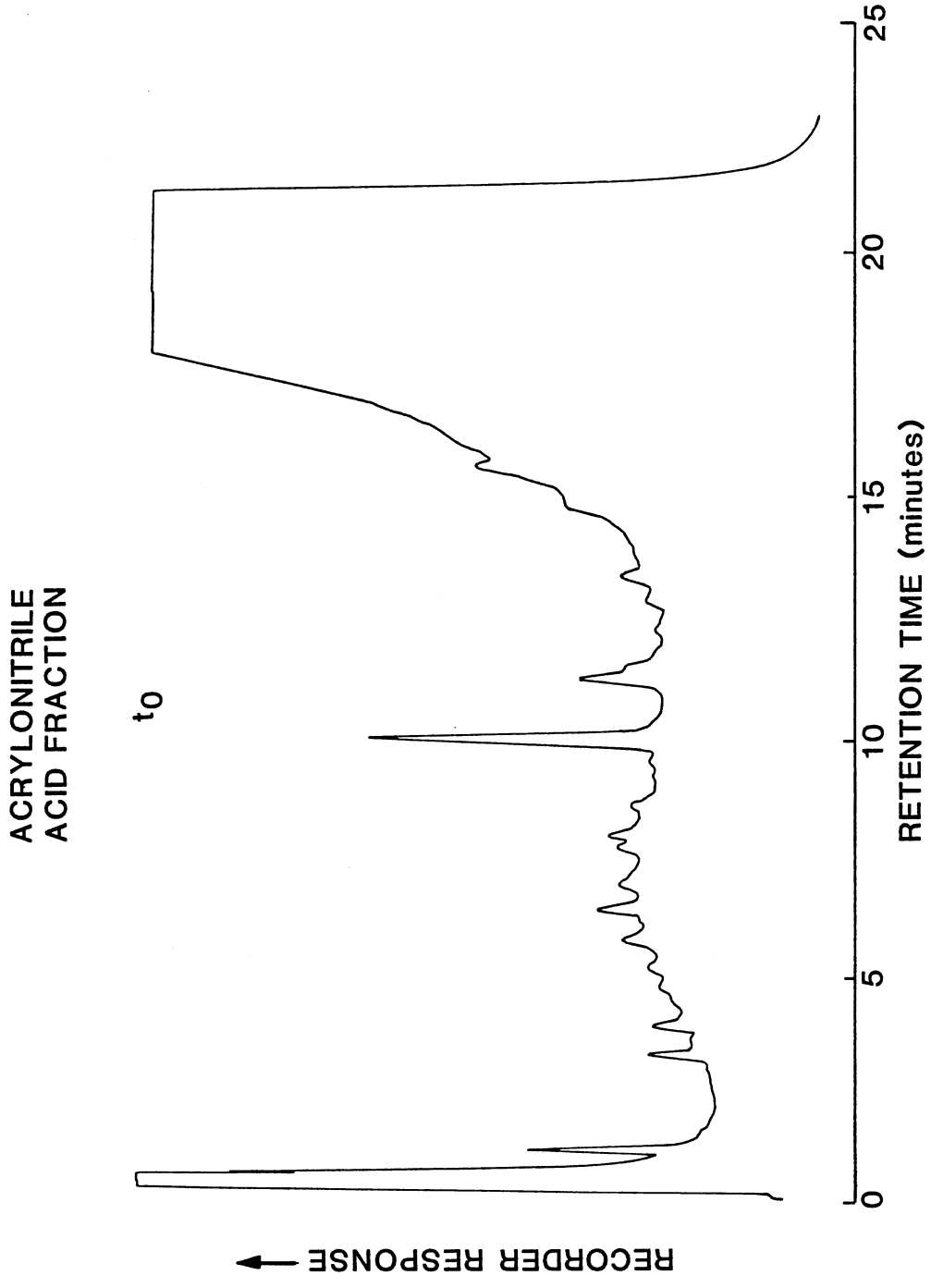

ACRYLONITRILE
ACID FRACTION
t_0
RECORDER RESPONSE
0
5
10
15
20
25
RETENTION TIME (minutes)

Figure 7. Chromatograph for the Acrylonitrile Acid Fraction Collected After Five Hours, t_5

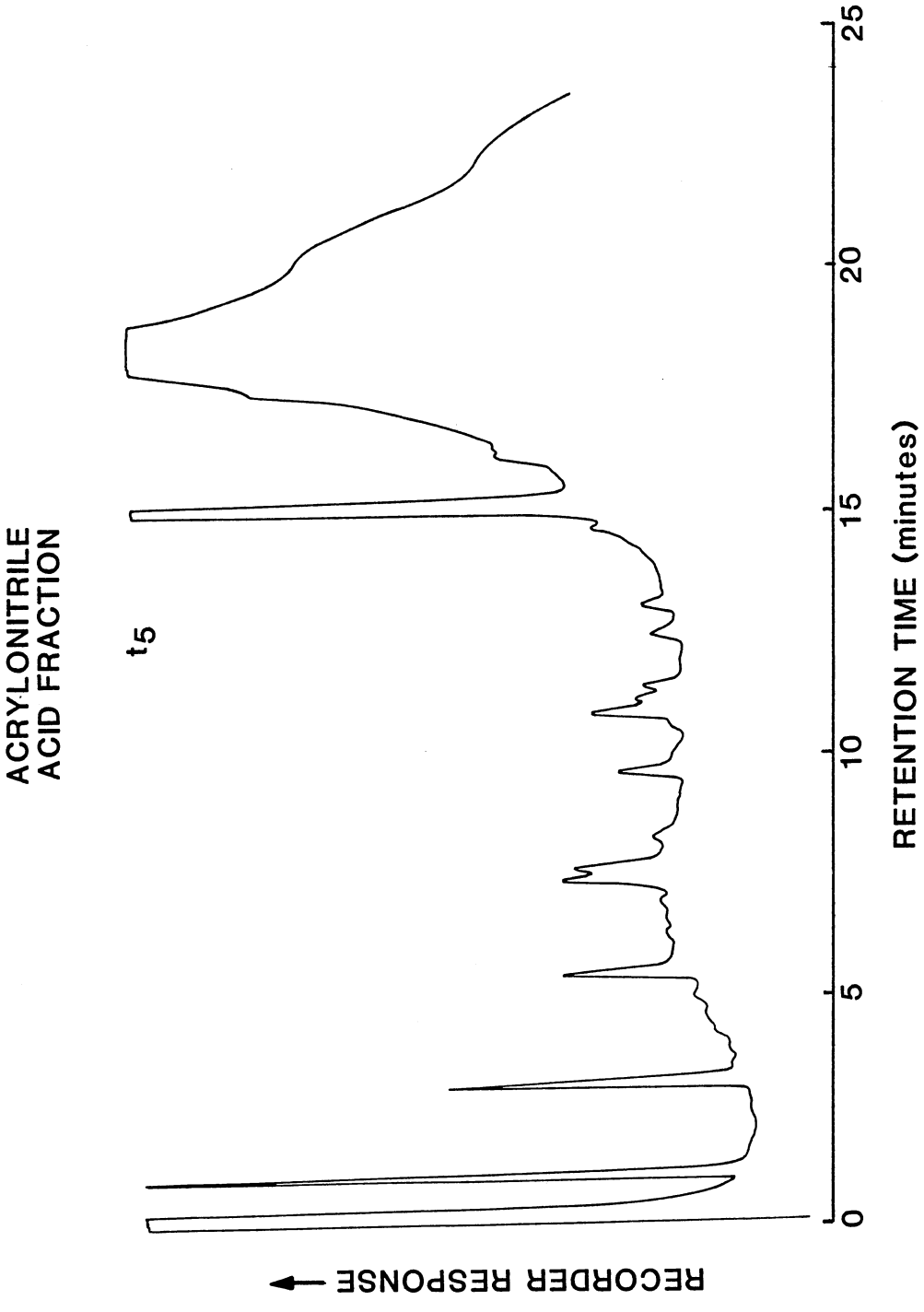

ACRYLONITRILE
ACID FRACTION
t_5
RECORDER RESPONSE
0
5
10
15
20
25
RETENTION TIME (minutes)

Figure 8. Chromatograph for the Acrylonitrile Acid Fraction Collected After Twenty-Four Hours, t_{24}

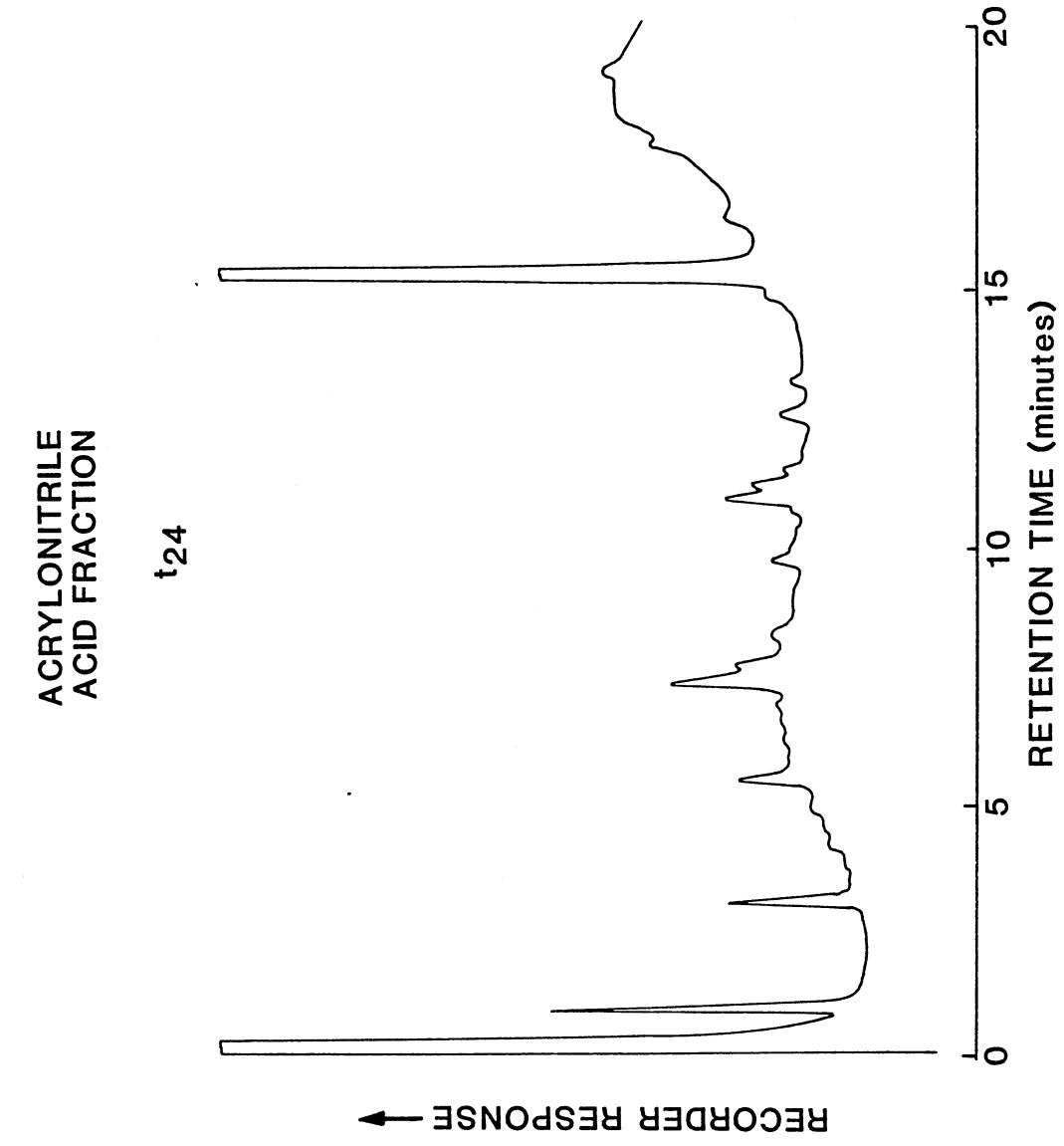
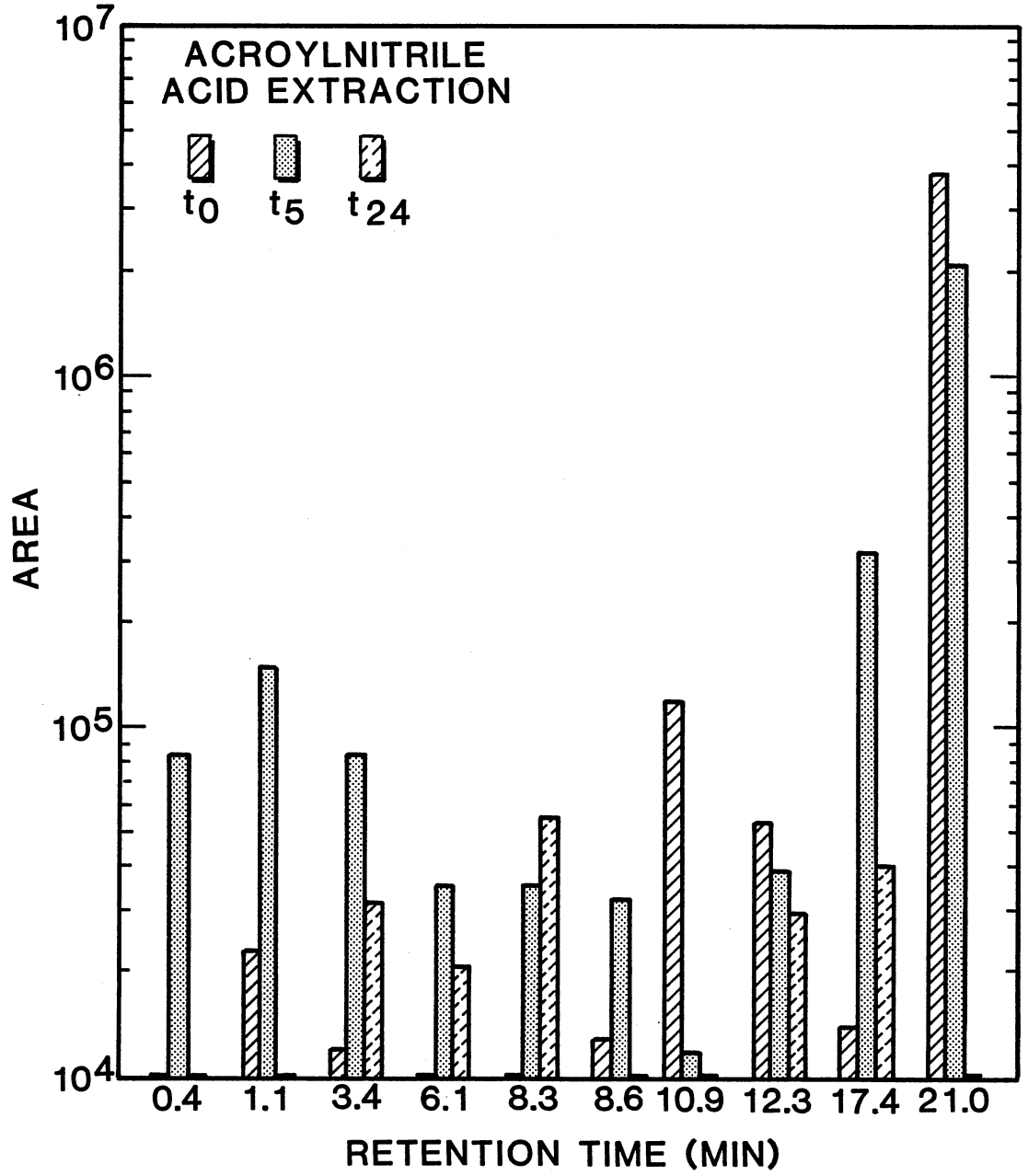

ACRYLONITRILE
ACID FRACTION
t_{24}
RECORDER RESPONSE
0
5
10
15
20
RETENTION TIME (minutes)

Figure 9. Bargraph of Acrylonitrile Acid Fraction Intermediates Demonstrating the Relative Quantity of Each Compound at t_0, t_5, and t_{24}


ACROYLNITRILE
ACID EXTRACTION
t_0 t_5 t_{24}
10^7
10^6
10^5
10^4
AREA
0.4 1.1 3.4 6.1 8.3 8.6 10.9 12.3 17.4 21.0
RETENTION TIME (MIN)

change in quantity of each compound. The length of the bar represents the relative amounts of the compounds detected.

Three compounds are noted at t_0 with an area response greater than 10^4. The response of the compound at a retention time (RT) of 21.0 minutes is clearly greater than the other compounds. All three compounds with a RT of 10.9, 12.3, and 21.0 decreased in concentration with time and were difficult to measure at t_{24}. At t_5, intermediates are noted at RT of 0.4, 1.1, 3.4, 6.1, 8.3, 8.6, and 17.4 minutes. It is noteworthy that the greater responses are at lower RT for t_5, while the greater response for t_0 was at higher RT. The area responses for t_{24} were generally very low and were essentially not measurable by the operating conditions used during the GC analysis. The GC analysis revealed no base/neutral compounds present at t_0, t_5, or t_{24}.

C. Aromatics

1. Benzene

The growth curve for benzene in Figure 10 reveals a rapid change in TSS and TOC during the first two hours of the growth study. However, after the second hour the concentration of these parameters remains relatively unchanged. The sludge yield was calculated at the end of substrate utilization, t_2, and was found to be 1.00. The concentration of benzene at t_0 was 54 mg/ℓ and decreased to less than 1 mg/ℓ after the first hour of operation. Benzene was not detected at any later time during the study.

The off-gases for benzene were collected at t_0, t_1, and t_3. The chromatographic analysis in Figure 11 reveals only benzene during the first hour, but a second volatile appears on the chromatograph for the samples

Figure 10. Growth Curve for Benzene Demonstrating TOC and Benzene Removal and TSS Production

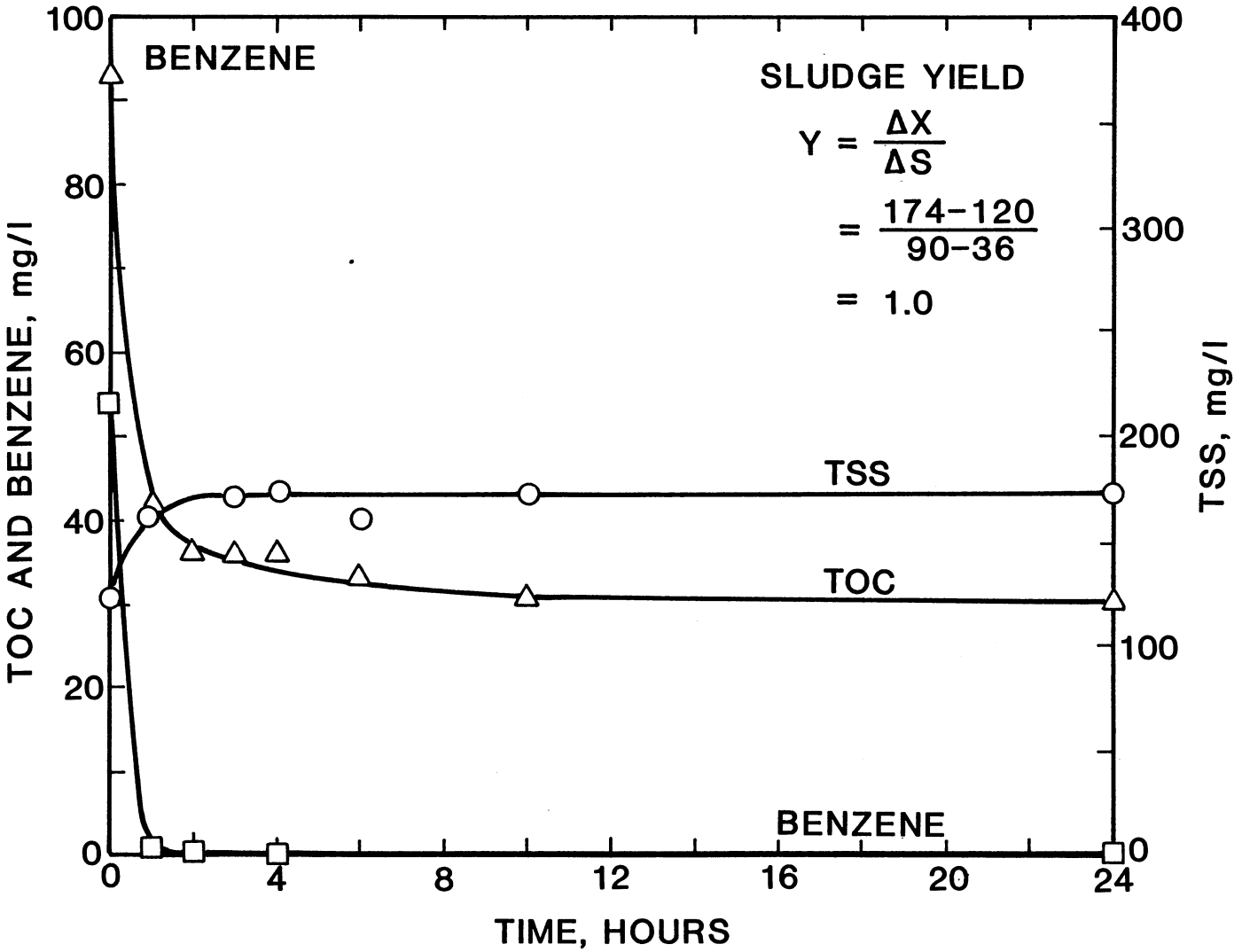
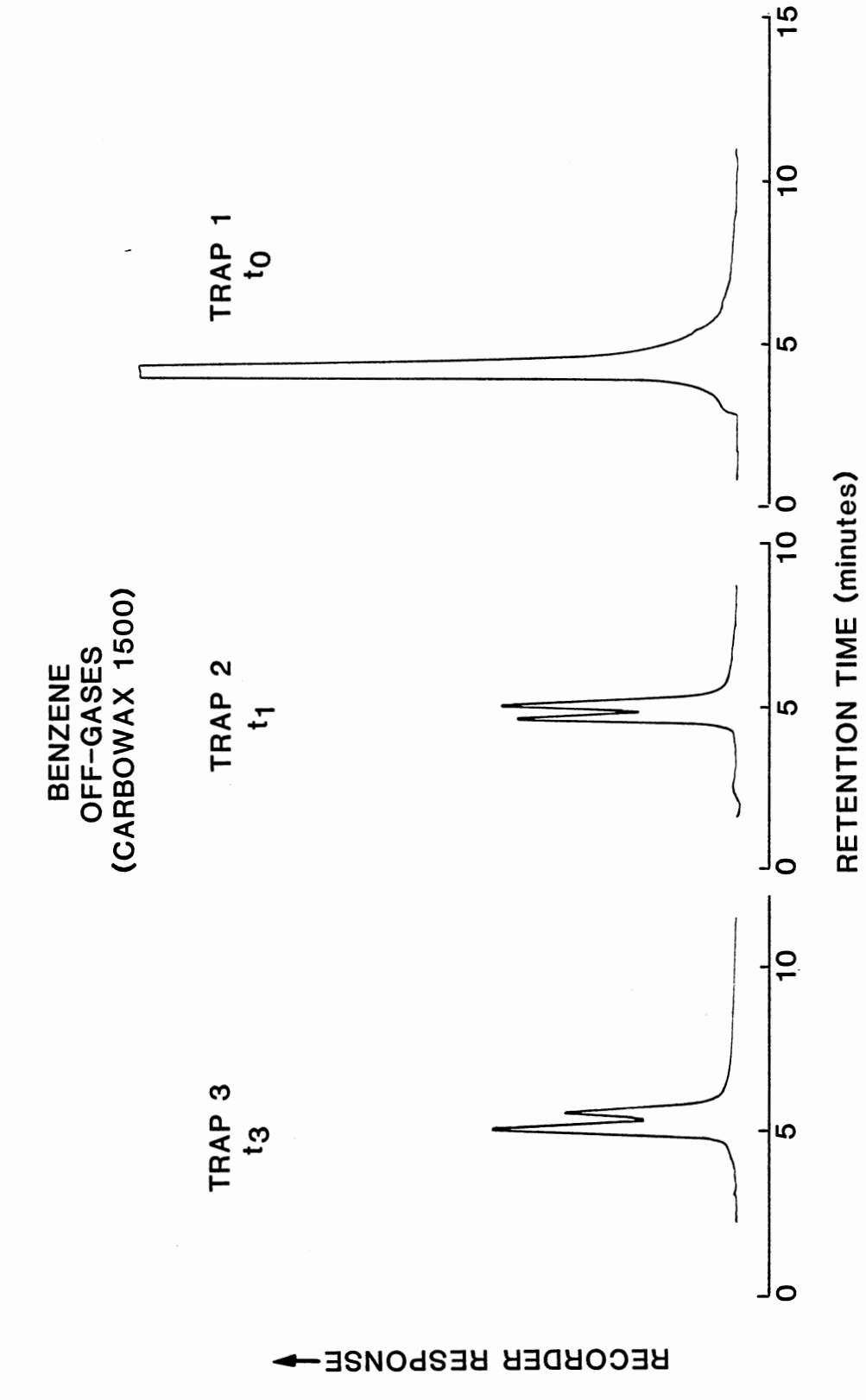

BENZENE
SLUDGE YIELD
Y = ΔX/ΔS
= 174−120/90−36
= 1.0
TSS
TOC
BENZENE
TOC AND BENZENE, mg/l
TSS, mg/l
TIME, HOURS
0
20
40
60
80
100
0
100
200
300
400
0
4
8
12
16
20
24

Figure 11. Chromatograph for Benzene and Other Volatile Intermediates Collected at t_0, t_1, and t_3


BENZENE
OFF-GASES
(CARBOWAX 1500)
TRAP 1
t_0
TRAP 2
t_1
TRAP 3
t_3
0
5
10
15
RETENTION TIME (minutes)
RECORDER RESPONSE

t_1 and t_3. These data are also displayed on the bar graph in Figure 12. Note the 10 percent volatilization of benzene during the first hour of operation as summarized in Table III. The amount of benzene could not be determined at t_1 or t_3.

GC analysis indicates a total absence of base/neutral compounds at t_0, t_8, and t_{24}; however, there were several acid extractable compounds observed with 12 having an area response greater than 10^4 (Figures 13 and 14). The bar graph in Figure 15 demonstrates the moderately large responses for the compounds in the collected samples. Note the compounds with the RT of 4.8, 10.3, 11.3, 15.4, and 21.5. Of these six, the compounds at 10.3, 11.3, 13.4, and 15.4 decrease in quantity at t_8 but increase rather significantly at t_{24}. The compounds at 4.8 and 21.5 produce a very low response at t_{24}. The sample at t_{24} reveals ten compounds with a relatively large response. Note the compounds with RT of 10.3, 11.3, 12.6, 13.0, 13.4, 14.0, 14.4, 15.4, 16.6, and 19.0. The compounds at 12.6, 13.0, 14.0, 14.4, 16.6, and 19.0 were not detected to any extent at t_0 or t_8; however, they produce a significant response at t_{24}.

2. 1,2-Dichlorobenzene

The growth curve for 1,2-dichlorobenzene is presented in Figure 16. The concentration of TSS is usually low but increased 80 mg/ℓ during the first eight hours. TOC concentration declined slowly the first eight hours and remained steady at 17 mg/ℓ for the remaining portion of the growth study. The sludge yield was calculated at t_8, and was found to be 1.37. The concentration of 1,2-dichlorobenzene was initially 54 mg/ℓ but decreased to 3 mg/ℓ during the first two hours of operation. The

Figure 12. Bargraph of Benzene and Volatile Intermediate Displaying the Relative Quantity of Each Compound at t_0, t_1, and t_3

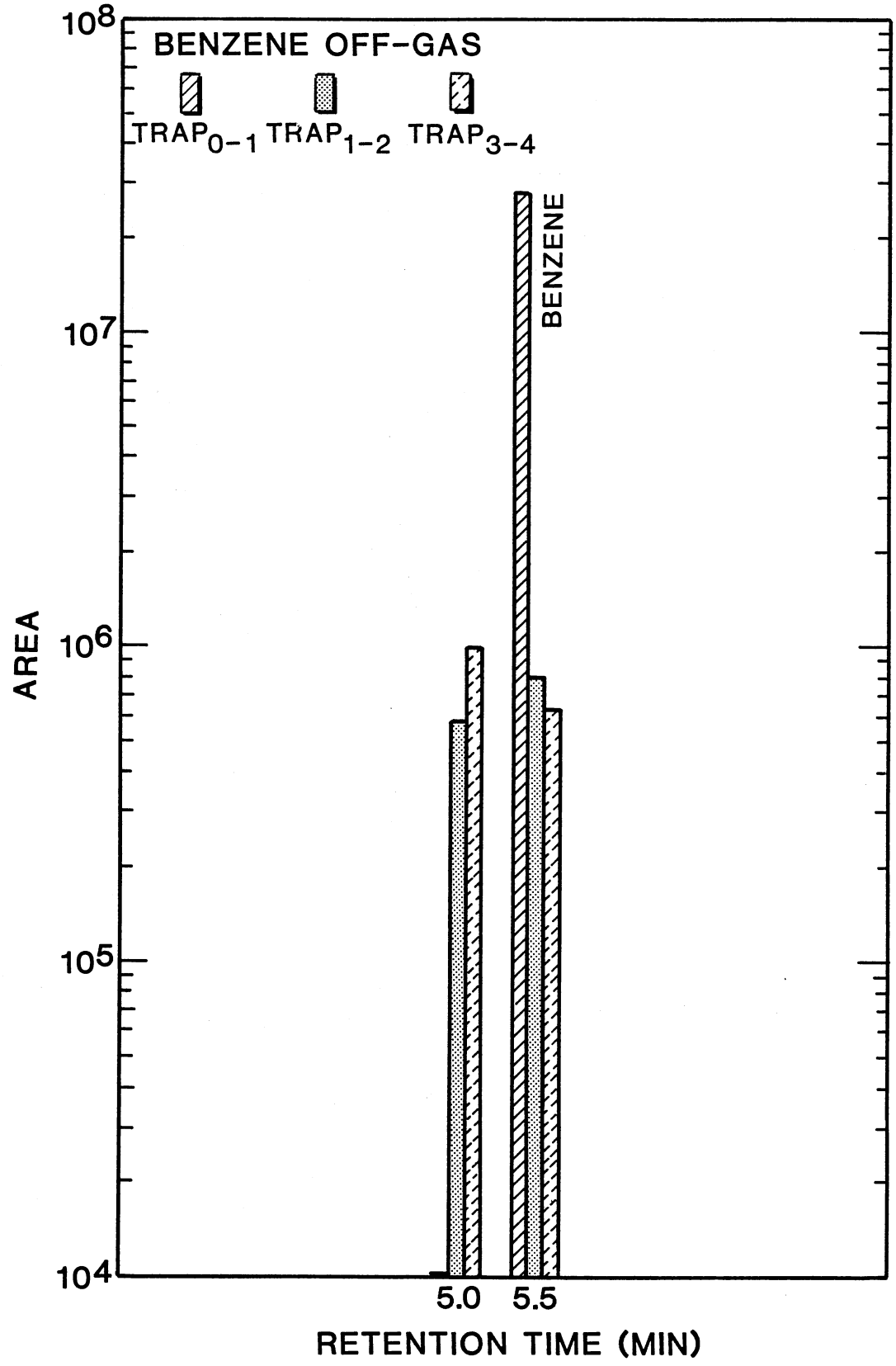

BENZENE OFF-GAS
TRAP0-1
TRAP1-2
TRAP3-4
BENZENE
AREA
10^8
10^7
10^6
10^5
10^4
5.0
5.5
RETENTION TIME (MIN)

TABLE III

VOLATILIZATION ANALYSIS FOR BENZENE

Time	*Area Response x 10^5	Mg	Dilution Factor x mg	Total Removal, mg	Volatilization, Percent
t_0	22.90	0.104	10.4	108	9.6
t_1	0.81	0.004	0.4	---	---
t_3	0.65	0.004	0.4	---	---

Air flow rates: Reactor rate = 2.0 l/min; trap rate = 20.0 ml/min.

Dilution factor: 20/2000 = 0.01.

Figure 13. Chromatograph for the Benzene Acid Fraction Collected at t_0 and t_8

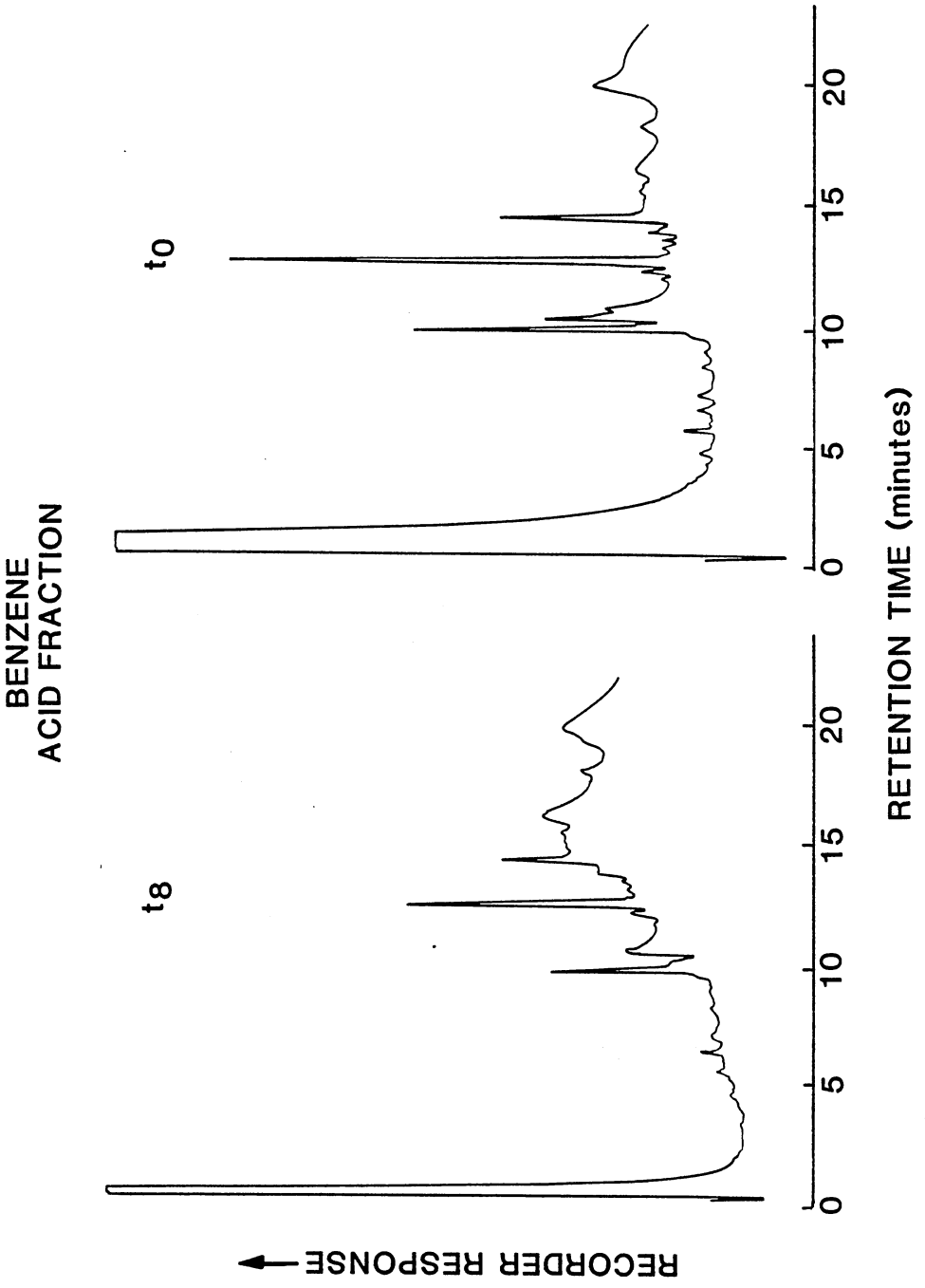

BENZENE
ACID FRACTION
t8
t0
RECORDER RESPONSE
0
5
10
15
20
RETENTION TIME (minutes)

Figure 14. Chromatograph for the Benzene Acid Fraction Collected at t_{24}

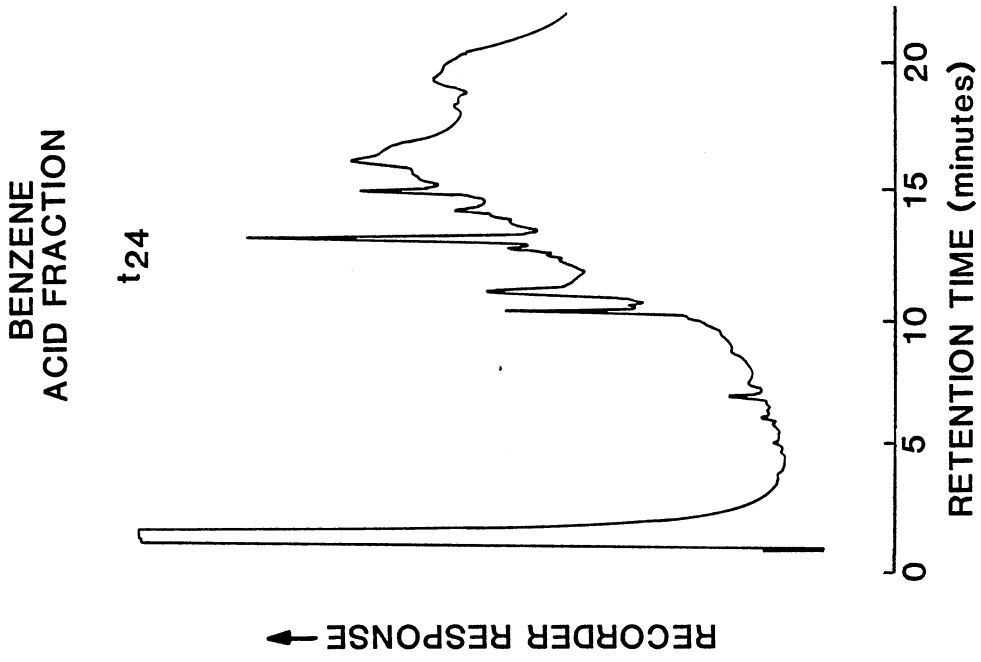

BENZENE
ACID FRACTION
t_{24}
RECORDER RESPONSE
0
5
10
15
20
RETENTION TIME (minutes)

Figure 15. Bargraph of Benzene Acid Fraction Intermediates Demonstrating the Relative Quantity of Each Compound at t_0, t_8, and t_{24}

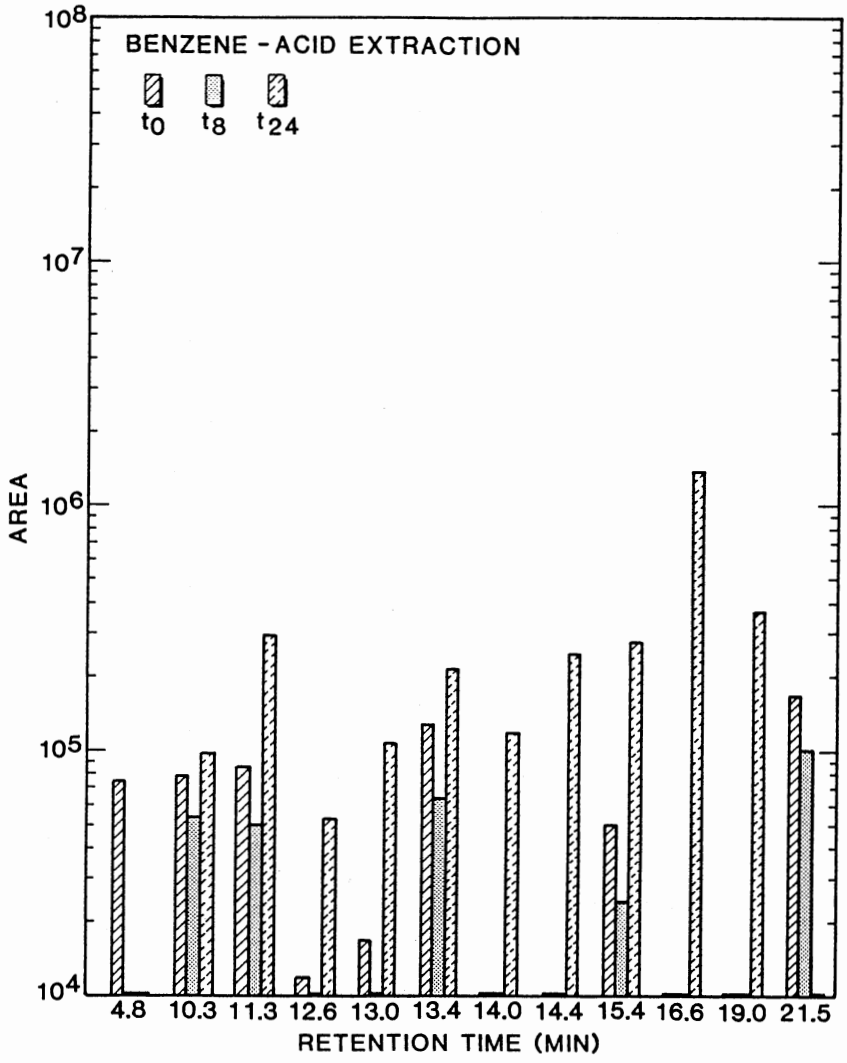
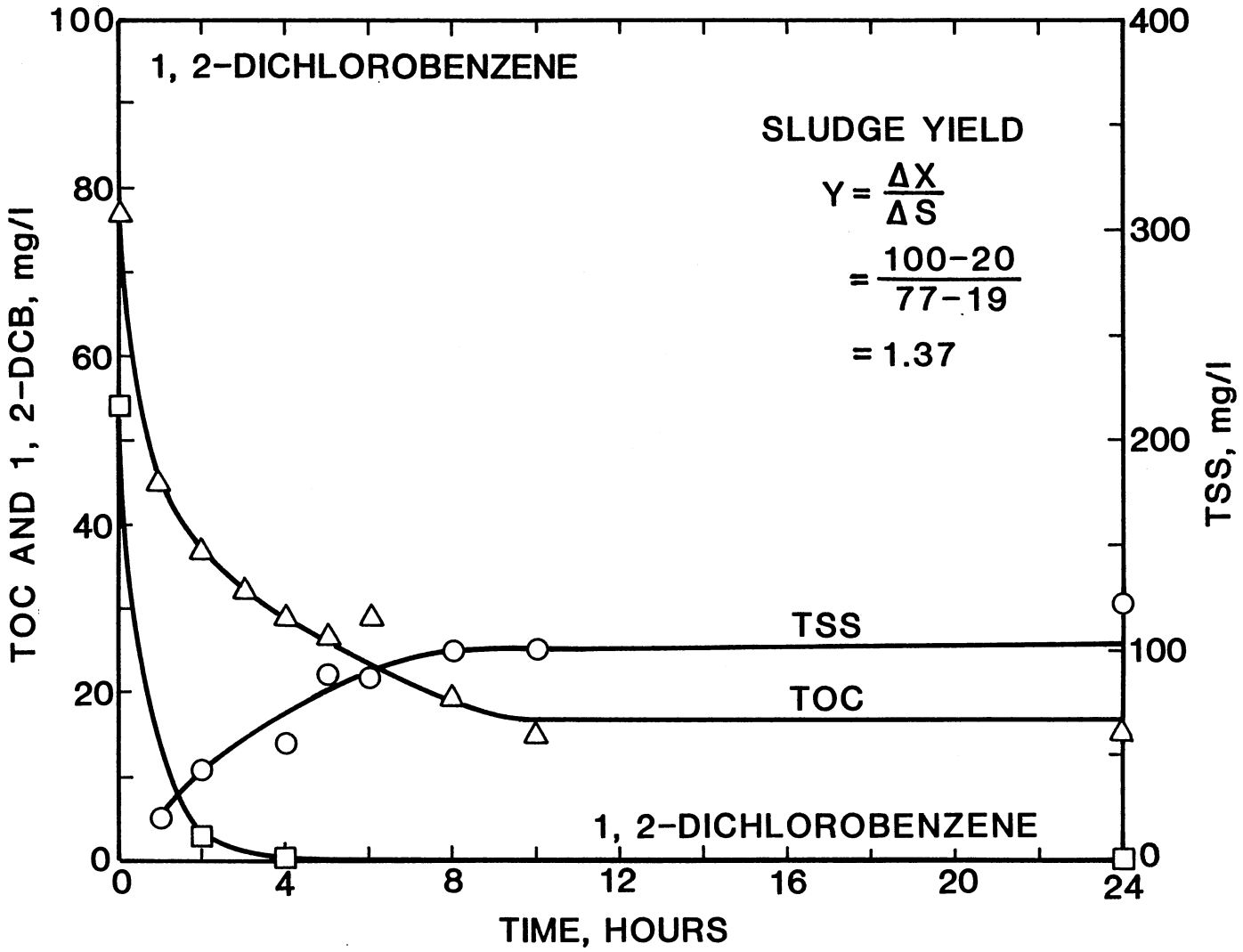

BENZENE - ACID EXTRACTION
t_0 t_8 t_{24}
AREA
10^8
10^7
10^6
10^5
10^4
4.8 10.3 11.3 12.6 13.0 13.4 14.0 14.4 15.4 16.6 19.0 21.5
RETENTION TIME (MIN)

Figure 16. Growth Curve for 1,2-Dichlorobenzene Demonstrating TOC and 1,2-Dichlorobenzene Removal and TSS Production


1, 2-DICHLOROBENZENE
SLUDGE YIELD
Y = ΔX/ΔS
= 100-20/77-19
= 1.37
TSS
TOC
1, 2-DICHLOROBENZENE
TOC AND 1, 2-DCB, mg/l
TSS, mg/l
TIME, HOURS
0
20
40
60
80
100
0
100
200
300
400
0
4
8
12
16
20
24

concentration dropped to less than 1 mg/ℓ at t_4 and apparently remained at this level through t_{24}.

The off-gases were collected at t_0 and t_1 and were analyzed as shown in Figure 17. The bar graph in Figure 18 displays 15 compounds at these two times with 8 registering a response greater than 10^6. Thirteen of the fifteen compounds were detected at t_0, and the area response of the compounds at 1.8, 2.2, 3.0, 5.8, 7.0, and 8.7 increased or remained constant. Compounds with a RT of 6.0, 6.2, 6.3, 7.2, 7.4, and 7.7 had a relatively high response but dropped below 10^4 at t_1. Table IV summarizes the volatilization data for 1,2-dichlorobenzene.

The chromatographs for the base/neutral compounds isolated at t_0, t_2, and t_{24} are presented in Figures 19 and 20. The bar graph in Figure 21 demonstrates 14 compounds with an area response greater than 10^4 with 1,2-dichlorobenzene producing the greater response. The graph also indicates that the initial sample, t_0, contained only three compounds, and all three decreased to less than a 10^4 response at t_2 and t_{24}. The compound with a RT of 4.6 produced a response greater than 10^6 at t_2 but was not detected at t_0 or t_{24}. It is noteworthy that nine compounds with a RT of 10.9, 11.9, 12.1, 12.4, 12.7, 12.9, 13.1, 13.6, and 14.1 were detected only at t_{24}.

The chromatographs in Figure 22 represent the acid extractable compounds isolated at t_0, t_2, and t_{24}. The bar graph in Figure 23 displays 13 compounds with an area response greater than 10^4. Five of the six compounds detected at t_0 did not produce a response greater than 10^4 at t_2 or t_{24}, and the compound with a RT of 14.9 did not produce the minimum response at t_{24}. It is also of interest that the compounds at 16.1, 16.3,

Figure 17. Chromatograph of 1,2-Dichlorobenzene and Other Volatile Intermediates Collected at t_0 and t_1

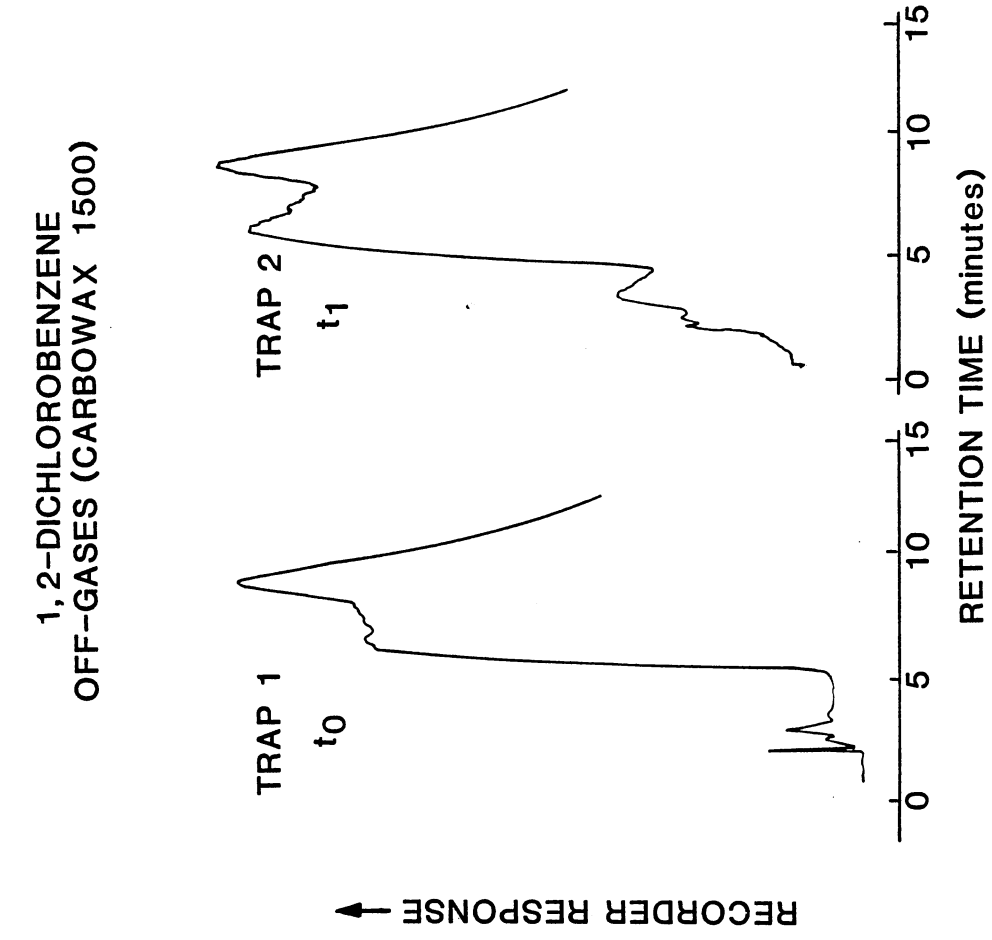

1,2-DICHLOROBENZENE
OFF-GASES (CARBOWAX 1500)
TRAP 1
t_0
TRAP 2
t_1
RECORDER RESPONSE
0
5
10
15
0
5
10
15
RETENTION TIME (minutes)

Figure 18. Bargraph of 1,2-Dichlorobenzene and Volatile Intermediates Displaying the Relative Quantity of Each Compound at t_0 and t_1

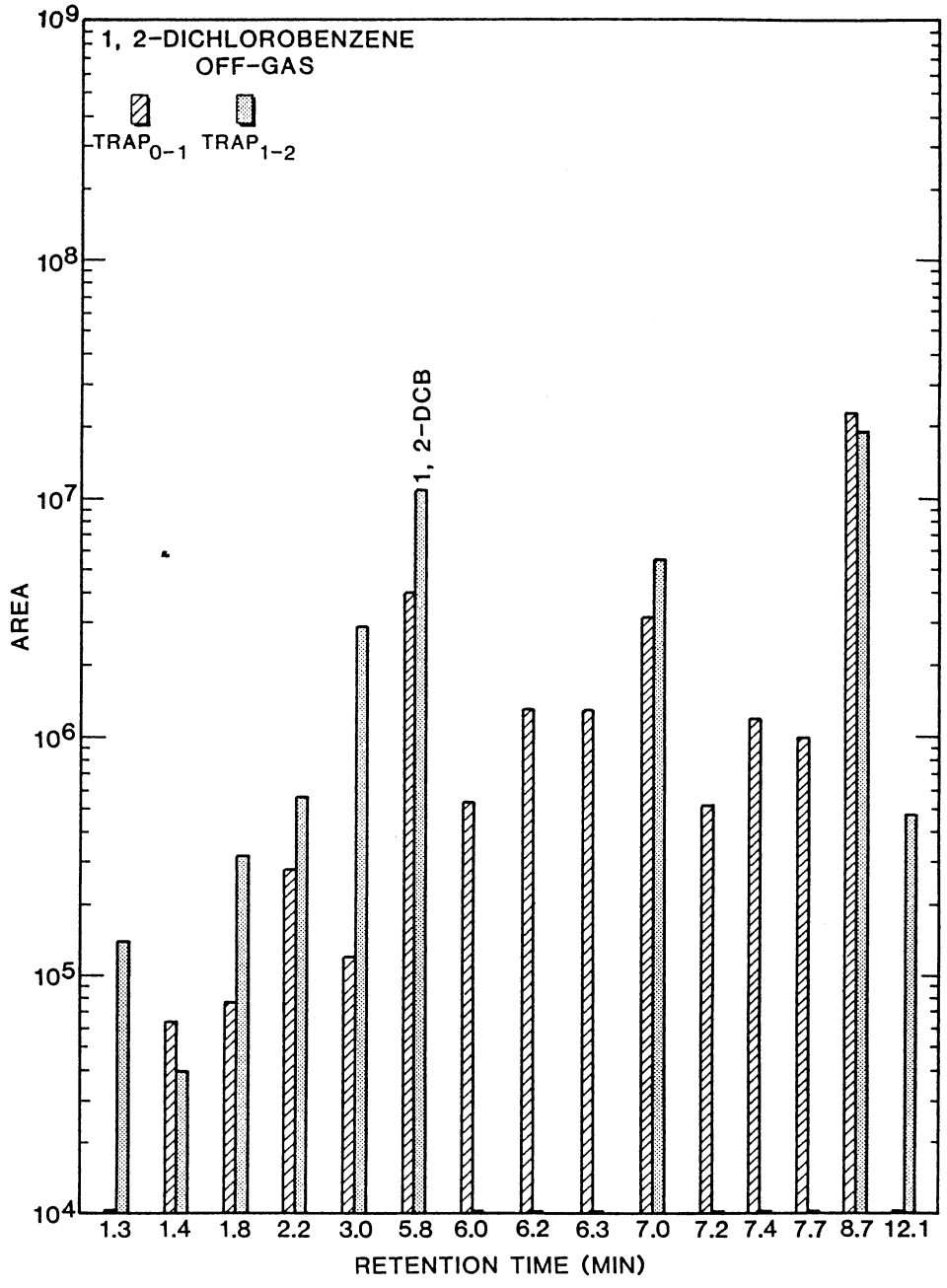

1, 2-DICHLOROBENZENE
OFF-GAS
TRAP0-1
TRAP1-2
1, 2-DCB
AREA
10^9
10^8
10^7
10^6
10^5
10^4
1.3
1.4
1.8
2.2
3.0
5.8
6.0
6.2
6.3
7.0
7.2
7.4
7.7
8.7
12.1
RETENTION TIME (MIN)

TABLE IV

VOLATILIZATION ANALYSIS FOR 1,2-DICHLOROBENZENE

Time	Area Response x 10^5	Mg	Dilution Factor x mg	Total Removal, mg	Volatilization, Percent
t_0	3.39	0.09	0.9	70	1.3
t_1	12.00	0.03	3.0	32	9.3

Air flow rates: Reactor rate = 2.0 l/min; trap rate = 20.0 ml/min.

Dilution factor: 20/2000 = 0.01.

Figure 19. Chromatograph for the 1,2-Dichlorobenzene Base/Neutral Fraction Collected at the Initial Time, t_0

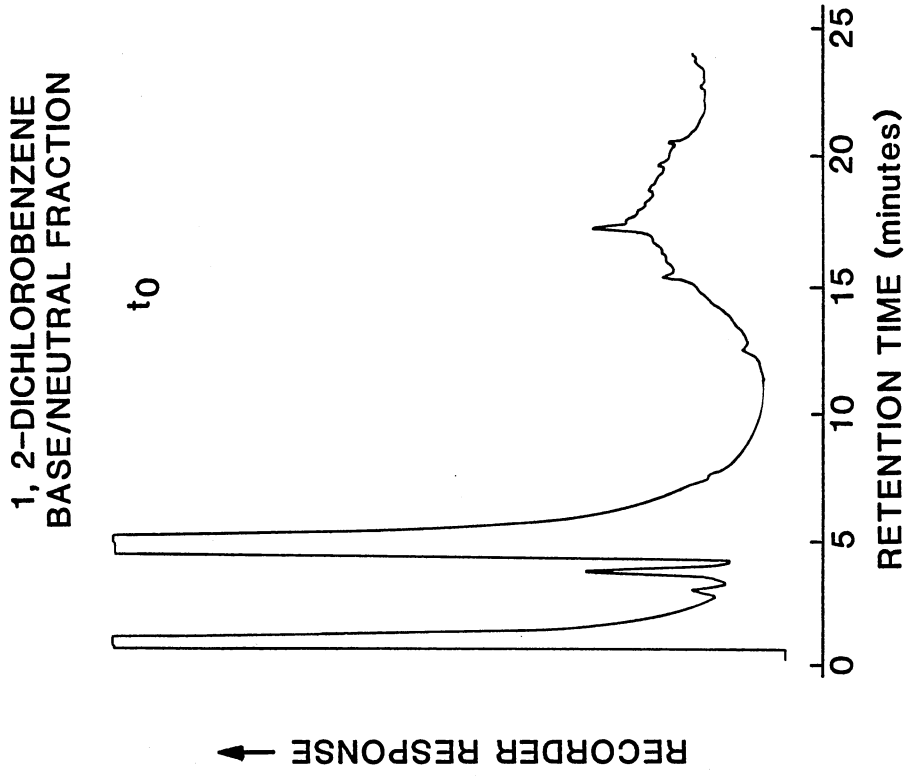

1, 2-DICHLOROBENZENE
BASE/NEUTRAL FRACTION
t_0
RECORDER RESPONSE
0
5
10
15
20
25
RETENTION TIME (minutes)

Figure 20. Chromatograph for the 1,2-Dichlorobenzene Base/Neutral Fraction Collected at t_2 and t_{24}

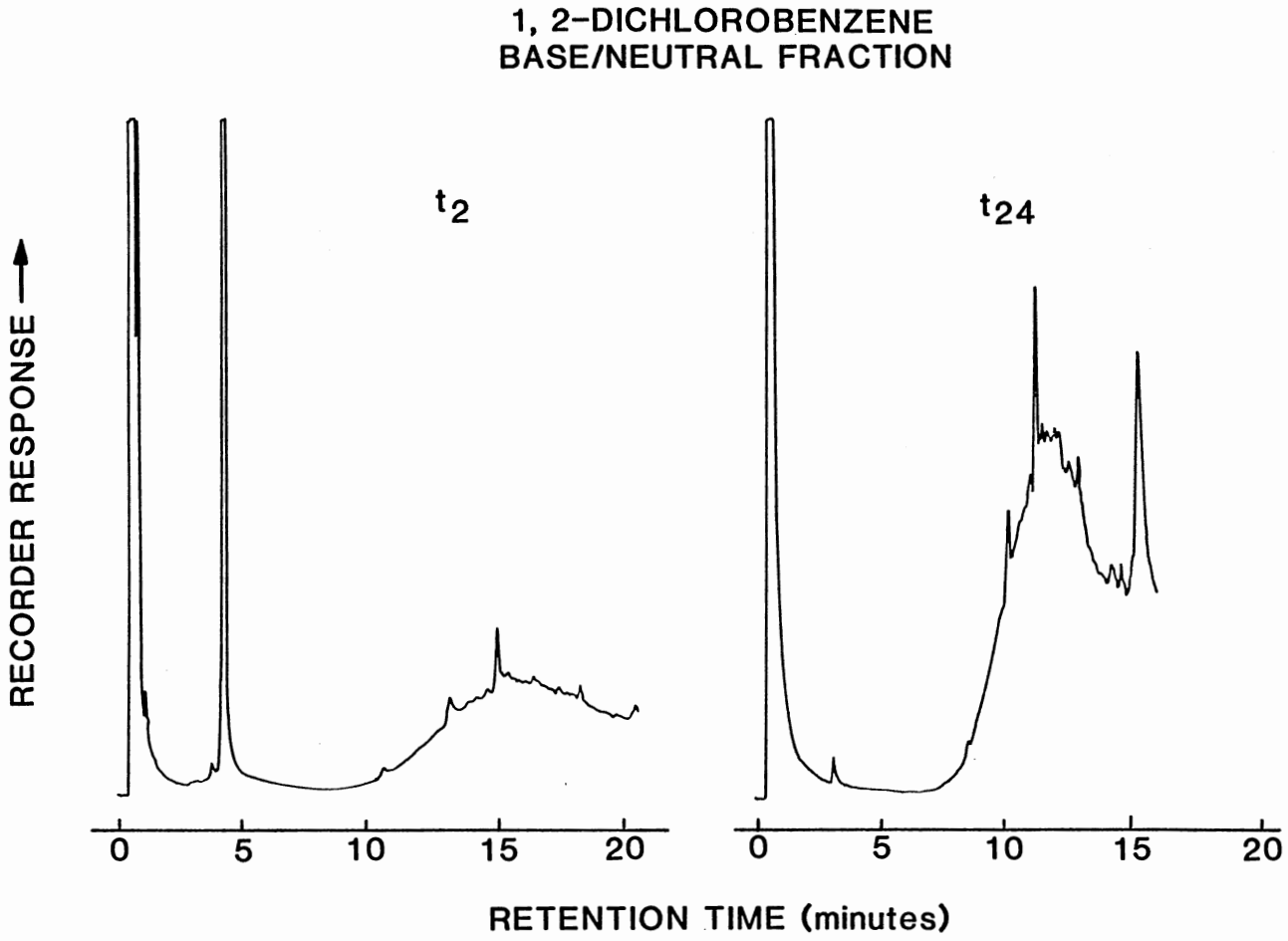

1, 2-DICHLOROBENZENE
BASE/NEUTRAL FRACTION
RECORDER RESPONSE
t_2
t_{24}
0
5
10
15
20
0
5
10
15
20
RETENTION TIME (minutes)

Figure 21. Bargraph of 1,2-Dichlorobenzene and Base/Neutral Intermediates Displaying the Relative Quantity of Each Compound at t_0, t_2, and t_{24}

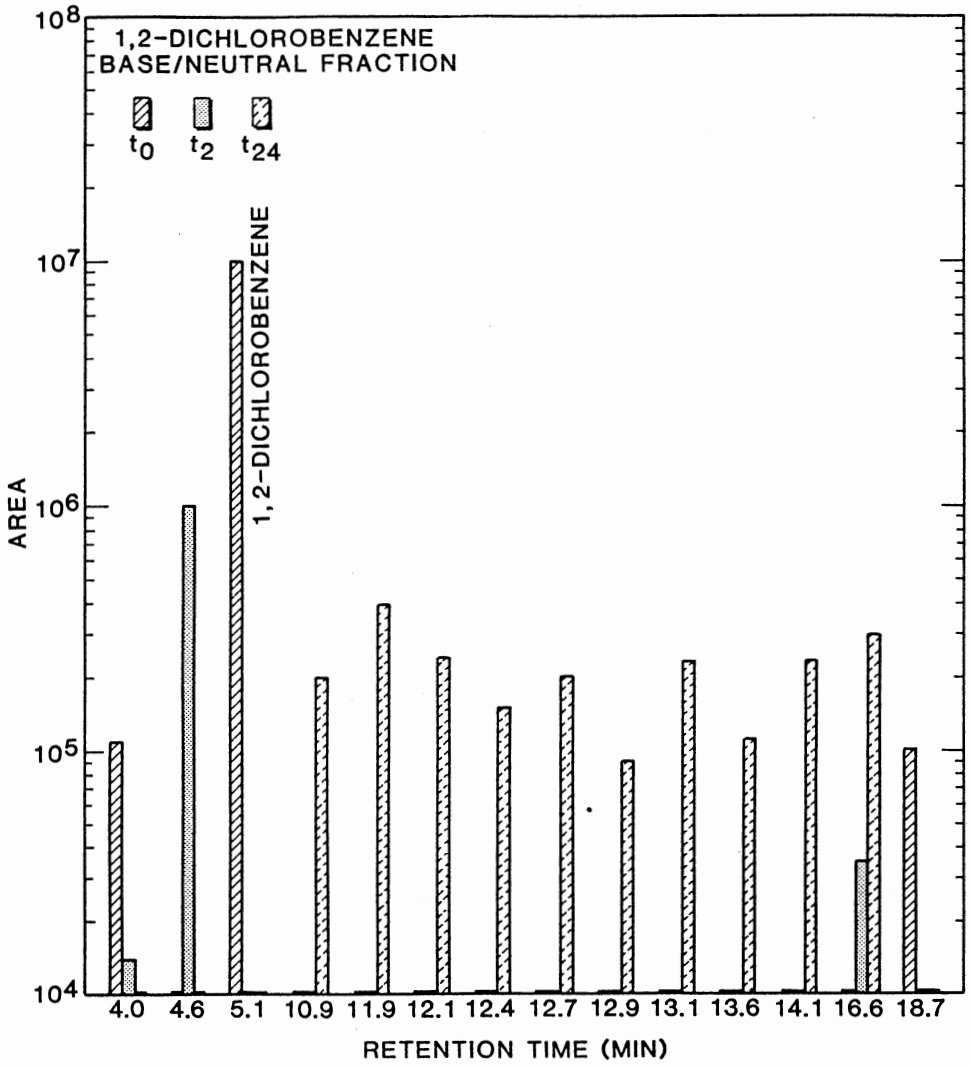

1,2-DICHLOROBENZENE
BASE/NEUTRAL FRACTION
t_0 t_2 t_{24}
1,2-DICHLOROBENZENE
AREA
10^8
10^7
10^6
10^5
10^4
4.0 4.6 5.1 10.9 11.9 12.1 12.4 12.7 12.9 13.1 13.6 14.1 16.6 18.7
RETENTION TIME (MIN)

Figure 22. Chromatographs for the 1,2-Dichlorobenzene Acid Fraction Collected at t_0, t_2, and t_{24}

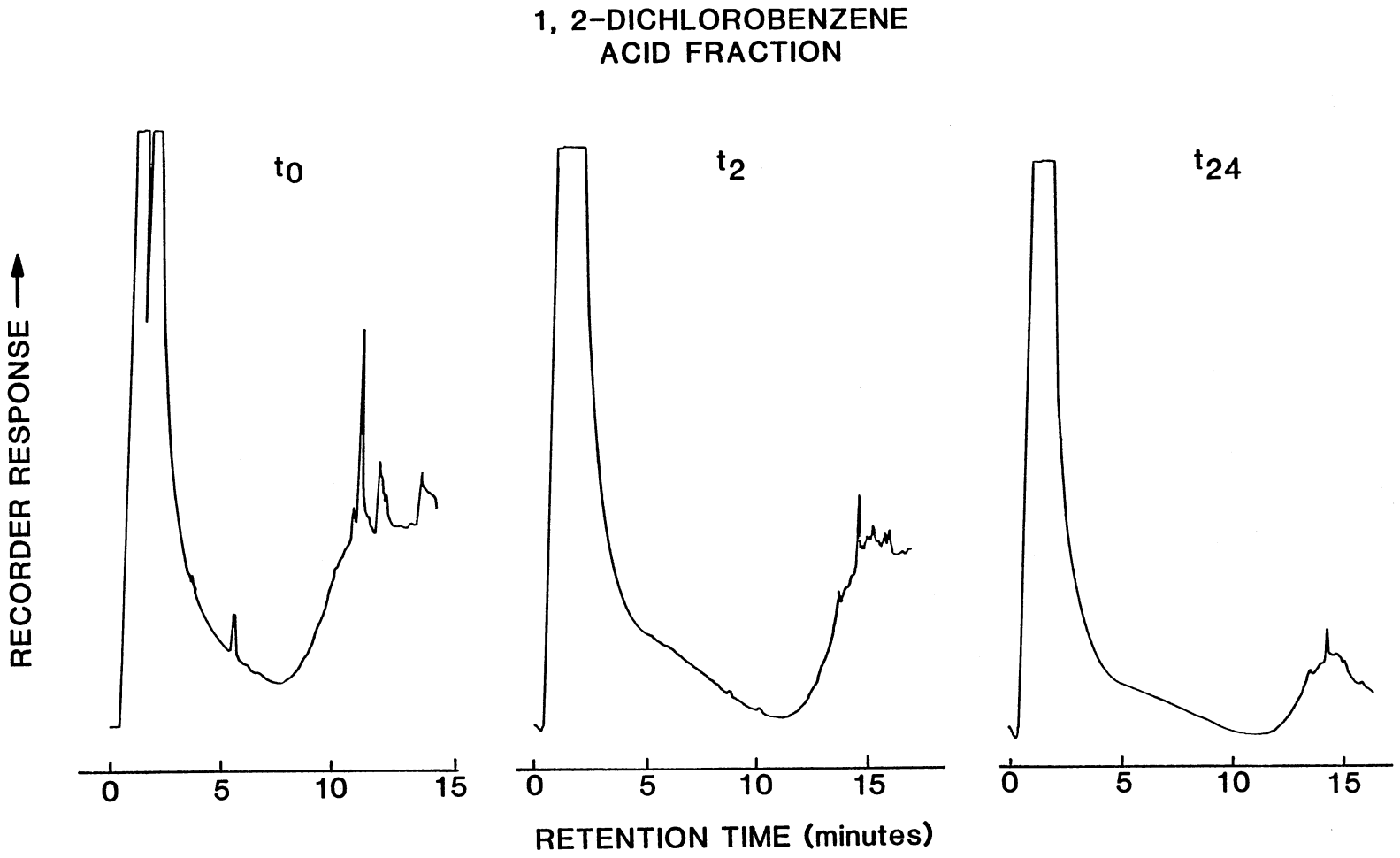
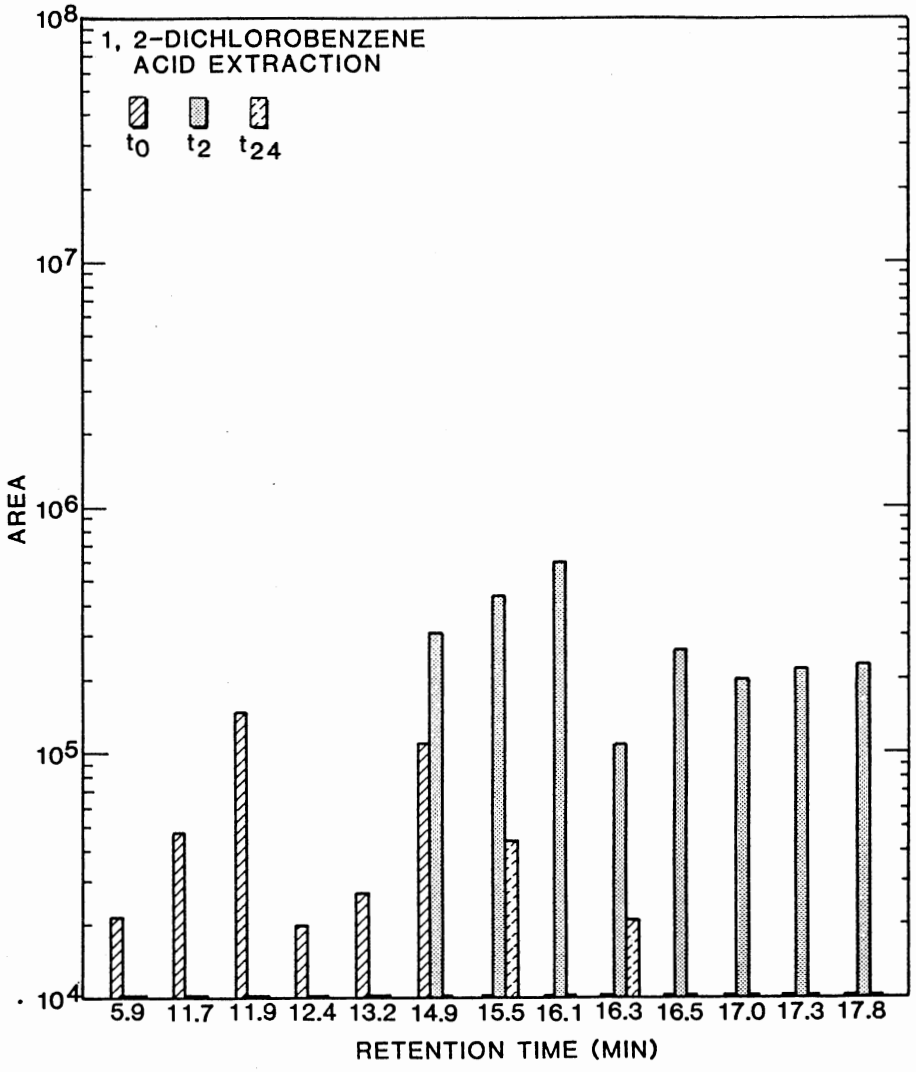

1, 2-DICHLOROBENZENE
ACID FRACTION
t_0
t_2
t_{24}
RECORDER RESPONSE
0
5
10
15
RETENTION TIME (minutes)

Figure 23. Bargraph of 1,2-Dichlorobenzene Acid Fraction Intermediates Demonstrating the Relative Quantity of Each Compound at t_0, t_2, and t_{24}


1, 2-DICHLOROBENZENE
ACID EXTRACTION
t_0 t_2 t_{24}
10^8
10^7
10^6
10^5
10^4
AREA
5.9 11.7 11.9 12.4 13.2 14.9 15.5 16.1 16.3 16.5 17.0 17.3 17.8
RETENTION TIME (MIN)

16.5, 17.0, 17.3, and 17.8 were detected only at t_2, and that only two responses greater than 10^4 were recorded for the sample t_{24}.

D. Phenols

1. Phenol

The growth curve for phenol is presented in Figure 24. The initial TOC concentration was 137 mg/ℓ and decreased to 23 mg/ℓ at the end of the eighth hour, t_8. The TOC concentration remained constant after t_8, but note the change in utilization rates between t_0 and t_8. The concentration of TSS also produced a similar increasing pattern on the growth curve. Sludge yield was determined at t_8 and was found to be 1.05. The concentration of phenol at t_0 was measured at 26 mg/ℓ and slowly declined to 3 mg/ℓ at t_8 where it remained constant throughout the experiment.

The off-gases were collected at t_1, t_3, and t_{15} and were analyzed by the GC as shown in Figure 25. The bar graph in Figure 26 displays the compounds producing a response greater than 10^4. All five compounds were detected at rather low RT with the compound at 2.8 producing the larger response. Four of the five compounds were detected at the later time, t_{15}. The volatilization rate of phenol was not determined but stripping samples were collected at t_0, t_2, and t_{14} and were analyzed on the SP 2250 base/neutral column. The results of this analysis are presented in Figure 27. The t_0 sample revealed a large area response at the RT of 3.32.

The GC analysis for the acid extractable organics revealed only phenol (Figures 28 and 29), but there were four compounds detected during the base/neutral analysis (Figures 30 and 31). The bar graph in Figure 32 displays these compounds which recorded a response greater than 10^4. The only

Figure 24. Growth Curve for Phenol Demonstrating TOC and Phenol Removal and TSS Production

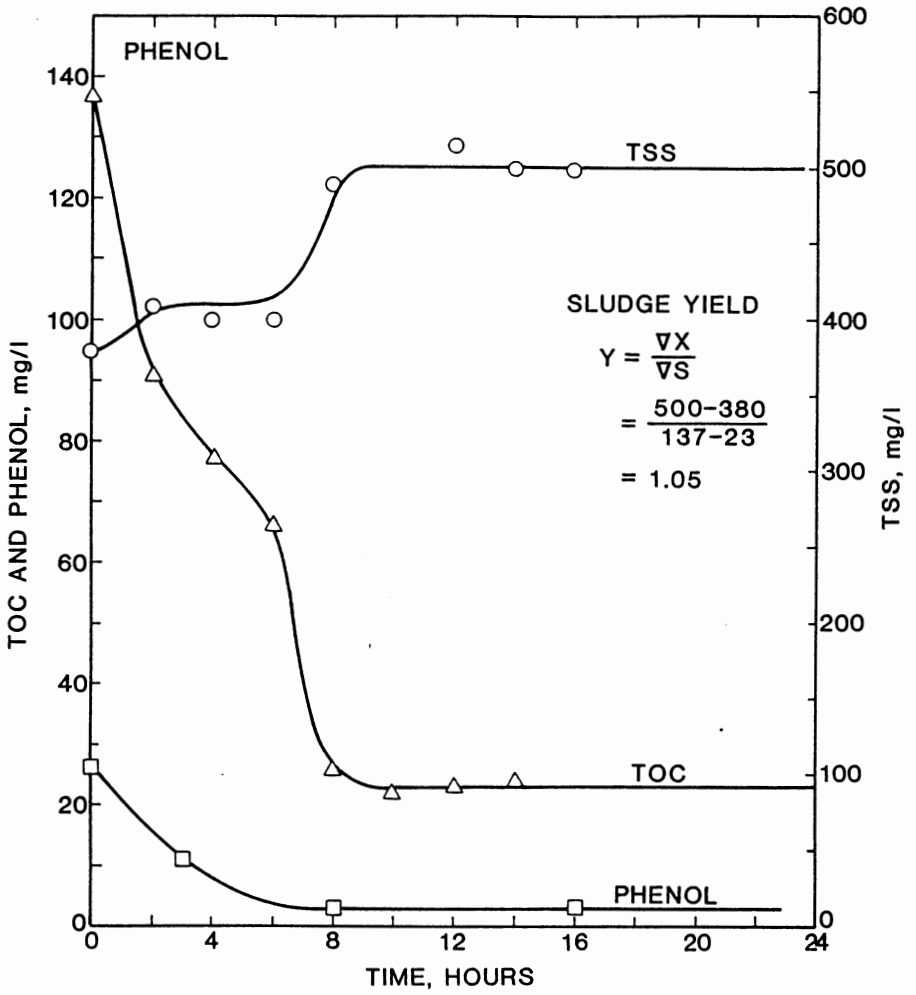

PHENOL
TSS
TOC
PHENOL
SLUDGE YIELD
Y = ∇X/∇S
= 500-380/137-23
= 1.05
TOC AND PHENOL, mg/l
TSS, mg/l
TIME, HOURS
0
4
8
12
16
20
24
0
20
40
60
80
100
120
140
0
100
200
300
400
500
600

Figure 25. Chromatographs for Phenol Off-Gases Collected at t_1, t_3, and t_{15} (Carbowax 1500 Column)

PHENOL
OFF-GASES (CARBOWAX 1500)

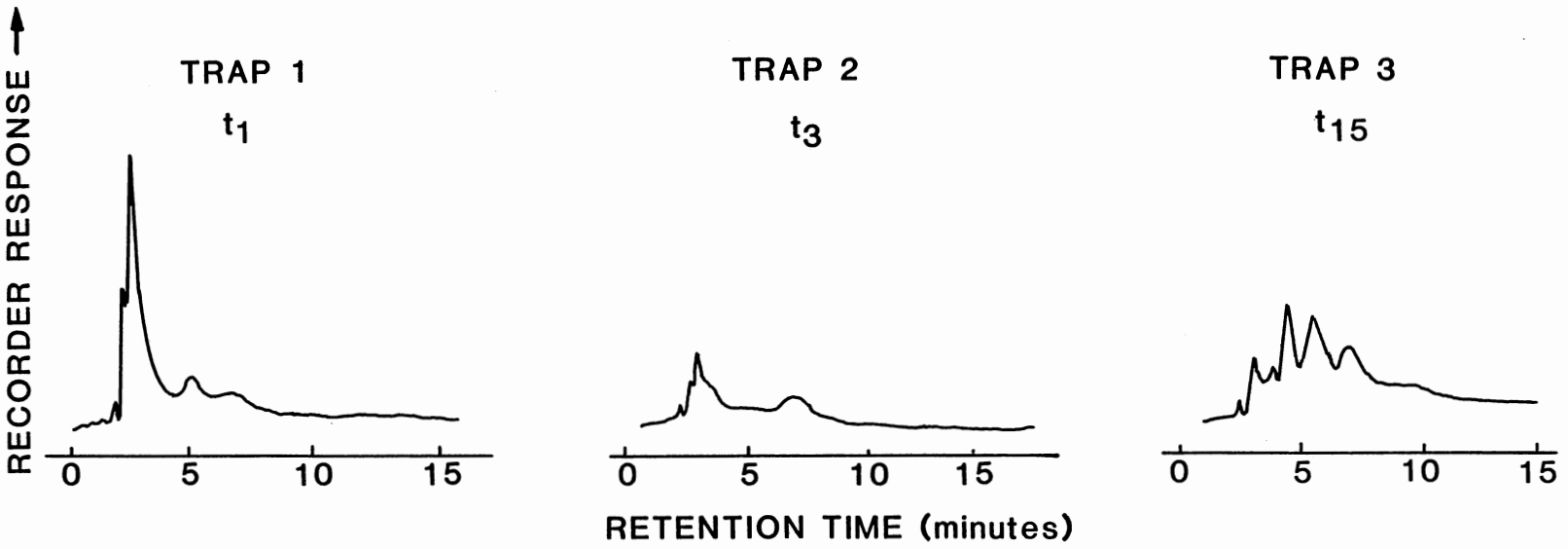


Figure 26. Bargraph for Phenol Off-Gases Displaying the Relative Quantity of Each Compound at t_1, t_3, and t_{15}

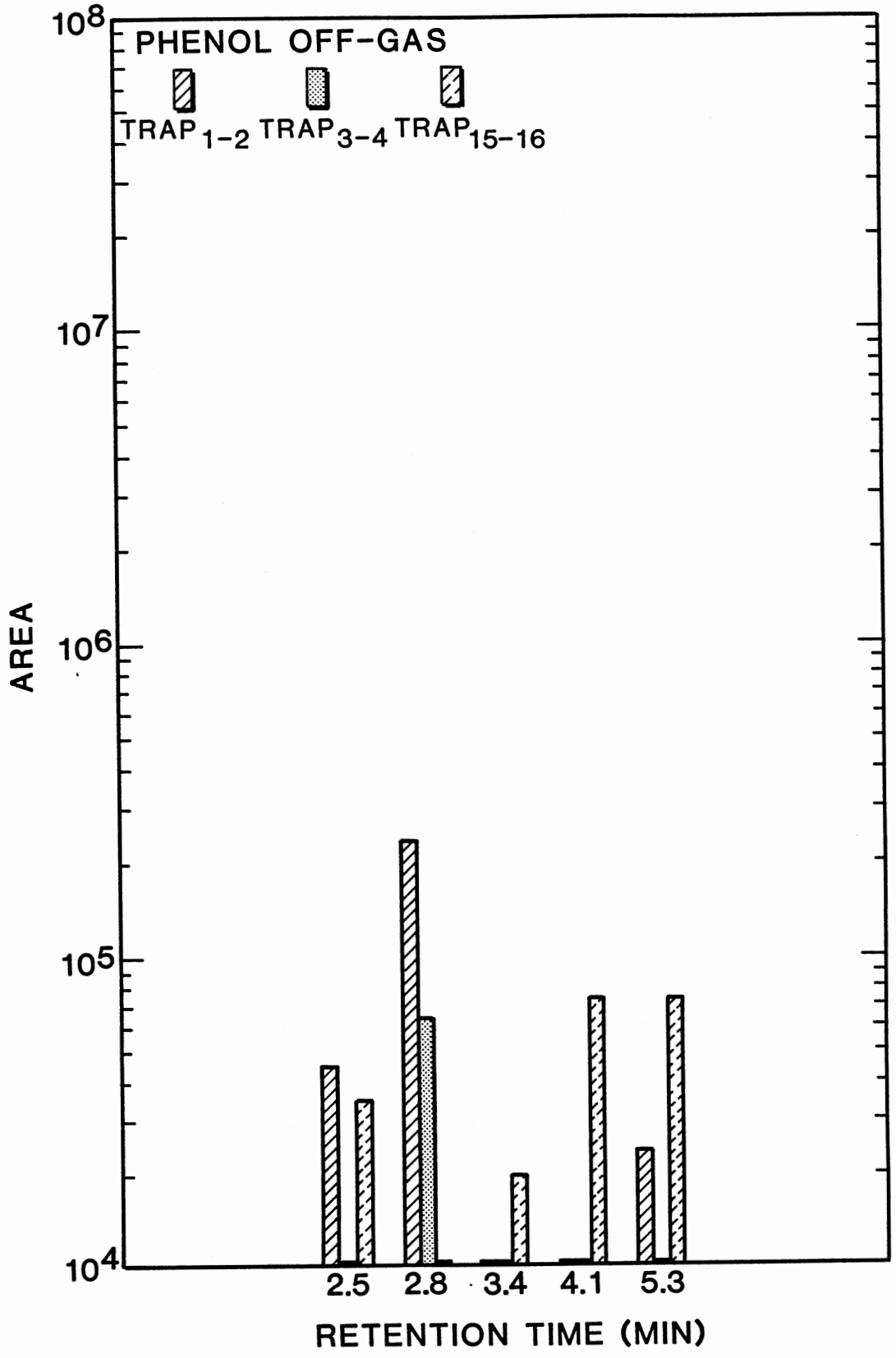

PHENOL OFF-GAS
TRAP 1-2
TRAP 3-4
TRAP 15-16
AREA
10^8
10^7
10^6
10^5
10^4
2.5
2.8
3.4
4.1
5.3
RETENTION TIME (MIN)

Figure 27. Chromatographs for Phenol Off-Gases Collected at t_0, t_2, and t_{14} (SP 2250 Column)

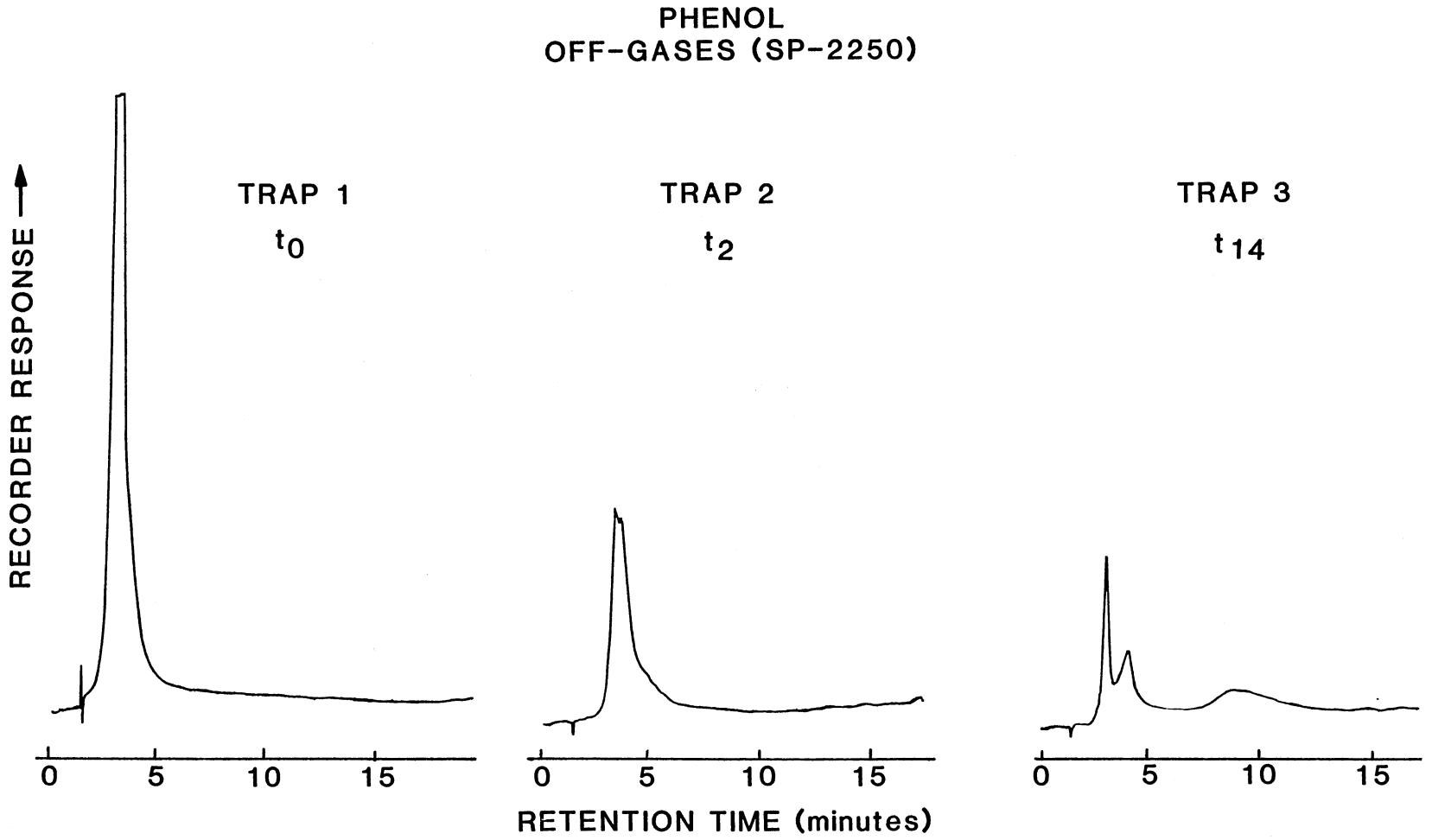

PHENOL
OFF-GASES (SP-2250)
RECORDER RESPONSE
TRAP 1
t_0
TRAP 2
t_2
TRAP 3
t_{14}
0
5
10
15
0
5
10
15
0
5
10
15
RETENTION TIME (minutes)

Figure 28. Chromatographs for the Phenol Acid Fraction Collected at t_0 and t_3

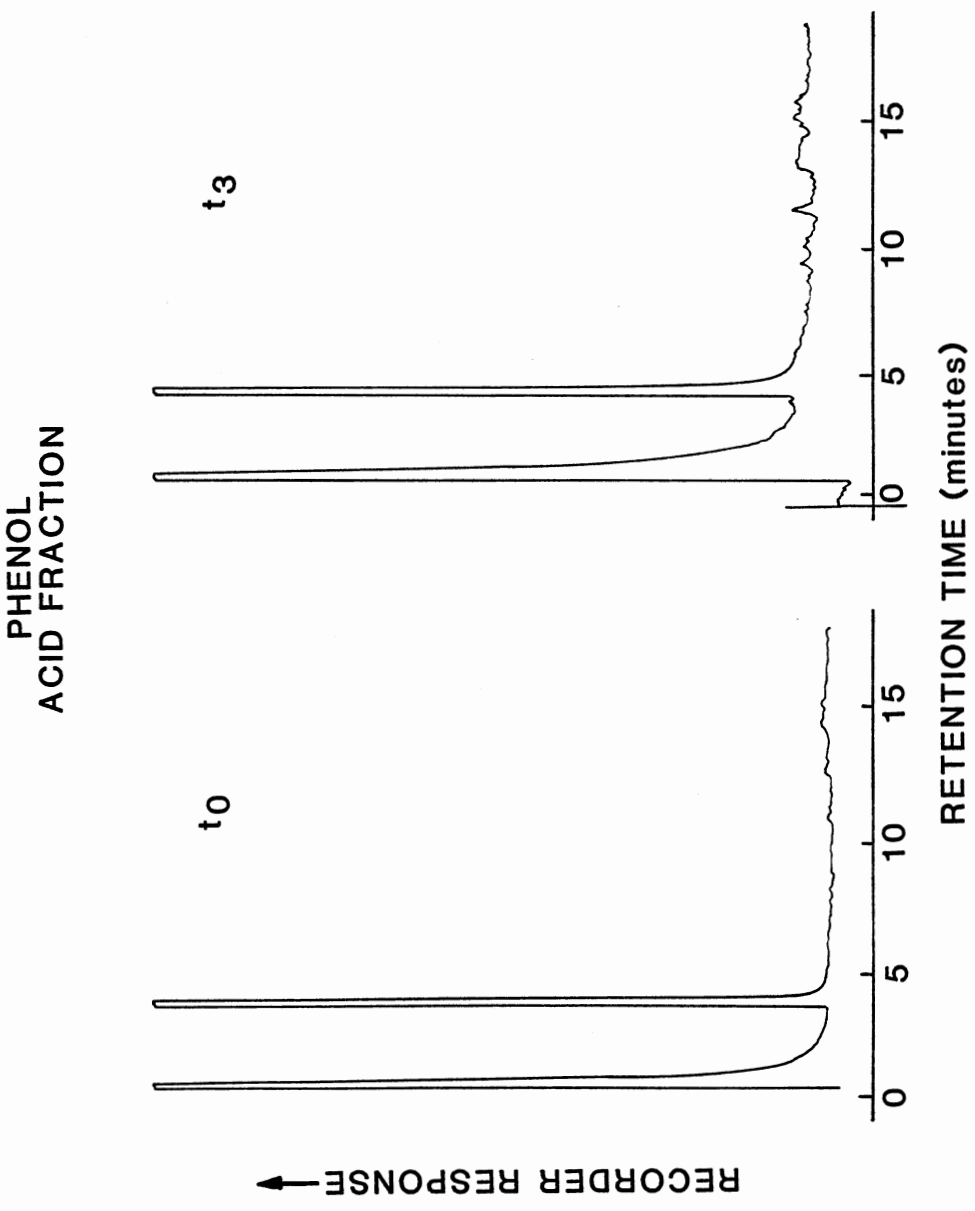

PHENOL
ACID FRACTION
t_0
t_3
0
5
10
15
RETENTION TIME (minutes)
RECORDER RESPONSE

Figure 29. Chromatographs for the Phenol Acid Fraction Collected at t_8 and t_{16}

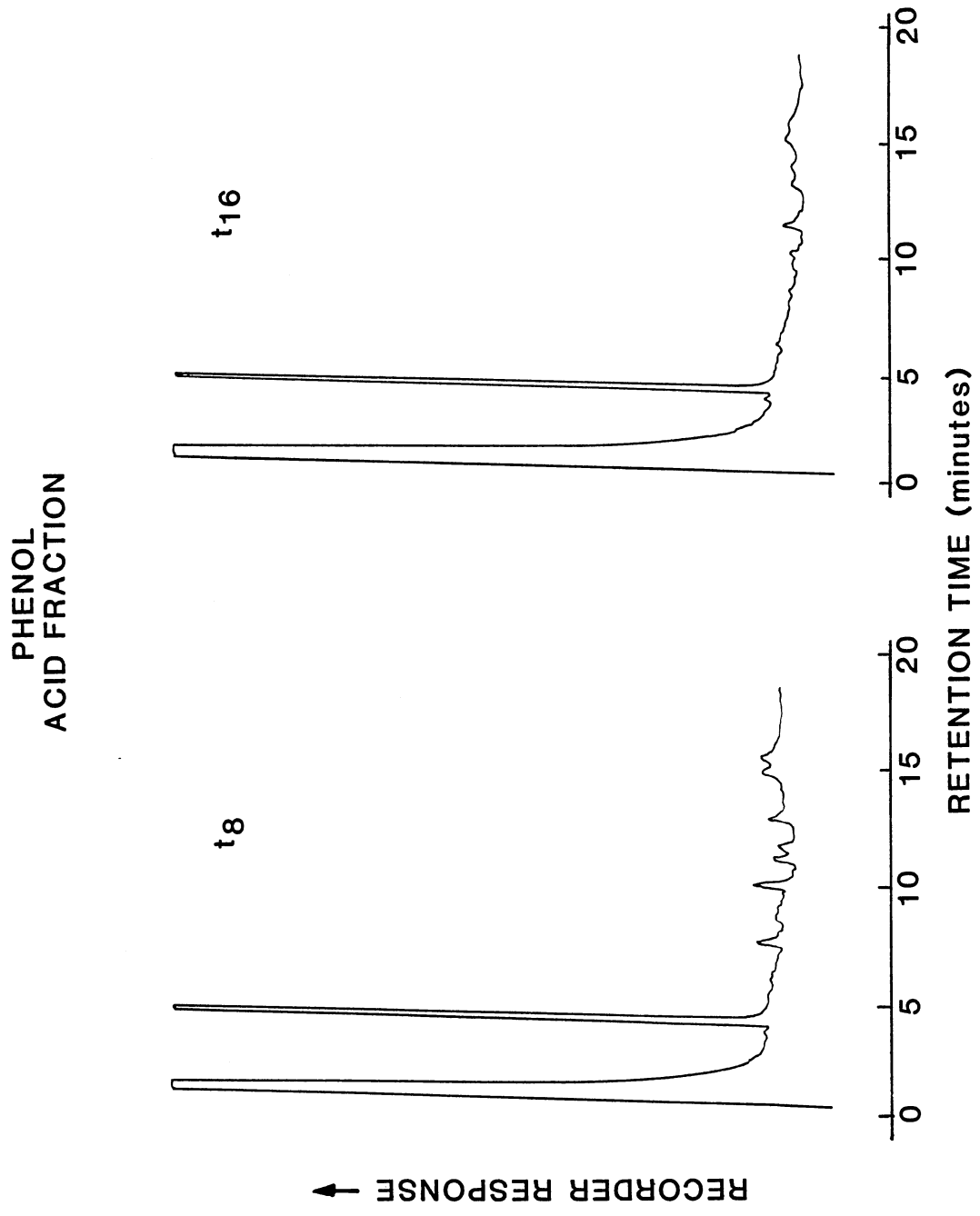

PHENOL
ACID FRACTION
t_{16}
t_8
0
5
10
15
20
RETENTION TIME (minutes)
RECORDER RESPONSE

Figure 30. Chromatographs for Phenol Base/Neutral Fraction Collected at t_0 and t_3

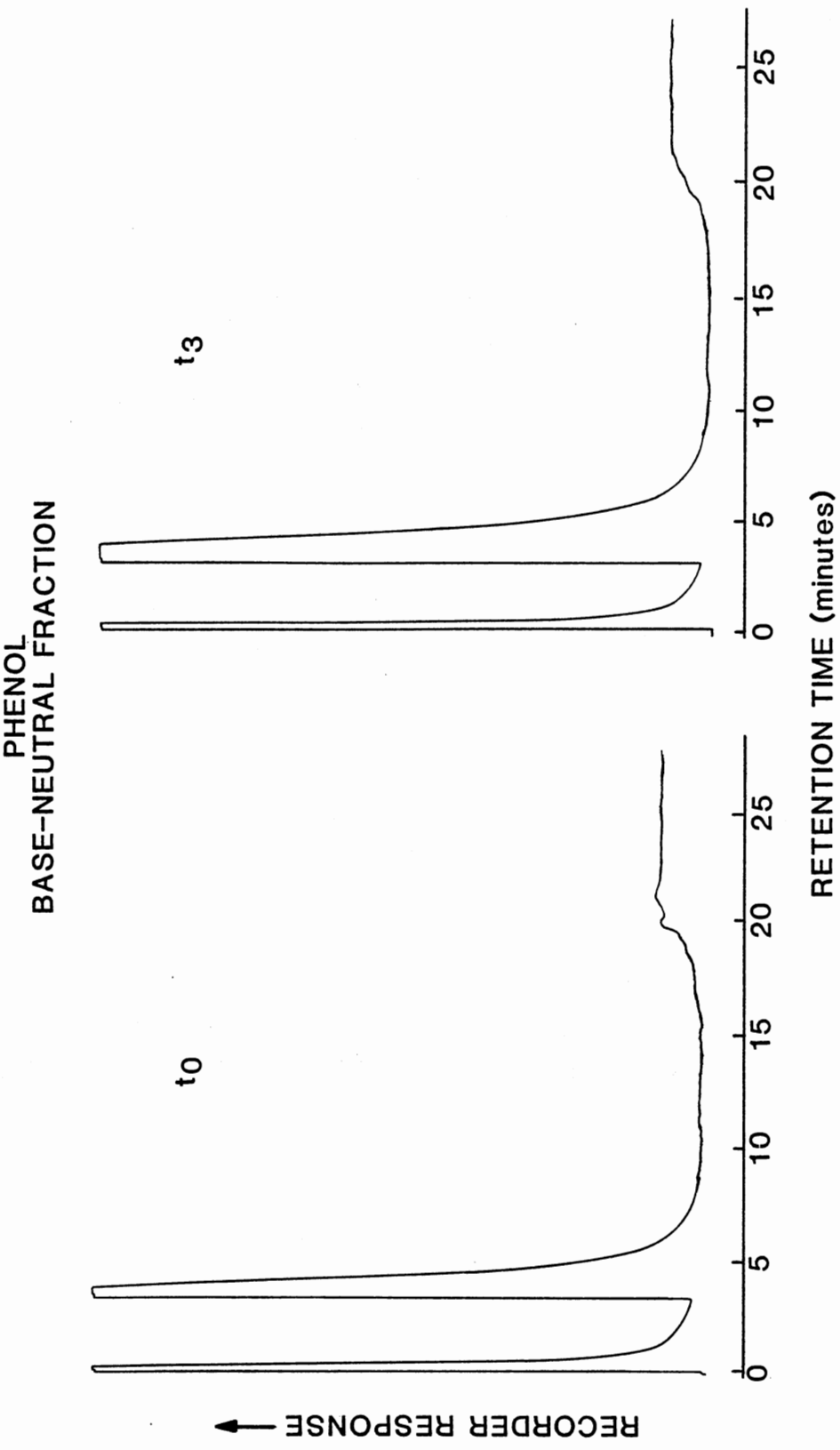

PHENOL
BASE-NEUTRAL FRACTION
t0
t3
RECORDER RESPONSE
0
5
10
15
20
25
RETENTION TIME (minutes)

Figure 31. Chromatographs for Phenol Base/Neutral Fraction Collected at t_8 and t_{16}

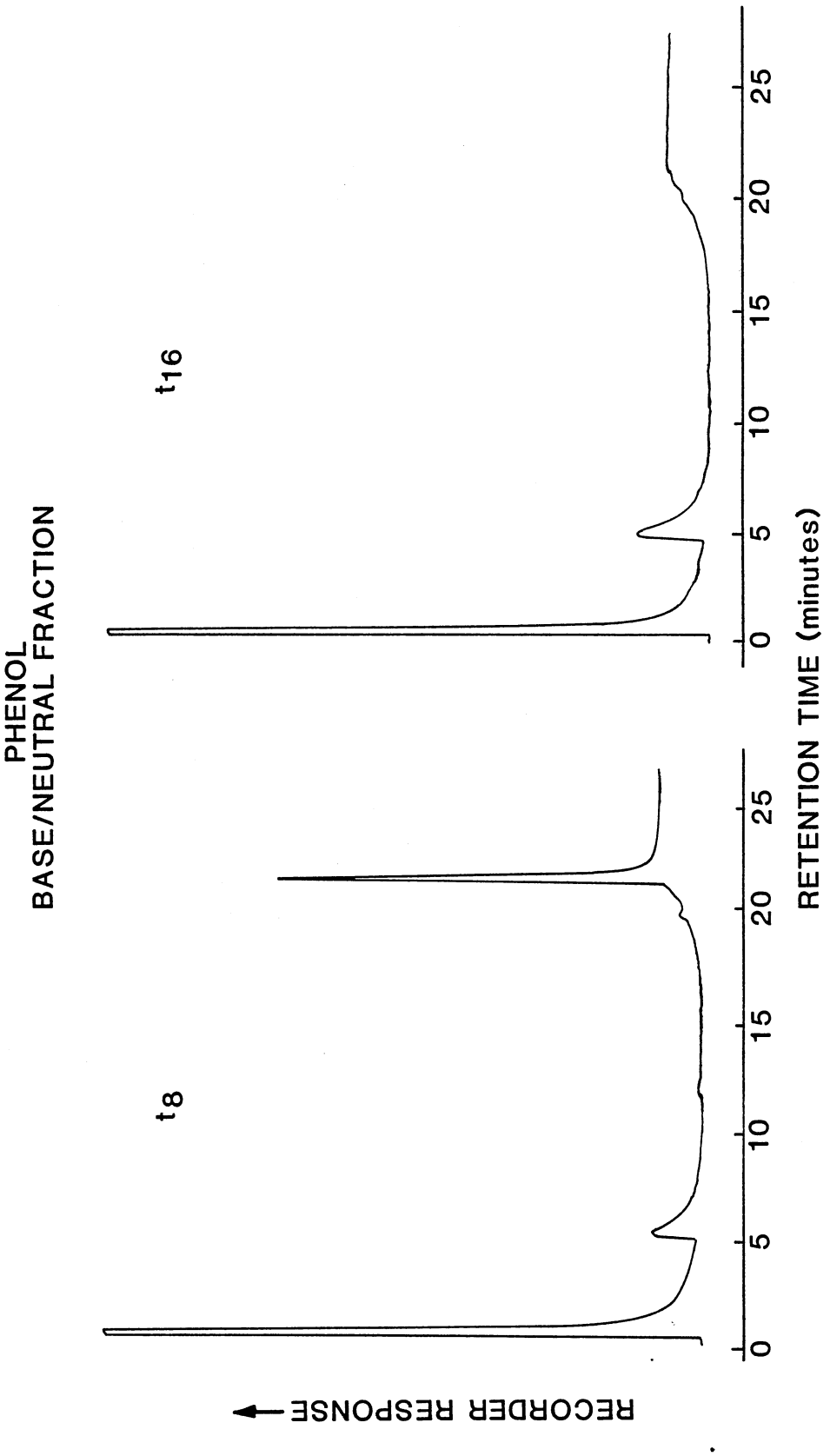
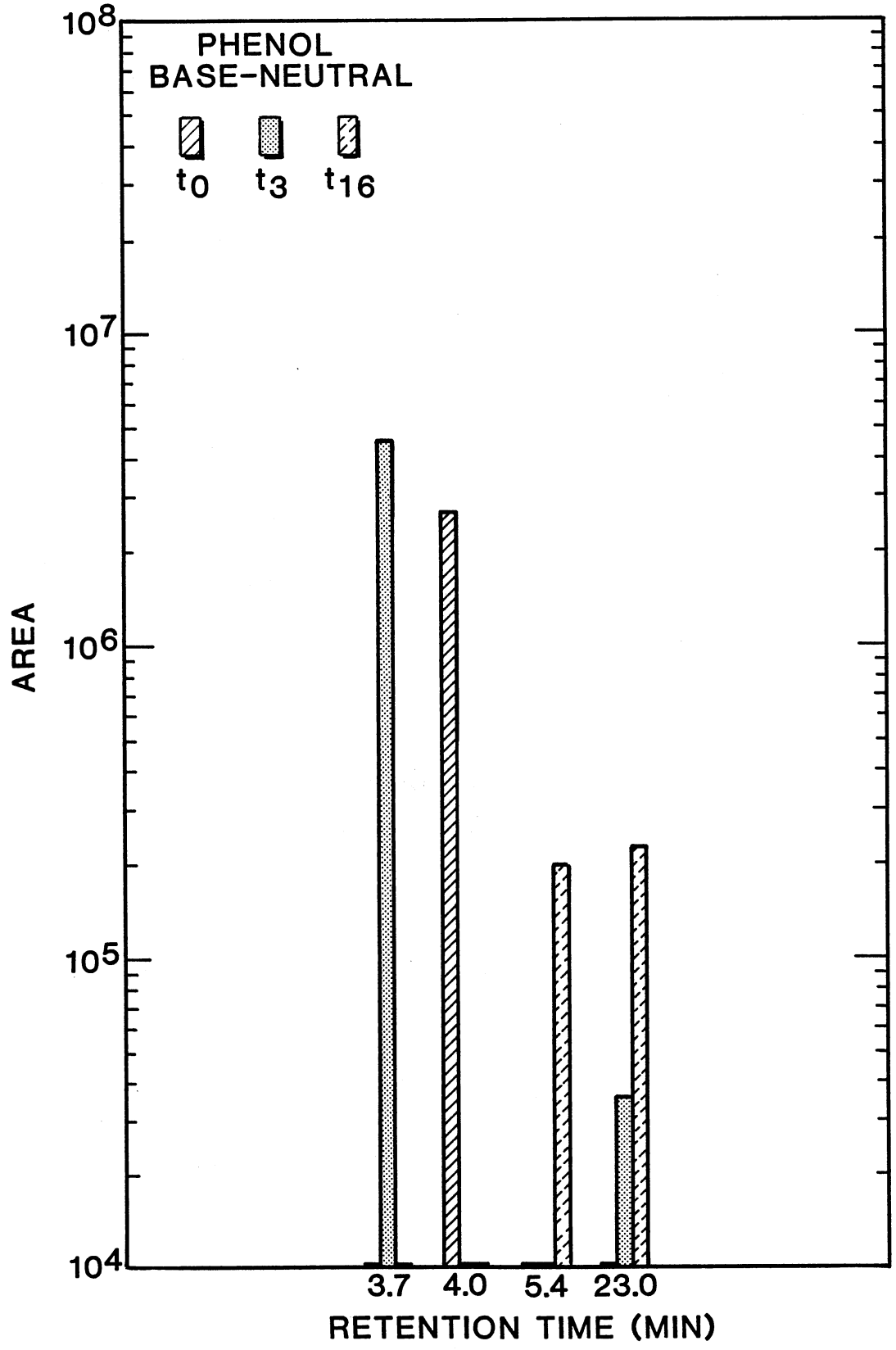

PHENOL
BASE/NEUTRAL FRACTION
t_8
t_{16}
0
5
10
15
20
25
RETENTION TIME (minutes)
RECORDER RESPONSE

Figure 32. Bargraph for Phenol and Base/Neutral Intermediates Displaying the Relative Quantity of Each Compound at t_0, t_3, and t_{16}


PHENOL
BASE-NEUTRAL
t_0 t_3 t_{16}
10^8
10^7
10^6
10^5
10^4
AREA
3.7 4.0 5.4 23.0
RETENTION TIME (MIN)

compound noted during the initial sample, t_0, was at the RT of 4.0, but it produced a significant response. Also note the large response recorded with sample t_3 but its absence at t_0 and t_{24}. The compounds at 5.4 and 23.0 produced a moderate response in sample t_{16}.

2. 2,4-Dinitrophenol

The growth curve for 2,4-dinitrophenol is presented in Figure 33. The TOC concentration decreases rapidly during the first two hours but remains constant at 40 mg/ℓ after t_2. TSS increased approximately 50 mg/ℓ during the first two hours but then remained constant after t_2. The sludge yield was calculated at the end of t_2, and was found to be 1.50. The 2,4-dinitrophenol concentration decreased rapidly to 48 mg/ℓ at the end of the fourth hour, and remained at this relatively high concentration during the growth study.

The off-gases were collected at t_1, t_5, and t_{13} and were analyzed as shown in Figure 34. The bar graph in Figure 35 displays only four compounds which produced a response greater than 10^4. The compound with a RT of 19.7 produced the greater response, but note the compound at 4.4. A large peak was observed on the chromatograph at 4.4 min (Figure 34) but the chart recorder failed to print the area. It is also of interest that both compounds were detected only at t_{13}. Stripping rates were not determined for 2,4-dinitrophenol, but volatile samples were collected and analyzed using the base/neutral column. The result of this chromatographic analysis is displayed in Figure 36.

The GC analysis for the base/neutrals is presented in Figures 37 and 38. No intermediates were detected with the exception of one compound in sample t_{17} where a small response was recorded at 23.1. The acid

Figure 33. Growth Curve for 2,4-Dinitrophenol Demonstrating TOC and 2,4-Dinitrophenol Removal and TSS Production

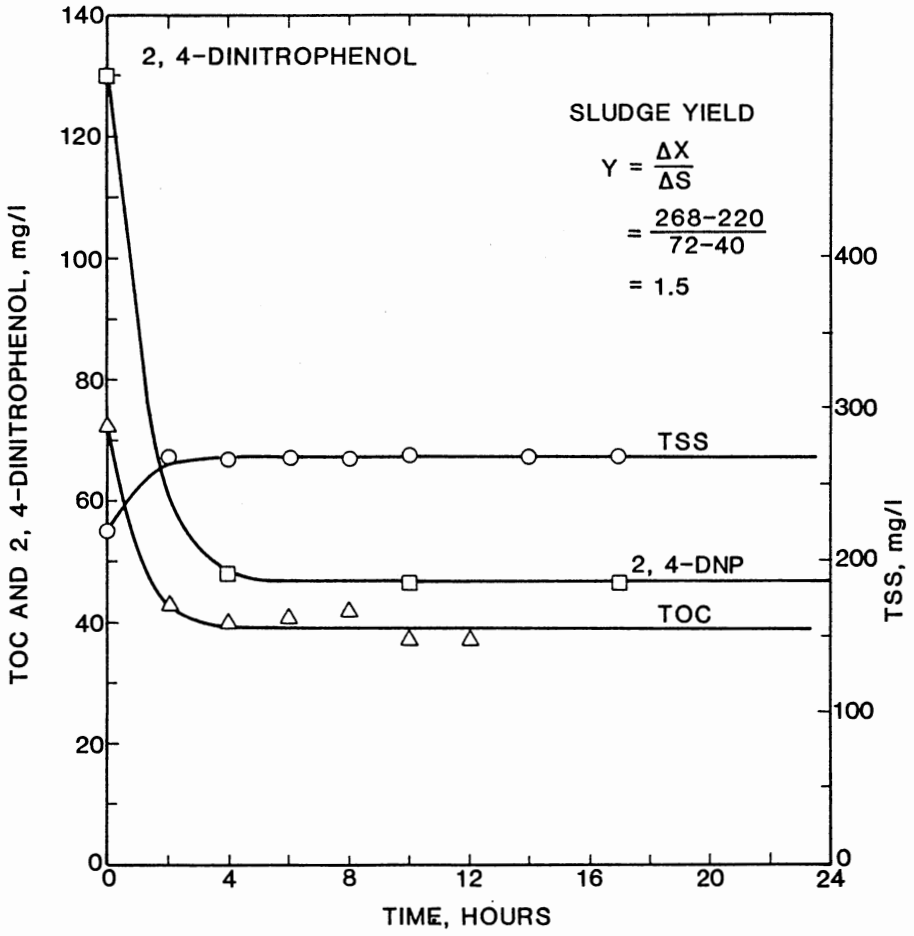
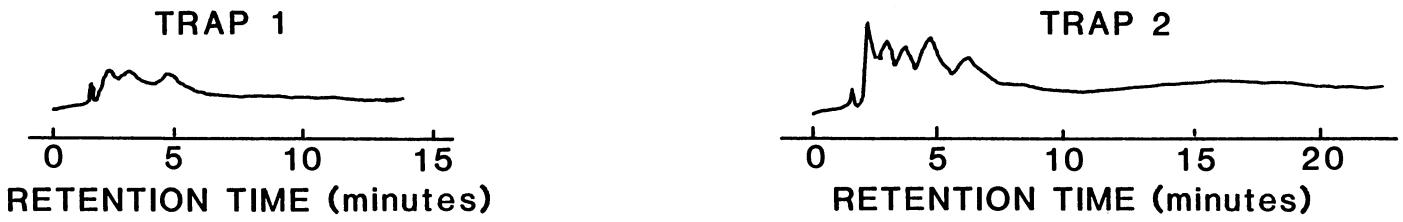

2, 4-DINITROPHENOL
SLUDGE YIELD
Y = ΔX/ΔS
= 268-220/72-40
= 1.5
TSS
2, 4-DNP
TOC
TOC AND 2, 4-DINITROPHENOL, mg/l
TSS, mg/l
TIME, HOURS
0
20
40
60
80
100
120
140
100
200
300
400
0
4
8
12
16
20
24

Figure 34. Chromatographs for 2,4-Dinitrophenol Off-Gases Collected at t_1, t_5, and t_{13} (Carbowax 1500 Column)

2, 4-DINITROPHENOL
OFF-GASES (CARBOWAX 1500)



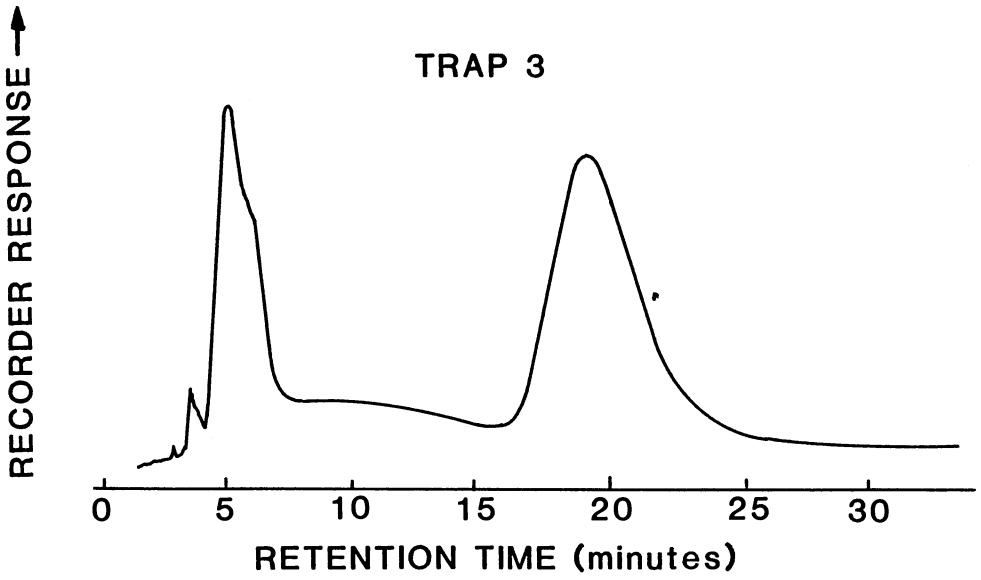


Figure 35. Bargraph for 2,4-Dinitrophenol Off-Gases Displaying the Relative Quantity of Each Compound at t_1, t_5, and t_{13}

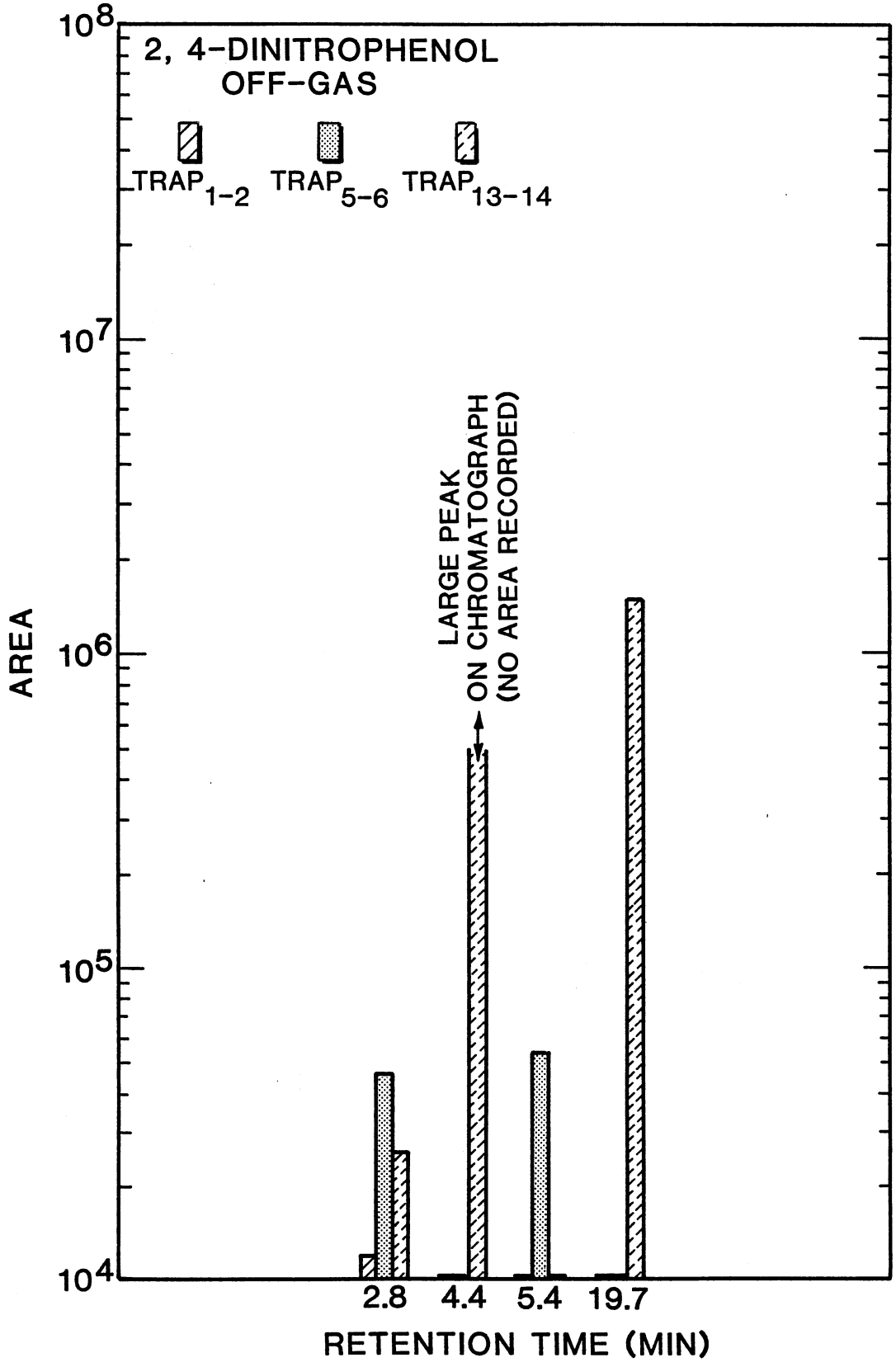

2, 4-DINITROPHENOL
OFF-GAS
TRAP1-2
TRAP5-6
TRAP13-14
10^8
10^7
10^6
10^5
10^4
AREA
LARGE PEAK
ON CHROMATOGRAPH
(NO AREA RECORDED)
2.8
4.4
5.4
19.7
RETENTION TIME (MIN)

Figure 36. Chromatographs for 2,4-Dinitrophenol Off-Gases Collected at t_0, t_4, and t_{12} (SP-2250 Column)

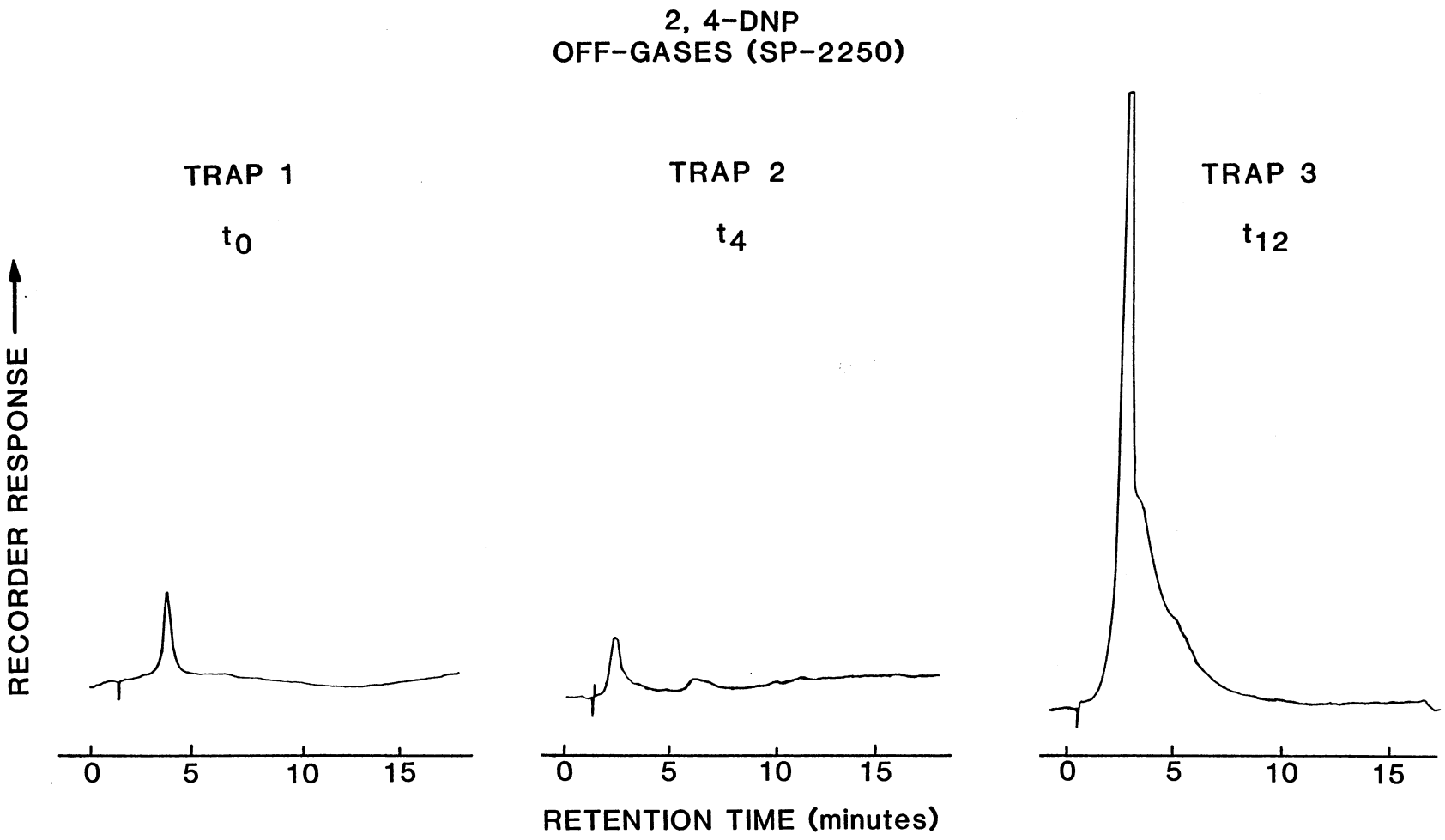

2, 4-DNP
OFF-GASES (SP-2250)
TRAP 1
t_0
TRAP 2
t_4
TRAP 3
t_{12}
RECORDER RESPONSE
0
5
10
15
0
5
10
15
0
5
10
15
RETENTION TIME (minutes)

Figure 37. Chromatographs for 2,4-Dinitrophenol Base/Neutral Fraction Collected at t_0 and t_4

2, 4-DNP
BASE/NEUTRAL FRACTION

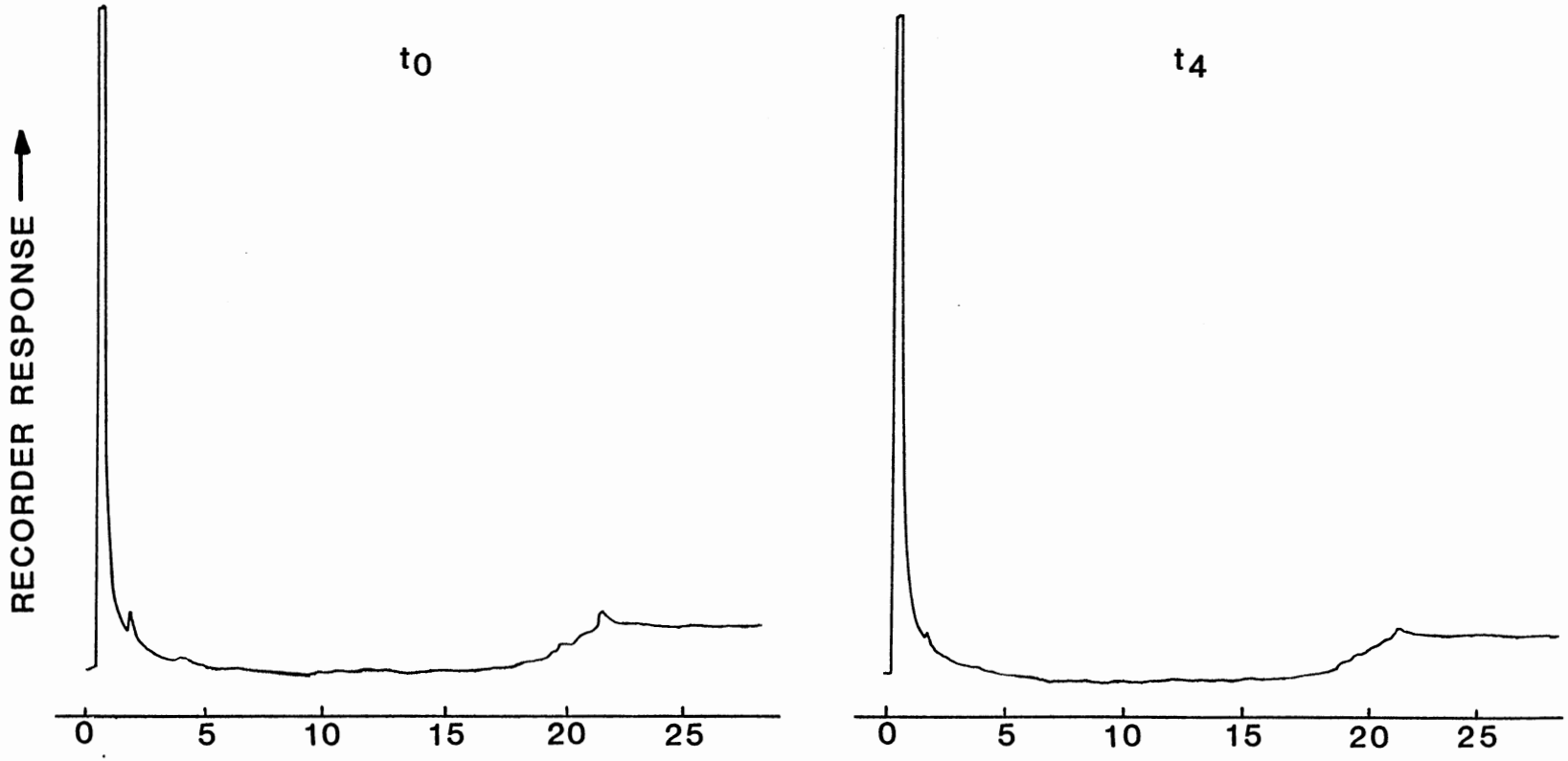
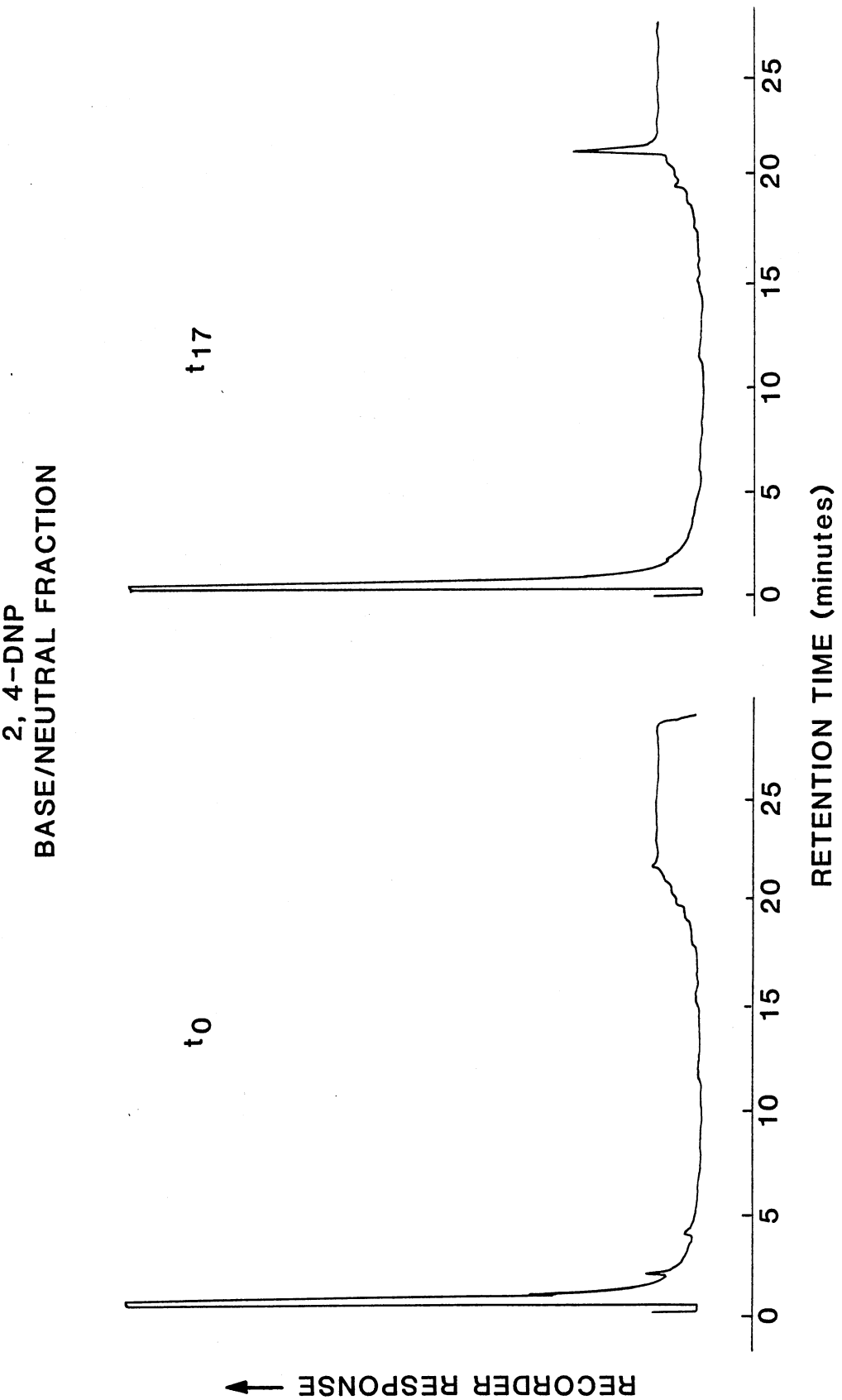


Figure 38. Chromatographs for 2,4-Dinitrophenol Base/Neutral Fraction Collected at t_{10} and t_{17}


2, 4-DNP
BASE/NEUTRAL FRACTION
t_0
t_{17}
0
5
10
15
20
25
RETENTION TIME (minutes)
RECORDER RESPONSE

extractable organics are displayed in Figures 39 and 40. The bar graph in Figure 41 shows five compounds with a response greater than 10^4, including 2,4-dinitrophenol. The compounds at 13.7, 15.8, and 16.6 all produced greater responses in sample t_{17}. Also note the moderate response detected at 12.7.

E. Halogenated Aliphatics

1. 1,2-Dichloropropane

The growth study for 1,2-dichloropropane is presented in Figure 42. Note the rapid decrease in TOC during the first hour accompanied by a gradual increase in TSS. Both TOC and TSS remain relatively constant after t_2. The sludge yield was calculated after t_2 and was found to be 1.30. The concentration of 1,2-dichloropropane was 98.5 mg/ℓ at t_0, but decreased rapidly to less than 1 gm/ℓ at t_1.

The chromatograph resulting from the off-gas analysis revealed only 1,2-dichloropropane. Since this compound is very volatile, the flow rate through the trap was adjusted to 10 mℓ/min and the collection time was lowered to five minutes. Because of this small time interval it was necessary to calculate the volatilization by applying the first-order equation, $C_R = C_0 (1 - e^{kt})$. The stripping summary in Table V and the plot of ln C_R/C_0 versus time in Figure 43 demonstrates that stripping of 1,2-dichloropropane does follow first order. The volatilization rate was determined to be 99.9 percent after the first hour of operation.

The base/neutral and acid extractable compounds were analyzed by installing the SP 2250 and SP 1240 DA columns into a new GC unit. The printout of the area response was different, but there was no change in

Figure 39. Chromatographs for 2,4-Dinitrophenol Acid Fraction Collected at t_0 and t_4

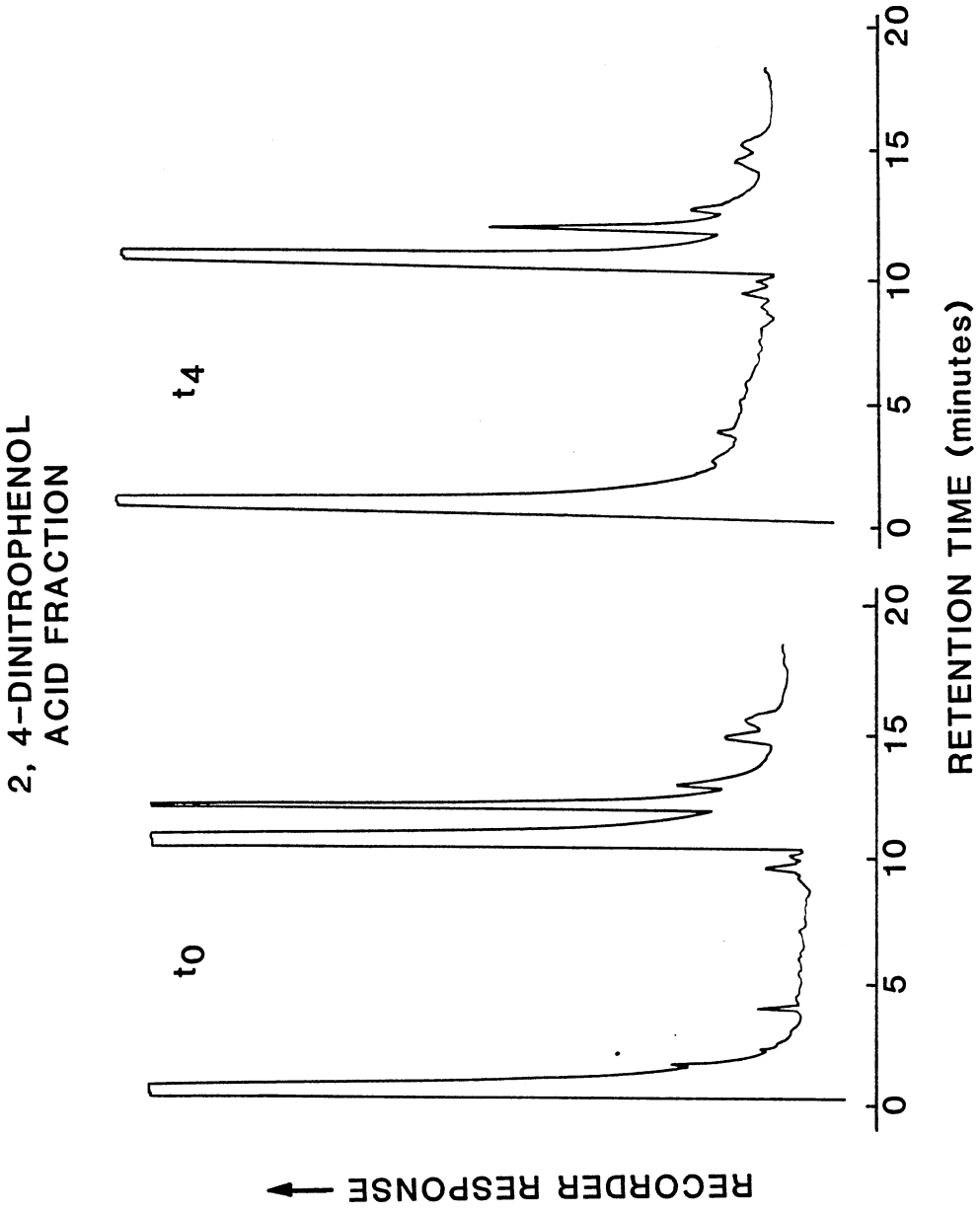

2, 4-DINITROPHENOL
ACID FRACTION
t_0
t_4
RECORDER RESPONSE
0
5
10
15
20
RETENTION TIME (minutes)

Figure 40. Chromatographs for 2,4-Dinitrophenol Acid Fraction Collected at t_{10} and t_{17}

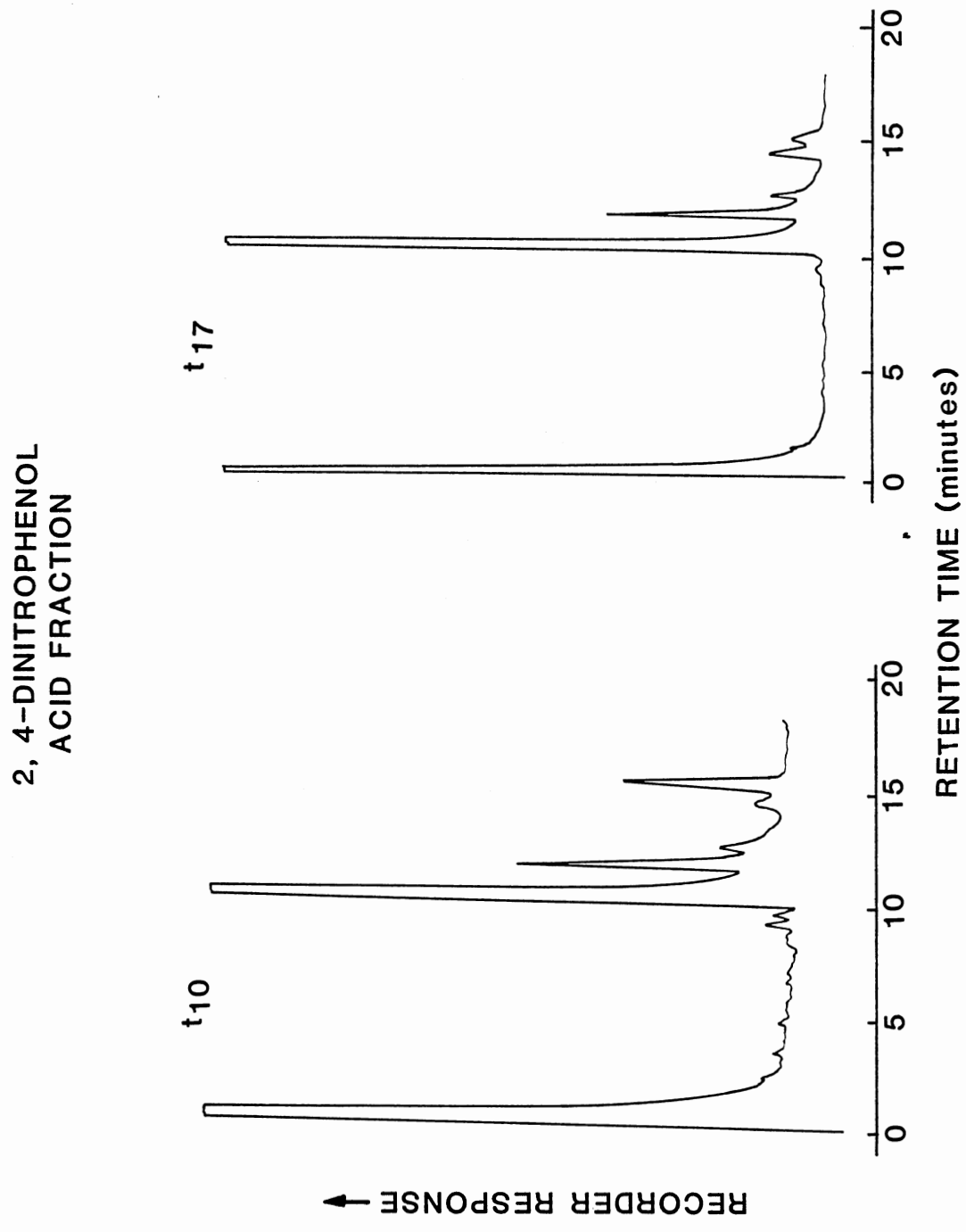

2, 4-DINITROPHENOL
ACID FRACTION
t10
t17
RECORDER RESPONSE
0
5
10
15
20
RETENTION TIME (minutes)

Figure 41. Bargraph for 2,4-Dinitrophenol Acid Fraction Displaying the Relative Quantity of Each Compound at t_0, t_4, and t_{17}

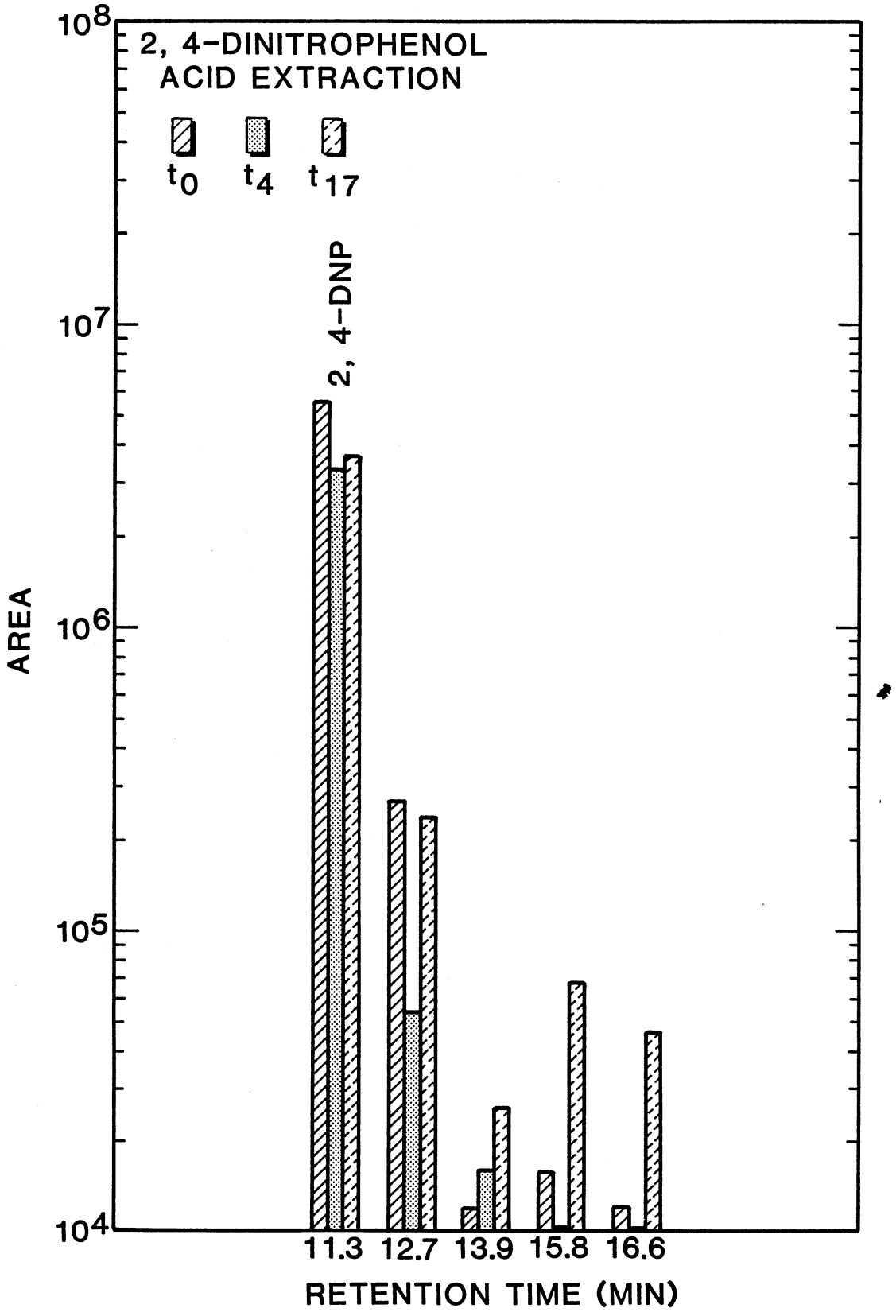

2, 4-DINITROPHENOL
ACID EXTRACTION
t_0 t_4 t_{17}
2, 4-DNP
10^8
10^7
10^6
10^5
10^4
AREA
11.3 12.7 13.9 15.8 16.6
RETENTION TIME (MIN)

Figure 42. Growth Curve for 1,2-Dichloropropane Demonstrating TOC and 1,2-Dichloropropane Removal and TSS Production

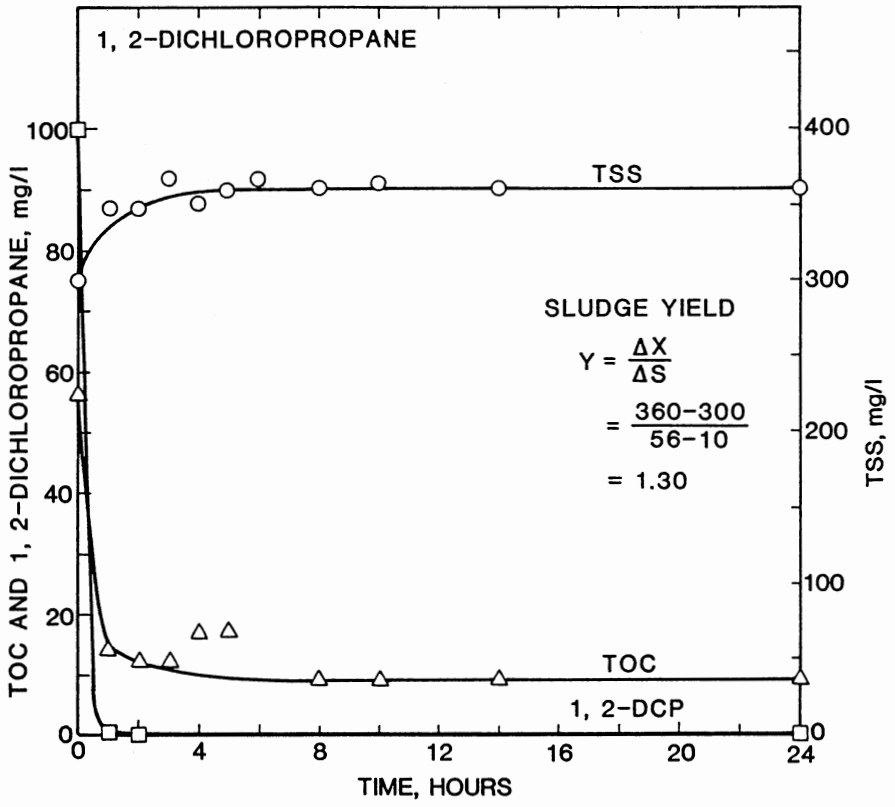

1, 2-DICHLOROPROPANE
TSS
SLUDGE YIELD
Y = ΔX/ΔS
= 360-300/56-10
= 1.30
TOC
1, 2-DCP
TOC AND 1, 2-DICHLOROPROPANE, mg/l
TSS, mg/l
TIME, HOURS
0
20
40
60
80
100
100
200
300
400
0
4
8
12
16
20
24

TABLE V

VOLATILIZATION ANALYSIS FOR 1,2-DICHLOROPROPANE

Trap	Area Response x 10^6	Mg	C_R mg x 200	ln C_R/C_0	Volatilization, Percent
1	7.50	0.284	56.8	-1.24	99.9 (first hour)
2	2.10	0.062	12.4	-2.76	
3	0.17	0.001	0.2	-6.89	

Air flow rates: Reactor rate = 2.0 l/min; trap rate = 10 ml/min.

Dilution factor: 10/2000 = 0.005.

Calculation: $C_R = C_0 (1 - e^{kt})$

where $C_0 = 197$ mg

$k = -7.5$

$t = 1$ hr

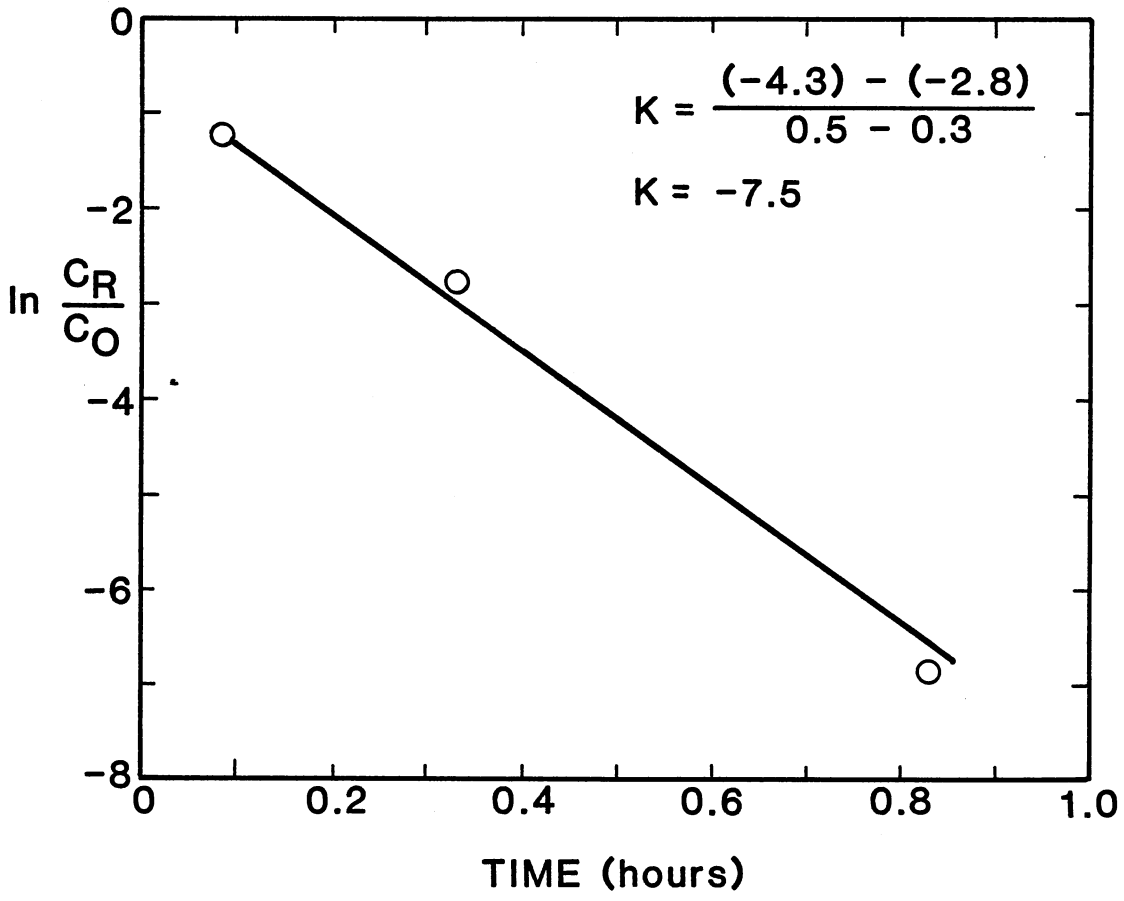
$e = 2.7183$

$C_R = 197 (1 - e^{-7.5})$

$C_R = 196.9$ mg.

Volatilization, %: 196.9/197 x 100 = 99.9%.

Figure 43. Plot of $\ln C_R/C_o$ Versus Time and the Determination of the Constant k in the First Order Equation, $C_R = C_o (1 - e^{Kt})$


0
-2
-4
-6
-8
ln $\frac{C_R}{C_O}$
0
0.2
0.4
0.6
0.8
1.0
TIME (hours)
K = $\frac{(-4.3) - (-2.8)}{0.5 - 0.3}$
K = -7.5

experimental technique. The analysis revealed a total absence of base/neutral compounds at t_0, t_1, and t_{24}. There were several acid extractables noted on the chromatograph in Figure 44, but only four produced a response greater than 10. These four compounds are displayed in Figure 45. Note that the compounds at a RT of 2.1, 2.8, and 6.3 all decreased at t_1 but increased significantly at t_{24}.

2. 1,1,2,2-Tetrachloroethane

The growth study for 1,1,2,2-tetrachloroethane is presented in Figure 46. The decrease in TOC and increase in TSS is similar to that of 1,2-dichloropropane. The sludge yield was determined after t_2 and was found to be 1.33. The concentration of tetrachloroethane was 97.2 mg/ℓ at t_0 but decreased rapidly to 10 mg/ℓ at t_2.

The chromatograph resulting from the off-gas analysis is shown in Figure 47. The collection procedure was altered since tetrachloroethane is also considered to be very volatile. The flow rate was adjusted to 20 mℓ/min, but the collection time was reduced to 15 to 20 minutes. The bar graph in Figure 48 displays the compounds with an area response greater than 10^4. The analysis of trap 1 indicates tetrachloroethane along with two additional compounds. Both compounds show a moderate area response greater than 10^5. Trap 2 shows an increase in the compound at 4.1, a significant decrease of the compound at 10.3, plus two additional compounds at 5.3 and 12.4. Table VI summarizes the volatilization of 1,1,2,2-tetrachloroethane. The volatilization was determined after each trap and was found to be 102 percent after 35 minutes into the experiment.

The chromatographic analysis for the acid extractable organics indicated no compounds at t_0, t_2, or t_{24}. The base/neutral analysis in Figure

Figure 44. Chromatograph for 1,2-Dichloropropane Acid Fraction Collected at t_0, t_1, and t_{24}

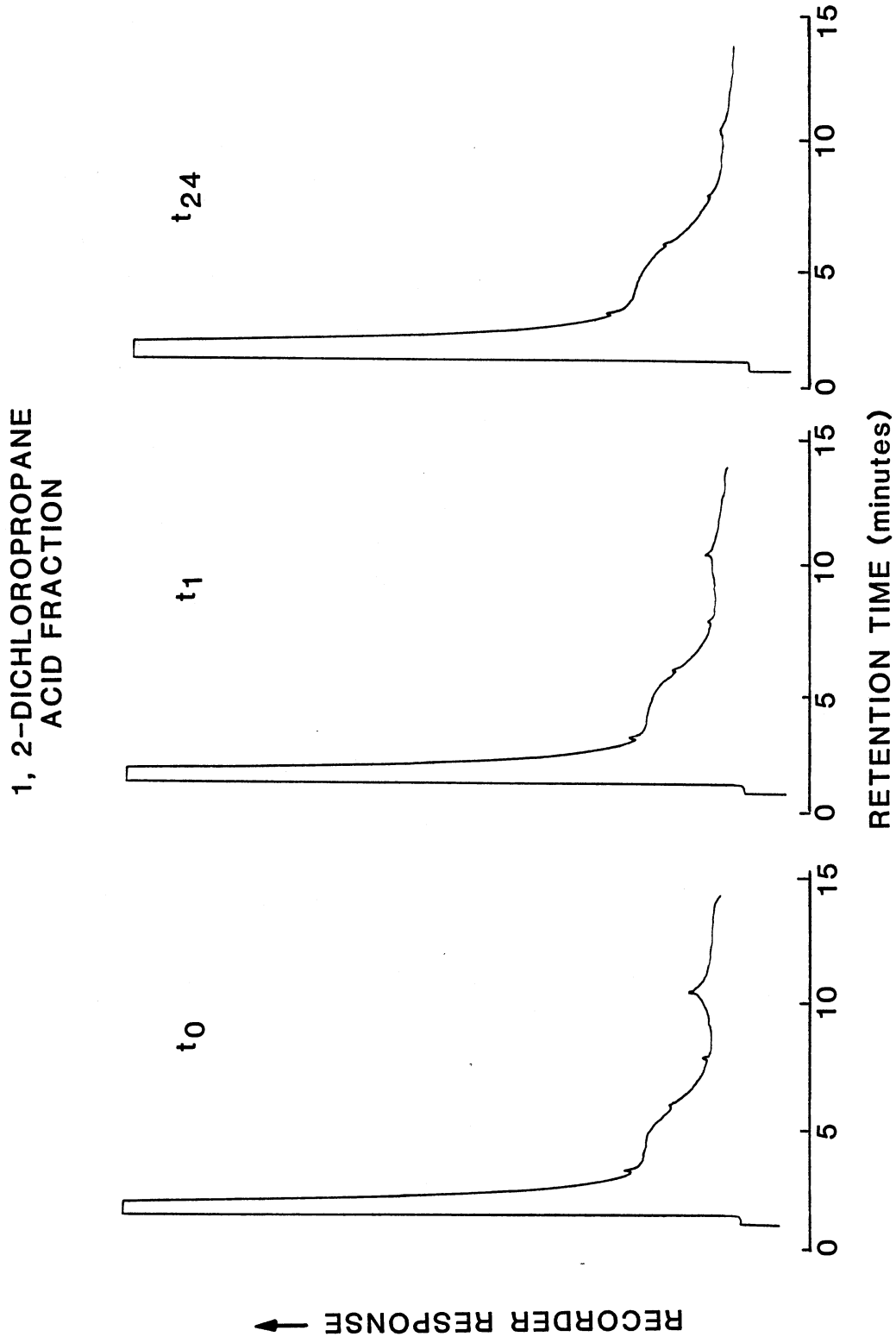

1, 2-DICHLOROPROPANE
ACID FRACTION
t_0
t_1
t_{24}
0
5
10
15
RETENTION TIME (minutes)
RECORDER RESPONSE

Figure 45. Bargraph for 1,2-Dichloropropane Acid Fraction Displaying the Relative Quantity of Each Compound at t_0, t_1, and t_{24}

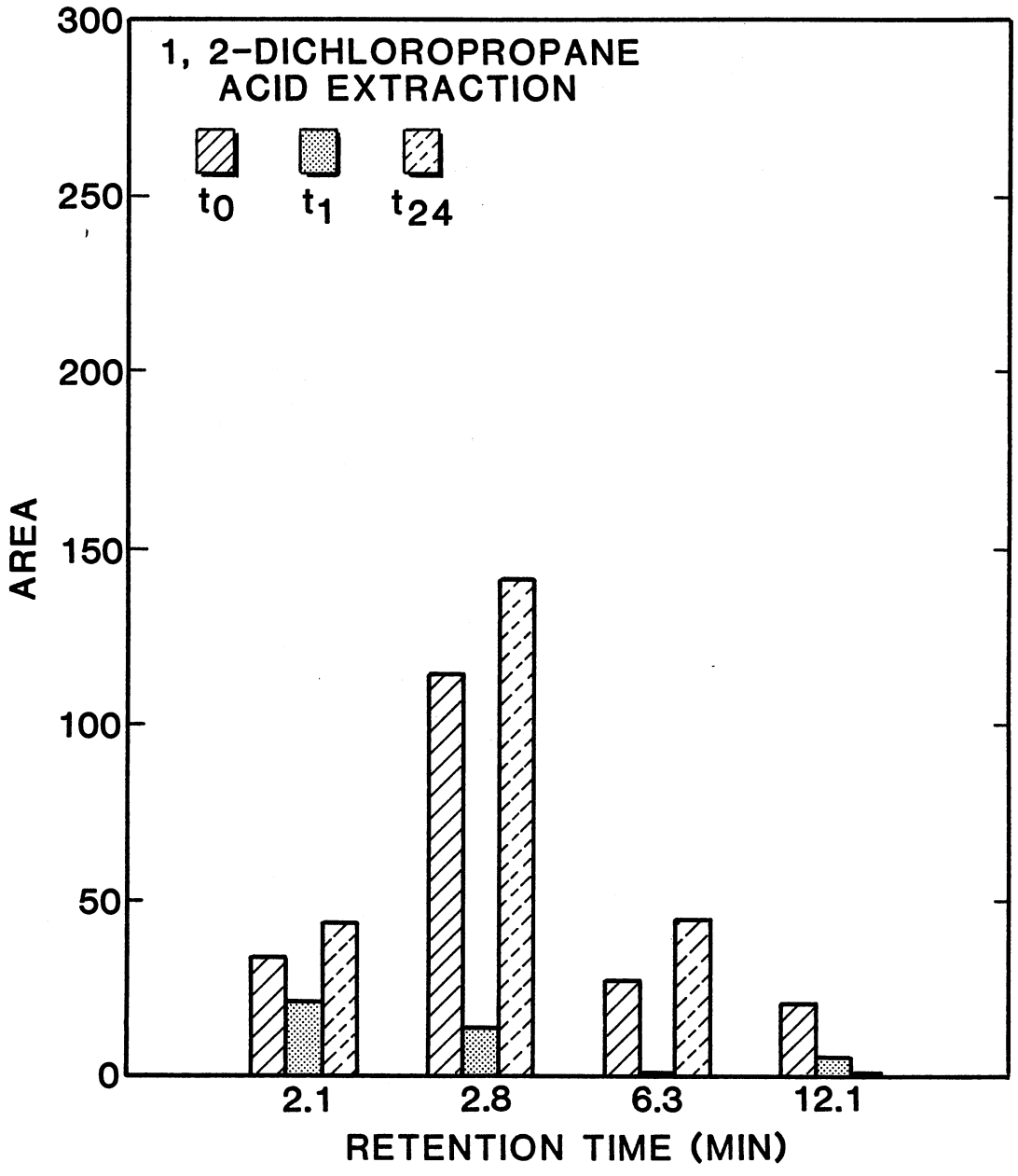

1, 2-DICHLOROPROPANE
ACID EXTRACTION
t_0
t_1
t_{24}
300
250
200
150
100
50
0
AREA
2.1
2.8
6.3
12.1
RETENTION TIME (MIN)

Figure 46. Growth Curve for 1,1,2,2-Tetrachloroethane Demonstrating TOC and 1,1,2,2-Tetrachloroethane Removal and TSS Production

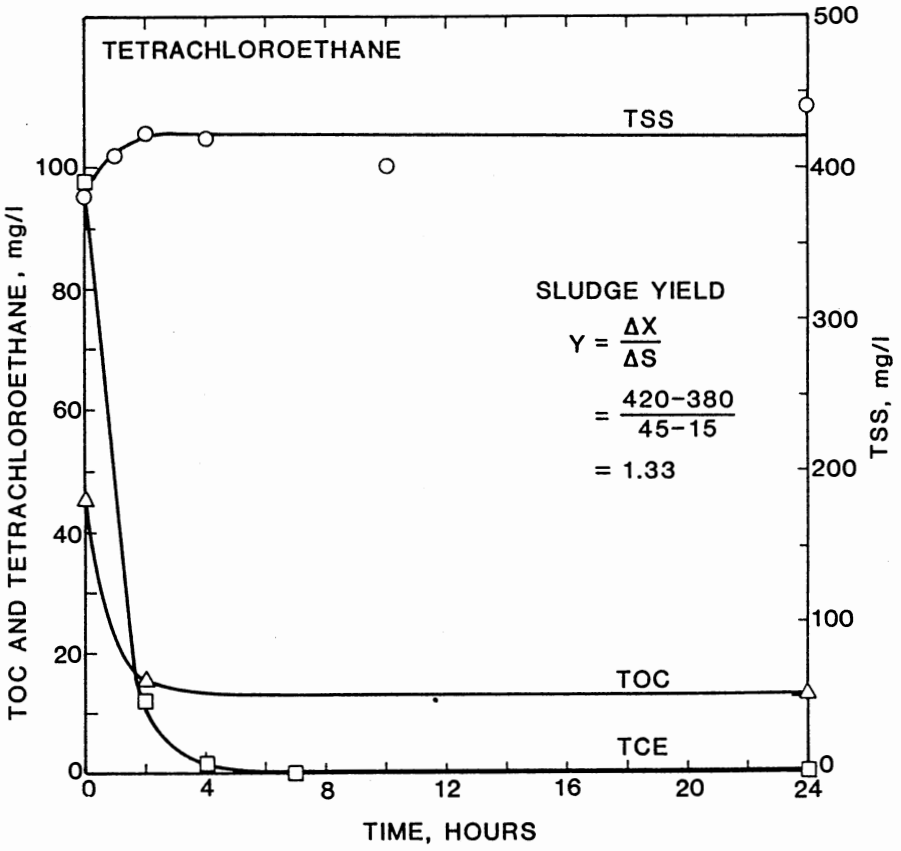

TETRACHLOROETHANE
TSS
TOC
TCE
SLUDGE YIELD
Y = ΔX/ΔS
= 420−380/45−15
= 1.33
TOC AND TETRACHLOROETHANE, mg/l
TSS, mg/l
TIME, HOURS
0
20
40
60
80
100
100
200
300
400
500
0
4
8
12
16
20
24

Figure 47. Chromatograph of 1,1,2,2-Tetrachloroethane and Volatile Intermediates Collected in Traps 1 and 2

1, 1, 2, 2-TETRACHLOROETHANE
OFF-GASES (CARBOWAX 1500)

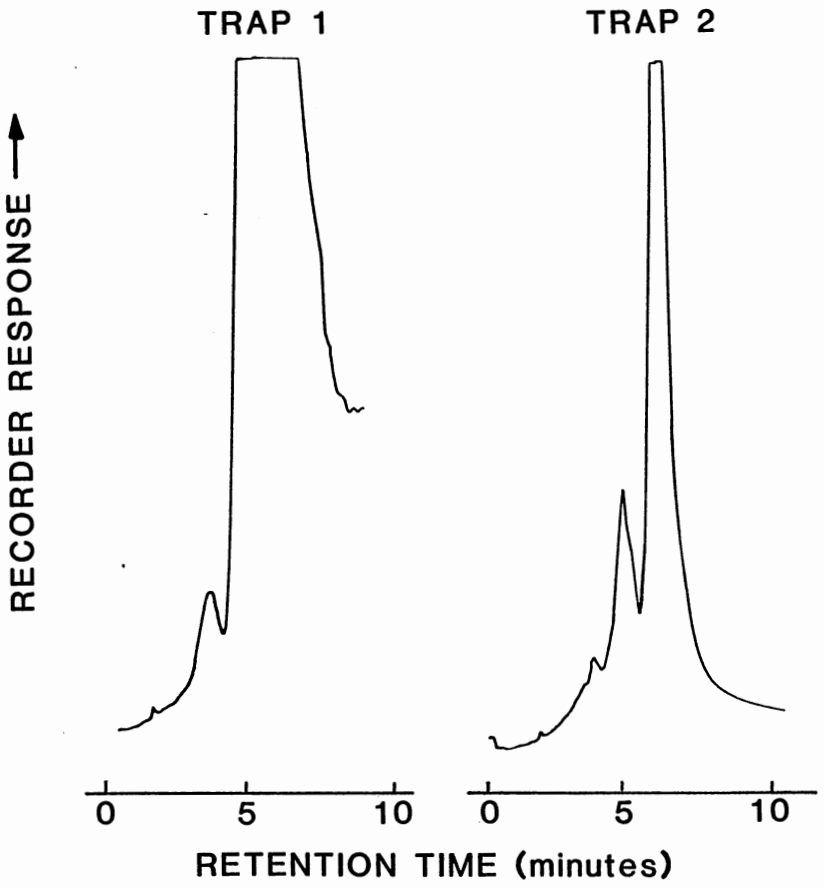


Figure 48. Bargraph for 1,1,2,2-Tetrachloroethane Off-Gases Displaying the Relative Quantity of Each Compound in Traps 1 and 2

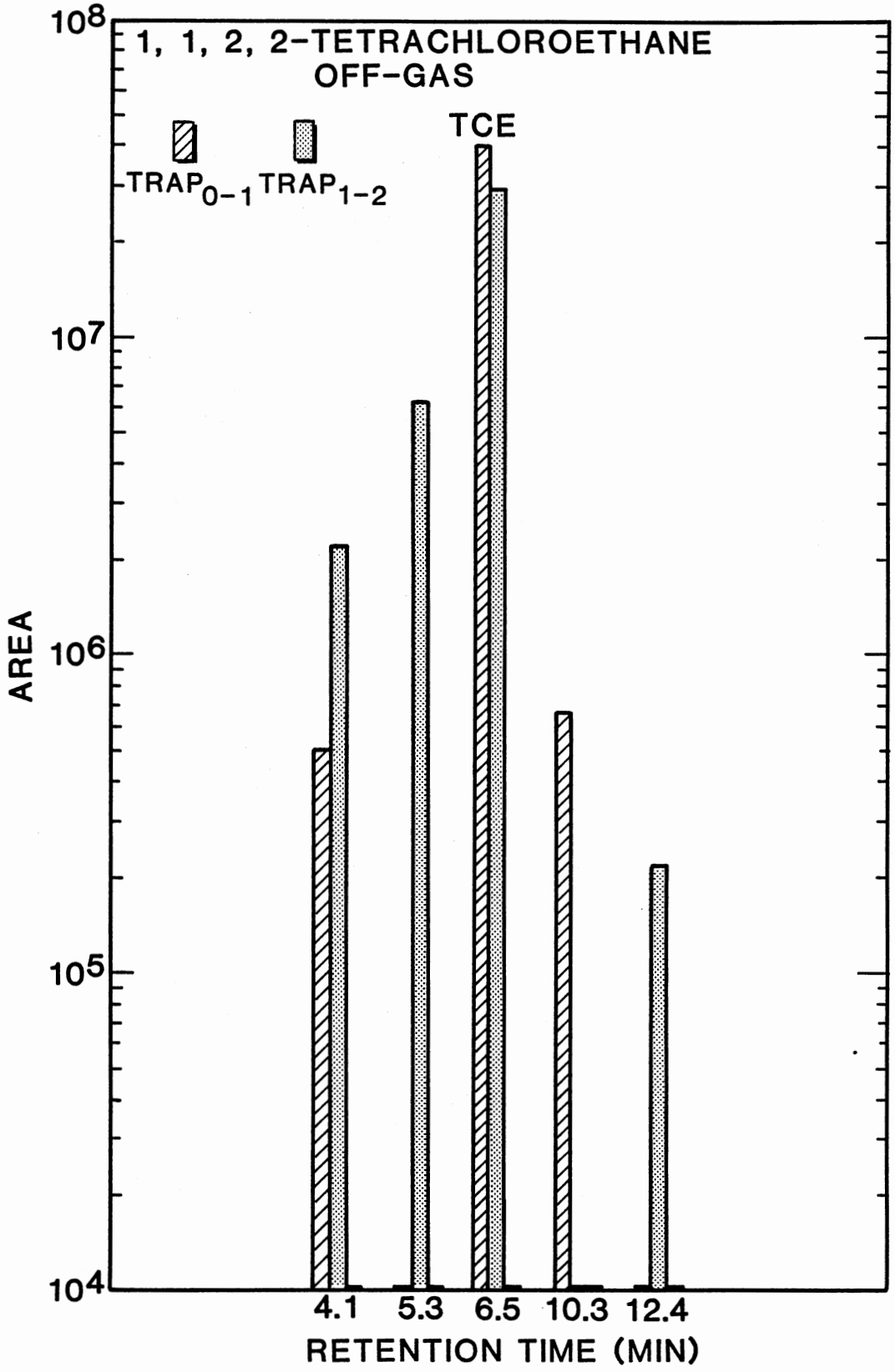

1, 1, 2, 2-TETRACHLOROETHANE
OFF-GAS
TCE
TRAP0-1
TRAP1-2
AREA
10^8
10^7
10^6
10^5
10^4
4.1
5.3
6.5
10.3
12.4
RETENTION TIME (MIN)

TABLE VI

VOLATILIZATION ANALYSIS FOR 1,1,2,2-TETRACHLOROETHANE

Trap	Area Response x 10^6	Mg	Dilution Factor x mg	Total Removal, mg	Volatilization, Percent
1	39.6	0.90	90	104.0	86.5
2	28.8	0.84	84	65.6	128.0
			174	170.0	102.0 (Avg)

Air flow rates: reactor rate = 2.0 ℓ/min; trap rate = 20.0 mℓ/min.

Collection time: trap 1 = 20 min; trap 2 = 15 min.

Dilution factor: 20/2000 = 0.01.

49 revealed four compounds which produced a response greater than 10^4. The bar graph in Figure 50 shows only one compound detected during the analysis of sample t_0. Also note that the compounds at 11.2, 11.6, and 17.7 were only found in sample t_{24}.

F. Summary

Table VII summarizes the overall research data. Eckenfelder's constant, $K = S_i - S_e / X S_e t$, was included to determine if serious design problems exist in the treatment of toxic priority pollutants. The values for the parameters S_e and X were selected at the end of substrate utilization. The tabular results revealed that K_{cpd} did vary, but the K_{cpd} for the aromatic group and the K_{cpd} for the phenols were relatively constant. All seven priority pollutants resulted in similar K_{TOC}. The chloroaliphatics were 100 percent stripped, the phenols were 100 percent biodegraded, and the aromatics and acrylonitrile were removed by a combination of volatilization and biological oxidation. Benzene and 1,2-dichlorobenzene are characterized as base/neutral compounds, but 25 intermediates were detected in their acid extracts. Likewise, phenol is known as an acid extractable compound, but at least four compounds were detected in the base/neutral extracts.

Figure 49. Chromatographs for 1,1,2,2-Tetrachloroethane Base/Neutral Fraction Collected at t_0, t_2, and t_{24}

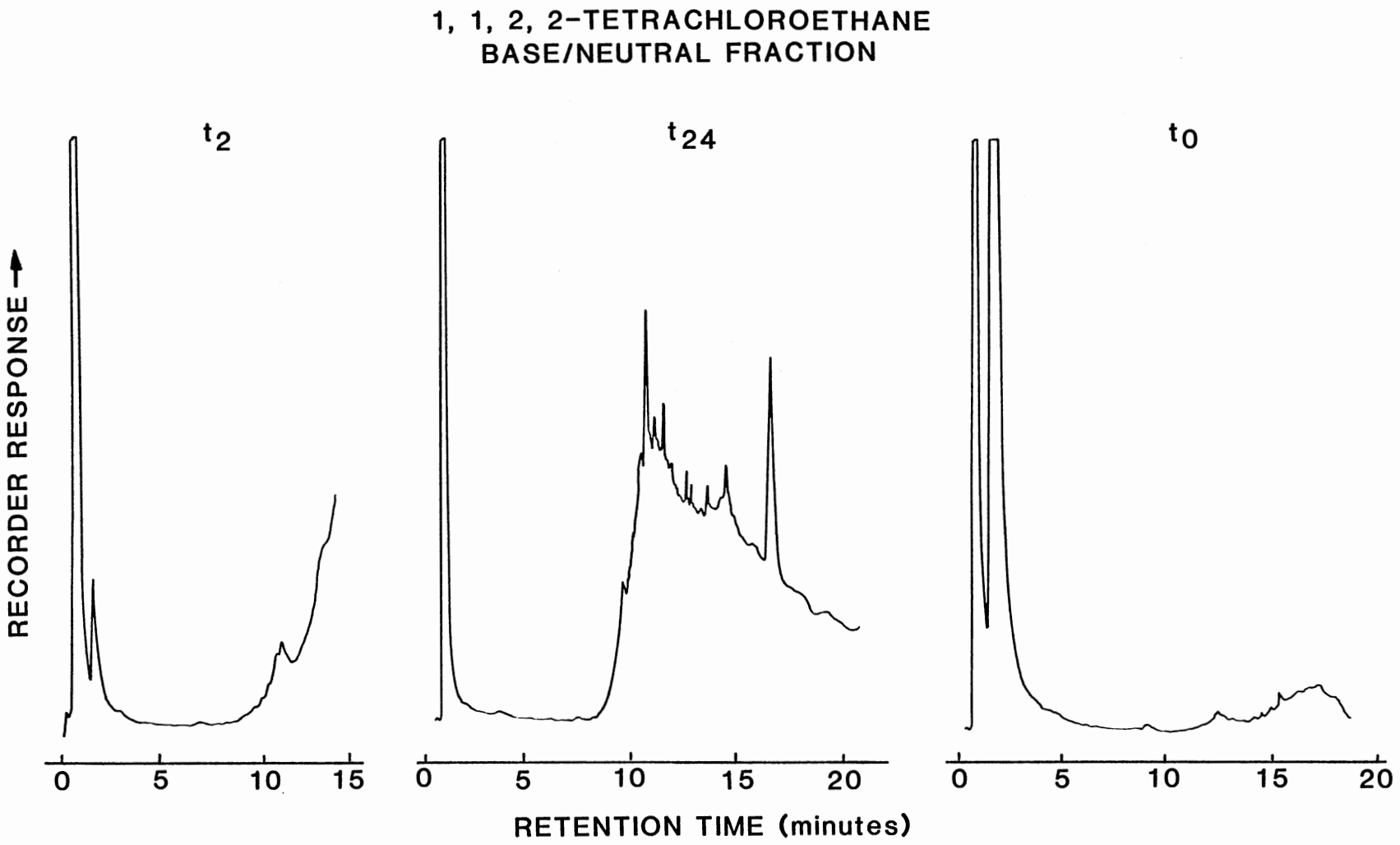

1, 1, 2, 2-TETRACHLOROETHANE
BASE/NEUTRAL FRACTION
t_2
t_{24}
t_0
RECORDER RESPONSE
0
5
10
15
20
RETENTION TIME (minutes)

Figure 50. Bargraph for 1,1,2,2-Tetrachloroethane Base/ Neutral Fraction Displaying the Relative Quantity of Each Compound at t_0, t_2, and t_{24}

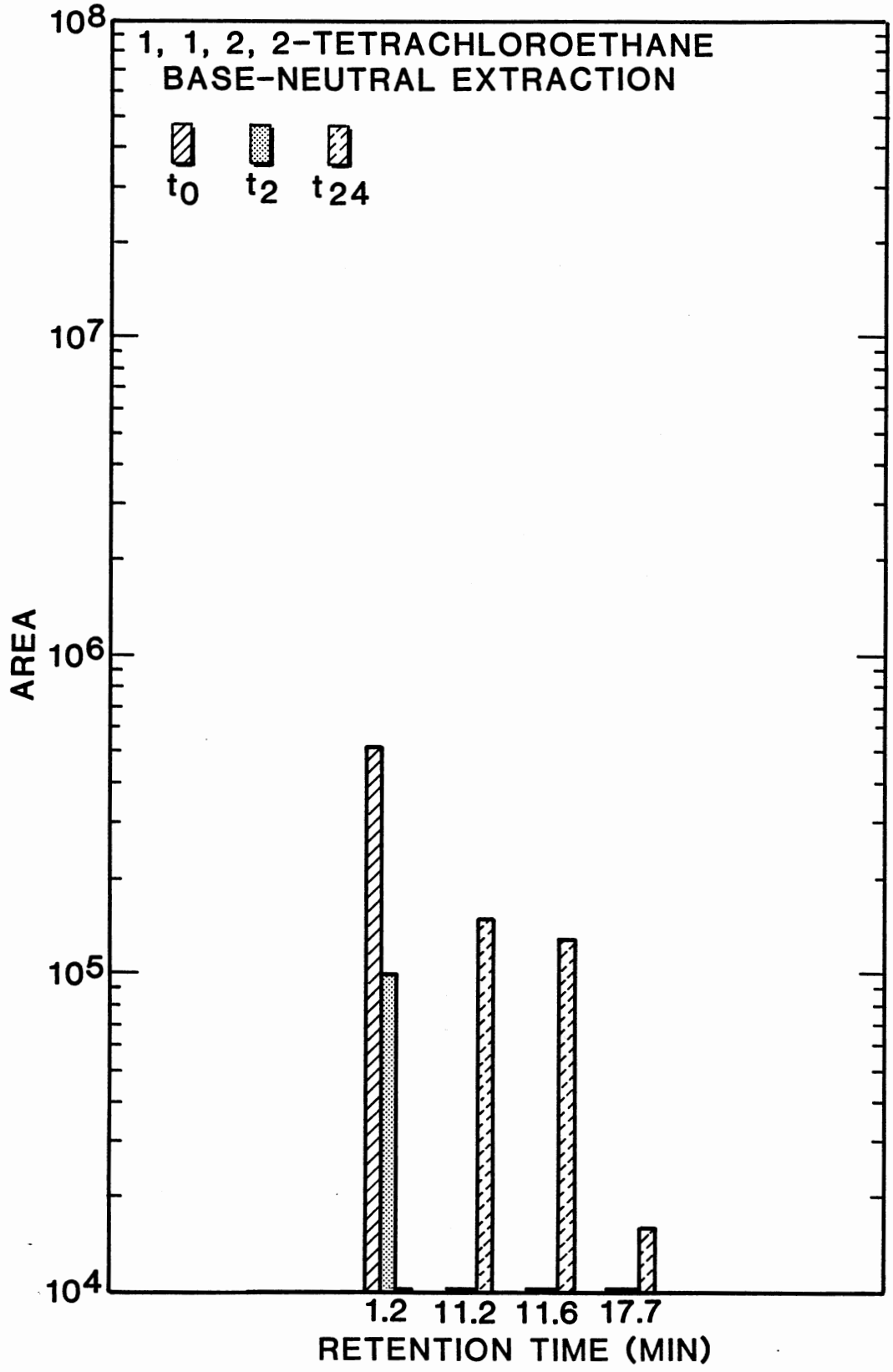

1, 1, 2, 2-TETRACHLOROETHANE
BASE-NEUTRAL EXTRACTION
t_0 t_2 t_{24}
10^8
10^7
10^6
10^5
10^4
AREA
1.2 11.2 11.6 17.7
RETENTION TIME (MIN)

TABLE VII

RESEARCH SUMMARY

Compound	$Y_{t(TOC)}$	K_{cpd}	K_{TOC}	Percentage Stripped	No. Volatile Inter-mediates	Percent-age Bio-degraded	No. Base/ Neutral Intermediates	No. Acid Inter-mediates
Acrylonitrile	2.41	4.6×10^{-3}	1.9×10^{-4}	25	---	75	---	10
Benzene	1.00	3.0×10^{-1}	4.3×10^{-3}	10	1	90	---	12
1,2-DCB	1.37	8.5×10^{-2}	3.8×10^{-3}	10	15	90	14	13
Phenol	1.05	1.9×10^{-3}	1.2×10^{-3}	---	5	100	4	---
1,2-DNP	1.50	1.6×10^{-3}	1.5×10^{-3}	---	4	100	---	5
1,2-DCP	1.30	2.7×10^{-1}	6.4×10^{-3}	100	---	---	---	4
1,1,2,2-TCE	1.33	5.7×10^{-2}	2.4×10^{-3}	100	5	---	4	---

Eckenfelder's constant: $K = \frac{S_i - S_e}{X S_e t}$, where: S_i = mg/l
S_e = mg/l
X = mg/l
t = hour

$K = (mg/l \times hour)^{-1}$

K_{cpd}	$\frac{K_{max}}{3.0 \times 10^{-1}}$	$\frac{K_{min}}{1.6 \times 10^{-3}}$	$\frac{K_{mean}}{1.03 \times 10^{-1}}$
K_{TOC}	6.4×10^{-3}	1.9×10^{-4}	2.83×10^{-3}

CHAPTER V

DISCUSSION

A. Introduction

The research data collected during these experiments indicated that numerous by-products were produced when biological oxidation was a major removal mechanism. Because the toxic priority pollutant was biodegraded, it is assumed that several of these intermediates were a derivative of the parent toxic compound. The metabolism of any substrate, including toxic substances, is due to specialized enzyme activity within the microorganisms. Microbial enzymes have the specificity and catalytic power to accelerate reactions under biological conditions which would have taken place only under extreme laboratory conditions. Factors which influence these enzymatically produced reactions are as follows:

1. Enzymes combine with substrates to form unstable intermediates.

2. Enzymes provide functional groups which are capable of bringing about general acid/base catalysis.

3. Enzymes may also produce a strain on the susceptible bond of the substrate, making the bond easier to break.

Nucleophiles are a very effective and versatile catalyst. Unstable intermediates can be formed when a nucleophilic group on the catalyst attacks an electrophilic atom on the substrate. The substrate has susceptible bonds and once the intermediate has been formed, one or more bonds

can be broken. Three nucleophilic groups are the serine hydroxyl group, the cysteine sulfhydryl group, and the histidine imidazole group.

General acid/base (proton donor/proton acceptor) catalysis occurs in many living cells: the addition of water to carbonyl groups, the hydrolysis of carboxylic and phosphoric esters, the elimination of water to form double bonds, and many substitution reactions. This type of catalysis enables the living organism to produce a chemical reaction that would otherwise require high temperatures and a very high concentration of H^+ or OH^- ions. For example, the breaking of peptide bonds requires a very high concentration of H^+ ions, high temperatures, and a long reaction time. However, the enzyme chymotrypsin can hydrolyze peptides rapidly and efficiently at neutral pH and biological temperatures. Enzyme molecules are known to contain several kinds of functional groups which can act as general acids or bases. Typical examples include the carboxyl, amino, phenolic hydroxyl, sulfhydryl, and the imidazole groups. There are other types of enzymatic mechanisms, but nucleophilic attack and general acid/base catalysis are thought to be the most common.

B. Acrylonitrile

There was slight evidence that acrylonitrile was removed by sorption; therefore, it has been assumed that the removal of acrylonitrile from the batch reactor occurred through biodegradation and volatilization. Cometabolism was essential for biological oxidation since it was demonstrated that a definite loss in biomass occurred when "Sego" was not added to the reactor. Seventy-five percent of the acrylonitrile removal was due to biodegradation and this occurred during the first three hours when the concentration of the cometabolites was relatively higher. The rate of

volatilization was measured to be 25 percent, but this could vary depending upon experimental conditions such as initial concentration, temperature, air flow rates, mixing, etc. The stripping of the acrylonitrile was suspected because of its high vapor pressure (100 torr at 22.8 C°), but the degree was unknown because of the solubility of the compound in water and its participation in hydrogen bonding with water. The increase or decrease in acid extractable intermediates during the 24-hour growth study also indicated biological activity.

The metabolism of acrylonitrile by microorganisms has not been investigated thoroughly, but chemical oxidation suggests a wide variety of reactions. Acrylonitrile contains a highly polar nitrile group, and therefore can be converted into an acid, ester, amide, or amine. The reaction usually involves an aqueous acid or base with a relatively high reaction temperature, but similar biochemical reactions are a distinct possibility. A typical reaction is as follows:

$$CH_2 = CHCN + 2H_2O \xrightarrow[\text{or } OH^-]{H^+} CH_2 = CHCOOH + NH_3$$

There are many compounds which contain a labile hydrogen atom which promotes the addition across the carbon-carbon activated double bond. The general reaction is called cyanoethylation, and involves a variety of alcohols, aldehydes, amines, amides, esters, ketones, and inorganic acids and their salts. The general reaction is as follows:

$$CH_2 = CHCN + HA \longrightarrow ACH_2CH_2CN$$

In an aquatic environment, the reaction between water and acrylonitrile would lead to 3-hydroxypropionitrile, and the treatment of acrylonitrile with chlorine-water leads to the formation of 2-chloro-3-hydroxypropionitrile. The reaction conditions of the latter could easily be met

during the chlorine disinfection procedure of a water treatment facility.

C. Benzene

Batch studies indicated that biodegradation was the major process responsible for removing benzene from a complex wastewater. The concentration of benzene decreased from 54 mg/ℓ to less than 1 mg/ℓ, and it was determined that 90 percent of this removal was due to biological oxidation. Benzene along with nitrogen and phosphorus was unable to support microbial growth, and it was concluded that cometabolites were necessary in the biodegradation of benzene. The biological oxidation also occurred during the first hour of operation when the concentration of the cometabolites was relatively high. The chromatographs from the off-gases and the acid extractable samples revealed biological activity throughout the 24-hour growth study. Since benzene was apparently degraded, the probability exists that several of the intermediates observed by chromatographic analysis may be aromatic in nature.

Since volatilization was measured at 10 percent, it was concluded that volatilization does not play a major role in the removal process. However, the volatilization of benzene cannot be ignored in planning for the treatment of a wastewater containing benzene. Volatilization and biodegradation data from this experiment supported the results of the continuous flow complete mix experiments conducted by Kincannon et al. (20), where volatilization was reported as 16 percent and biodegradation as 84 percent.

Benzene has the molecular formula C_6H_6. The molecule is described as a cyclohexatriene with equivalent carbon to carbon bonds. The reactions of similar compounds such as cyclohexadiene and cyclohexene are

similar to all alkenes, but this is not the case for benzene or cyclohexatriene. The reactions of benzene are substitution reactions rather than the expected addition reactions of the alkenes. The most important substitution reactions include nitration, sulfonation, and halogenation. In each reaction only one monosubstitution product is formed, and the benzene ring system is preserved which retains the characteristic properties of benzene. Since the benzene ring maintains its basic electron arrangement, this leads to future reactions involving the aromatic structure.

The cloud of electrons which are above and below the planar benzene molecule are loosely held and provide a source of electrons for any electrophilic reagent. The general electrophilic substitution is summarized for the reagent YZ below:

1. $C_6H_6 + Y^+ \longrightarrow C_6H_5\overset{\oplus}{\underset{Y}{H}}$

2. $C_6H_5\overset{\oplus}{\underset{Y}{H}} + :Z^- \longrightarrow C_6H_5Y + H:Z$

Step 1 involves the attack by the electrophilic reagent to form the intermediate carbonium ion, $C_6H_5\overset{\oplus}{\underset{Y}{H}}$, and step 2 is the abstraction of the hydrogen ion from the carbonium ion by some base. While metabolism of benzene has not been investigated, the biochemical reactions should be electrophilic substitution reactions similar to the above organic mechanism.

D. 1,2-Dichlorobenzene

The removal of 1,2-dichlorobenzene, 1,2-DCB, from a complex wastewater in a batch reactor was similar to the removal data collected on

benzene. The concentration of 1,2-DCB decreased from 54 mg/ℓ to 3 mg/ℓ. Ninety percent of the removal was due to biological oxidation which occurred during the first three hours of operation. 1,2-DCB, nitrogen, and phosphorus were unable to support microbial growth, and it was concluded that the presence of metabolites was also necessary for the removal of this toxic compound. The chromatographic analysis involving the off-gases, base/neutral, and acid extractable samples indicated extensive biological activity throughout the 24-hour growth study. This may be due to the activating and deactivating groups which become attached to the benzene ring structure. These groups not only affect the activity of the ring, but they also direct other groups to certain positions on the ring structure. Each group and each orientation provide for numerous possibilities. Also, organic reactions may occur between the by-products and 1,2-DCB.

The volatilization of 1,2-DCB was measured at less than 10 percent, and should not be considered as a major removal process. However, 15 compounds were detected in the off-gases with several of these recording significant responses on the chromatograph. This suggests that stripping cannot be overlooked in the design and treatment of a wastewater containing this priority pollutant. Kincannon et al.'s (20) research with continuous flow units indicated 22 percent stripping and 78 percent biodegradation which supports the data collected during batch studies of this research.

The theoretical organic reactions of 1,2-DCB are similar to chlorobenzene or other aryl halides. These reactions include electrophilic aromatic substitution and nucleophilic aromatic substitution which can be accomplished through bimolecular displacement or by elimination-

addition. The reactions are complex due to the electron arrangement of the aryl halides. These compounds have a resonance effect which tends to release electrons to the ring; and in the case of chlorobenzene, five different theoretical structures must be drawn to represent the hybrid molecule. At the same time the molecule experiences an inductive effect where the halide atom withdraws electrons from the ring and acts to deactivate the ring.

The attack by electrophilic agents on 1,2-DCB is assumed to be similar to the attack on benzene and chlorobenzene. The electron-seeking agents react with benzene to form the intermediate carbonium I ion, and the attack on the chlorobenzene forms the carbonium II ion which leads to 1,2-DCB.

1. $C_6H_6 + Y^+ \longrightarrow C_6H_5\overset{\oplus}{\underset{Y}{\overset{H}{}}} \xrightarrow{:Z^-} C_6H_5Y + HZ$

Carbonium I

2. $C_6H_5Cl + Y^+ \longrightarrow C_6H_5\overset{\oplus}{Cl}\overset{H}{\underset{Y}{}} \longrightarrow C_6H_4ClY + HZ$

Carbonium II

The electron withdrawing effect of chlorine intensifies the positive charge on the carbonium II ion, makes the ion unstable, and causes a slower reaction. The resonance effect tends to release electrons to the ring, stabilizes the intermediate, and causes a faster reaction.

Nucleophilic aromatic substitution through elimination-addition is accomplished through the benzyne intermediate. However, this type of reaction may not be an important metabolic reaction since it involves a very strong base and/or very high temperatures. The bimolecular displacement, however, could lead to metabolic reactions, including several

possible nucleophilic agents. The general nucleophilic aromatic substitution on chlorobenzene is as follows:

1. $C_6H_5Cl + :Z \longrightarrow C_6H_5(\overset{\ominus}{Cl})Z$

Carbanion ion

2. $C_6H_5(\overset{\ominus}{Cl})Cl(Z) \longrightarrow C_6H_5Z + :Cl$

Step 1 involves the attack by the nucleophilic agent :Z to form the intermediate carbanion ion, $C_6H_5(\overset{\ominus}{Cl})Z$. Step 2 is the expulsion of the Cl^- forming the final product, C_6H_5Z. The reaction of dichlorobenzene is thought to be very similar. However, it cannot be assumed that biochemical activity on 1,2-DCB follows the same mechanism. The metabolism may be similar in part, but usually the enzymatic-induced reactions are quite different.

E. Phenol

Current research literature indicated that the volatilization of phenol is minimal, and this was supported by the continuous flow experiments conducted by Kincannon et al. (20). Therefore, volatilization was not considered to be a major removal mechanism for phenol. However, off-gas analysis was conducted on two separate columns to determine if volatile biodegradation by-products were being produced. The small number of intermediates detected along with their insignificant GC responses suggests that volatilization is not a major removal process.

Biological oxidation of phenol has been well established over a period of years. This research with batch reactors revealed a 90 percent removal by biodegradation. Initially 100 mg/ℓ of phenol was added to the reactor, but only 26 mg/ℓ was detected after the extraction

process. This low concentration may be the result of photooxidation within the stock solution of phenol. Although the phenol was stored in the dark, the daily exposure to the light over a two- to three-month period may have produced unwanted by-products such as hydroquinone, catechol, and several dihydroxybiphenyls. It is possible that the compound with RT of 4.0 in the base/neutral extraction analysis (Figure 29) could be one of the irradiation by-products of phenol.

The multiple decreasing rates of TOC and the corresponding increasing rates of TSS could be the result of biological oxidation of the irradiation intermediates. The changing rates could also be explained by the biodegradation of phenol and the subsequent metabolic activity on the phenolic by-products. It appears that phenol is metabolized by several hydroxylative enzymes to catechol and possibly quinol and/or resorcinol. All three compounds can be further hydroxylated to form o-diol compounds which is acted upon by a ring-cleaving enzyme, catechol 1,2-oxygenase. Once the ring structure has been cleaved, then the resulting molecule may undergo other types of enzymatic reactions. The metabolism of phenols is described completely by Neujahr and Varga (55), Buswell (26), and Buswell and Twomey (56).

It is also possible that phenol may undergo direct oxidation within the biological reactor. Phenol is active chemically because of the high activity of its ring toward electrophilic substitution. Once the ring structure has attracted a substituent, the substituent may also activate or deactivate the ring. Typical organic reactions include acid/base reactions, ether and ester formation, nitration, sulfonation, and halogenation.

F. 2,4-Dinitrophenol

The literature review did not reveal the vapor pressure of 2,4-DNP, but did indicate that it should be less than 4-nitrophenol which was reported as 2.2 torr at 146°C (32). An extremely low vapor pressure was also demonstrated by Kincannon et al.'s (20) work with continuous flow units. Therefore, volatilization was not considered to be a major removal process for 2,4-DNP in the batch reactor. However, an off-gas analysis was completed similar to that conducted for phenol. Two compounds of interest were detected at RT 4.4 and 19.7 (Figure 32). These compounds may be the result of either photooxidation or biological oxidation.

It is apparent that 2,4-DNP is relatively persistent in this aquatic environment. 100 mg/ℓ was initially added to the reactor, but 130 mg/ℓ was measured at the starting time of t_0. The residual concentration was measured at 45 mg/ℓ after five hours, t_5, and remained constant throughout the experiment. The decrease in concentration along with the detection of intermediates supported the degradation of this toxic compound. Nakagawa and Crosby (57) studied the photooxidation of 4-nitrophenol and detected two principal by-products: hydroquinone and 4-nitrocatechol. Raymond and Alexander (52) also exposed 4-nitrophenol to different forms of irradiation and confirmed the presence of 4-nitrocatechol. It is possible that 2,4-DNP may undergo similar photolysis resulting in such compounds as 4-nitrocatechol, 2-nitrohydroquinone, and 3,5-dinitrocatechol (32).

These batch study experiments, along with the continuous flow research of Kincannon et al. (20) and Medley (21), revealed that a large heterogeneous microbial population does biodegrade 2,4-DNP. Simpson and

Evans (59) were able to isolate a soil microorganism which utilized 2,4-DNP. The metabolism of 2,4-DNP has not been confirmed, but it is suspected to be similar to that of 4-nitrophenol. Simpson and Evans proposed that the catabolism of 4-nitrophenol proceeded through hydroquinone. This was supported by Munnecke and Hsieh (60), who proposed a tentative pathway starting with hydroquinone to 1,3,4-benzenetriol and finally ring cleavage. It has also been suggested that strong biological reducing agents such as ferredoxin can reduce 2,4-DNP to 2-amino-4-nitrophenol (61). Madhosingh (62) proposes that bacteria may not utilize 2,4-DNP as a carbon source, but detoxifies the compound by converting it to 4-amino-2-nitrophenol.

It has been well established that 2,4-DNP inhibits microbial growth, at least on pure cultures. The nitrophenols accomplish this inhibitory action by uncoupling the oxidative phosphorylation process. The reduced enzymes such as NADH and the cytochromes are reoxidized but without the production of ATP. As a result the microbes do not have an energy source to continue metabolic activity and eventually die. However, most of this inhibitory research was conducted with pure cultures.

G. 1,2-Dichloropropane and 1,1,2,2-Tetrachloroethane

Organic reactions involving alkyl halides are nucleophilic substitution or elimination. The halides are very reluctant to share their electrons with carbon and therefore can be replaced by several nucleophilic groups. The general substitution reaction is: $R{:}X + {:}Z \longrightarrow RZ + {:}X^-$. This basic reaction is used by organic chemists to synthesize alcohols, ethers, esters, alkynes, nitriles, amines, and many other useful compounds. The experimental conditions are so severe, however, that is is

very unlikely the above reactions will take place under environmental conditions.

This research with batch reactors, along with Kincannon et al. (20) and Medley's (21) studies with continuous flow units, have demonstrated that 1,2-DCP and 1,1,2,2-TCE are not biodegraded. All literature investigated indicated no evidence for photolysis, oxidation, hydrolysis, sorption, or biodegradation. Volatilization was the only removal mechanism suggested by the literature, and this proved to be the case in both batch and continuous flow units. 99.9 percent of the 1,2-DCP was removed from both the batch and continuous flow units, and this was accomplished completely by volatilization. The lack of intermediates in the off-gases, acid, and base/neutral extracts also demonstrated that biodegradation was not a major removal process. 100 percent of the 1,1,2,2-TCE was removed by volatilization from the batch reactor as compared to 94.5 percent for the continuous flow units of Kincannon et al. (20). The acid and base/neutral samples for 1,1,2,2-TCE also revealed a limited number of intermediates. However, the off-gas analysis revealed several volatile by-products (Figure 45). These compounds may be the result of some biological oxidation of the toxic compound, or it may be the result of microbial activity on the "Sego."

CHAPTER VI

CONCLUSIONS AND SUGGESTIONS

A. Conclusions

The results of this research lead to the following conclusions.

1. General

1. All selected priority pollutants were successfully removed from the complex wastewater in a batch system. However, 2,4-dinitrophenol did persist in the solution at a concentration of 45 mg/ℓ.

2. Eckenfelder's constant K_{TOC} was similar for all seven pollutants.

3. There was not close agreement for Eckenfelder's constant, K_{cpd}.

4. The production of intermediates may be the result of microbial activity on the ingredients of "Sego."

5. Intermediates detected at the initial time, t_0, may have been present from the remaining solution of the previous day.

6. Results from the batch system concerning biodegradation and volatilization were in close agreement with the results obtained in Kincannon's continuous flow experiments.

2. Acrylonitrile

1. The aquatic fate includes biological oxidation and volatilization.

2. Volatile intermediates are not produced.

3. A relatively large number of aqueous metabolic by-products were produced.

4. A large cell yield is observed with acrylonitrile.

3. Aromatics

1. The aquatic fate includes biological oxidation and volatilization.

2. Volatile intermediates do not appear to be a serious problem with benzene as the pollutant. However, 1,2-dichlorobenzene produced a large number of volatile by-products.

3. Eckenfelder's constant, K_{TOC}, determined for benzene and 1,2-DCB, were in close agreement.

4. Eckenfelder's constant, K_{cpd}, for benzene and 1,2-dichlorobenzene were also similar.

5. Benzene and 1,2-dichlorobenzene produced a large number of aqueous metabolic by-products.

6. A large value for the cell yield was not observed for benzene or 1,2-DCB.

4. Phenols

1. Removal of phenol and 2,4-dinitrophenol results only from biological oxidation.

2. Values of Eckenfelder's constant, K_{TOC}, were in close agreement.

3. K_{cpd} also was very similar for both phenol and 2,4-DNP.

4. Volatile intermediates were produced for both phenol and 2,4-DNP.

5. A small number of aqueous intermediates were also observed from both compounds.

6. A reduced cell yield for phenol may have been the result of the low initial concentration of phenol.

5. Chloroaliphatics

1. The removal of 1,2-DCP and 1,1,2,2-TCE resulted only from volatilization.

2. K_{TOC} and K_{cpd} for these two compounds were not in close agreement.

3. No volatile intermediates were detected for 1,2-DCP; however, a few volatiles were noted for 1,1,2,2-TCE.

4. A limited number of aqueous intermediates were observed for both compounds.

5. The cell yield for both compounds were very similar.

B. Suggestions

The research conclusions lead to the following experimental proposals:

1. Identify the volatile and aqueous intermediates noted in this research.

2. Investigate other halogenated aromatics, phenols, and halogenated aliphatics to determine if their ultimate fate is similar to those compounds selected in this study.

3. Investigate the extensive volatile and aqueous intermediates of 1,2-DCB.

4. Research the isomers 1,3-DCB and 1,4-DCB to compare the results to that of 1,2-DCB.

5. Investigate multiple ring structures, insecticides, and other agricultural chemicals in a similar manner to this research.

6. Research sorption studies and cell yields or sludge production for each group.

7. Investigate the K_{TOC} for other selected priority pollutants to support or negate the results of this research.

8. Investigate K_{cpd} for special groups, i.e., aromatics, phenols, etc., to determine if they are constant.

9. Research the metabolism of toxic priority pollutants.

10. Identify the microbes involved in the treatment of each group.

11. Investigate the cometabolites which participate in the biological process and determine their ultimate fate.

12. Investigate the treatability of volatile compounds such as 1,2-DCP and 1,1,2,2-TCE under anaerobic conditions.

13. Investigate the utilization of sequential batch systems to lower the concentration of 2,4-DNP and other toxic priority pollutants which are not treated to an acceptable level by the single closed batch reactor.

14. Conduct toxicity studies comparing effects of the priority pollutant and the final effluent from the batch reactor.

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