BIOLOGICAL TREATMENT OF TANK-BOTTOM SLUDGES IN AEROBIC BATCH REACTORS

By

SASI KUMAR PULLOTTUPADATH

Bachelor of Technology

Indian Institute of Technology

Madras, INDIA

1983

Submitted to the Faculty of the
Graduate College of the
Oklahoma State University
in partial fulfillment of
the requirements for
the Degree of
MASTER OF SCIENCE
July, 1995

BIOLOGICAL TREATMENT OF TANK-BOTTOM SLUDGES IN AEROBIC BATCH REACTORS

Thesis Approved:

J. N. Veenstra

Thesis Advisor

Gregory G. Wilber

Wm Clarkson

Thomas C. Collins

Dean of Graduate College

ACKNOWLEDGMENTS

I wish to express my sincere appreciation to my major advisor, Dr. John Veenstra for his supervision, constructive guidance, assistance, and friendship throughout my masters program. I also wish to thank the other members of my committee, Dr. William Clarkson and Dr. Greg Wilber for all of their guidance and advice. Other members of the Oklahoma State University staff, including Don Spoonemore, Fred Myers, and Beverly Vencill, also deserve my gratitude. In addition to those mentioned above, there are many more people who had directly and indirectly supported and assisted me, and without their help this research could not have been performed.

None of this research or class work could have been possible without the constant encouragement from my parents and in laws. Last but not least, I would like to give my special appreciation to my wife Sheeja, for her valuable suggestions and love and understanding throughout this whole process. I would also like to mention my daughter Malavika, who provided the relief from all the tensions.

TABLE OF CONTENTS

Chapter Page

LIST OF TABLES

Table Page

Table Page

LIST OF FIGURES

Figure Page

CHAPTER I

INTRODUCTION

The U.S. petroleum refining industry annually produces a large quantity of storage tank sludges. These wastes consist of sediments, water, and oil emulsions which are periodically pumped or drained from the bottom of crude oil, intermediate product, or refined product storage tanks. Due to the lack of consistent data, it is difficult to accurately estimate the annual amount of tank-bottoms generated. One source estimated 85,500 wet metric tons of hazardous tank-bottom sludges as being produced annually [Conner, 1990]. Williams Pipe Line Co., Tulsa, which has about 630 active storage tanks, generates approximately 15,000 billion barrels of tank-bottom material annually [Emery, 1993]. For conversion purposes, six barrels of tank-bottom material is approximately equal to one ton [Spitzer, 1994]. This shows that the reported values are highly inconsistent and cannot be interconverted with any degree of accuracy.

The composition of tank-bottom sludges varies from facility to facility, and from tank to tank within the same facility. The nature of the sludge is dependent upon the composition of the stored product, the storage conditions, the length of storage time, and the condition of the storage tank. Williams Pipe Line Co., Tulsa, has reported the typical composition of tank-bottoms from their storage tanks to be 5-20% refined petroleum product, 5-20% solids, and 60-90% water [Emery, 1993]. The solids in the sludges were mainly constituted of sand, dirt, scale, solidified fuel, and rust. Similarly, the laboratory analysis of a crude oil tank-bottom sludge at Mobil Oil Corporation's Paulsboro, NJ, refinery indicated that the sludge was constituted of approximately 70% recoverable hydrocarbons, 16% solids, and the remainder water [Davis *et al.*, 1993].

Environmental Regulations Controlling the Disposal of Tank-Bottom Sludges

In December 1970, the Environmental Protection Agency (EPA) was created by executive order. Since then, EPA has been administering the major environmental statutes enacted by the U.S. Congress. In 1976, to identify and regulate hazardous waste, the Resource Conservation and Recovery Act (RCRA) was passed. By 1980, EPA had promulgated regulations that govern the day-to-day management of hazardous wastes. Under these regulations, wastes are defined as hazardous if they are listed, or if they exhibit certain hazardous characteristics.

EPA lists a waste as hazardous when the waste exhibits certain hazardous characteristics, has been found to be acutely toxic, or contains certain toxic constituents [Pierce, 1991]. Currently, only tank-bottom sludge from the storage of leaded gasoline is a listed hazardous waste (K052). In response to a litigation by Environmental Defense Fund Inc., EPA agreed to determine by October 31, 1996, whether to list crude storage tank sludge, and unleaded storage tank sludge as hazardous waste [Pierce, 1991].

Even if a waste is not on one of the EPA's lists of hazardous wastes, it still may be classified as hazardous if it exhibits any of the four hazardous characteristics of ignitability, corrosivity, reactivity, and toxicity. A waste is ignitable if it can cause a fire, or exacerbate a fire once started. A corrosive waste can corrode metals, or is a liquid with a pH less than 2.0 or greater than 12.5. A waste is considered reactive if it is unstable, and tends to react violently when mixed with water or other chemicals. If upon the application of specified test methods, the waste produces an extract that contains specified contaminants at or above EPA-established concentrations, then the waste is considered to exhibit the characteristic of toxicity. In 1990, EPA added 25 organic chemicals to the existing toxicity characteristic list of 14 chemicals. Of particular concern to the petroleum industry is the 0.5 mg/L toxicity characteristic regulatory level for benzene [Pierce, 1991].

The Hazardous and Solid Waste Amendments (HSWA) of 1984 to RCRA mandated that land disposal of untreated hazardous wastes be prohibited. HSWA also required EPA to develop treatment standards for all hazardous wastes. By 1990, EPA had promulgated treatment standards, known as Best Developed Available Technology (BDAT) standards, for the listed wastes such as leaded tank-bottoms. Similar standards can be expected to be issued for wastes that are found to be hazardous by virtue of exhibiting a hazardous characteristic [Oolman *et al.*, 1992].

In 1980, the Comprehensive Environmental Response, Compensation, and Liability Act (CERCLA) was enacted. CERCLA imposes cleanup liability on the current owner or operator of a facility where there is a release or threat of a release of a hazardous substance. Liability is also applicable to the owner or operator of the facility at the time the hazardous substance was placed at the facility. Liability is also imposed on those who disposed of their hazardous substances at the facility, and those who transported hazardous substances to the facility. In addition to RCRA hazardous wastes, any substance listed as hazardous under air pollution, water pollution, and toxic chemical manufacturing laws is also a hazardous waste under CERCLA [Pierce, 1991].

Specifically excluded from CERCLA's definition of a hazardous substance is petroleum (including crude oil or any fraction thereof), natural gas, natural gas liquids, liquefied natural gas, and synthetic gas usable for fuel, which is not otherwise specifically listed or designated as a hazardous substance under any of the environmental laws. This is commonly referred to as the "petroleum exclusion". Although this "petroleum exclusion" has been generously interpreted by the courts to exclude gasoline leaking from underground storage tanks, it is not expected to offer any protection when dealing with oil-contaminated waste streams [Pierce, 1991]. In August, 1993, the United States Court of Appeals, Ninth Circuit, reversed the decision of U.S. District court in "Cose vs. Getty Oil Company", and held that crude oil tank-bottoms did not fall within CERCLA's "petroleum exclusion" [Kennedy, 1993]. This sets a precedent, and could lead to similar decisions which may affect the disposal of tank-bottoms.

Treatment Options for Tank-Bottom Sludges

Prior to 1984, tank-bottoms were primarily disposed in landfills or land farms. The enactment of HSWA in 1984 prevented direct land disposal of hazardous wastes, and required prior to land disposal, the reduction of hazardous constituents to levels achievable using BDAT. This has led to the development of alternate methods to treat or dispose of oily sludges. In this regard, various physical, chemical, and biological methods have been tried.

One of the more common physical treatment methods is the use of a filter press to separate the solids from the liquid in a tank-bottom sludge. The filtrate thus obtained is sent to an oil-water separator. The filter cake generated may still contain additional hydrocarbons which may make it hazardous under the toxicity characteristic rule. The filter cake can be subjected to heat treatment [Emery, 1993], or biological treatment [Engelder *et al.*, 1990] to render it nonhazardous and permissible for land disposal.

Thermal desorption is another common physical treatment method used in the treatment of oily sludges. In this method, the sludge is heated to 600-850^{0}F in an oxygen deficient atmosphere, whereby the hydrocarbons are desorbed from the sludge. The desorbed hydrocarbons are carried to an offgas treatment system, where the hydrocarbons are condensed and recovered [Abrishamian *et al.*, 1992 and Rasmussen, 1994]. A variation of the thermal desorption process is the High Temperature Reprocessing (HTR) of tank-bottom sludges [Hahn, 1994]. In HTR, the sludge is heated above the boiling point of water, and then allowed to flash in a separation tower, where steam and light hydrocarbons are subsequently extracted. Heavier hydrocarbons and inorganic material are removed from the separation tower as a slurry. Light hydrocarbons and water are recovered by condensation, while heavier hydrocarbons are recovered by the liquid/solid separation of the slurry.

Solvent extraction using a light hydrocarbon such as propane or butane recovers the hydrocarbons in the sludge, thereby rendering the sludge nonhazardous [Bryant and Moores, 1991]. Mobil Oil Corp., with the help of Nalco Chemical Co., has developed a patented process for extracting the hydrocarbons and water from the tank-bottoms in-situ [Davis *et al.*, 1993]. In this process, an aqueous chemical solution along with a diluent is added to the tank containing the sludge. The lighter diluent forms the upper layer, while the water containing the solvent forms the middle layer. Steam is then injected to the middle layer to initiate the dissolution of the hydrocarbons in the sludge. In the end, the non-hydrocarbon solids remain at the bottom, while the hydrocarbons rise into the uppermost diluent layer.

The use of biosurfactants, produced by a proprietary bacterial strain, has been demonstrated in the clean-up of storage tank-bottoms [Banat *et al.*, 1991]. The process involved the introduction of a blend of the produced biosurfactant, brackish water, and fresh product, into the storage tank. The mixture is then circulated, to lift the sludge from the bottom of the tank, and thereby resulting in its emulsification. An emulsion breaker is then added, to separate the emulsion into oil and water layers. The oil layer is pumped out, and the water layer and the remaining inorganic impurities are removed for subsequent disposal.

Although the biodegradability of the organic constituents of petroleum and petrochemical products has been extensively investigated, tank-bottom sludges have not been widely subjected to biological treatment. Composting is a biological process suitable for the treatment of tank-bottom sludges. A pilot scale study of the composting of non-hazardous petroleum production sludges has shown a reduction in the sludge Total Petroleum Hydrocarbon (TPH) level from 10% to less than 1% in 40 days [Fyock *et al.*, 1991]. Studies have also shown the applicability of using batch reactors for the treatment of listed petroleum sludges such as oil/water separator sludges [Oolman *et al.*, 1992 and Abrishamian *et al.*, 1992].

The physical and chemical methods of treatment described above focus on the recovery and reuse of the organic content of the sludge. As tank-bottom sludges are generated intermittently, the owners and operators of small storage facilities may not find it economically viable to recycle the organic content of such sludges. In such cases the other options available are incineration and disposal in landfills, but increasing costs and restrictive regulations necessitate the development of other environmentally sound and cost effective methods to treat and dispose of these wastes. In this regard, the biological treatment of such sludges in aerobic batch reactors is one alternative that shows promise. Although the use of aerobic batch reactors has been shown to be effective in reducing the organic content of listed petroleum sludges, very little study has been done on their applicability with tank-bottom sludges.

Research Objectives

Considering the above facts, a research project was started to investigate the biodegradation of storage tank-bottom sludges. The study specifically focused on the biodegradation of a distillate tank-bottom sludge and a gasoline tank-bottom sludge. To simulate a likely treatment scenario, these two sludges were also mixed on a volumetric basis to investigate the effect on the biodegradation of the individual components. Specific goals of the research were to:

(i) Determine the reduction in the organic content of each of the sludges during sixty days of reactor operation, and arrive at the rate of degradation; and,

(ii) Determine any changes in the toxicity levels of the sludges due to biodegradation.

A review of the literature relevant to this study is presented in Chapter II. The materials and the analytical methods used in this study are described in Chapter III. In Chapter IV the results obtained from this study are presented. Chapter V gives the conclusions that can be drawn from this study and offers suggestions for further study.

CHAPTER II

LITERATURE REVIEW

Biodegradation of Petroleum Hydrocarbons

Biodegradation is the transformation of an organic compound to another form, by microorganisms [Grady, 1985]. Depending on various factors, the transformation can proceed a single step, or to the complete mineralization of the original compound. In this process, the microorganisms make use of the organic compound as a source of food and energy. In certain cases, organic compounds that do not serve as a source of food and energy can be transformed by microorganisms in the obligate presence of other growth substrates. Considering this, the fundamental principle of a biological treatment process for hazardous organic compounds is to create favorable conditions under which microorganisms can grow and utilize the compounds as substrates [Hahn and Loehr, 1992].

In the 1940's, it was demonstrated that many microorganisms that are widely distributed in nature have the ability to utilize hydrocarbons as sole sources of food and energy [Sisler and Zobell, 1947]. It was also recognized that the microbial utilization of hydrocarbons was highly dependent on the chemical nature of the compound and various other environmental factors. Since then, several studies have been performed to determine the metabolic pathways for the degradation of petroleum hydrocarbons.

Petroleum hydrocarbons can be divided into four classes: the saturates, the aromatics, the

asphaltenes, and the resins [Leahy and Colwell, 1990]. Hydrocarbons in the saturate fraction include alkanes, branched alkanes, and cyclo alkanes. Resins, which are oxygen-, nitrogen-, and sulfur-containing compounds, and asphaltenes, which are partially oxygenated and highly condensed organic compounds, occur in small amounts only in crude petroleum [Bartha, 1986]. Biodegradation rates have generally been shown to be highest for the saturates, followed by the light aromatics, with high-molecular-weight aromatics exhibiting extremely low rates of degradation [Leahy and Colwell, 1990]. There is relatively very little information on the biodegradation of resins and asphaltenes. During biodegradation, the relative and sometimes the absolute amounts of resins and asphaltenes tend to increase. This indicates that they not only tend to resist biodegradation, but also may be formed as byproducts of biodegradation [Bartha, 1986].

Biodegradation of petroleum hydrocarbons is almost always an aerobic process, requiring dissolved oxygen as a cosubstrate. The oxygen is utilized by hydrocarbon-degrading bacteria in initial activation of the substrate and in respiration [Rittmann, 1994]. The initial activation involves the insertion of oxygen into the hydrocarbon molecule, creating an alcohol. In the case of alkanes, the alcohol formed is oxidized further to an aldehyde, and finally to a fatty acid. Further degradation of the fatty acid by oxidation leads to the eventual liberation of carbon dioxide [Atlas, 1981]. In the case of branched alkanes, extensive branching interferes with the oxidation of the fatty acids that are formed, thereby inhibiting the complete biodegradation of such hydrocarbons [Bartha, 1986].

Cycloalkanes are transformed to a corresponding cyclic alcohol, which is then dehydrogenated to form a ketone. Degradation then proceeds with ring cleavage [Bartha, 1986]. Degradation of substituted cycloalkanes appears to occur more readily than the degradation of the unsubstituted forms, particularly if there is an alkane substituent of adequate chain length [Perry, 1979]. The bacterial degradation of aromatic compounds normally involves the formation of a diol or double alcohol, followed by ring cleavage and the formation of a diacid [Atlas, 1981]. Polycyclic

aromatic hydrocarbons are degraded, one ring at a time, but biodegradability tends to decline with the number of rings. Biodegradation also declines with the number of alkyl substituents on the aromatic nucleus [Bartha, 1986].

In crude oil as well as in refined products, the hydrocarbons occur in complex mixtures, and influence each other's biodegradation [Bartha, 1986]. In addition, there are several environmental factors that influence the biodegradation of petroleum hydrocarbons. The first and most important factor is the composition and the inherent biodegradability of the pollutant [Bartha, 1986]. The physical state of the petroleum hydrocarbons determines the initial surface area where biodegradation can be initiated, and increased surface area, as in the case of oil-water emulsions, leads to enhanced biodegradation [Atlas, 1981]. Another factor affecting the biodegradation rates is the concentration and aqueous solubility of the hydrocarbons. The mineralization of higher-molecular-weight hydrocarbons is more governed by their aqueous solubilities than by their total concentrations [Leahy and Colwell, 1990].

The other factors that influence petroleum hydrocarbon degradation are temperature, oxygen, nutrients, and pH. Temperature affects degradation by its effect on the physical nature and chemical composition of the oil, the rate of hydrocarbon metabolism by the microorganisms, and the composition of the microbial community [Atlas, 1981]. Since rapid biodegradation of hydrocarbons does not occur under anaerobic conditions, an oxygen rich environment is essential for microbial oxidation of hydrocarbons [Leahy and Colwell, 1990]. Also, as hydrocarbons have very reduced carbon, complete mineralization has a high total oxygen demand [Rittmann, 1994]. Similarly, the available concentrations of nitrogen and phosphorous severely limit the extent of hydrocarbon degradation, requiring the addition of nutrients to facilitate degradation [Atlas, 1981]. Extremes in pH will have a negative influence on the ability of microbial populations to degrade hydrocarbons, and most microorganisms favor a pH near neutrality [Leahy and Colwell, 1990].

Landfarming of Oily Sludges

Prior to the enactment of HSWA in 1984, landfarming was the widely practiced biological process for the disposal of refinery sludges such as tank-bottoms. It offered a cost-effective yet environmentally acceptable treatment alternative [Bryant and Moores, 1991]. Dibble and Bartha [1979] conducted a laboratory study on the effect of controlled environmental parameters on oil hydrocarbon biodegradation under simulated landfarming conditions. They used an actual refinery sludge and soil from a prospective disposal site, and evaluated the effects of soil moisture content, soil pH, nutrient levels, sludge loading rates, application frequencies, and temperature on hydrocarbon degradation in the landfarming process.

To eliminate the water content and to facilitate handling, the sludge used for the study was air dried until sufficiently friable to pass through a 3-mm sieve. The air dried sludge consisted of 24% extractable hydrocarbons, 72% ash, and 4% water. The initial hydrocarbon composition was determined to be 57% saturated hydrocarbons, 29% aromatic hydrocarbons, and 14% asphaltic hydrocarbons. Although the soil for the study was collected from a prospective landfarming site, due to its poor quality, it was conditioned with 0.1% (weight/weight) good-quality agricultural soil that had been preincubated with 0.5% (weight/weight) oil sludge hydrocarbons.

The experiments were carried out in Biometer flasks containing 10 g of the conditioned soil, 10 g of sand, and 2.08 g of the air dried sludge containing 0.5 g of extractable hydrocarbons. Water was added to 60% of the soil holding capacity, and the soil pH was adjusted to 7.5. Mineral nutrients were added to maintain a carbon : nitrogen : phosphorous : potassium ratio of 800 : 13 : 1 : 2. While maintaining all other parameters constant, individual parameters were varied to study their effect on degradation rates. The soil pH was varied from 5.0 to 7.8, and the soil moisture was varied 30 to 90% of the soil holding capacity. The sludge loading rate was varied from 0.25g to 3.00 g of extractable hydrocarbons per flask, and the nutrient ratio varied from 200 : 13 : 1 : 2 to

4000 : 13 : 1 : 2. Temperature effects were studied by varying the incubation temperature from 5°C to 30°C. The duration of the experiment was 285 days. At the end of 130 days, the flasks received a second sludge dosage corresponding to the initial one.

When pH was varied, the greatest extent of hydrocarbon conversion, 57%, was noted at the highest pH of 7.80. Similarly,when the nutrient ratio was varied, the hydrocarbon degradation percentage was found to be highest at 55% with a nutrient ratio of 800 : 13 : 1 : 2. For the various sludge loading rates tested, the greatest percentage of degradation (48.7%) occurred at the lowest sludge loading rate of 0.25g. A reduction in percentage of biodegradation was observed at loading rates above 1.0g of sludge per flask. Similarly, it was noted that smaller increments of sludge loading resulted in a higher rate and a higher total hydrocarbon biodegradation than a single large loading. The study of the temperature effects showed that there was no appreciable bacterial activity at 5°C, and that there was no increase of the biodegradation activity above 20°C.

Based on the above study [Dibble and Bartha, 1979], the following conclusions were made:

- Biodegradation is maximum at 50 to 70% of the soil water holding capacity.
- Extremes of pH are inhibitory to the majority of microbial degradation processes.
- A carbon : nitrogen : phosphorous ratio of 100 : 10 : 1, based on the microbial cellular composition, is acceptable for 100% conversion of hydrocarbons into biomass.
- Smaller and more frequent application of sludge yields higher biodegradation rates than does the infrequent application of the large batches of sludge.

Although the effects of a large number of environmental parameters were studied, other important parameters such as tillage and soil texture were not tested in the laboratory. It was felt that the laboratory studies may be quite limited in reproducing field situations, and may need validation and possible adjustments in the field. With the advent of the land ban regulations of HSWA, it is highly unlikely that landfarming process will still be used for treatment of tank-bottoms, and other oily sludges.

Biological Treatment of Sludges in Batch Reactors

Biological treatment of sludges in batch reactors is a process in which the sludge is introduced into an aeration unit where it contacts microorganisms that are maintained in suspension by aeration and mixing. Such reactors are also known as liquid/solid contact reactors, and the process is called slurry biodegradation. The process is similar to conventional biological suspended growth processes such as activated sludge treatment and aerated lagoons, but functions with much higher solid loadings [Hahn and Loehr, 1992]. Other advantages of sludge treatment in batch reactors are the reduced risk of off-site contamination, and better control over environmental conditions. The process provides favorable conditions for biodegradation since contaminated solid materials are suspended in a completely wetted environment which provides for maximum surface exposure. Furthermore, through mixing and aeration, high mass transfer rates of oxygen to the aqueous phase are effected [Hahn and Loehr, 1992].

The batch reactors used for the biological treatment of sludges are designed to relieve the environmental factors that limit microbial growth and activity [Stroo, 1989]. Aeration is provided by floating or submerged aerators, or by compressors and spargers. Mixing can be provided by aeration alone, or by aeration and mechanical mixing. To support microbial activity, nutrients and other neutralizing agents are supplied as necessary. Materials such as surfactants and dispersants may also be added to the reactor, to increase substrate availability for biodegradation [Stroo, 1989].

There are various factors that can limit the biodegradation rate in a liquid/solid contact reactor [U. S. EPA, 1990]. They are:

- Variable waste composition;
- Non-uniform particle size;

- Biodegradability of the waste;
- Temperature outside 15-35°C range;
- Oxygen deficiency;
- Nutrient deficiency;
- pH outside 4.5-8.8 range;
- Insufficient microbial population; and,
- Presence of elevated levels of heavy metals, highly chlorinated organics, pesticides and herbicides, and inorganic salts.

These factors will have to be taken care of while designing the reactor. In addition, possible water and air emissions from the reactor will have to be controlled to avoid potential environmental and health effects.

Aerobic Treatment of Petroleum Sludges

In 1988, under the direction of HSWA, the EPA established treatment standards for five listed petroleum refining wastes. These standards give contaminant concentration levels that must be attained prior to land disposal of the waste. Although the standards for refinery listed wastes were based on solvent extraction or incineration as the BDAT, any treatment technology that is not specifically prohibited can be used to meet the BDAT standards. This has led to several studies on the feasibility of using aerobic biological treatment of petroleum sludges to achieve the BDAT standards.

Prince and Sambasivam [1993] conducted a series of shaker flask experiments to determine the feasibility of bioremediation as a viable treatment for petroleum sludges. The sludge used in the study was obtained from the production of a highly refined lubricant oil. The sludge initially had 429,000 ppm Total Organic Carbon (TOC), and 37% of the dry solid weight of the sludge was Oil and Grease (O&G). Bushnell-Haas media was used to provide the necessary nutrients. The sludge concentration in the media was approximately 30 weight percent. Aeration of the sludge-media mix was achieved by rotating the flasks on a shaker table. In one series of shaker flasks, in addition to the sludge, wastewater obtained from a sewage treatment plant was used as an additional microbial source. Even though there was growth of bacteria over the course of the experiment, which was six weeks, except in one series with low sludge concentration, no significant reduction in the TOC and O&G was observed. This was believed to be due to the rapid depletion of nutrients from the media. An independent study showed that the microbial population present in the sludge was capable of degrading Polycyclic Aromatic Compounds (PAHs) such as naphthalene, phenanthrene, and pyrene [Prince and Sambasivam, 1993].

As an alternative to BDAT treatment of listed hazardous petroleum sludges, Amoco Research Center in Naperville, Illinois, investigated the liquid/solids biotreatment of pressure filtered sludge [Engelder *et al.*, 1990]. In the study, a composite refinery sludge was pressure filtered, and the resulting filter cake was slurried to a 10% solids loading. The slurry was placed in 1L electrolytic respirometer vessels which served as the reactors. Mixing of the slurry was carried out using magnetic stir bars. The reactors were operated for up to 50 days. Analysis at the end of the treatment period indicated a total O&G removal half life of 42 days. Half lives of individual base-neutral organics, such as phenol, di-n-butyl phthalate, naphthalene, phenanthrene, etc., ranged from 5 to 27 days. Total cyanide and Volatile Organic Compounds (VOCs) were also found to be removed [Engelder *et al.*, 1990].

The Biotechnology Applications Center of IT Corporation studied the biodegradation of a crude oil storage tank-bottom sludge using liquid/solid contact reactors [Brown *et al.*, 1991]. The sludge used was determined to be 100% Total Petroleum Hydrocarbon (TPH) in accordance with EPA Method 418.1. Benzene and o-cresol concentrations obtained from the sludge using the Toxicity Characteristic Leaching Procedure (TCLP) were 1.05 mg/L, and 0.05 mg/L, respectively. EIMCO Biolift slurry reactors of 60 L capacity were used for the experiment. Two reactors were each loaded with 20 L of distilled water and 30 L of tank-bottom sludge. To determine if biokinetics was improved, one of the reactors was inoculated with a naturally isolated, petroleum degrading culture. The solids loading to both the reactors was approximately 15 to 20 percent. Both the reactors were maintained at a pH of 7, a dissolved oxygen level of 10 mg/L, and a temperature of 25°C. At the end of the eighth week of operation, both the reactors demonstrated 100 percent removal of compounds containing less than or equal to 8 carbon atoms. The seeded reactor demonstrated 97 and 14 percent removal of compounds containing 8 to 12 carbon atoms, and 12 to 14 carbon atoms, respectively, with no reduction of higher molecular weight compounds. During the same period, the other reactor demonstrated 98, 60, 53, and 30 percent removal of compounds containing 8 to 12 carbon atoms, 12 to 14 carbon atoms, 14 to 18 carbon atoms, and greater than 18 carbon atoms, respectively. This corresponded to a 10 percent TPH reduction in the seeded reactor and a 61 percent TPH reduction in the other reactor. No explanation was offered for the reduced performance of the seeded reactor [Brown *et al.*, 1992].

ENSR Consulting & Engineering carried out full scale biodegradation testing at Murphy Oil USA Inc., refinery storm-water basin at Meraux, La. [Vail, 1991]. The objective was to treat approximately 9,000 cu. yd of oil and grease sludge that had accumulated in the storm-water basin. For this purpose, a 120 ft x 100 ft x 9 ft reactor was built in the storm-water basin by constructing two sheet pile walls across the basin. Mixing and aeration in the reactor was carried out using six 20 hp aspirator-type floating aerators. Aerobic treatment was initiated using indigenous bacteria. Nutrient levels were ensured to be at sufficient level throughout the experimental stage, by monitoring the operating variables. The total PAHs in the sludge reduced from 2,710 mg/kg to 0.62 mg/kg at the end of the 16 week treatment period. Similarly, the oil and grease in the sludge decreased from 16.9 dry weight % to 5.1 dry weight % [Vail, 1991].

Laboratory and pilot scale studies were carried out by Salameh and Kabrick [1992], with the objective of determining the kinetics of PAH removal using liquid/solid contact reactors.

Laboratory studies were carried out in 20 L reactor vessels maintained at a constant temperature of 20°C. The reactors were mechanically mixed and aerated with bubble diffusers at a rate of 20 L/min. The reactors had 15 L of oily sludge and 5 L of acclimated microorganisms. Nutrients were periodically added to maintain a C : N : P ratio of 100 : 5 : 1. The reactors were operated for 64 days. At the end of operation the PAH levels were found to be reduced by as much as 93%. PAHs with up to four rings were degraded within the treatment period. However, five and six-ring PAHs were not removed significantly [Salameh and Kabrick, 1992].

A Continuously Stirred Tank Reactor (CSTR) has been used to treat API separator sludge in the laboratory [Field *et al.*, 1991]. The CSTR had an operating volume of one liter, a hydraulic residence time of 4.3 days, and a mixed liquor suspended solids concentration of 5,000 to 10,000 mg/L. An acclimated, diverse microbial culture was used to initiate biodegradation. The degradation of a five-ring PAH compound, benzo(a)pyrene, was monitored and an overall degradation of 95.6% was reported [Field *et al.*, 1991].

Castaldi and Ford [1992] conducted a laboratory biodegradation study of tarry sludges in batch reactors. Each test reactor had a volume of 20 L. Mechanical mixing was provided and aeration carried out through a diffuser stone. Acclimated microorganisms were used to inoculate the reactors. Reactors were maintained at different substrate-to-microorganism ratios, and had an initial solids content ranging from 3.5% to 7.5%. Adequate nutrients were maintained in the reactors during the test period. Temperature was maintained at 22-24°C. During a 90 day test period, the volatile constituents were at negligible concentrations within the first 15 days of testing. The semivolatile constituents were at nondetectable levels between 15 and 30 days after the start of the experiment. The waste oil and grease content was reduced from 23.3 percent to 18.5 percent [Castaldi and Ford, 1992].

A pilot-scale demonstration of liquid/solids treatment was conducted at a major refinery to

evaluate biological treatment as a remedial option for petroleum impoundment sludges [Coover *et al.*, 1994]. A single batch treatment of sludge at a nominal solids loading of 10% was conducted for a period of 56 days in a 3.8 x 10^3 m^3 (1.34 x 10^5 ft^3) bioreactor. Aeration was supplied using 19 kW updraft float-mounted aerators, and mixing was provided by two 30 kW float-mounted mixers. Nutrients and lime were premixed and added incrementally to the reactor. The reactor was also seeded with a mixed culture of hydrocarbon-degrading microorganisms. Volatile hydrocarbons in the sludge were lost rapidly through a combination of volatilization and biodegradation. Polycyclic Aromatic Hydrocarbons (PAHs) in the mixed liquor solids were below the analytical detection limits after four weeks of treatment. Analysis at the end of the treatment period indicated an Oil & Grease (O&G) removal half life of 80 to 90 days [Coover *et al.*, 1994].

Summary

From the studies described above, it can be seen that the hazardous constituents in different kinds of petroleum sludges can be degraded in aerobic batch reactors. However, no studies have been conducted on the applicability of using aerobic batch reactors for reducing the organic content of refined product tank-bottom sludges. None of the studies described above looked at the metals content and toxicity levels of the sludges before and after treatment. Also, very little has been done to determine the order and rate of degradation of tank-bottom sludges in aerobic batch reactors. Another aspect that has not been tried before is the effect of mixing two different types of sludges on the biodegradation rate.

In the studies described below, the biodegradation of sludges from refined petroleum product storage tanks in aerobic batch reactors are investigated and their rate of degradation determined. It is also investigated whether there is a reduction in the metals content and toxicity levels of the sludges as a result of biological treatment. One specific focus of this study is on the effect of mixing two different types of sludges on the rate of biodegradation.

CHAPTER III

EXPERIMENTAL PROCEDURES AND ANALYTICAL METHODS

This chapter describes and explains the experimental setup, and the experimental and analytical techniques used to conduct the study. All the chemicals used in the study were of an analytical grade. Hexane and acetone that were used as extraction solvents were of pesticide grade, and were obtained from Fisher Scientific (Fairlawn, NJ).

Preliminary Analyses

For the purpose of this study, distillate storage tank-bottom sludge was obtained from a local Oklahoma refinery. Similarly, gasoline storage tank-bottom sludge was provided by a local Oklahoma pipeline company. Acclimated, hydrocarbon-degrading microorganisms were obtained from the wastewater treatment plant at the same refinery from where the distillate tank-bottom sludge was collected. In order to properly design the experimental reactors, preliminary experiments were carried out to characterize both sludges. The results thus obtained were used to determine the operating conditions for the batch reactors.

The sludges were analyzed for their density, the total and volatile solids content, the Chemical Oxygen Demand (COD), the Total Kjeldahl Nitrogen (TKN), the phosphorous content (as P), the Total Petroleum Hydrocarbon (TPH) content, and the concentrations of metals such as arsenic, cadmium, chromium and lead. The densities of the sludges were determined by weighing a known volume. The total and volatile solids fraction in the sludges were determined according to

Method No. 2540 G of Standard Methods [APHA *et al.*, 1992]. For determining the COD, a small weighed sample of the sludge was taken in an Erlenmeyer flask and a known volume of deionized water was added to it. The flask was then placed on a shaker table, and the contents mixed for twenty four hours. After mixing was completed, a sample of the liquid portion in the flask was taken, digested, and colorimetrically analyzed for its COD using High Range COD Reagent vials from Hach Company (Loveland, CO).

In order to determine the TKN content, about 1 g of the sludge was first digested as described in Method No. 4500-N_{org} B of Standard Methods [APHA *et al.*, 1992]. The final ammonia measurement was carried out titrimetrically according to Method No. 4500-NH_3 E of Standard Methods [APHA *et al.*, 1992]. For measuring the phosphorous content, a weighed sample of the sludge was first digested with sulfuric acid and nitric acid as per Method No. 4500-P 4 of Standard Methods [APHA *et al.*, 1992]. The phosphorous content of the digested sludge was then colorimetrically analyzed on a Hach DR/3000 Spectrophotometer, using "PhosVer 3 Phosphate Reagent Powder Pillows" from Hach Company [Hach Company, 1989].

In order to prepare the sludge for analysis of metals, a representative sample of about 1 g was digested in nitric acid and hydrogen peroxide and then refluxed with hydrochloric acid as described in Method No. 3050 of "Test Methods for Evaluating Solid Waste" [U. S. EPA, 1986]. The digested sample was then analyzed for the concentrations of arsenic, cadmium, chromium, and lead. The initial analysis of the digested samples was carried out by Bates Lab, Sand Springs, OK.

For the determination of TPH, the hydrocarbon contents of the sludge were extracted as per Method No. 3540 of "Test Methods for Evaluating Solid Waste" [U. S. EPA, 1986]. A 10 g sludge sample was mixed with 10 g of anhydrous sodium sulfate, and extracted with a 1:1 (volume/volume) mixture of acetone and hexane in a Soxhlet extractor for 24 hours. The extract was then concentrated over a water bath in a Kuderna-Danish apparatus fitted with a three-ball Snyder

column, to a final volume of 10 ml. From this, a 10 μl sample was injected into a Perkin Elmer Sigma 3B Gas Chromatograph (GC) equipped with a Flame Ionization Detector (FID). Component separation took place on a Supelco 3.2 mm ($1/_8$ inch) diameter, 1.8 m (6 feet) long, stainless steel column containing 10% SP-2100 on 80/100 mesh Supelcoport. Nitrogen was the carrier gas, with a flow rate of 30 cc/min and a head pressure of 30 psi. The column was run in a nonisothermal mode, and was heated over the period of analysis from 75^o C to 275^o C. The initial temperature was held constant for 5 minutes, and then increased at 10^o C per minute to 275^oC, where it was held constant for 20 minutes. The injector temperature was at 100^o C, and the detector temperature was at 300^o C. The area under the resulting chromatogram was then compared with an appropriate standard curve to calculate the TPH of the sample.

For distillate tank-bottom sludge, a TPH standard was prepared containing 6626.5 mg of pure Number Two fuel oil (diesel) and enough acetone/hexane extraction solvent to bring the total volume to 10 ml. Volumes ranging from 1 to 10 μl of this standard were then injected into the GC, and based on the area under the chromatogram that corresponds to the amount of diesel in the volume injected, a TPH standard curve was developed. Similarly, for gasoline tank-bottom sludge, a TPH standard was prepared containing 212.6 mg of pure gasoline and enough acetone/hexane extraction solvent to bring the total volume to 10 ml. Using this standard, a TPH standard curve for gasoline tank-bottom sludge was developed.

In addition to the above analyses, the pH of the sludges was also measured using a Fisher Scientific "Accumet 900" pH meter and probe. The results obtained from these preliminary analyses are discussed in Chapter IV. Based on the results of the sludge solids analysis, in order to maintain the solids in suspension, it was decided to add the sludge and the acclimated seed to the reactor in the ratio of 1:1 (volume/volume). Also, based on the nitrogen and phosphorous analysis of the sludges, it was decided to add nutrients in the form of ammonium chloride and potassium phosphate monobasic to the reactor, in order to maintain an overall

Carbon : Nitrogen : Phosphorous ratio of 100 : 10 : 1.

Experimental Setup

As stated before, the purpose of this study was to evaluate the biodegradation of refined product tank-bottom sludges in aerobic batch reactors. A schematic of the experimental setup used for the study is shown in Figure 1. The test reactor was made of glass, and had a total capacity of 2.5 liters. The main body of the reactor had an internal diameter of 10.2 cm (4 inches). As shown in Figure 1, the reactor diameter increased to 15.2 cm (6 inches) at the top, thereby forming a one inch wide channel surrounding the main reactor body. The reactor had a 1.3 cm ($1/_2$ inch) tube at the top, and a 0.64 cm ($1/_4$ inch) tube at the bottom. The reactor was clamped to a ring stand.

The reactor was covered at the top with a removable plexiglass cover. This was done to contain and collect the volatile gases that are liberated during the aeration and mixing of the sludges. A 1 mm thick rubber gasket was placed between the cover and the reactor top, and the gaps filled with vacuum grease. Prior to reactor start up, the air tightness was tested by applying a vacuum. The vacuum gauge showed a pressure of 50.8 cm (20 inches) of mercury, thereby indicating a fairly tight seal.

For the purpose of mixing the contents of the reactor, a 0.64 cm ($1/_4$ inch) diameter steel rod was introduced into the reactor through a set of ball bearings that were seated on the reactor cover. The rod extended from 2.54 cm (1 inch) above the reactor bottom to at least 20.32 cm (8 inches) above the top of the reactor. Two 1 mm thick steel plates with dimensions of 1.91 cm x 7.62 cm ($3/_4$ inch x 3 inches) were welded onto the rod, and served as paddles for mixing. One of the plates was fixed to the bottom of the rod, and the other one, 15.24 cm (6 inches) above it. The paddles and the rod were given a coat of rust proof paint. The top of the rod was attached to a $1/_{50}$ hp mixer from Mixing Equipment Co. Inc. (Rochester, NY). The speed of the mixer was controlled

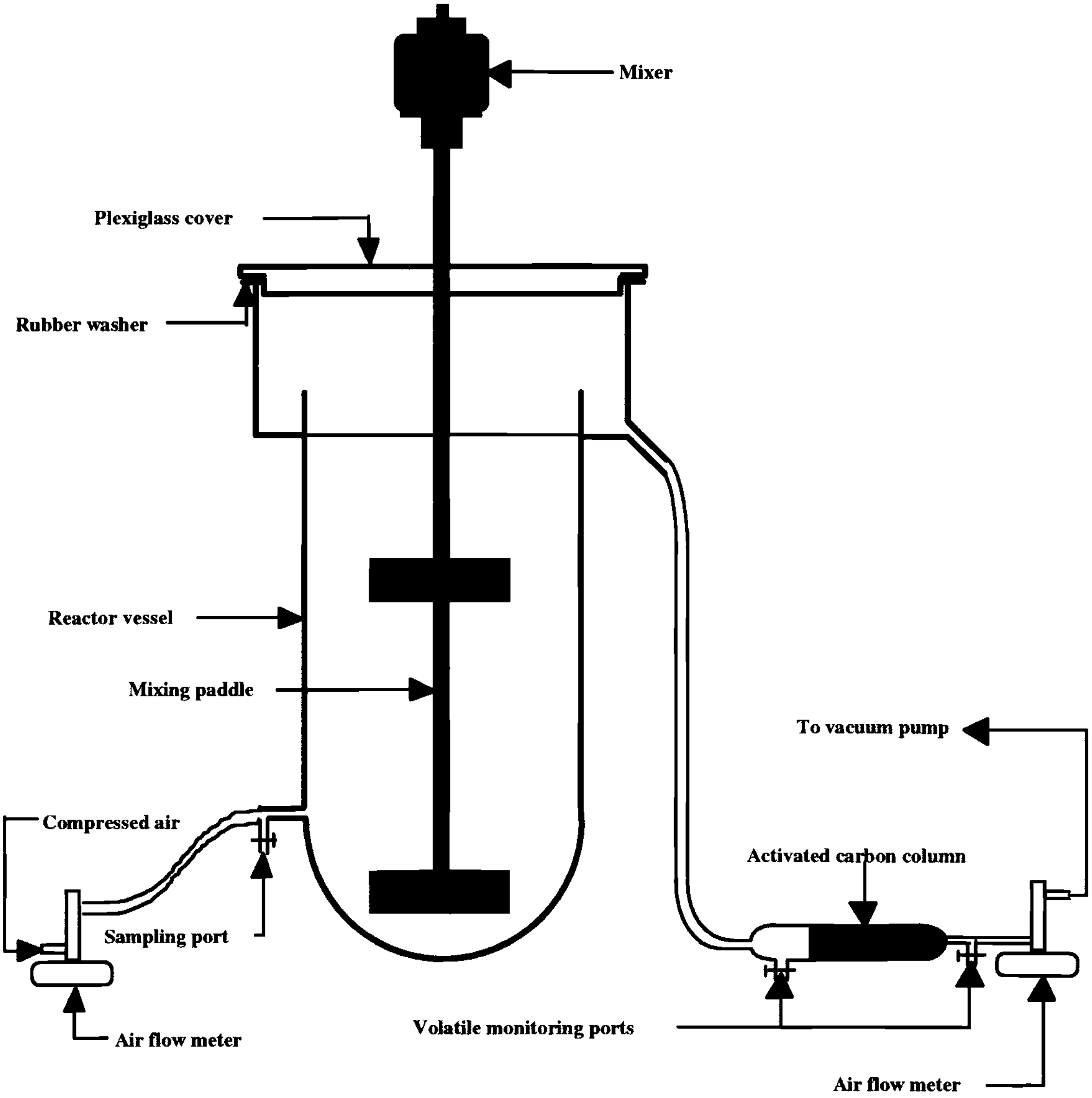


Figure 1. Schematic of Experimental Setup

by controlling the power supply to the mixer through a variable voltage transformer (Variac). Each reactor had its own mixer and Variac.

The air supply to the reactor was provided by an air compressor. The air supply from the compressor was taken through a Gelman Air Flowmeter, and connected to the tube at the bottom of the reactor. Tygon tubing was used to connect the air supply point to the air flowmeter, and the air flow meter to the reactor tube. A tee connector was provided between the reactor tube and the Tygon tubing, and it served as a sampling port. When not in use, the sampling port was clamped off.

The volatile organic compounds that were released by the mixing and aeration of the sludge were captured in a granular activated carbon column. The column was made of glass, 2.54 cm (1 inch) in diameter, and had a volume of 150 cc. Both ends of the column tapered off to 0.32 cm ($1/_8$ inch) in diameter, and were plugged with glass wool. The column had a 0.64 cm ($1/_4$ inch) capped outlet on one side, to facilitate the addition and removal of activated carbon from the column. Forty grams of washed and dried granular activated carbon, GAC 830 from Atochem Inc., was loaded into the column. The influent end of the column was connected with Tygon tubing to the upper tube of the reactor. The effluent end of the column was connected to a Gelman airflow meter that was connected through a manifold to a vacuum pump. The valves in the manifold were adjusted such that the rate of vacuum extraction was maintained to be the same as the rate of aeration. This was confirmed by the flow rates in the flow meters connected to the aeration line and the vacuum line. The manifold was also fitted with a vacuum pressure gauge. When all three reactors were in operation, the gauge showed a vacuum pressure of 20.32 cm (8 inches) of mercury. A tee connector was also provided to the effluent end of the carbon column, to enable the monitoring of volatile hydrocarbons in the effluent air stream. The capped outlet in the column was made use of for monitoring the volatile hydrocarbons in the influent air stream to the activated carbon. A pictorial representation of the experimental setup prior to the start of the reactors is shown in Figure 2.

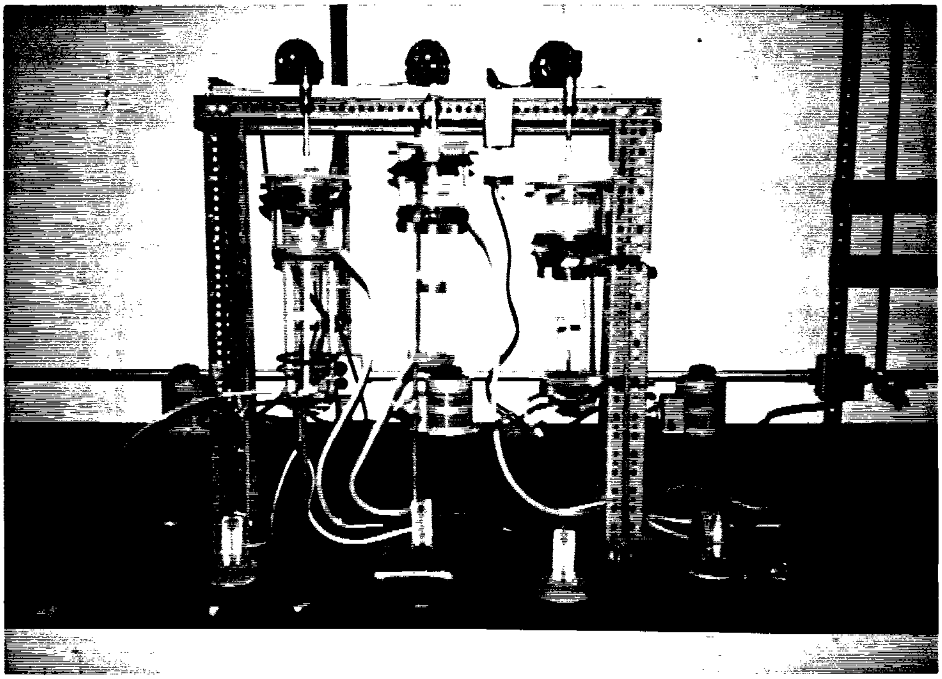


Figure 2. Setup of Experimental Reactors

Reactor Operation and Sampling Procedures

Although it is desirable to operate the reactors with as high a solids content as possible, the ability to achieve adequate mixing and aeration, and to sustain a reasonable suspension, limits the reactor solids loading to a maximum of thirty to forty percent [Stroo, 1989]. To achieve this, it was necessary to dilute both the sludges. Based on the results of the preliminary analyses, it was decided to mix 1 liter of sludge with 1 liter of acclimated seed, and the combined mixture was placed in the reactor. One reactor had distillate tank-bottom sludge, and another one had the gasoline tank-bottom sludge. A third reactor had a 1:1 (volume/volume) mixture of distillate and gasoline tank-bottom sludges. The preliminary sludge analysis also showed the necessity of nutrient addition to maintain a carbon : nitrogen : phosphorous ratio of 100:10:1 in the reactor. This was achieved by dissolving the requisite amounts of ammonium chloride and potassium phosphate monobasic in 100 ml of deionized water, and adding the resulting solution into the reactor. A sample calculation for the nutrient requirements of the reactor containing distillate tank-bottom sludge is shown in Appendix A. The pH of the mixture in the reactor was lowered by the addition of the nutrient solution, but it was brought back to neutrality with the addition of a few drops of ammonium hydroxide. The total volume of the mixture in the reactor was now 2.1 liters.

After noting the level of the mixture, the reactor was closed with the cover, and the gaps filled with vacuum grease. The mixer was then started, and its speed so adjusted that the paddles were rotating at 90 rpm. After thoroughly mixing the contents of the reactor, the sampling port at the bottom was opened, and about 60 ml of the mixture was collected for carrying out various analyses. Then the sampling port was clamped, and the air flow to the reactor started. Simultaneously, the vacuum pump was also switched on, and the air in the head space extracted through the activated carbon column. The air flow through the reactors and the activated carbon columns was maintained at a rate of 3 liters per minute. In the case of the reactor containing the gasoline tank-bottom, due to excessive foaming initially, the air flow through the reactor and the

activated carbon column had to be lowered to 1 liter per minute. Once the foaming had subsided, the air flow rate was increased to 3 liters per minute.

Immediately after the start of aeration of the reactors, the air flow to and from each of the activated carbon columns was monitored periodically for their total volatile organic contents. This was done to ensure that breakthrough of the activated carbon columns did not occur. The monitoring was carried out using a DL-101 portable photoionization analyzer from HNU Systems Inc. (Newton, MA). Before use, the analyzer was calibrated using isobutylene of known concentration. It was also zeroed using the influent air stream to the reactors. The analyzer was capable of measuring volatile organic concentrations up to 2000 ppm. Initially, when the liberation of volatile organic compounds was at its peak, the influent and effluent air streams were monitored every 15 minutes. With the passage of time, the amount of volatiles liberated decreased, and correspondingly, the monitoring also became less frequent. Within five to seven days from the start of the reactors, the amount of organics that were present in the air stream had become negligible, and from then onwards the monitoring was stopped. The activated carbon columns were disconnected from the reactors at the twentieth day of operation, and the vacuum pump stopped. Plots of the analyzer readings for the three reactors are given in Appendix B.

The reactors were operated continuously for sixty days. Every tenth day, the aeration was stopped briefly to collect a sample from the reactor. At this time, any drop in the level of the reactor contents due to evaporation and sampling was made up with tap water. Salameh and Kabrick [1992] had followed a similar procedure, but used distilled water in place of tap water to compensate for evaporative losses. The aeration and mixing of the reactor was then restarted, and the reactor continued to operate until it was time to take the next sample. During the course of the experiment, the room temperature varied between 10 to 20°C. Figure 3 shows a pictorial view of the reactors in operation.

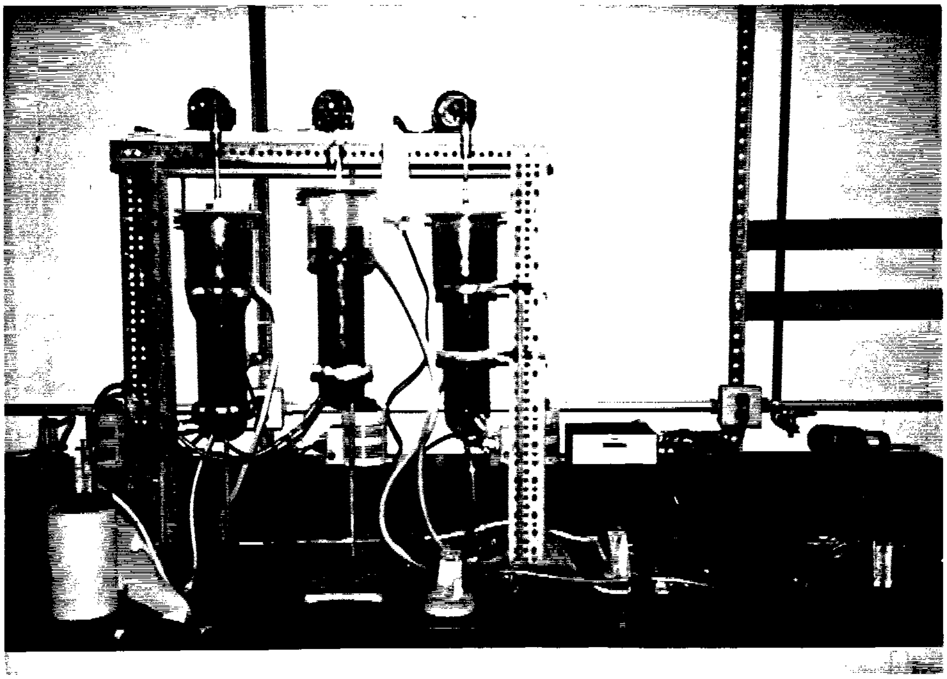


Figure 3. Reactors in Operation

Samples collected from the reactors were immediately analyzed for pH and Dissolved Oxygen (DO) levels. Dissolved oxygen content was measured using a YSI Model 5739 DO probe connected to a YSI Model 54A DO meter. Afterwards a portion of the sample was removed and refrigerated for toxicity and microbial determination at a later date. The remaining sample was acidified to a pH below 2 using concentrated hydrochloric acid, and refrigerated at $4^{o}C$, until the various analyses were performed. This procedure was followed for all the samples.

Analytical Methods

All the samples from each reactor were analyzed for total and volatile solids content, chemical oxygen demand, total petroleum hydrocarbons content, and oil and grease content. These samples were also analyzed to estimate the number of hydrocarbon-utilizing microorganisms.

The initial, the thirty-day, and the sixty-day samples from each reactor were analyzed for the metals cadmium, chromium, and lead. The total Kjeldahl nitrogen and phosphorous content in these samples were also determined. The initial, the twenty-day, the forty-day, and the final samples from each reactor were analyzed for toxicity using a Microtox instrument. In addition to these tests, the activated carbon used to capture the volatile organic compounds (VOCs) liberated from each reactor was analyzed to determine the amount of VOCs adsorbed.

The total and volatile solids content, the chemical oxygen demand, and the total petroleum hydrocarbon content of each sample were determined in the same way as in the preliminary analyses. The total and volatile solids analysis was done in triplicate for each sample, and the average of the three values reported. In the case of COD analysis, the dissolution process was carried out in duplicate for each sample. The COD determination of the liquid portion thus obtained was also carried out in duplicate, and the average of the four values reported as the COD of the sample.

For determining the TPH of a sample, the extract of the sample was injected three times into the GC, and the mean of the three values was taken as the TPH of the sample. Typical gas chromatograms of the initial and final samples from each reactor are shown in Appendix C. For the samples from the reactor containing the distillate and gasoline tank-bottom mixture, a TPH standard was prepared containing 366.4 mg of a 1:1 (volume/volume) mixture of pure gasoline and diesel and enough acetone/hexane extraction solvent to bring the total volume to 10 ml. Varying volumes of this standard were then injected into the GC, and based on the area under the chromatogram that corresponds to the amount of diesel/gasoline mixture in the volume injected, a TPH standard curve was developed. The TPH standard curves developed for each reactor are given in Appendix D.

The TKN and phosphorous content of the samples were determined as described before. The concentrations of cadmium, chromium, and lead in the digested samples were determined using a Perkin Elmer 5000 Atomic Absorption Spectrophotometer. The initial arsenic concentrations in both the sludges were lower than the chromium and lead concentrations. Moreover, the furnace absorption spectrophotometer necessary for arsenic analysis was not functioning properly, and hence the arsenic content of the samples was not determined. Oil and Grease content of the samples was determined as per Method No. 5520 E of Standard Methods [APHA *et al.*, 1992]. The extraction solvent used for this procedure was an 80:20 (volume / volume) mixture of hexane and methyl-tert-butyl ether.

For estimating the number of hydrocarbon-utilizing microorganisms in the sample, a five-tube Most Probable Number (MPN) technique was used [Song and Bartha, 1990 and APHA *et al.*, 1992]. The growth medium used was Bushnell-Haas broth (Difco Laboratories, Detroit, MI) containing 1 mg/l of resazurin. For each sample, three sets of five screw cap tubes containing 5 ml of the autoclaved medium, were prepared. All the tubes in a set were then inoculated with 1 ml of the appropriately diluted sample. The dilutions chosen for the three sets were 0.1, 0.01, and 0.001, respectively. All the tubes were also given a supplement of hydrocarbon fuel. If the tubes contained sample from the distillate tank-bottom reactor, then the fuel supplement consisted of 50 µl of diesel. Fifty (50) µl of gasoline was added to the tubes containing samples from the gasoline tank-bottom reactor. In the case of samples from the reactor containing the distillate/gasoline tank-bottom mixture, the tubes were supplemented with 50 µl of a 1:1(volume/volume) mixture of diesel and gasoline. After incubating the tubes at 27^{o} C for 3 weeks, the positive tubes in each set were counted. As a result of the reduction of the resazurin by microbial oxygen consumption, the color of positive tubes ranged from pink to colorless. The MPN values for the samples were then obtained from the MPN index for five tubes [APHA *et al.*, 1992].

For determining the toxicity of the samples, an extraction fluid was first prepared by diluting 5.7 ml of glacial acetic acid with deionized water to a volume of 1 liter. About 2 grams of the sample was placed in an Erlenmeyer flask, and extraction fluid of an amount equivalent to twenty times the weight of the sample taken was added to it. The flask was then placed in a shaker table and the contents mixed at a rate of 30 rpm for 20 hours. Following the extraction, the material in the flask was separated into its component solid and liquid fractions by filtering through a 0.8 µm glass fiber filter. The liquid portion thus obtained was evaluated for its toxicity levels using the Microtox Basic Test from Microbics Corporation (Carlsbad, CA). The test exposes bioluminescent bacteria to the samples, and measures the toxic effect of the sample on the organism. The Microtox test system measures the light output of the luminescent bacteria after they have been challenged by a sample of unknown toxicity, and compares it to the light output of a control that contains no sample. The degree of light loss is an indication of the degree of toxicity of the sample. The toxicity of the sample is expressed as an effective concentration of the sample causing a 50% decrease (EC50) in the Microtox Reagent light output under defined conditions of exposure time and test temperature [Microbics Corporation, 1992]. The smaller the EC50 value the more toxic the sample is.

In order to determine the amount of volatile organics adsorbed by the activated carbon, the contents of the carbon column were emptied and separated into three equal piles representing the top 1/3, the middle 1/3, and the bottom 1/3 of the column. A composite 3 gram sample was then prepared by taking one gram of activated carbon from each pile. The activated carbon sample thus obtained was placed in an Erlenmeyer flask, and 10 ml of carbon disulfide added to it. After stoppering the flask, the contents were mixed for an hour by keeping the flask on a shaker table. The carbon particles were then filtered out using a 0.8 μm glass fiber filter. Ten (10) μl of the filtered solution was injected into the GC that was operating under the same conditions as that for the TPH analysis. The resulting chromatogram was compared with the corresponding TPH standard curve, and the total volatile organic concentration adsorbed by the activated carbon calculated. This procedure was carried out in quadruplicate, and the mean of the four values reported as the TPH of the volatile organics adsorbed by the activated carbon column of each reactor. Typical gas chromatograms obtained for each reactor are shown in Appendix E.

CHAPTER IV

RESULTS AND DISCUSSION

Preliminary Studies

As mentioned earlier, for the purpose of the study, a distillate tank-bottom sludge sample and a gasoline tank-bottom sludge sample were obtained. In order to design the experimental batch reactors, preliminary studies were carried out to characterize the sludges. Both the sludges were analyzed for their density and pH, their solids content, their chemical oxygen demand, their nitrogen and phosphorous levels, and their total petroleum hydrocarbon content. The sludges were also analyzed for the concentrations of four metals: arsenic, cadmium, chromium, and lead. The results obtained from these experiments are given in Table 1. Similarly, the acclimated seed that was used for this study was tested for its pH and solids content. Table 2 gives the results obtained from the analysis of the acclimated seed.

As can be seen from the results, the solids content of both the sludge samples were very high. In order to maintain the solids in suspension, it was essential that the feed to the batch reactor had a solids content of less than 40% [Stroo, 1989]. To achieve this, it was necessary to dilute the sludges before they were added to the batch reactor. Based on the results obtained, it could be seen that a 1:1 (volume/volume) mixture of the sludge and the seed would have a solids content of less than 35%. Hence, the experimental reactors were each filled with a 1:1 (volume/volume) mixture of sludge and seed.

Table 1. Results of the Preliminary Experiments on Tank-Bottom Sludges

Parameter	Distillate Tank-Bottom	Gasoline Tank-Bottom
Density (g/l)	1143.27	1267.38
pH	8.2	8.6
Total Solids Content (% wet weight)	43.50	49.50
Volatile Solids Content (% wet weight)	16.00	12.50
Chemical Oxygen Demand (mg/g wet weight)	415	19
Total Kjeldahl Nitrogen (mg N/g wet weight)	2	5
Phosphorous (mg P/g wet weight)	0.02	0.03
Total Petroleum Hydrocarbons (mg/g wet wt)	748	18
Arsenic (mg/g wet weight)	0.09	0.35
Cadmium (mg/g wet weight)	ND	ND
Chromium (mg/g wet weight)	0.15	0.40
Lead (mg/g wet weight)	0.26	0.68

ND - Not Detected

Table 2. Results of the Analysis of the Acclimated Seed

Parameter	
Total Suspended Solids (mg/l)	7,860
Volatile Suspended Solids (mg/l)	5,960
pH	7.2

Another fact that became evident from the preliminary study of the sludges was that the gasoline tank-bottom sludge had a much lower organic content than the distillate tank-bottom sludge. This was clear from the volatile solids, the COD, and the TPH analysis of the sludges. Also, the concentrations of metals in the gasoline tank-bottom sludge were more than that in the distillate tank-bottom sludge. Based on the results of the solids analysis, it could be seen that whereas 36.8% of the total solids content of the distillate tank-bottom sludge consisted of volatiles, only 25.3% of the total solids content of the gasoline tank-bottom sludge consisted of volatiles. This should suggest that the solids in the gasoline tank-bottom sludge consisted primarily of inorganic material such as sand, dirt, rust, etc.

In order to have proper conversion of the hydrocarbons into biomass, it was necessary to maintain a carbon : nitrogen : phosphorous ratio of 100 : 10 : 1 in the batch reactors. The distillate resembled diesel fuel in hydrocarbon composition, and so based on the typical composition of diesel fuel [Riser-Roberts, 1992], a carbon content of 0.856 gram per gram of distillate was calculated [Sieck, 1993]. Similarly, based on the typical composition of gasoline [Riser-Roberts, 1992], a carbon content of 0.866 gram per gram of gasoline was calculated. Based on these, and the TPH, nitrogen, and phosphorous contents that were obtained by the preliminary tests, it was clear that both sludge samples were nutrient deficient. This necessitated the addition of nutrient supplements to the batch reactors so as to maintain a carbon : nitrogen : phosphorous ratio of 100 : 10 : 1.

Results of the Batch Reactor Sample Analyses

After the preliminary studies were completed, the main objectives of this report were addressed. The results obtained from the preliminary studies were utilized to set up three batch reactors. As stated earlier, the primary objectives of these experiments were to study the reduction in the organic content of distillate tank-bottom sludge, gasoline tank-bottom sludge, and a 1:1 (volume/volume) mixture of distillate/gasoline tank-bottom sludges, in aerobic batch reactors. Prior

to start up, a 60 ml sample was taken from each reactor. After starting the reactors, similar volumes of samples were taken from them every tenth day, until they were stopped at the sixtieth day. The samples were analyzed for various parameters that describe the degree of destruction of the organics in the sludges. The samples were also analyzed for other factors that may have an effect on the biodegradability of the sludges. The results of these experiments are presented in both graphical and tabular forms, and are discussed in the sections below.

Between the sampling periods, there was a reduction in the volume of the contents of each reactor due to evaporation, which was compensated for using tap water. Similarly, the reduction in volume of the reactor contents due to sampling was also replenished with tap water. Although the addition of tap water to compensate for evaporative losses would not have an effect on the results of the study, the addition of tap water to replace the sample volumes removed might lead to the dilution of the reactor contents, and thereby affecting the final results. By the sixtieth day, nearly 360 ml, which corresponded 17% of the initial volume of each reactor, had been removed for analytical purposes and was replaced with tap water. Assuming that the tap water had a density of 1 g/ml, by the sixtieth day, the net weight loss in the reactors ranged from 22.87 g in the distillate tank-bottom reactor to 42.68 g in the gasoline tank-bottom reactor. This represented a 1 to 1.8% reduction in the initial mass of the reactor contents. The densities of the final samples showed that the mass of the reactor contents at the end of operation was close to the theoretical value obtained by taking dilution effects into account. As all the results were expressed per unit weight of the wet sludge, the dilution of the reactor contents should not unduly affect the final results. The effect of dilution was taken into consideration while carrying out the mass balances on the major parameters of the study.

The monitoring of subsequent samples showed that the pH in all the three reactors remained at 7.0 ± 0.2 during the course of the experiments. Before starting the aeration of the reactors, the contents had a dissolved oxygen concentration of about 2 mg/l. After the start of aeration, the dissolved oxygen levels in all the reactors rose to 8 to 9 mg/l, and remained at that level during the entire duration of the experiments. This indicated that all the three reactors were at an oxygen saturation level during the entire duration of the reactor operation. This is probably due to the fact that aeration was carried out at a rate of 3 liters per minute through a net volume of 2.1 liters.

Analysis of Total Petroleum Hydrocarbons

The Total Petroleum Hydrocarbon content of each reactor was the main parameter used to evaluate the extent of biodegradation of the tank-bottom sludge. It was evaluated using gas chromatography. The sample was extracted using a hexane/acetone mixture as the solvent, and the extract obtained was injected into the gas chromatograph. The area under the resulting chromatogram was compared with an appropriate standard curve to calculate the TPH of the sample. Each sample extract was injected three times into the GC, and the mean of the three gas chromatogram area values was used to determine the TPH of the sample. Appendix F gives the data obtained from the GC analysis of the samples.

Because the TPH components were different in each of the sludges, a different standard curve was used for determining the TPH of samples from each of the reactors. Each TPH standard was so chosen such that its composition resembled closely that of the respective raw sludge extract. Accordingly, Number Two Fuel Oil (diesel) was used as the TPH standard for the distillate tank-bottom, unleaded gasoline was used as the TPH standard for the gasoline tank-bottom, and a 1:1 (volume/volume) mixture of unleaded gasoline and diesel was used as the TPH standard for the mixed sludge. From the TPH standard curves, which are shown in Appendix D, it can be seen that the GC response is different for each of the three standards. For example, a GC area count of 500 corresponds to a TPH of 843 μg on the distillate standard curve, a TPH of 153 μg on the gasoline standard curve, and a TPH of 452 μg on the mixture standard curve, respectively. Due to the difference in the TPH composition of each reactor, a different standard curve was used for

determining the TPH of samples from each of the reactors, and hence the absolute values of TPH obtained for each reactor cannot be compared with each other. Only the relative changes in the TPH of each reactor over the course of time can be compared.

The TPH values of the initial samples from the distillate, gasoline, and mixed tank-bottom reactors were 279 mg/g, 14mg/g, and 48 mg/g, respectively. Theoretically, the initial TPH value of the mixed tank-bottom reactor sample should be midway between the initial TPH values of the distillate and gasoline tank -bottom reactor samples. However, as the TPH values obtained were based on three different standard curves, they cannot be directly compared with each other.

The raw distillate tank-bottom sludge had a TPH content of 748 mg/g, and the initial TPH value of the distillate tank-bottom reactor contents was 279 mg/g. Similarly, the raw gasoline tank-bottom sludge had a TPH content of 18 mg/g, and the corresponding initial TPH value of the gasoline tank-bottom reactor contents was 14 mg/g. This takes into account the mixing of 1 liter of the raw sludge with 1.1 liter of seed and nutrient solution. Another fact to be considered was that the raw waste was hand mixed before samples were taken for preliminary analysis and for placing in the reactors This might have lead to slight differences in the homogeneity of the raw sludge used in each case.

The variation of TPH with time in each reactor is graphically shown in Figure 4. As can be seen from the figure, there was a progressive reduction in the TPH levels of all the three reactors. However, a spike was observed in the forty-day samples from the distillate and gasoline tank-bottom reactors. There seems to be no explanation for these increases other than their being the result of nonrepresentative samples. TPH levels in the distillate tank-bottom reactor were reduced by 50% at the end of the sixty-day period of operation. Similarly, the gasoline tank-bottom reactor had a TPH reduction of 99% and the mixed tank-bottom reactor had a TPH reduction of 65% at the end of sixty days. The almost complete removal of TPH from the gasoline tank-bottom reactor is

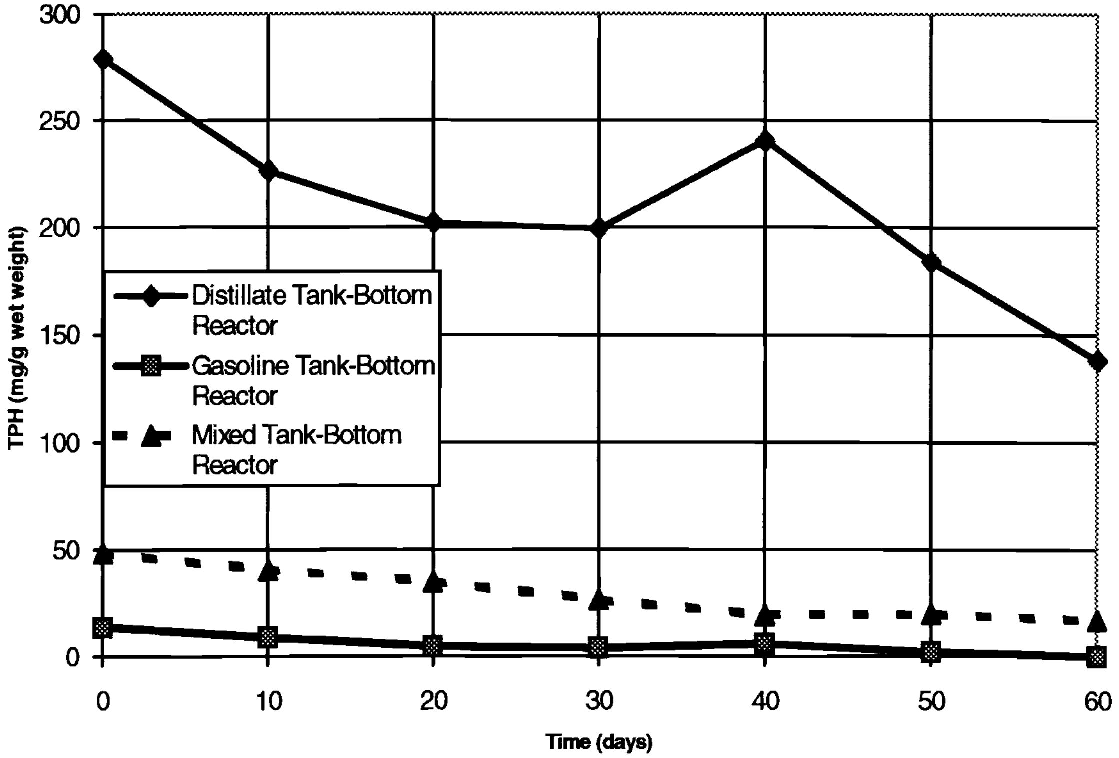
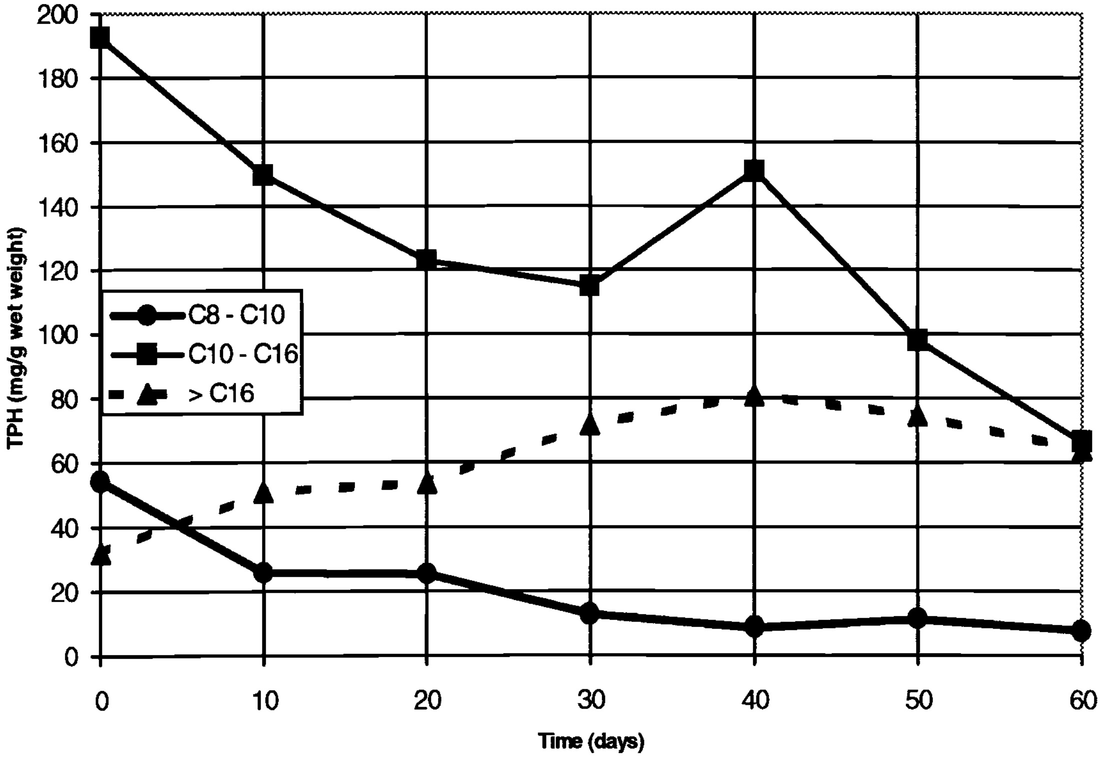


Figure 4. Variation of Reactor TPH Levels with Time

probably due to the low initial organic loading in the reactor. In the distillate tank-bottom reactor 57% of the total TPH reduction occurred in the first thirty days, whereas in the case of the gasoline tank-bottom reactor, 72% of the total TPH reduction had occurred in the first thirty days. In the mixed tank- bottom reactor, 69% of the total TPH reduction had occurred in the first thirty days. These results show that the mixing of the two tank-bottom sludges did not have any appreciable effect on the degradability of the TPH component.

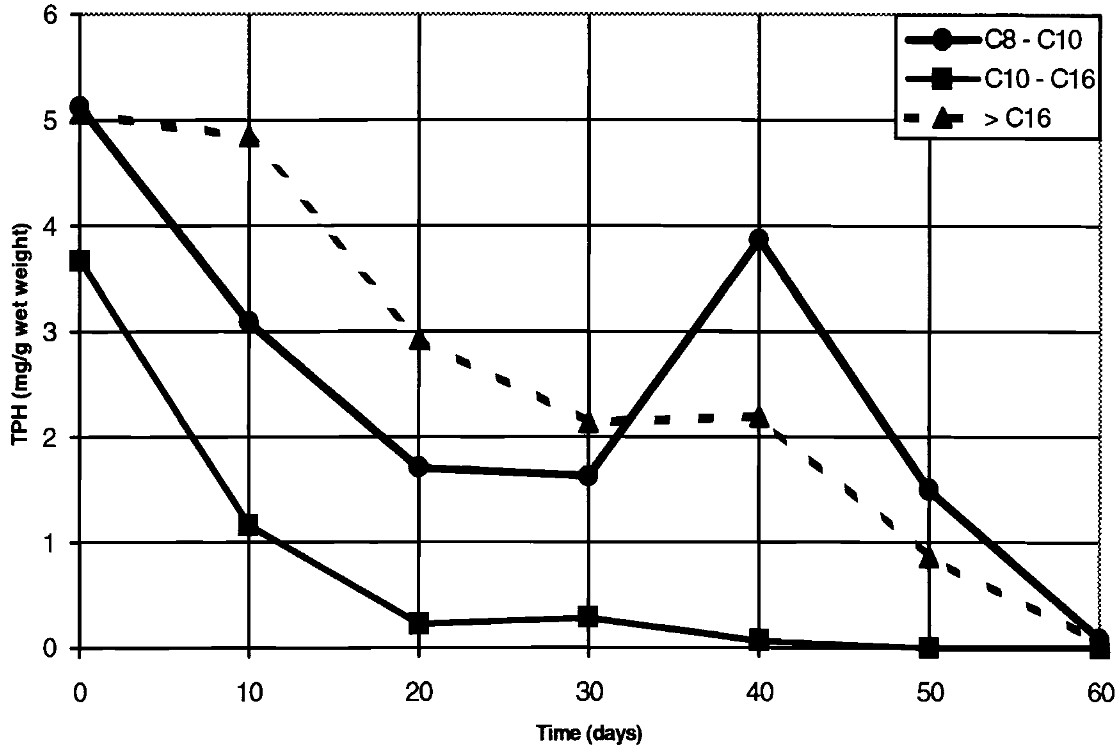
For the purpose of differentiating the various compounds in the TPH gas chromatogram on the basis of the number of carbon atoms, pure hydrocarbon compounds were injected into the GC, and their time of elution noted. The compounds used were trimethyl pentane, o-xylene, decahydronaphthalene, and n-hexadecane, containing 8, 8, 10 and 16 carbon atoms, respectively. Accordingly, it was determined that compounds containing 8 carbon atoms eluted between 5 and 10 minutes, those containing 10 carbon atoms eluted by 16 minutes, and those containing 16 carbon atoms eluted by 23 minutes. Based on this information, it was assumed that all compounds on the TPH chromatogram that eluted by 5 minutes contained less than 8 carbon atoms. Similarly, those that eluted between 5 and 16 minutes were assumed to contain 8 to 10 carbon atoms, and those that eluted between 16 and 23 minutes were assumed to contain 10 to 16 carbon atoms. All compounds that eluted after 23 minutes were assumed to contain more than 16 carbon atoms. Based on these, the gas chromatogram areas obtained for each sample were partitioned, and the TPH of the compounds in each group determined by apportioning the total TPH of the sample in the same proportions as the corresponding GC areas. The data used for this analysis are given in Appendix G.

Figure 5, Figure 6, and Figure 7 depict the variation in the TPH levels on the basis of the different hydrocarbon groups in the distillate, the gasoline, and the mixed tank-bottom reactors, respectively. From Figure 5 and Figure 7, it can be seen that 69% of the initial distillate tank-bottom reactor TPH and 54% of the initial mixed tank-bottom reactor TPH consisted of



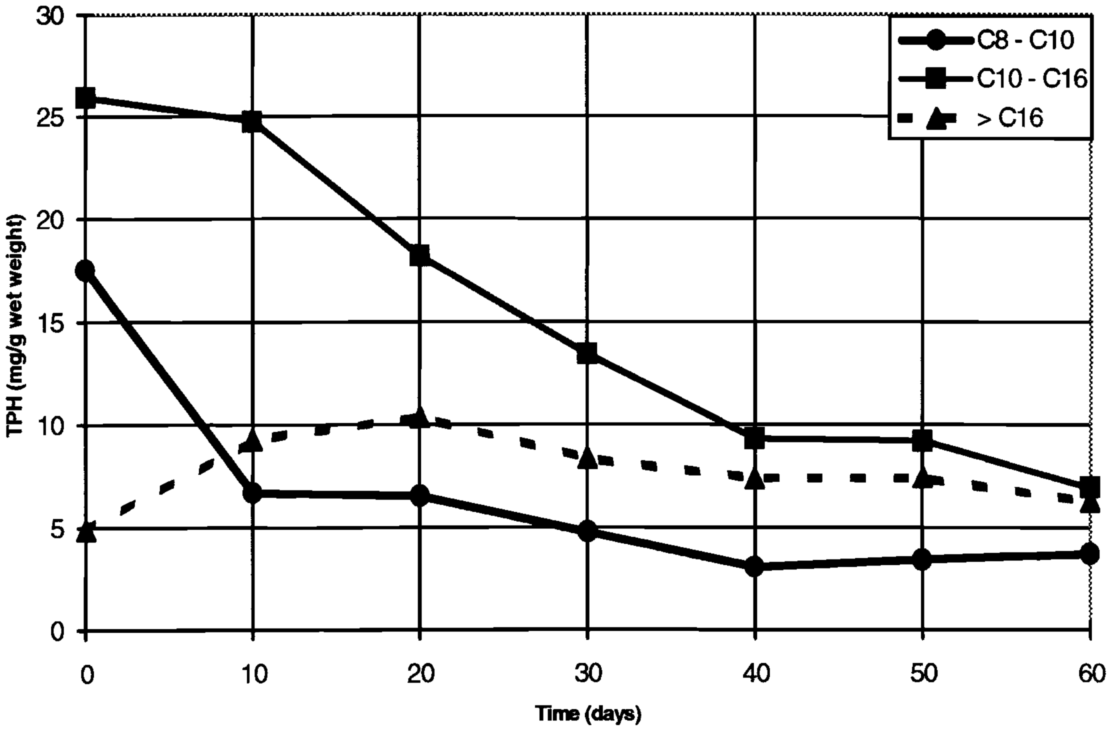
Note: Initial total petroleum hydrocarbon content of the reactor = 278.97 mg/g

Figure 5. Variation of Distillate Tank-Bottom TPH Levels with Time



Note: **Initial total petroleum hydrocarbon content of the reactor = 13.88 mg/g**

Figure 6. Variation of Gasoline Tank-Bottom TPH Levels with Time



Note: Initial total petroleum hydrocarbon content of the reactor = 48.39 mg/g

Figure 7. Variation of Mixed Tank-Bottom TPH Levels with Time

hydrocarbons with 10 to 16 carbon atoms. Similarly, it can be noted from Figure 6 that 37% of the initial gasoline tank-bottom reactor consisted of hydrocarbons with 8 to 10 carbons, and another 37% constituted of hydrocarbons with more than 16 carbon atoms. Although there were compounds eluting at less than 4 minutes, they could not be differentiated from the solvent, and hence it was assumed that none of the three reactors had any hydrocarbons containing less than 8 carbon atoms.

In all the three reactors in the first ten days, there was a sharp decrease in the TPH of hydrocarbons containing 8 to 10 carbon atoms. This decrease ranged from 62% in the mixed tank-bottom reactor to 52.5% in the distillate tank-bottom reactor, and 40% in the gasoline tank-bottom reactor. By the 20th day of operation, the level of hydrocarbons containing 8 to 10 carbon atoms had dropped by 67% in the gasoline tank-bottom reactor. These rapid decreases in the lower molecular weight hydrocarbons could be attributed to the volatilization and biodegradation of such compounds.

From Figures 5 and 7, it can be noted that the levels of the higher molecular weight hydrocarbons containing more than 16 carbon atoms increased from the initial level. In the case of the distillate tank-bottom reactor, this increase continued until the 40th day before starting to decline, whereas in the mixed tank-bottom reactor, the increase was observed only up to the 20th day. These increases might be due to the lower degradation and increased solubilization of the heavier hydrocarbons following the volatilization and biodegradation of the lighter compounds. At the end of the 60th day, the reduction in the amount of hydrocarbons containing more than 16 carbon atoms from the maximum level attained was 20% for the distillate tank-bottom reactor and 39 % for the mixed tank-bottom reactor. These low levels of degradation of the heavier hydrocarbons are probably due to the presence of more readily degradable lighter hydrocarbons. In the case of the gasoline tank-bottom reactor, due to the volatilization and biodegradation of the lower molecular weight compounds in the first ten days, the degradation of the hydrocarbons containing greater than 16 carbon atoms greatly increased after the 10th day. By the end of the operation period, nearly 99% of the heavier hydrocarbons had been degraded in the gasoline tank-bottom reactor. This is probably due to the low initial TPH values in the gasoline tank-bottom reactor.

As few studies done on the aerobic biodegradation of refined product tank-bottom sludges could be found, it was difficult to compare the results obtained above. The only study available made use of crude oil tank-bottoms [Brown *et al.*, 1992]. In that, it was reported that there was a 61% reduction in the reactor TPH levels in eight weeks of operation. This can be compared to the 65% TPH reduction obtained in sixty days in the mixed tank-bottom reactor, the contents of which are assumed the closest in composition to a crude oil tank-bottom sludge. Similarly, it was reported by Brown and coworkers [1992] that there was a 100, 98, 60, 53, and 30 percent removal of compounds containing less than 8 carbon atoms, 8 to 12 carbon atoms, 12 to 14 carbon atoms, 14 to 18 carbon atoms, and greater than 18 carbon atoms, respectively. In the present study, for the mixed tank-bottom sludge, 79 and 73 percent removal was obtained for compounds containing 8 to 10 carbon atoms, and 10 to 16 carbon atoms, respectively.

The volatile organic compounds that were liberated from the reactors due to aeration and mixing were captured in activated carbon columns for later quantification. The influent and effluent air streams to and from the carbon columns were monitored initially (first seven days) using a photo-ionization analyzer, to ensure that column breakthrough did not occur before all the volatiles had been adsorbed. From the plots of the analyzer readings for the three reactors that are given in Appendix B, it can be seen that a small fraction of the volatiles had escaped without being adsorbed by the carbon. This occurred mainly when the volatile concentration was maximum in the influent air to the carbon column, and must have occurred because the columns were not completely packed with carbon to their full capacity. An effort was made to correlate the analyzer readings to the TPH values obtained by GC analysis. This was done by injecting the analyzer calibration gas, isobutylene, into the GC to obtain the corresponding TPH value. However, due to the low concentration of the calibration gas, a proper response was not obtained from the GC.

The volatile organics that were adsorbed by the activated carbon were extracted using carbon disulfide, and the extract injected into the GC to quantify the amount captured by the activated carbon. Appendix H gives the data obtained by the GC analysis of the extracts. Based on the GC analyses of the extracts, it was determined that 301.89 mg of TPH was adsorbed by the activated carbon column attached to the distillate tank-bottom reactor. Similarly, 1363.07 mg of TPH was adsorbed by the activated carbon column attached to the gasoline tank-bottom reactor, and 6626.20 mg of TPH by the activated carbon column attached to the mixed tank-bottom reactor. Since these values were obtained using three different TPH standard curves, they cannot be compared with each other. As the activated carbon columns were disconnected from the respective reactors on the twentieth day of operation, an approximate mass balance of the TPH contents of each reactor on the twentieth day was done to determine the extent of volatilization and degradation of the lower molecular weight hydrocarbons. Appendix I gives the detailed mass balance calculations. Based on the mass balance, 99.8% of the hydrocarbons removed in the first twenty days from the distillate tank-bottom reactor was biodegraded, and 0.2% volatilized. Similarly, 93.7% of the hydrocarbons removed in the first twenty days from the gasoline tank-bottom reactor, and 78.6% of the hydrocarbons removed in the first twenty days from the mixed tank-bottom reactor, was biodegraded and 6.3% and 21.4% was volatilized, respectively. This indicates that the volatilization was comparatively higher in the mixed tank-bottom reactor.

Assuming that there had been no additional losses over the sixty days of operation, an approximate mass balance of the TPH contents of each reactor was done to determine the amount of hydrocarbons that was biologically degraded. The detailed mass balance calculations are given in Appendix J. Table 3 summarizes the results of the mass balance calculations. Based on the mass balance, it was found that 99.9% of the TPH that was removed from the distillate tank-bottom

reactor was biologically degraded. Similarly, 95.8% of the TPH that was removed from the gasoline tank-bottom reactor, and 90.9% of the TPH that was removed from the mixed tank-bottom reactor were biologically degraded. Assuming that all the TPH volatilized was captured and accurately measured, the mass balance results show that biodegradation was the predominant mechanism of removal of hydrocarbons in all the three reactors.

Table 3. Summary of Reactor TPH Mass Balance

	Distillate Tank-Bottom Reactor	Gasoline Tank-Bottom Reactor	Mixed Tank-Bottom Reactor
Initial TPH (mg)	625,800	32,850	111,550
Final TPH (mg)	307,650	300	38,550
TPH Removed (mg)	318,150	32,550	73,000
% of Initial TPH Removed	50.84	99.09	65.44
TPH Volatilized (mg)	300	1350	6650
% of Initial TPH Volatilized	0.05	4.11	5.96
TPH Biodegraded (mg)	317,850	31,200	66,350
% of Initial TPH Biodegraded	50.79	94.98	59.48
% of TPH removed that was Biodegraded	99.90	95.80	90.90

Solids, COD, and Oil & Grease Analysis

All the samples collected from the three reactors were analyzed for their total and volatile solids content. The analysis of each sample was done in triplicate, and the average of the three values reported. The data obtained from the solids analysis of the samples are given in Appendix K. The variation of the reactor total solids content during the course of the experiments is shown in Figure 8. As can be seen from the figure, in sixty days of operation, the total solids content reduced from 35% to 27.5% in the distillate tank-bottom reactor, from 27.3% to 21.7% in the gasoline tank-bottom reactor, and from 32.7% to 25.5% in the mixed tank-bottom reactor. In the case of the distillate tank-bottom reactor, 35% of the reduction in the total solids content occurred in the first thirty days, whereas in the case of the gasoline tank-bottom reactor nearly 80% of the reduction had taken place during the same period. In the mixed tank-bottom reactor, the reduction in the total solids content was more or less uniform during the course of the experiment, with 50% of the reduction occurring in the first thirty days. The overall reduction in the total solids content of all the three reactors were around 20%. As shown in the calculations in Appendix L, nearly 70% of the reductions in the total solids content in all the three reactors were the result of sampling. Clingage to the reactor sides, which was observed in all the three reactors, might account for a major portion of the remaining 30% reduction in the total solids content.

Figure 9 shows the variation of the reactor volatile solids content during the course of the experiments. As is evident from the figure, in sixty days of operation, the volatile solids contents were reduced from 17% to 12.3% in the distillate tank-bottom reactor, from 11.3% to 6.2% in the gasoline tank-bottom reactor, and from 14.9% to 9.8% in the mixed tank-bottom reactor. In the case of the distillate tank-bottom reactor, 90% of the reduction in the volatile solids content occurred in the first thirty days, whereas in the case of the gasoline tank-bottom reactor nearly 55% of the reduction had taken place during the same period. In the mixed tank-bottom reactor, 85% of the reduction in the volatile solids content occurred in the first thirty days. This reduction of the

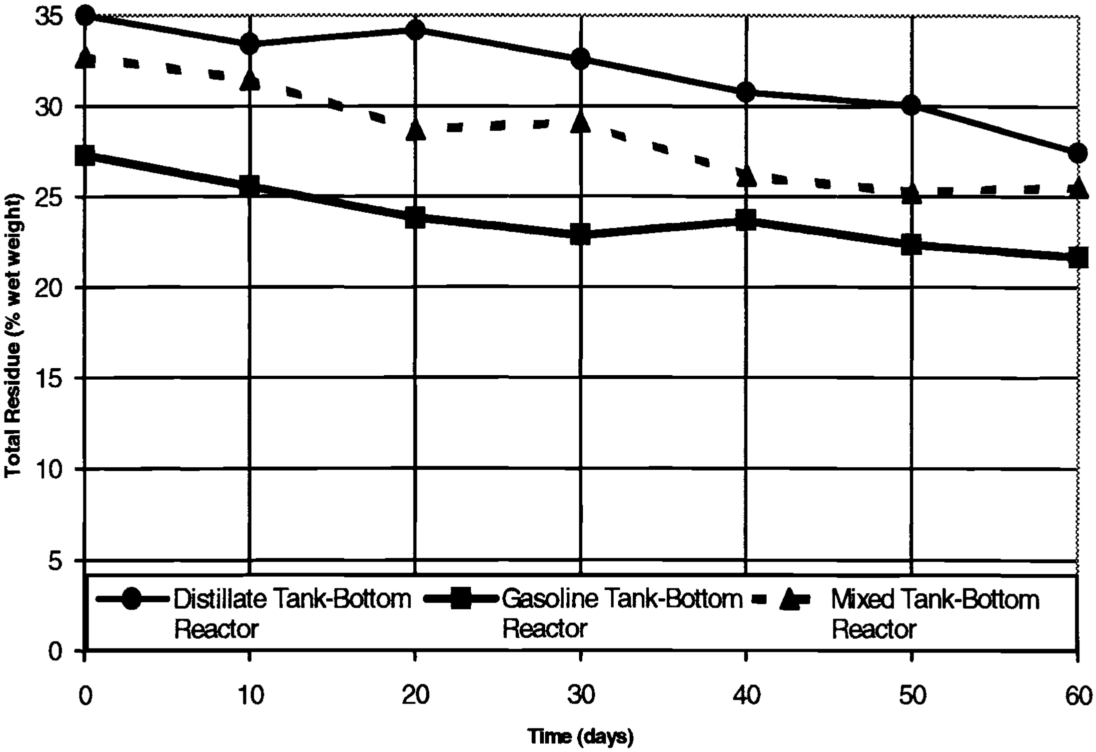


Figure 8. Variation of Reactor Total Solids Content with Time

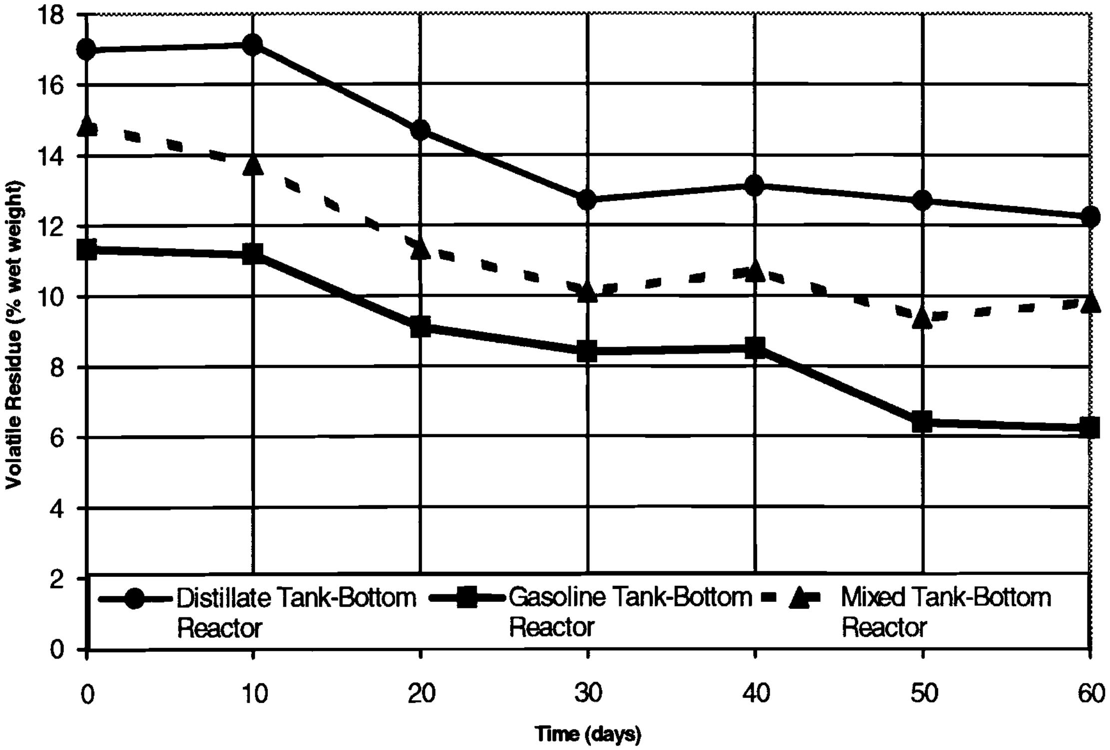


Figure 9. Variation of Reactor Volatile Solids Content with Time

volatile solids content is an indication of the reduction in the organic content of the reactor by biodegradation and to a lesser extent by volatilization.

The COD analysis of the samples was done in quadruplicate as described in Chapter III, and the mean of the four values was taken. Appendix M gives the data obtained from the COD analysis of the samples. The COD value of the initial sample from the mixed tank-bottom reactor should be midway between the COD values of the initial samples from the distillate and gasoline tank-bottom reactors. The value obtained was much higher than expected and this most probably must be due to the differences in the homogeneity of the raw sludges used in each reactor.

The variation of the COD of the reactor contents during the sixty-day operation is shown in Figure 10. In sixty days, the COD of the distillate tank-bottom reactor reduced from 562.6 mg/g to 238.4 mg/g. During the same period, the COD of the gasoline tank-bottom reactor reduced from 41.0 mg/g to 5.3 mg/g, and from 468.8 mg/g to 88.5 mg/g for the mixed tank-bottom reactor. In the case of the distillate tank-bottom reactor, 50% of the reduction in the COD levels occurred in the first ten days. Similarly, 68% of the reduction in the COD levels of the mixed tank-bottom reactor took place during the first ten days. In the case of the gasoline tank-bottom reactor, 66% of the reduction in the COD levels took place within the first twenty days of operation.

For determining the COD, the samples from the reactors were extracted with deionized water. Generally, lower molecular weight hydrocarbons are more soluble in water than heavier hydrocarbons. Hence, the COD determined using the extract, might be representative of the lighter hydrocarbons present in the samples. This is indicated by the fact that similar sharp decreases were observed in the TPH levels of hydrocarbons with 8 to 10 carbon atoms in all the reactors in the first ten to twenty days of operation. Hence, these increased rates of reduction in the COD levels of the reactors during the initial stages of operation can be attributed to the reduction in the organic

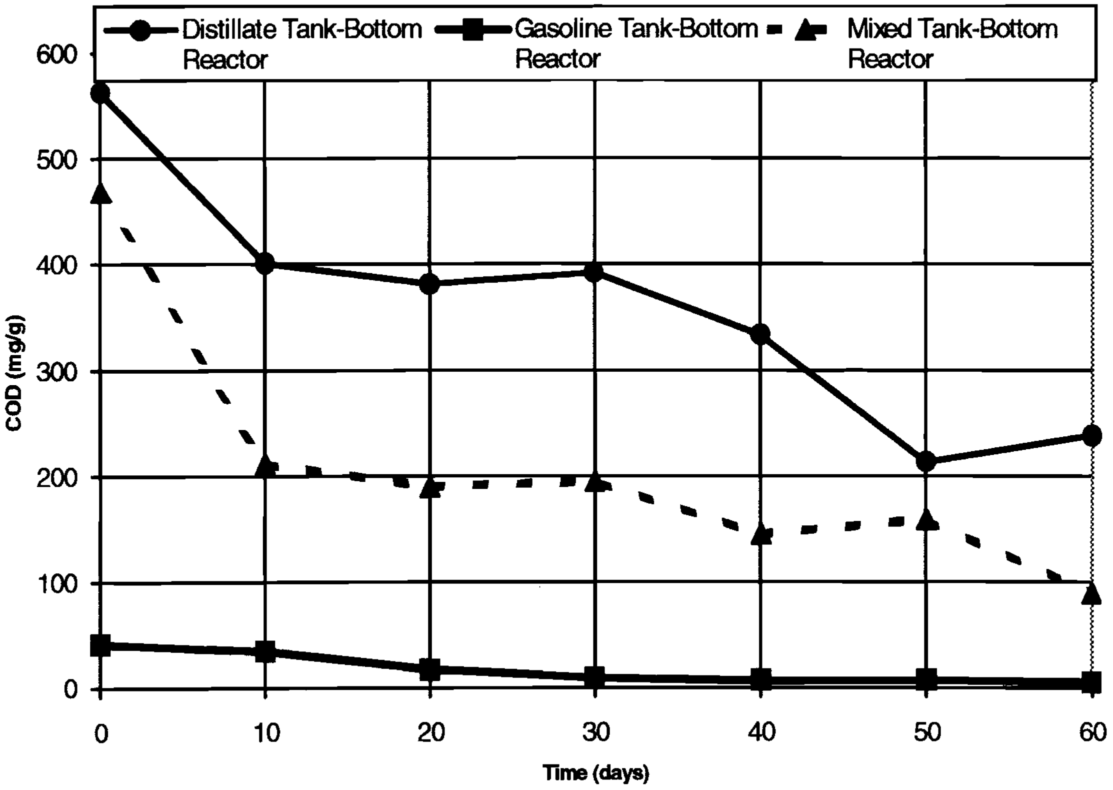


Figure 10. Variation of Reactor COD with Time

content due to the degradation and to a lesser extent to the volatilization of the lower molecular weight hydrocarbons. In the case of the distillate tank-bottom reactor, there was a further reduction of 48% in the COD level between the 30th and 50th day of operation, and must be an indication of increased microbial degradation of the organic contents.

"Oil and grease" is defined as any material recovered as a substance soluble in an organic solvent [APHA *et al.*, 1992]. It was used as another parameter to evaluate the extent of biodegradation in each of the reactors. All the samples from the reactors were analyzed for their oil and grease content, and the results obtained are graphically shown in Figure 11. From the figure it can be seen that in sixty days the oil and grease levels decreased from 25.4% to 14.3% in the distillate tank-bottom reactor, from 5.7% to 1.7% in the gasoline tank-bottom reactor, and from 19.7% to 9.2% in the mixed tank-bottom reactor. In the distillate tank-bottom reactor, 68% of the reduction in the oil and grease levels occurred in the first thirty days. Similarly, in the case of the gasoline tank-bottom reactor, 60% of the reduction in the oil and grease levels occurred in the first thirty days. In the case of the mixed tank-bottom reactor as much as 84% of the reduction in the oil and grease levels occurred within the first thirty days. Similar comparable reductions were observed in the TPH levels in all the three reactors during the first half of operation, and this may be an indication of first order hydrocarbon removal from the reactors.

The overall percentage reductions in the TPH, COD, and oil and grease levels in all the three reactors are summarized in Table 4. From the table it can be seen that percentage reduction in the oil and grease levels are lower than the corresponding percentage reductions in the TPH levels. Gasoline tank-bottom reactor, which had the highest amount of lighter hydrocarbons, showed the largest difference in the percentage levels, and distillate tank-bottom reactor, which had the lowest amount of lighter hydrocarbons, showed the smallest difference in the percentage levels. This can be attributed to the fact that certain lighter hydrocarbons that are detected by the TPH analysis may not be measured by the oil and grease analysis.

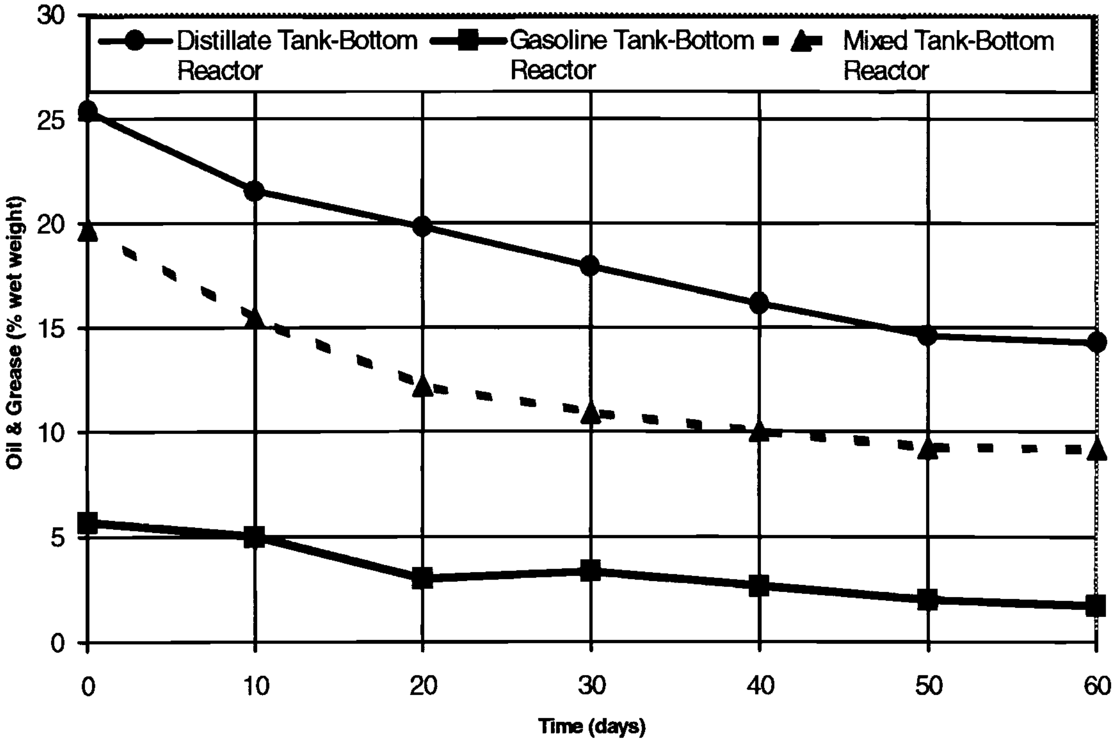


Figure 11. Variation of Reactor Oil and Grease Levels with Time

Analysis of Nutrients and Metals

The initial and final samples as well as the thirty-day samples from the reactors were analyzed for the Total Kjeldahl Nitrogen and phosphorous contents. Table 5 gives the results obtained from the nitrogen analysis of the samples, and Table 6 shows the results of the phosphorous analysis. Although the nutrient supplement was provided to maintain a nitrogen : phosphorous ratio of 10 : 1, the results obtained from the analysis of the initial samples show a nitrogen : phosphorous ratio ranging from 2.65 : 1 to 4.92 : 1. This difference might be either due to insufficient mixing of the reactor contents prior to the taking of the initial sample, and/or due to the erroneous results obtained in the preliminary waste characterization studies. As has been shown by the results, there was a gradual decrease in the nitrogen and phosphorous levels in all the three reactors. If there were no losses from the reactors, then there should not be any reduction in their nutrient levels. Hence the source of error must be either in the digestion procedure or in the sample collected. As only a single grab sample was collected from the reactors at each time, the source of the error could not be determined.

The initial, the thirty-day, and the final samples from each reactor were analyzed for the concentrations of three metals; cadmium, chromium, and lead. The results obtained from the cadmium, chromium, and lead analyses are shown in Table 7, Table 8, and Table 9, respectively. The atomic adsorption spectrophotometer had a detection limit of 15 µg/L for cadmium. The values obtained for the cadmium levels in all the samples, as given in Table 7, are very close to this value and cannot be considered to be accurate. Hence, it is clear that the cadmium levels in all the three reactors were negligible.

Considering the fact that the sludges were diluted with seed, the initial levels of chromium and lead in all the reactors were consistent with the results obtained from the preliminary sludge characterization analyses. At the end of the sixty-day operation period, there was a 23% reduction

Table 4. Overall Percentage Reduction in Reactor TPH, COD, and O & G Levels

Parameter	Distillate Tank-Bottom Reactor	Gasoline Tank-Bottom Reactor	Mixed Tank-Bottom Reactor
TPH	50	99	65
COD	58	87	81
Oil and Grease	44	70	53

Table 5. Total Kjeldahl Nitrogen Levels (mg N/g of wet sludge) in the Reactors

Time (days)	Distillate Tank-Bottom Reactor	Gasoline Tank- Bottom Reactor	Mixed Tank-Bottom Reactor
0	4.73 mg/g	6.69 mg/g	6.19 mg/g
30	4.32 mg/g	5.18 mg/g	5.15 mg/g
60	2.96 mg/g	3.32 mg/g	3.60 mg/g

Table 6. Phosphorous Levels (mg PO_4 - P/g of wet sludge) in the Reactors

Time (days)	Distillate Tank-Bottom Reactor	Gasoline Tank- Bottom Reactor	Mixed Tank-Bottom Reactor
0	1.78 mg/g	1.36 mg/g	1.62 mg/g
30	0.95 mg/g	1.09 mg/g	1.13 mg/g
60	0.79 mg/g	0.64 mg/g	0.88 mg/g

Table 7. Cadmium Levels (mg Cd/g of wet sludge) in the Reactors

Time (days)	Distillate Tank-Bottom Reactor	Gasoline Tank- Bottom Reactor	Mixed Tank-Bottom Reactor
0	0.003 mg/g	0.004 mg/g	0.003 mg/g
30	0.003 mg/g	0.004 mg/g	0.004 mg/g
60	0.003 mg/g	0.003 mg/g	0.001 mg/g

Note: The above values are very close to the AA detection limits for cadmium, and hence cannot be taken as accurate

Table 8. Chromium Levels (mg Cr/g of wet sludge) in the Reactors

Time (days)	Distillate Tank-Bottom Reactor	Gasoline Tank- Bottom Reactor	Mixed Tank-Bottom Reactor
0	0.086 mg/g	0.264 mg/g	0.230 mg/g
30	0.070 mg/g	0.248 mg/g	0.185 mg/g
60	0.066 mg/g	0.225 mg/g	0.168 mg/g

Table 9. Lead Levels (mg Pb/g of wet sludge) in the Reactors

Time (days)	Distillate Tank-Bottom Reactor	Gasoline Tank- Bottom Reactor	Mixed Tank-Bottom Reactor
0	0.152 mg/g	0.400 mg/g (0.533 mg/g)*	0.350 mg/g
30	0.123 mg/g	0.292 mg/g	0.267 mg/g
60	0.132 mg/g	0.159 mg/g (0.169 mg/g)*	0.165 mg/g

*** Values obtained by Bates Lab, Sand Springs, OK.**

in the chromium concentration of the distillate tank-bottom reactor, a 15% reduction in the gasoline tank-bottom reactor, and a 27% reduction in the mixed tank-bottom reactor. Similarly, during the same period, the lead levels dropped by 13% in the distillate tank-bottom reactor, by 60% in the gasoline tank-bottom reactor, and by 53% in the mixed tank-bottom reactor.

Metals cannot be degraded. Hence, the reductions in the lead and chromium content of all the three reactors must be mainly due to some sort of experimental error. To verify if the error had occurred while analyzing using the AA, two of the digested samples were analyzed by an external laboratory (Bates Lab, Sand Springs, OK), and similar results were obtained. Mass balances of the chromium, and lead content of each reactor was carried out to discount dilution effects. The detailed mass balance calculations are given in Appendix N. As can be seen from the mass balance calculations, the elimination of the dilution effect did not significantly change the percentage reduction in the chromium and lead contents of all the three reactors. The source of error must be either in the digestion procedure or in the sample collected. As only a single grab sample was collected from the reactors at each time, the source of the error could not be determined.

MPN and Toxicity Analysis

The estimation of the number of hydrocarbon utilizing microorganisms in each reactor was carried out using a five-tube Most Probable Number technique. Appendix O gives the data, and Figure 12 shows the results, obtained for the three reactors during the sixty-day period of operation. In all the three reactors, after an initial lag phase, the number of microorganisms peaked at 30 to 40 days from start of operations, and then declined. No clear correlation could be found between the TPH removal and the growth of microorganisms in the reactors.

The toxicity of the samples was determined using the Microtox Basic test procedure. In the procedure, the toxicity of the sample is expressed as an effective concentration of the sample causing

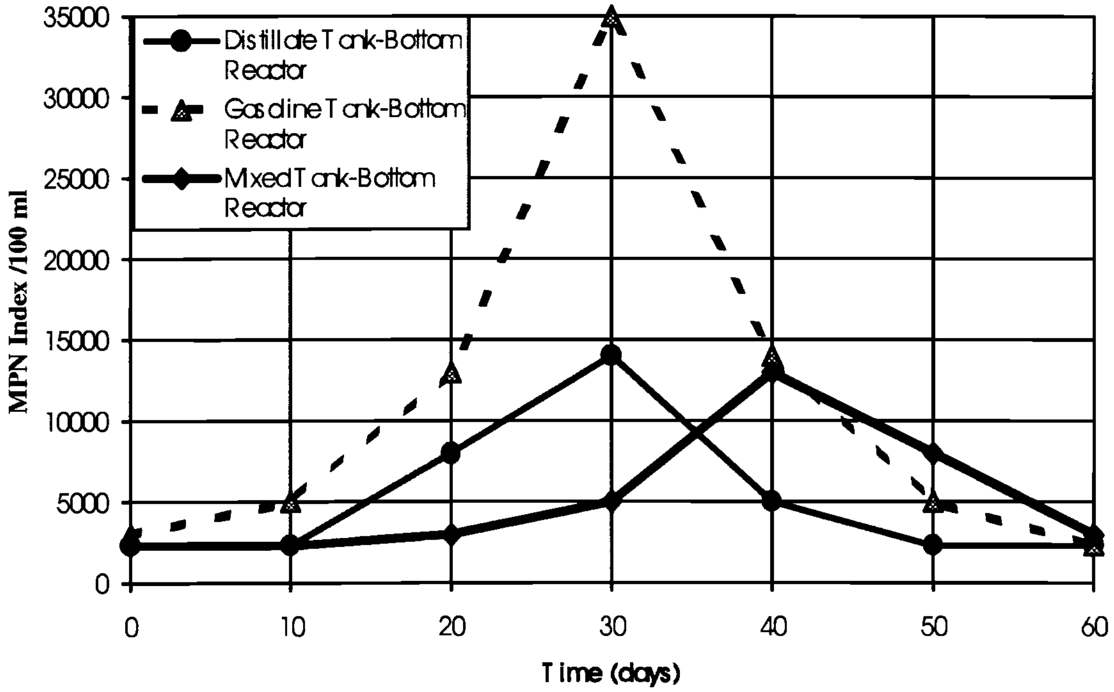


Figure 12. Variation of Reactor MPN Index with Time

a 50% decrease (EC50) in the Microtox Reagent light output under defined conditions of exposure time and test temperature. The smaller the EC50 value, the more toxic the sample. As the samples were extracted with an acetic acid solution, the pH of the extract was around 3. To eliminate the toxic effects of such low pH, the extracts were neutralized to pH 7 with sodium hydroxide solution. The extraction solvent also was neutralized, and its toxicity was checked to find if there was any background toxicity in the sample extracts. The neutralized solvent did not exhibit any toxicity.This was also confirmed by carrying out the test with a sodium acetate solution of equivalent concentration. A phenol standard (reference toxicant) was also run along with the other tests, and the toxicity value obtained for it (27.24% and 25.51% dilution) was compared with reported values. Phenol ordinarily exhibits an EC50 between 13 to 30% dilution, and a value within this range was obtained from the Microtox test. Appendix P gives the data from the toxicity experiments, and Figure 13 shows the variation of the EC50 values for the samples from the three reactors. From the figure, it can be seen that both in the distillate and mixed tank-bottom reactors there was practically no change in the toxicity levels and this might be due to the continued presence of the heavier hydrocarbons. In the case of the gasoline tank-bottom reactor, there was a noticeable decrease in the toxicity levels (increase in EC50 values). It cannot be said for sure whether the reactor contents would still be considered toxic under the TCLP rule.

Analysis of Degradation Kinetics

The data obtained from the TPH analyses of the samples from the reactors were used to determine the rate of degradation. As the plot of TPH against time was not a straight line for any of the reactors, it was evident that the TPH degradation was not a zero order reaction. Generally, microbial degradation processes have been found to follow first order kinetics. The rate expression for a first order reaction is:

$$\frac{dC}{dt} = -k[C] \qquad (1)$$

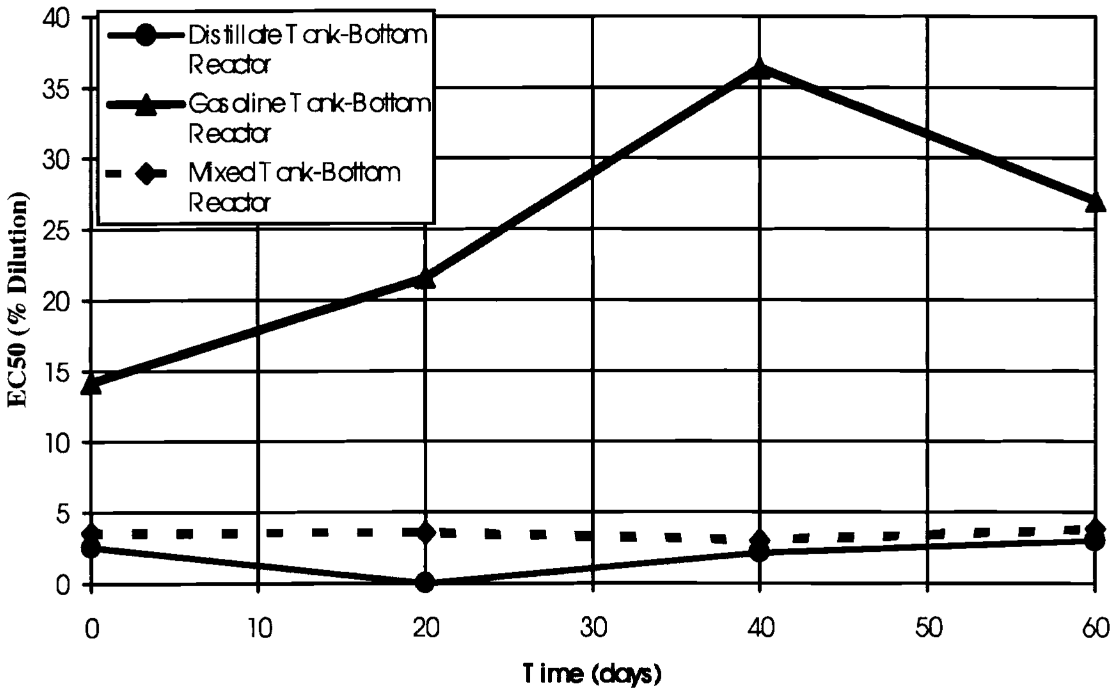


Figure 13. Variation of Reactor Toxicity Levels with Time

where 'C' is the concentration at time 't', and 'k' is the first-order rate constant. Rearranging and integrating yields:

$$\ln(\frac{C}{C_o}) = -kt \qquad (2)$$

where 'C_0' is the concentration at t = 0. Hence, if the plot of 'ln C' against 't' is a straight line, the reaction is first order, and the slope of the line gives the reaction rate constant.

To determine if the TPH degradation follows first order kinetics, 'ln (TPH)' was plotted against the corresponding 't'. Figure 14, Figure 15, and Figure 16, gives the first-order plot for distillate, gasoline, and mixed tank-bottom reactor. From the figures it can be seen that the TPH degradation in the distillate and gasoline tank-bottom reactors are not best fit by first-order reactions (probably due to previously mentioned scatter in the data). However, for the purpose of comparison of rates, they were taken to be of first-order. Based on the plots, the first-order rate constants for the distillate, gasoline, and mixed tank-bottom reactors are 0.00835 day^{-1}, 0.05856 day^{-1}, and 0.01834 day^{-1}, respectively. This corresponds to half lives of 83 days, 11.8 days, and 37.8 days for the distillate, gasoline, and mixed tank-bottom reactors, respectively. This shows that the mixing of the two sludges does not have any significant effect on the degradation rate

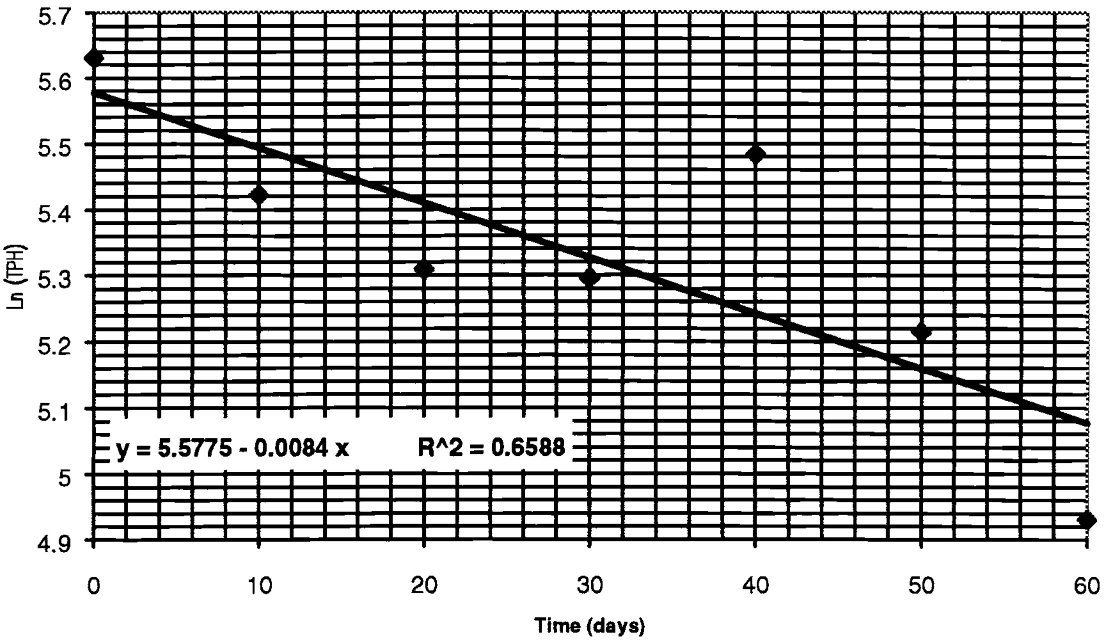


Figure 14. First-Order Plot for TPH Removal in Distillate Tank-Bottom Reactor

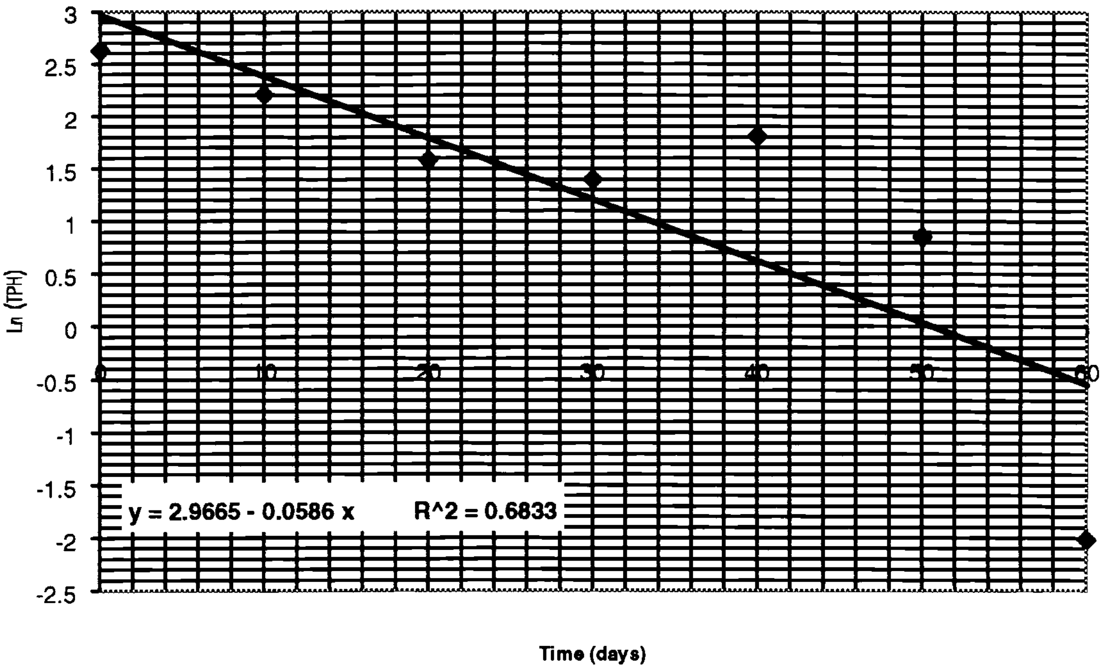


Figure 15. First-Order Plot for TPH Removal in Gasoline Tank- Bottom Reactor

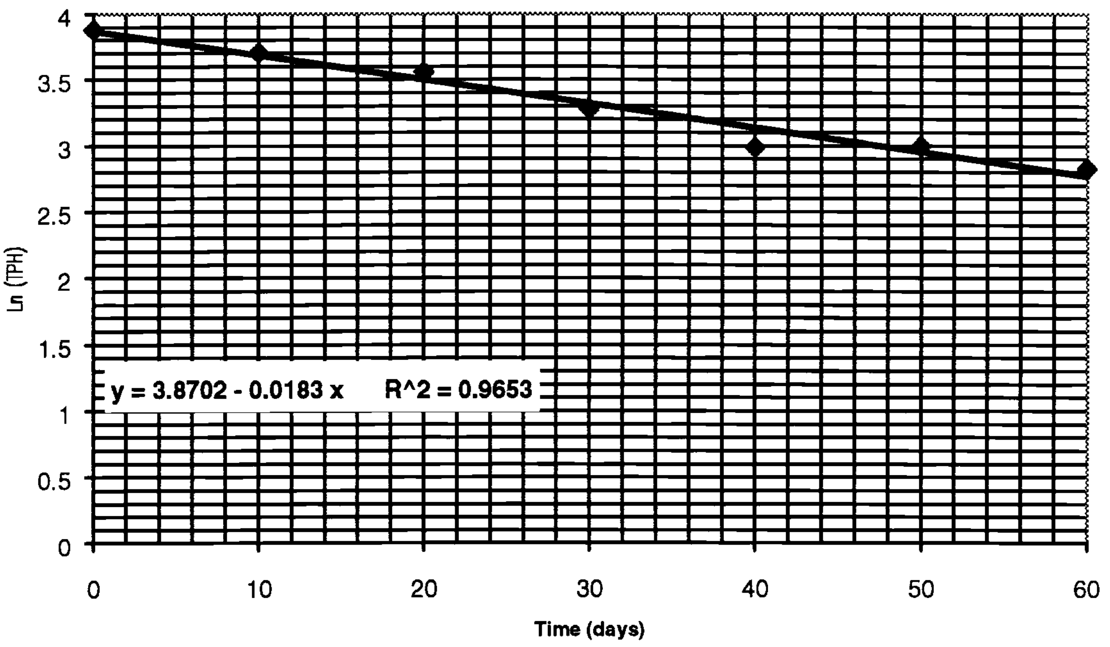


Figure 16. First-Order Plot for TPH Removal in Mixed Tank-Bottom Reactor

CHAPTER V

CONCLUSIONS AND RECOMMENDATIONS

The purpose of this study was to investigate if the biodegradation in aerobic batch reactors was an effective method for the treatment of tank-bottom sludges. In this regard, two different types of sludges were utilized to study their degradability. A mixture of the two sludges was also evaluated to determine whether the combination enhanced the degradability of the individual components. The degradation was monitored using certain gross parameters such as Total Petroleum Hydrocarbons, Chemical Oxygen Demand, and Oil and Grease. In addition, certain other factors such as the metals content and the toxicity levels, which could adversely affect the disposal of the treated sludge, were also studied.

Conclusions

The following conclusions can be drawn from the results of this study.

1. Based on the TPH analysis, tank-bottom sludge degradation of 50% in the distillate tank-bottom reactor, 65% in the mixed tank-bottom reactor, and 99% in the gasoline tank-bottom reactor was observed. Similar results were obtained by the COD and Oil & Grease analyses. The degradability and the extent of degradation of the tank-bottom sludge were dependent on the nature of the stored product in the tank from which the sludge was obtained. The degradability and the rate of degradation decrease as the percentage of heavier hydrocarbons present in the sludge increases.

2. TPH analysis showed that from 0.1 to 9.1% of the hydrocarbons that were removed, was

volatilized. Hence, volatilization was not the major mechanism of removal of hydrocarbons from the tank-bottom sludges tested. Biodegradation was dominant in all the three reactors.

3. The half lives of TPH removal in the reactors showed that the mixing of two different tank-bottom sludges did not significantly affect the degradation rates.

4. Results of the Microtox test showed that the toxicity level of the sludge does not change if, after treatment, the sludge still contains a significant amount of hydrocarbons. As in the case of gasoline tank-bottom reactor, when the hydrocarbons were removed significantly, the toxicity level of the sludge decreased.

The results obtained from the study show that the use of aerobic batch reactors may be a feasible and effective alternative for the reduction of organic content in tank-bottom sludges. Further work is needed to optimize the reactor performance, and to determine the economical viability of full-scale operations.

Recommendations

1. The experiments need to be conducted in a reactor of larger capacity so that multiple samples can be taken, thereby reducing the effect of sampling and experimental errors on the results.

2. The capture and quantification of the volatile organics from the reactor need to be modified so that the amount of volatiles captured can be properly correlated to the amount of volatiles liberated.

3. More experiments need to be done to optimize the operating parameters of the reactors such as the organic loading, nutrient levels, mixing and aeration requirements, etc.

4. Studies using proprietary bacterial strains and surfactants are also suggested to see if they can enhance the rate of biodegradation.

REFERENCES

Abrishamian, R., Kabrick, R., and Swett, G., "Two On Site Treatment Methods Reduce Sludge Waste Quantities." *Oil & Gas Journal,* November 2, 1992. pp. 51-56.

American Public Health Association., American Water Works Association., *Standard Methods for the Examination of Water and Wastewater,* 18th Edition, 1992.

Atlas, R., "Microbial Degradation of Petroleum Hydrocarbons: an Environmental Perspective." *Microbiological Reviews,* Vol. 45, 1981. pp. 180-209.

Banat, I.M., Samarah, N., Murad, M., Horne, R., and Banerjee, S., "Biosurfactant Production and Use in Oil Tank Clean-up." *World Journal of Microbiology and Biotechnology,* Vol. 7 (1), 1991.

Bartha, R., "Biotechnology of Petroleum Pollutant Biodegradation." *Microbial Ecology,* Vol. 12, 1986. pp. 155-172.

Brown, K.L., Rightmyer, J.N., and Leavitt, M.E., "Bioremediation of Waste Products from Petroleum Storage Facilities." *Proceedings of the Center for Environmental Research and Technology Symposium: Treatment and Disposal of Petroleum Sludges.,* University of Tulsa, 1991. pp. 83-93.

Brown, K.L., Haught, R., Hershey, D., Rightmyer, J.N., Lang, C., and Sansverino, J., "Biodegradation of Crude Oil Sludges." *Proceedings of the IT Corporation Technical Symposium,* Scottsdale, Arizona, 1992. pp. 1-14.

Bryant, J.S., and Moores, C.W., "Disposal of Hazardous Wastes from Petroleum Refineries." *45th Purdue Industrial Waste Conference Proceedings.,* Lewis Publishers, 1991. pp. 703-709.

Castaldi, F.J., and Ford, D.L., "Slurry Bioremediation of Petrochemical Waste Sludges." *Water Science & Technology,* Vol. 25 (3), 1992. pp. 207-212.

Conner, J.R., *Chemical Fixation and Solidification of Hazardous Wastes.,* Van Nostrand Reinhold, 1990.

Coover, M.P., Kabrick, R.M., Stroo, H.F., and Sherman, D.F., "In Situ Liquid/Solids Treatment of Petroleum Impoundment Sludges: Engineering Aspects and Field Applications." *Bioremediation - Field Experience.,* Lewis Publishers, 1994. pp. 197-224.

Davis, G.B., Goss, M.L., Schoemann P., and Tyler, S.L., "Crude Oil Tank Cleaning Process Recovers Oil, Reduces Hazardous Wastes." *Oil & Gas Journal,* December 13, 1993. pp. 35-39.

Dibble, J., and Bartha, R., "Effect of Environmental Parameters on the Biodegradation of Oil Sludge." *Applied and Environmental Microbiology,* Vol. 37, 1979. pp. 729-739.

Emery, G.E., "Tank-Bottoms Reclamation Unit Upgraded to Meet Stricter Rules." *Oil & Gas Journal,* April 12, 1993. pp. 40-66.

Engelder, C.L., Grieves C.G., and Weber J.M., "Liquid/Solids Biotreatment of Pressure Filtered Refinery Waste Sludge." *44th Purdue Industrial Waste Conference Proceedings.,* Lewis Publishers, 1990. pp. 703-709.

Field, S., Marks, R., and Wojtanowicz, A., "Advance Biological Treatment and Separation of Hazardous Constituents from Petrochemical Sludges." *Journal of Hazardous Materials,* Vol. 28, 1991. pp. 101-114.

Fyock, O.L., Nordrum, S.B., Fogel, Samuel., and Findlay, Margaret., "Pilot Scale Composting of Petroleum Production Sludges." *Proceedings of the Center for Environmental Research and Technology Symposium: Treatment and Disposal of Petroleum Sludges.*, University of Tulsa, 1991. pp. 21-32.

Grady, C.P.L., "Biodegradation: Its Measurement and Microbiological Basis." *Biotechnology and Bioengineering,* Vol. 27, 1985. pp. 660-674.

Hach Company, Loveland, CO. *Water Analysis Handbook,* 1989.

Hahn, W.J., and Loehr, R.C., "Biological Treatment of Petroleum Oily sludges." *Proceedings of Permian Basin Oil & Gas Recovery Conference,* Midland, Texas, 1992. pp. 519-530.

Hahn, W.J., "High-Temperature Reprocessing of Petroleum Oily Sludges." *SPE Production and Facilities,* August 1994. pp. 179-182.

Kennedy, J., "Hazardous Substances from Tank Bottom Dump Sites Considered No component of Petroleum." *American Oil & Gas Report,* October 1993. pp. 123.

Leahy, J., and Colwell, R., "Microbial Degradation of Hydrocarbons in the Environment." *Microbiological Reviews,* Vol. 54, 1990, pp. 305-315.

Microbics Corporation, Carlsbad, CA. *Microtox Operation Manual,* 1992.

Oolman, T., Castaldi, C.J., Behrens, G.P., and Owen, M.L., "Biotreat Oily Refinery Wastes." *Hydrocarbon Processing,* August 1992. pp. 67-69.

Perry, J.J., "Microbial Cooxidations Involving Hydrocarbons." *Microbiological Reviews,* Vol. 43, 1979. pp. 59-72.

Pierce, D.E., "The Law of Petroleum Sludges & Slop Oils." *Proceedings of the Center for Environmental Research and Technology Symposium: Treatment and Disposal of Petroleum Sludges.*, University of Tulsa, 1991. pp. 2-20.

Prince, M., and Sambasivam, Y., "Bioremediation of Petroleum Wastes from the Refining of Lubricant Oils." *Environmental Progress,* Vol. 12 (1), February 1993. pp. 5-11.

Rasmussen, G.P., "New Desorption Process Treats Refinery K and F Wastes in Demo Trial." *Oil & Gas Journal,* January 10, 1994. pp. 48-51.

Riser-Roberts, E., *Bioremediation of Petroleum Contaminated Sites,* C. K. Smoley, 1992.

Rittmann, B.E., "Aerobic Biodegradation of Petroleum Hydrocarbons." *Proceedings of the International Petroleum Environmental Conference,* Houston, Texas, 1994.

Salameh, M.F., and Kabrick, R.M., "Treatment of Petroleum Oily Sludges in Liquid/Solids Contact Reactors - Results of Batch Testing." *46th Purdue Industrial Waste Conference Proceedings.,* Lewis Publishers, 1992. pp. 55-64.

Sieck, J.F., "Applicability of Composting Techniques to Enhance the Bioremediation of Soils Contaminated with Refined Petroleum Products." *Masters Thesis,* Oklahoma State University, 1993. pp. 101.

Sisler, F., and Zobell, C., "Microbial Utilization of Carcinogenic Hydrocarbons." *Science,* Vol. 106, 1947. pp. 521-522.

Song, H.G., and Bartha, R., "Effects of Jet Fuel Spills on Microbial Community of Soil." *Applied and Environmental Microbiology,* March 1990. pp. 646-651.

Spitzer, W.G., "Treatment Options for Crude Oil Storage Tank Bottoms." *Creative Component Report.,* Oklahoma State University, Unpublished.

Stroo, H.F., "Biological Treatment of Petroleum Sludges in Liquid/Solids Contact Reactors." *Environmental Waste Management World,* Vol. 3, 1989. pp. 9-12.

U.S. EPA, *Test Methods for Evaluating Solid Waste: Physical/Chemical Methods,* September 1986.

U.S. EPA, "Slurry Biodegradation." *Engineering Bulletin,* September 1990. pp. 1-8.

Vail, L.R., "Refiner Biodegrades Separator-type Sludge to BDAT Standards." *Oil & Gas Journal,* November 11, 1991. pp. 53-57.

APPENDIX A

Sample Calculation of the Nutrient Requirements for the Distillate Tank-Bottom Reactor

Density of distillate tank-bottom sludge = 1143.27 g/l

Volume of distillate tank-bottom sludge in the reactor = 1 liter

∴ Mass of sludge in the reactor = 1143.27 g

TPH content of the distillate tank-bottom sludge =0.0982 g/g

∴ Total TPH content of the sludge in the reactor = 1143.27 x 0.0982 = 112.27 g

Assuming that distillate has the same composition as diesel fuel, and contains 0.856 g of carbon per gram of distillate,

∴ Total carbon content of the sludge in the reactor = 112.27 x 0.856 = 96.1 g

∴ Total nitrogen requirement to maintain a C : N ratio of 10 : 1 = 96.1/10 = 9.61 g

Nitrogen content of the distillate tank-bottom sludge = 0.0022 g/g

∴ Total Nitrogen content of the sludge in the reactor = 1143.27 x 0.0022 = 2.52 g

∴ Nitrogen supplement required for the reactor = 9.61 - 2.52 = 7.09 g

∴ Total phosphorous requirement to maintain a C : P ratio of 100 : 1 = 96.1/100 = 0.96 g

Phosphorous content of the distillate tank-bottom sludge =0 g/g

∴ Phosphorous supplement required for the reactor = 0.96 g

APPENDIX B

Concentration Plots of Volatile Organic Emissions from the Reactors

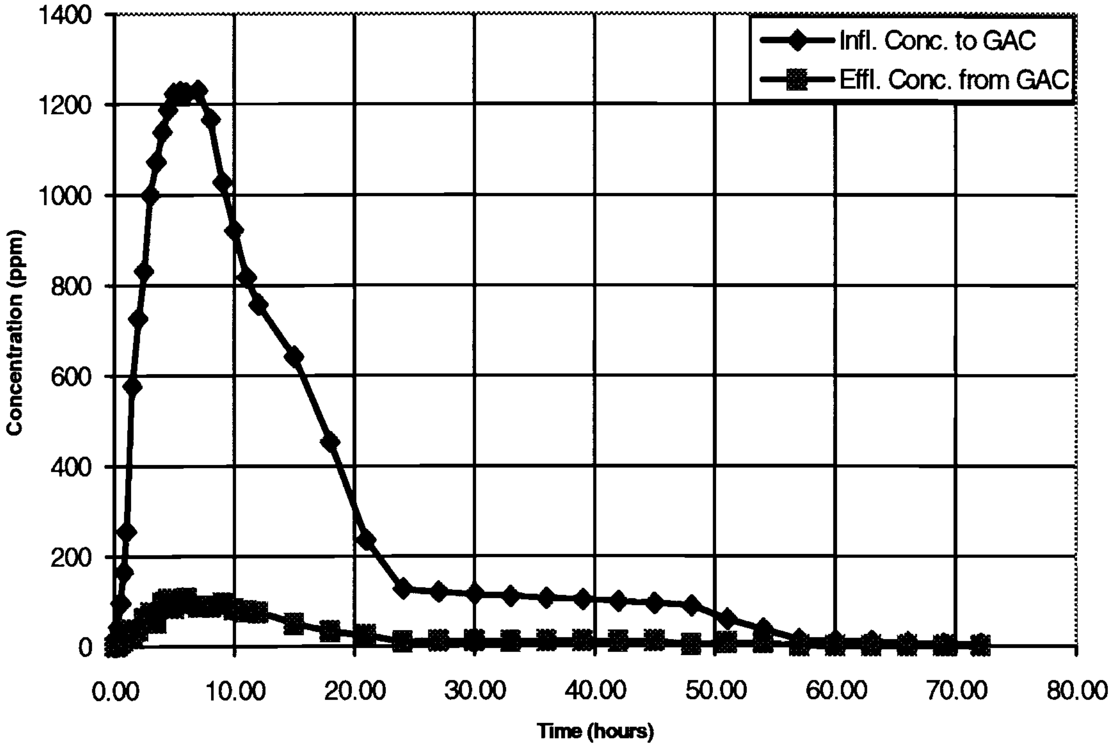


Figure 17. Concentration Plots of Volatile Organic Emissions from the Distillate Tank-Bottom Reactor

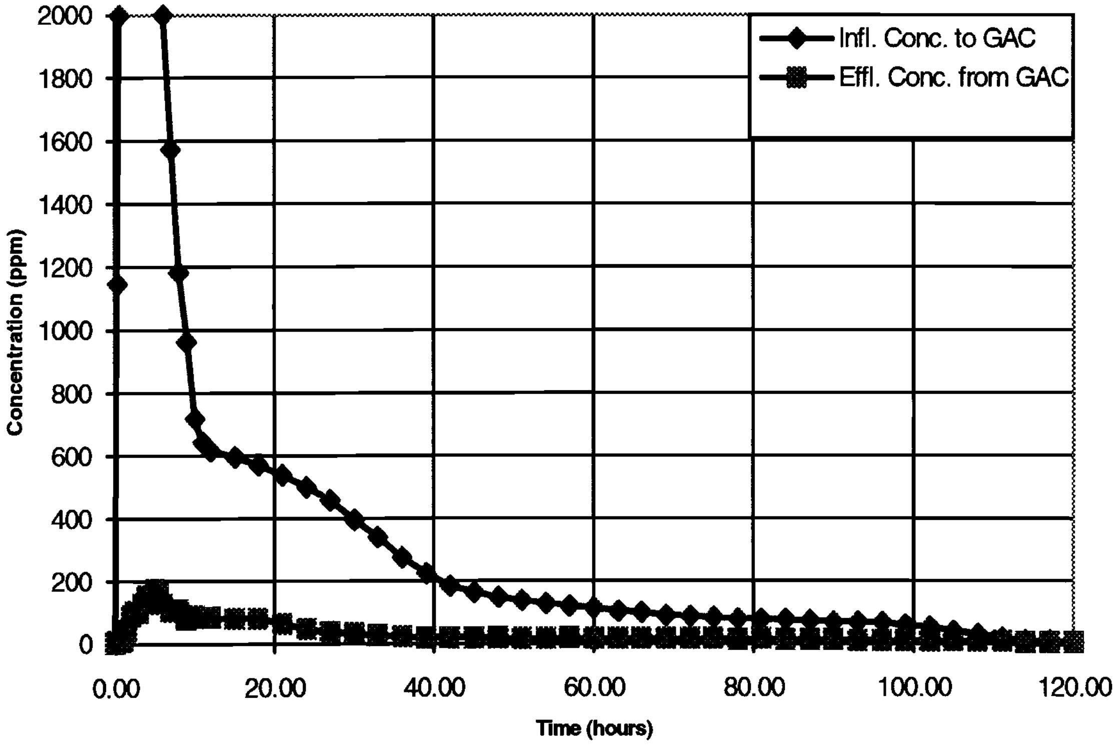


Figure 18. Concentration Plots of Volatile Organic Emissions from the Gasoline Tank-Bottom Reactor

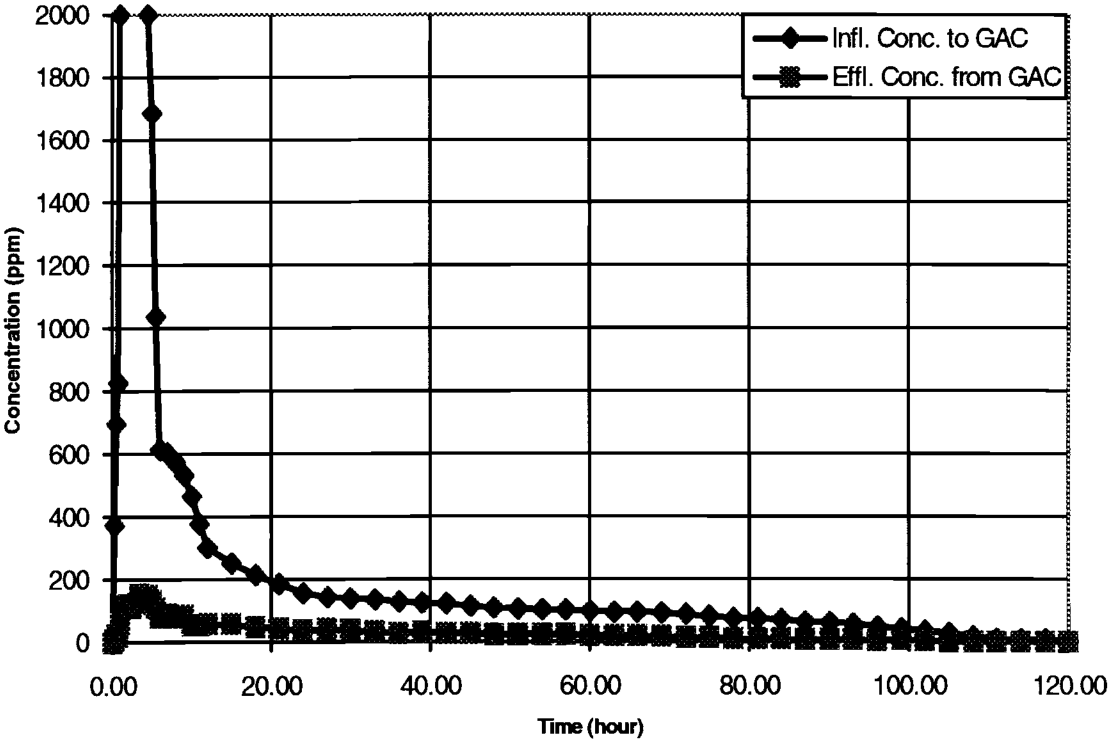
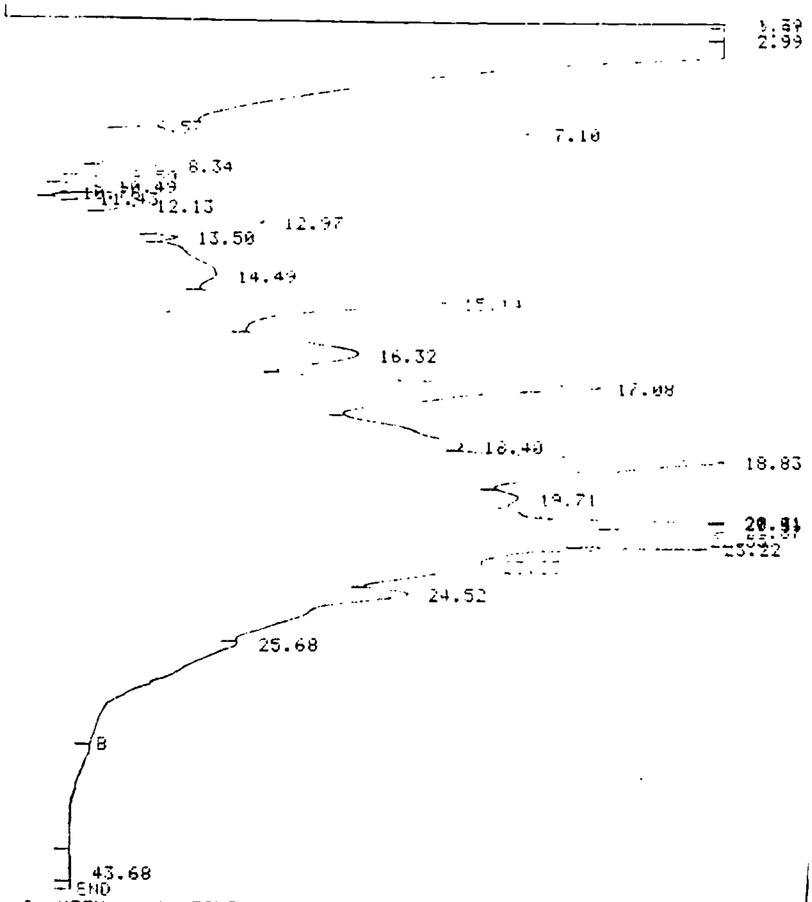


Figure 19. Concentration Plots of Volatile Organic Emissions from the Mixed Tank-Bottom Reactor

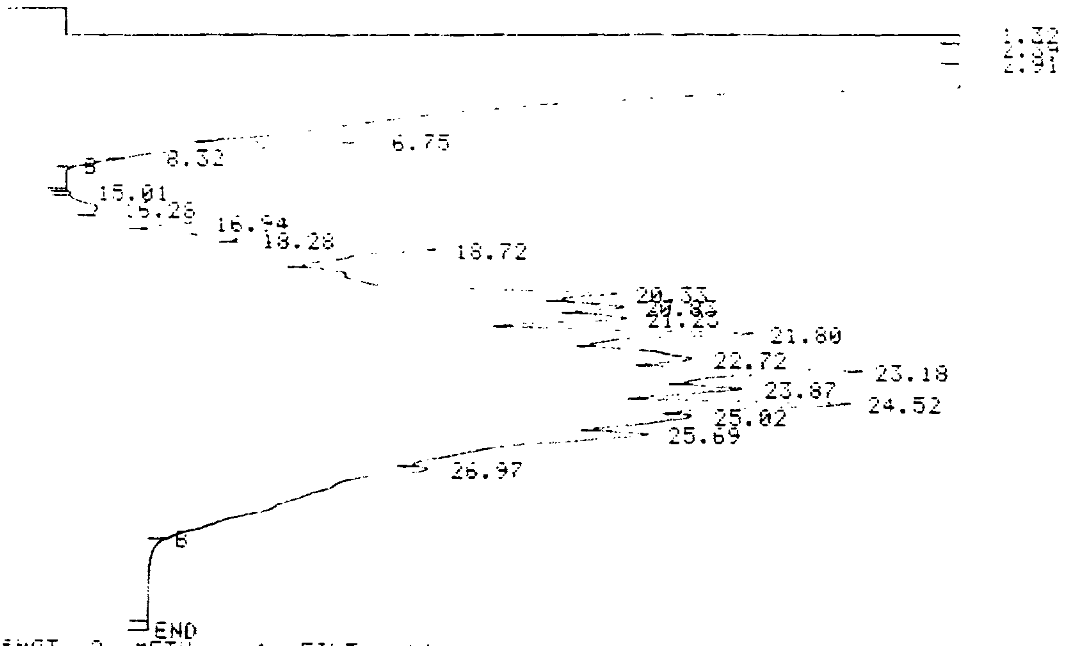
APPENDIX C

Typical Gas Chromatograms for the Initial and Final Samples from the Reactors



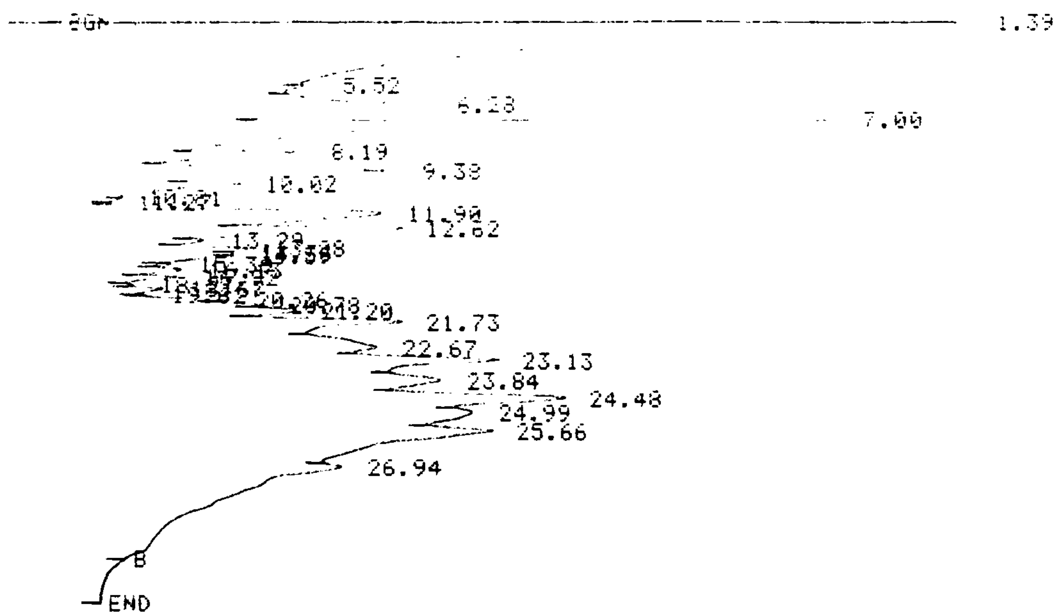
```
INST  2  METH  2 1  FILE  0 9  0 /  0
SENSITIVITIES    100   5
   TIME        AREA  BC     RRT       RF           C   NAME
   1.39  1540.7513 T     0.139    1.000      35.4477 !
   2.51  1189.6012 T     0.251    1.000      27.3689 !
   2.99   658.0134 T     0.299    1.000      14.6786 !
   6.57     4.7220 T     0.657    1.000       0.1086 !
   7.10    43.0515 T     0.710    1.000       0.9905 !
   8.34    12.2272 T     0.834    1.000       0.2813 !
   9.50     6.9110 T     0.950    1.000       0.1590 !
  10.49     5.3401 T     1.049    1.000       0.1229 !
  10.76     0.9860 T     1.076    1.000       0.0227 !
  11.43     2.8296 T     1.143    1.000       0.0651 !
  12.13    10.5062 T     1.213    1.000       0.2417 !
  12.97    18.2028 T     1.297    1.000       0.4188 !
  13.50     8.2588 T     1.350    1.000       0.1900 !
  14.49    28.4108 T     1.449    1.000       0.6536 !
  15.14    41.3657 T     1.514    1.000       0.9517 !
  16.32    40.4288 T     1.632    1.000       0.9301 !
  17.08    62.7404 T     1.708    1.000       1.4435 !
  18.40    47.0502 T     1.840    1.000       1.0825 !
  18.83    89.1699 T     1.883    1.000       2.0515 !
  19.71    30.5945 T     1.971    1.000       0.7039 !
  20.41    78.5630 T     2.041    1.000       1.8075 !
  20.91    55.1733 T     2.091    1.000       1.2686 !
  21.31    57.4310 T     2.131    1.000       1.3213 !
  21.87    82.8595 T     2.187    1.000       1.9063 !
  22.55    65.5027 T     2.255    1.000       1.5070 !
  23.22    66.7212 T     2.322    1.000       1.5350 !
  23.85    31.5417 T     2.385    1.000       0.7257 !
  24.52    51.3817 T     2.452    1.000       1.1821 !
  25.68    36.2342       2.568    1.000       0.8336 !
```

Figure 20. Typical Gas Chromatogram for the Initial Sample of Distillate Tank-Bottom Reactor



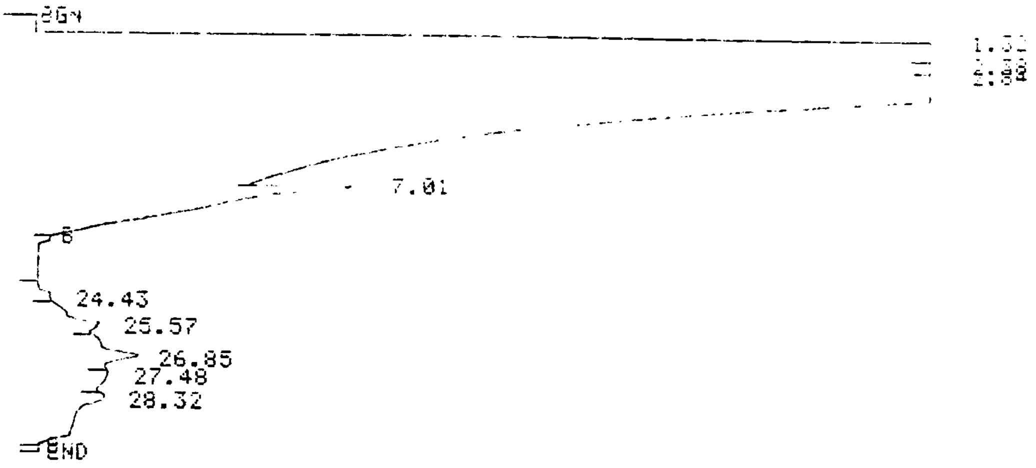
```
RUN  2  METH  6 1 4FILE  014  0 / 0
SENSITIVITIES    100   5
   TIME       AREA  BC     RRT       RF           C   NAME
   1.32  1375.6006  T    0.132    1.000     32.3843  !
   2.39  1375.6825  T    0.239    1.000     32.3862  !
   2.91   849.5104  T    0.291    1.000     19.9991  !
   6.75    26.8096  T    0.675    1.000      0.6311  !
   8.32     5.7670       0.832    1.000      0.1358  !
  15.01     0.0916  V    1.501    1.000      0.0022  !
  16.28     1.6320  T    1.628    1.000      0.0384  !
  16.94     9.0521  T    1.694    1.000      0.2131  !
  18.28    12.9619  T    1.828    1.000      0.3051  !
  18.72    32.1100  T    1.872    1.000      0.7559  !
  20.33    53.7651  T    2.033    1.000      1.2657  !
  20.83    30.1286  T    2.083    1.000      0.7093  !
  21.23    32.9561  T    2.123    1.000      0.7759  !
  21.80    49.3132  T    2.180    1.000      1.1609  !
  22.72    51.4560  T    2.272    1.000      1.2114  !
  23.18    58.8160  T    2.318    1.000      1.3846  !
  23.87    49.2902  T    2.387    1.000      1.1604  !
  24.52    53.4118  T    2.452    1.000      1.2574  !
  25.02    44.7488  T    2.502    1.000      1.0535  !
  25.69    68.1625- T    2.569    1.000      1.6047  !
  26.97    66.4806       2.697    1.000      1.5651  !
```

Figure 21. Typical Gas Chromatogram for the Final Sample of Distillate Tank-Bottom Reactor



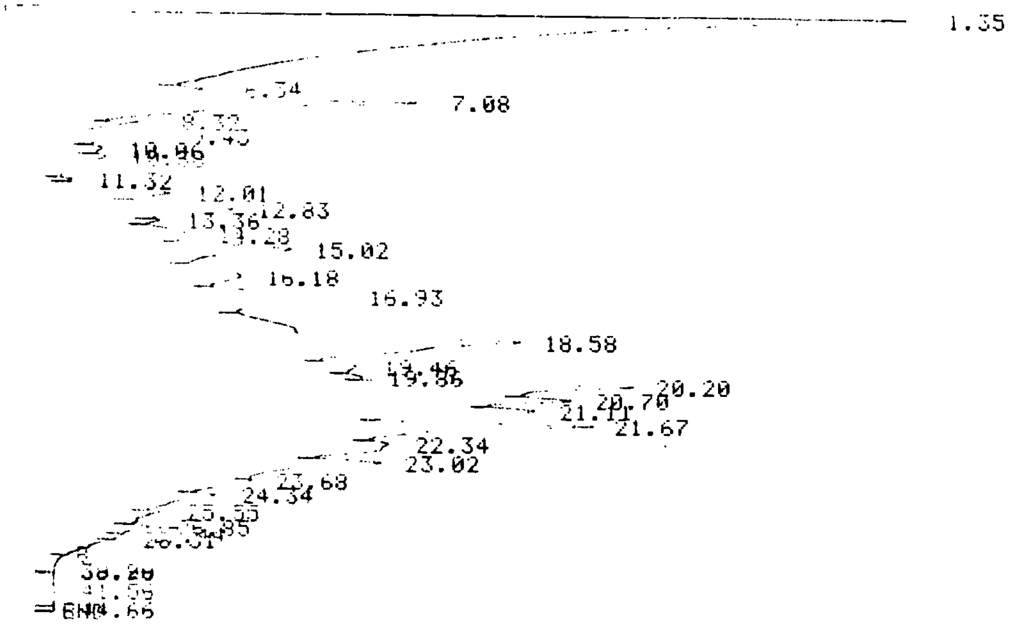
```
RUN#   2 METH 12 1 SLOPE  020   0 /  0
SENSITIVITIES    100   5
   TIME          AREA  BC      RRT        RF               C    NAME

   1.39  2054.6355 T     0.139    1.000        79.2563 !
   5.52     9.6828 T     0.552    1.000         0.3735 !
   6.28    33.6460 T     0.628    1.000         1.2979 !
   7.00    50.5702 T     0.700    1.000         1.9507 !
   8.19    15.5302 T     0.819    1.000         0.5991 !
   9.38    20.5056 T     0.938    1.000         0.7910 !
  10.02    12.1305 T     1.002    1.000         0.4679 !
  10.81     1.8612 T     1.081    1.000         0.0718 !
  11.27     0.9513 T     1.127    1.000         0.0367 !
  11.90    24.2700 T     1.190    1.000         0.9362 !
  12.62    20.5222 T     1.262    1.000         0.7916 !
  13.29     7.5564 T     1.329    1.000         0.2915 !
  13.98     9.2499 T     1.398    1.000         0.3568 !
  14.36     6.0115 T     1.436    1.000         0.2319 !
  14.59    10.4051 T     1.459    1.000         0.4014 !
  15.39     4.5057 T     1.539    1.000         0.1738 !
  16.13     8.5398 T     1.613    1.000         0.3294 !
  16.92     7.1532 T     1.692    1.000         0.2759 !
  18.21     3.5195 T     1.821    1.000         0.1358 !
  18.67     6.3884 T     1.867    1.000         0.2464 !
  19.62     2.1525 T     1.962    1.000         0.0830 !
  20.26     7.3833 T     2.026    1.000         0.2848 !
  20.78     9.4252 T     2.078    1.000         0.3636 !
  21.20    11.6089 T     2.120    1.000         0.4478 !
  21.73    20.4486 T     2.173    1.000         0.7888 !
  22.67    23.4393 T     2.267    1.000         0.9042 !
  23.13    29.2300 T     2.313    1.000         1.1275 !
  23.84    25.4617 T     2.384    1.000         0.9822 !
  24.48    31.5929 T     2.448    1.000         1.2187 !
  24.99    29.0636 T     2.499    1.000         1.1211 !
  25.66    49.6793 T     2.566    1.000         1.9164 !
  26.94    45.2761       2.694    1.000         1.7465 !
```

Figure 22. Typical Gas Chromatogram for the Initial Sample of Gasoline Tank-Bottom Reactor



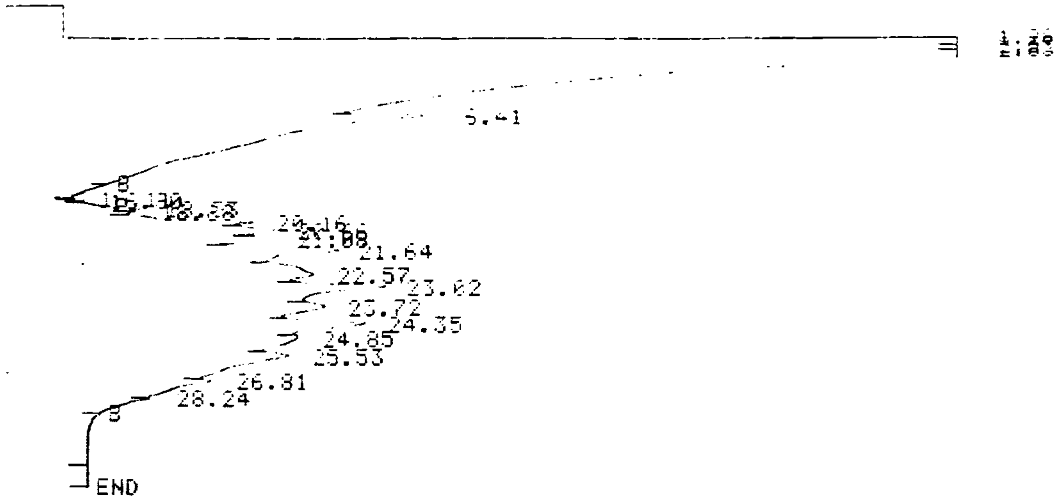
```
INST  2  METH    1  FILE    6

RUN      1  1  0 :  5.8   0 /  0 /  0

SENSITIVITIES    100   5

  TIME        AREA   BC     RRT        RF           C    NAME

  1.32  1268.9408  T     0.132    1.000     39.6580 !
  2.38   935.9360  T     0.238    1.000     29.2506 !
  2.84   889.4464  T     0.284    1.000     27.7977 !
  7.01    62.2182        0.701    1.000      1.9445 !
 24.43     0.6192  T     2.443    1.000      0.0194 !
 25.57     7.0496  T     2.557    1.000      0.2203 !
 26.85    12.6336  T     2.685    1.000      0.3948 !
 27.48     7.2902  T     2.748    1.000      0.2278 !
 28.32    15.5808        2.832    1.000      0.4869 !
```

Figure 23. Typical Gas Chromatogram for the Final Sample of Gasoline Tank-Bottom Reactor



```
RUN  3  METH  4 1 46IBE  012   0 /  0
[illegible]         100    5
   TIME        AREA  BC     RRT       RF             C   NAME
   1.35   1661.0918  T    0.135    1.000       74.1383 !
   6.34     19.6851  T    0.634    1.000        0.8786 !
   7.08     32.1420  T    0.708    1.000        1.4346 !
   8.32     10.2912  T    0.832    1.000        0.4593 !
   9.43     10.7168  T    0.943    1.000        0.4783 !
  10.06      3.4376  T    1.006    1.000        0.1534 !
  10.38      6.4489  T    1.038    1.000        0.2878 !
  11.32      2.0264  T    1.132    1.000        0.0904 !
  12.01     12.9638  T    1.201    1.000        0.5786 !
  12.83     16.4217  T    1.283    1.000        0.7329 !
  13.36      6.7232  T    1.336    1.000        0.3001 !
  14.28     20.7020  T    1.428    1.000        0.9240 !
  15.02     24.5529  T    1.502    1.000        1.0959 !
  16.18     21.7331  T    1.618    1.000        0.9700 !
  16.93     30.4499  T    1.693    1.000        1.3590 !
  18.58     70.8377  T    1.858    1.000        3.1617 !
  19.46     18.2515  T    1.946    1.000        0.8146 !
  19.86     10.3667  T    1.986    1.000        0.4627 !
  20.20     38.2745  T    2.020    1.000        1.7083 !
  20.70     25.4169  T    2.070    1.000        1.1344 !
  21.11     29.8009  T    2.111    1.000        1.3301 !
  21.67     39.7209  T    2.167    1.000        1.7728 !
  22.34     28.6963  T    2.234    1.000        1.2808 !
  23.02     25.9456  T    2.302    1.000        1.1580 !
  23.68     13.5398  T    2.368    1.000        0.6043 !
  24.34     19.8508  T    2.434    1.000        0.8860 !
  25.55     14.3507  T    2.555    1.000        0.6405 !
  26.85      8.2643  T    2.685    1.000        0.3689 !
  27.44      6.0678  T    2.744    1.000        0.2708 !
  28.31     11.5392       2.831    1.000        0.5150 !
```

Figure 24. Typical Gas Chromatogram for the Initial Sample of Mixed Tank-Bottom Reactor



```
INST  2  METH     1  FILE    21
RUN      15  1 13 : 34.2   0 /   0 /  0

SENSITIVITIES    100    5
   TIME         AREA  BC     RRT       RF          C   NAME
   1.28  1139.2614  T    0.128    1.000     32.0067 !
   2.33  1221.3043  T    0.233    1.000     34.3117 !
   2.83   912.5888  T    0.283    1.000     25.6385 !
   6.41    67.4918       0.641    1.000      1.8961 !
  16.11     0.0570  V    1.611    1.000      0.0016 !
  16.80     0.7929  T    1.680    1.000      0.0223 !
  18.53     5.2694  T    1.853    1.000      0.1480 !
  18.88     3.3150  T    1.888    1.000      0.0931 !
  20.16    14.6508  T    2.016    1.000      0.4116 !
  20.66    12.3180  T    2.066    1.000      0.3461 !
  21.08    11.9488  T    2.108    1.000      0.3357 !
  21.64    18.8537  T    2.164    1.000      0.5297 !
  22.57    20.1696  T    2.257    1.000      0.5667 !
  23.02    23.8720  T    2.302    1.000      0.6707 !
  23.72    17.9436  T    2.372    1.000      0.5041 !
  24.35    19.7772  T    2.435    1.000      0.5556 !
  24.85    16.7865  T    2.485    1.000      0.4716 !
  25.53    25.5257  T    2.553    1.000      0.7171 !
  26.81    17.0329  T    2.681    1.000      0.4785 !
  28.24    10.4896       2.824    1.000      0.2947 !
```

Figure 25. Typical Gas Chromatogram for the Final Sample of Mixed Tank-Bottom Reactor

APPENDIX D

TPH Standard Curves for the Reactors

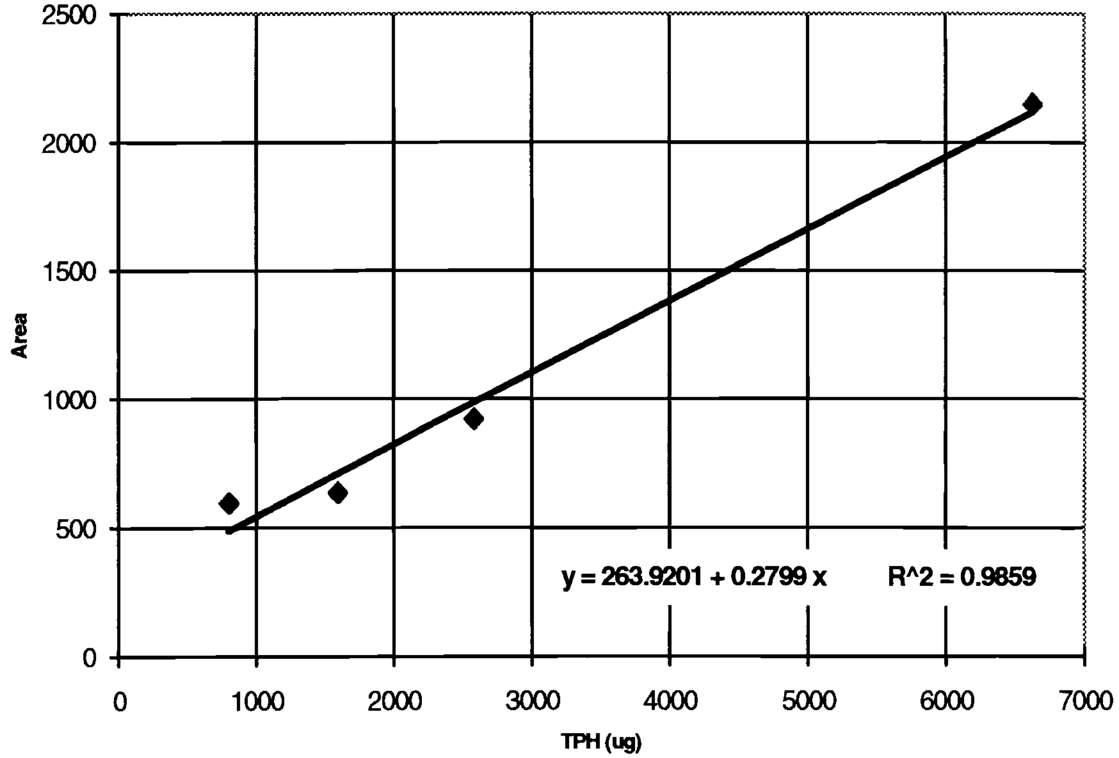


Figure 26. TPH Standard Curve for Distillate Tank-Bottom Reactor

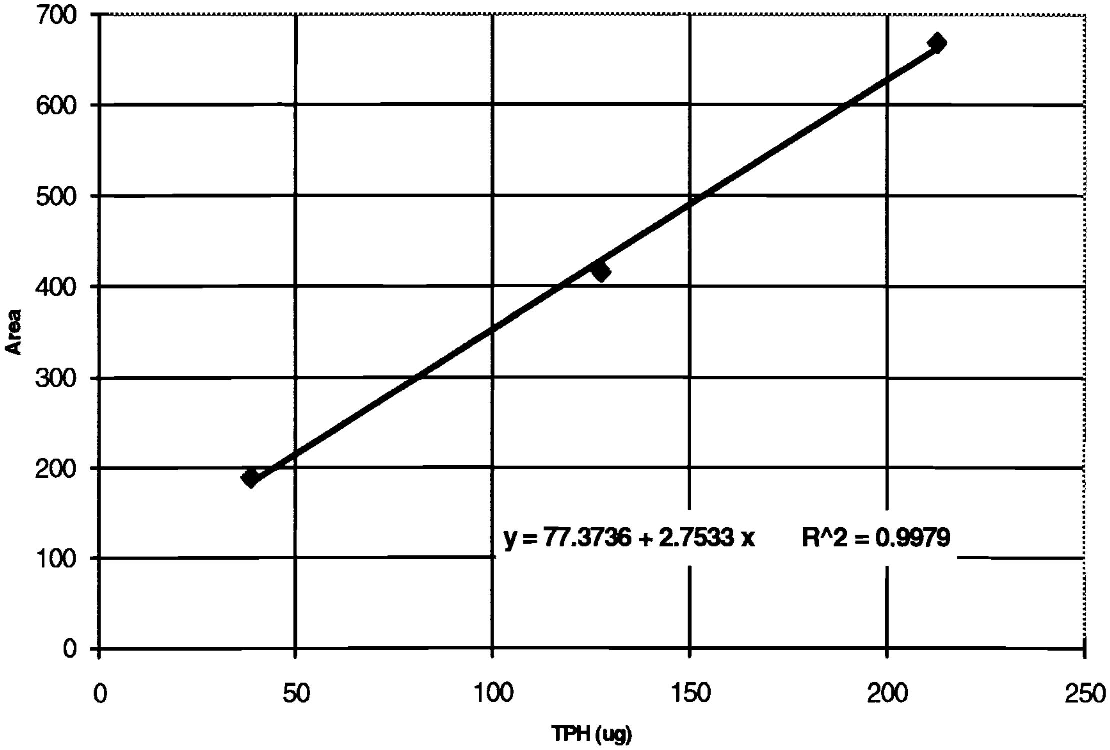


Figure 27. TPH Standard Curve for Gasoline Tank-Bottom Reactor

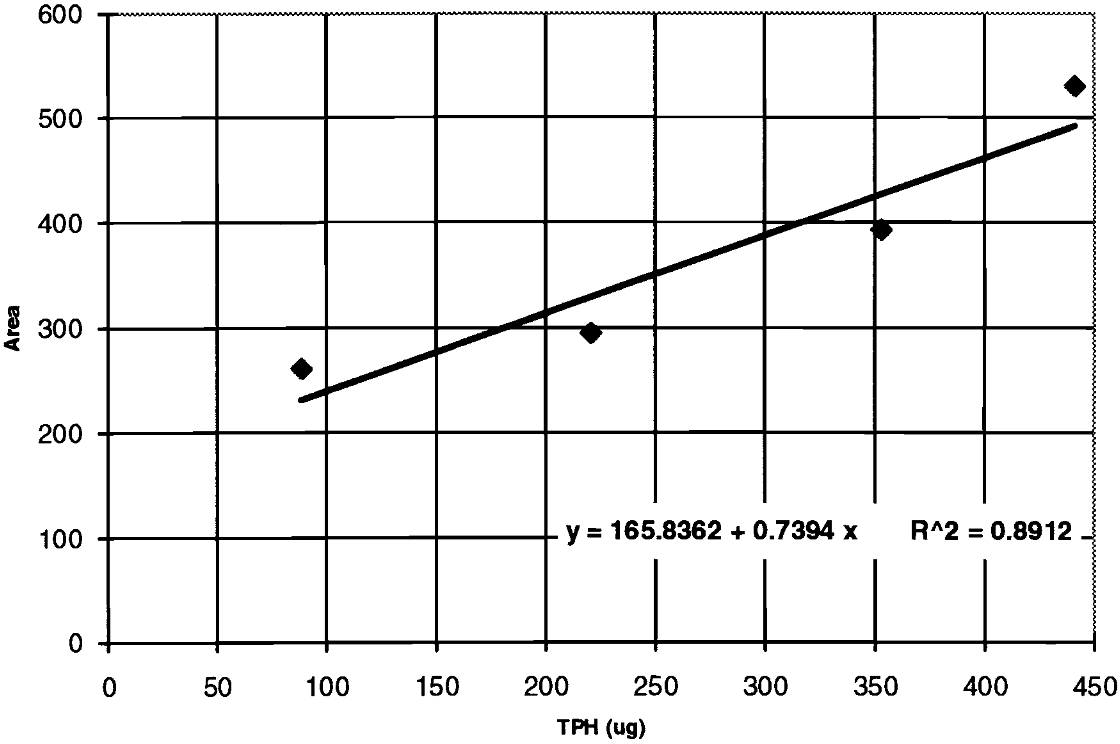
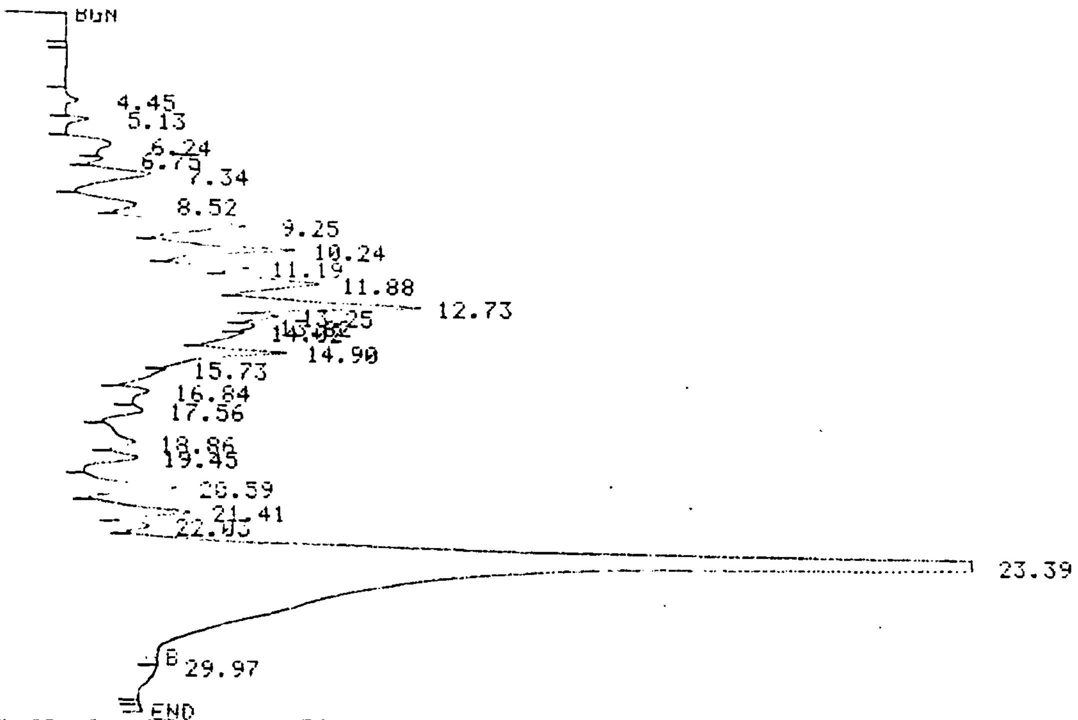


Figure 28. TPH Standard Curve for Mixed Tank-Bottom Reactor

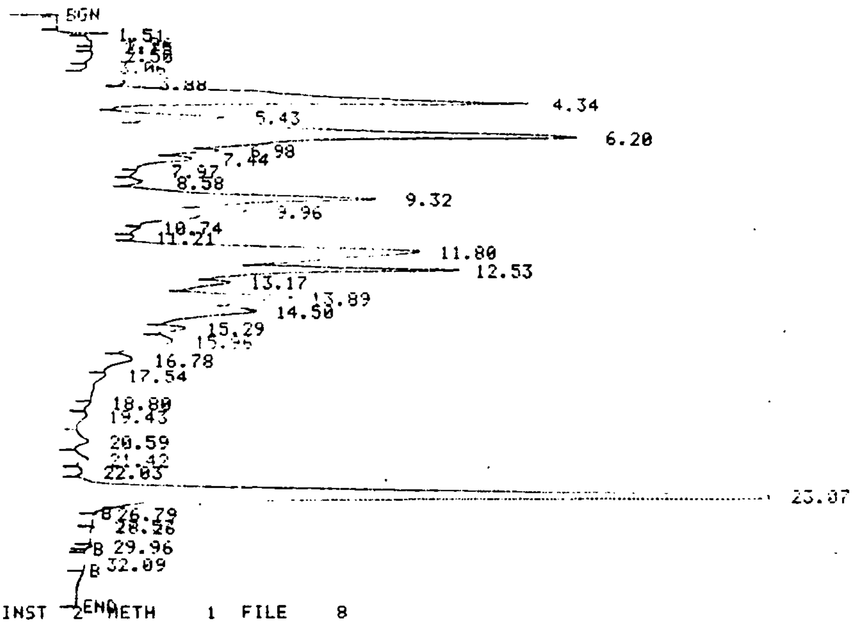
APPENDIX E

Typical Gas Chromatograms for the Extract from the Activated Carbon Column Attached to Each Reactor



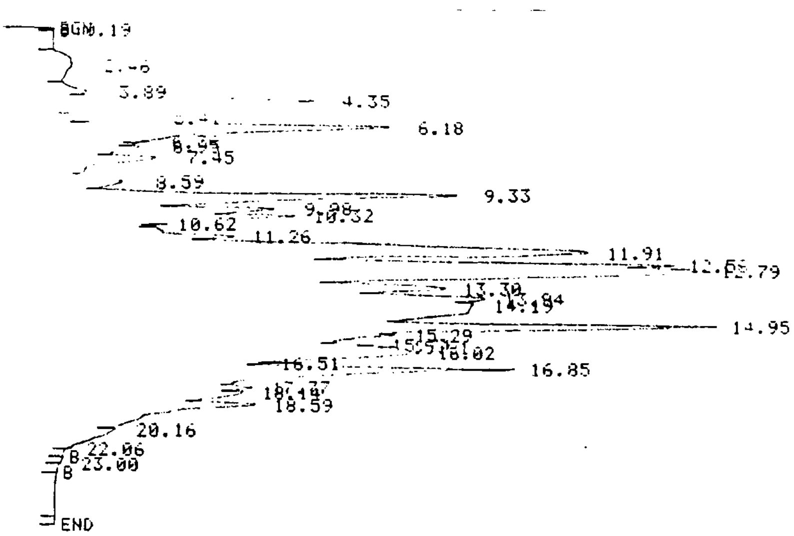
```
RUN#  2  DEC/0  + 1 30IBE  012  0 /  0
SENSITIVITIES    100    5
   TIME          AREA  BC     RRT       RF             C   NAME

   4.45        0.3392 V     0.445    1.000        0.0473 !
   5.13        0.7616 V     0.513    1.000        0.1063 !
   6.24        2.9772 T     0.624    1.000        0.4154 !
   6.75        1.5612 T     0.675    1.000        0.2178 !
   7.34        4.9720 T     0.734    1.000        0.6936 !
   8.52        4.6707 T     0.852    1.000        0.6516 !
   9.25       13.4214 T     0.925    1.000        1.8724 !
  10.24       18.5043 T     1.024    1.000        2.5815 !
  11.19       13.4009 T     1.119    1.000        1.8696 !
  11.88       21.2044 T     1.188    1.000        2.9582 !
  12.73       24.2944 T     1.273    1.000        3.3893 !
  13.25       10.4876 T     1.325    1.000        1.4631 !
  13.62        8.5916 T     1.362    1.000        1.1986 !
  14.02       14.6918 T     1.402    1.000        2.0496 !
  14.90       19.1852 T     1.490    1.000        2.6765 !
  15.73        8.3184 T     1.573    1.000        1.1605 !
  16.84        5.4041 T     1.684    1.000        0.7539 !
  17.56        4.7006 T     1.756    1.000        0.6558 !
  18.86        5.3494 T     1.886    1.000        0.7463 !
  19.45        2.9632 V     1.945    1.000        0.4134 !
  20.59        4.1044 V     2.059    1.000        0.5726 !
  21.41        4.8750 T     2.141    1.000        0.6801 !
  22.03        3.3854 T     2.203    1.000        0.4723 !
  23.39      518.6355       2.339    1.000       72.3543 !
TIME OF COMPUTED ZERO AREA PEAK
  29.97
```

Figure 29. Typical Gas Chromatogram for the Extract from the Activated Carbon Column Attached to the Distillate Tank-Bottom Reactor



```
INST    2   METH      1   FILE     8

RUN        2  1   1 :  3.5   0 /  0 /  0

SENSITIVITIES    100   5

    TIME         AREA  BC     RRT       RF             C   NAME
    1.51       0.1499 T     0.151    1.000        0.0338 !
    1.96       2.8585 T     0.196    1.000        0.6443 !
    2.15       1.4004 T     0.215    1.000        0.3157 !
    2.50       3.0646 T     0.250    1.000        0.6908 !
    3.06       1.8820 T     0.306    1.000        0.4242 !
    3.88       5.2649 T     0.388    1.000        1.1867 !
    4.34      29.5206 T     0.434    1.000        6.6540 !
    5.43      11.2953 T     0.543    1.000        2.5460 !
    6.20      51.6992 T     0.620    1.000       11.6530 !
    6.98       9.0659 T     0.698    1.000        2.0435 !
    7.44      10.7795 T     0.744    1.000        2.4297 !
    7.97       4.4585 T     0.797    1.000        1.0050 !
    8.58       6.6962 T     0.858    1.000        1.5098 !
    9.32      26.8646 T     0.932    1.000        6.0553 !
    9.96      19.6582 T     0.996    1.000        4.4310 !
   10.74       4.4534 T     1.074    1.000        1.0038 !
   11.21       3.1404 T     1.121    1.000        0.7079 !
   11.80      37.0073 T     1.180    1.000        8.3415 !
   12.53      30.6291 T     1.253    1.000        6.9038 !
   13.17      12.7168 T     1.317    1.009        2.8664 !
   13.89      19.3766 T     1.389    1.000        4.3675 !
   14.50      20.5113 T     1.450    1.000        4.6233 !
   15.29       7.9753 T     1.529    1.000        1.7976 !
   15.96      13.4713 T     1.596    1.000        3.0364 !
   16.78       6.9123 T     1.678    1.000        1.5580 !
   17.54       5.5443 T     1.754    1.000        1.2497 !
   18.80       1.3303 T     1.880    1.000        0.2999 !
   19.43       1.2121 T     1.943    1.000        0.2732 !
   20.59       0.9766 V     2.059    1.000        0.2201 !
   21.42       0.7291 V     2.142    1.000        0.1643 !
   22.03       0.3013 V     2.203    1.000        0.0679 !
   23.07      91.6070       2.307    1.000       20.6482 !
   26.79       0.1431 V     2.679    1.000        0.0323 !
   27.57       0.0864 T     2.757    1.000        0.0195 !
   28.26       0.2727 T     2.826    1.000        0.0615 !
   29.96       0.5264       2.996    1.000        0.1191 !
   32.09       0.0695       3.209    1.000        0.0157 !
```

Figure 30. Typical Gas Chromatogram for the Extract from the Activated Carbon Column Attached to the Gasoline Tank-Bottom Reactor



```
INST  2  METH     1  FILE   15
RUN       9  1 11 : 15.3   0 /  0 /  0
SENSITIVITIES    100   5
  TIME       AREA  BC     RRT      RF            C   NAME

   0.19     0.0017       0.019   1.000        0.0003 !
   2.46     3.6904 T     0.246   1.000        0.6364 !
   3.89     2.5233 T     0.389   1.000        0.4351 !
   4.35    14.7398 T     0.435   1.000        2.5418 !
   5.41     5.3312 T     0.541   1.000        0.9193 !
   6.18    29.6921 T     0.618   1.000        5.1202 !
   6.76     1.0852 T     0.676   1.000        0.1871 !
   6.95     4.5966 T     0.695   1.000        0.7927 !
   7.45     8.3049 T     0.745   1.000        1.4321 !
   8.59     5.9219 T     0.859   1.000        1.0712 !
   9.33    29.3427 T     0.933   1.000        5.0599 !
   9.98    11.6934 T     0.998   1.000        2.0165 !
  10.32    13.0688 T     1.032   1.000        2.2536 !
  10.62     1.1246 T     1.062   1.000        0.1939 !
  11.26    14.7712 T     1.126   1.000        2.5472 !
  11.91    57.3670 T     1.191   1.000        9.8925 !
  12.58    26.9529 T     1.258   1.000        4.6479 !
  12.79    31.5993 T     1.279   1.000        5.4491 !
  13.30    26.8992 T     1.330   1.000        4.6386 !
  13.94    35.2332 T     1.394   1.000        6.0757 !
  14.19    39.8246 T     1.419   1.000        6.8675 !
  14.95    41.9033 T     1.495   1.000        7.2259 !
  15.29    19.7574 T     1.529   1.000        3.4070 !
  15.74     5.9673 T     1.574   1.000        1.0290 !
  15.81     4.0329 T     1.581   1.000        0.6955 !
  16.02    34.9081 T     1.602   1.000        6.0197 !
  16.51     1.0784 T     1.651   1.000        0.1860 !
  16.85    44.4544 T     1.685   1.000        7.6659 !
  17.77    15.3849 T     1.777   1.000        2.6530 !
  18.14    11.0969 T     1.814   1.000        1.9136 !
  18.59    28.9100 T     1.859   1.000        4.9853 !
  20.16     8.4890 T     2.016   1.000        1.4637 !
  22.06     0.1169       2.206   1.000        0.0202 !
  23.00     0.0384       2.300   1.000        0.0066 !
```

Figure 31. Typical Gas Chromatogram for the Extract from the Activated Carbon Column Attached to the Mixed Tank-Bottom Reactor

APPENDIX F

Data from Gas Chromatograph Analysis of TPH in the Samples from the Reactors

Table 10. Data from GC Analysis of Samples from Distillate Tank-Bottom Reactor

Sample	GC Area	Mean	Standard Deviation	TPH (mg/g)
Initial	1024.25	1044.6520	64.2373	278.97
	978.1883			
	1131.518			
10-day	806.3743	897.4514	110.5929	226.38
	832.8768			
	1053.103			
20-day	835.2006	829.5443	5.275	202.11
	830.9289			
	822.5035			
30-day	874.4592	822.2887	45.8225	199.52
	829.4937			
	762.9133			
40-day	890.4031	937.7256	132.2785	240.77
	804.6487			
	1118.125			
50-day	833.8639	778.8408	49.9797	183.99
	789.7489			
	712.9077			
Final	679.2658	651.6780	20.8657	138.56
	646.9531			
	628.815			

Note:
Slope of Standard curve = 0.2799
Intercept of Standard Curve = 263.9201
Weight of Sample = 10 g

TPH = ((Mean-263.9201)/(0.2799x10))

Table 11. Data from GC Analysis of Samples from Gasoline Tank-Bottom Reactor

Sample	GC Area	Mean	Standard Deviation	TPH (mg/g)
Initial	452.9630	459.4256	32.9722	13.88
	422.6641			
	502.6498			
10-day	352.0000	327.6928	20.7989	9.09
	329.8837			
	301.1948			
20-day	222.5010	210.8142	12.5063	4.85
	193.4741			
	216.4674			
30-day	196.5587	188.5958	16.9603	4.04
	204.2083			
	162.0205			
40-day	309.0502	246.0484	50.2656	6.13
	186.0340			
	243.0611			
50-day	140.9929	142.1072	6.7227	2.35
	150.8411			
	134.4875			
Final	105.3916	81.0555	17.3365	0.13
	66.3093			
	71.4656			

Note:
Slope of Standard curve = 2.7533
Intercept of Standard Curve = 77.3736
Weight of Sample = 10 g

TPH = ((Mean-77.3736)/(2.7533x10))

Table 12. Data from GC Analysis of Samples from Mixed Tank-Bottom Reactor

Sample	GC Area	Mean	Standard Deviation	TPH (mg/g)
	337.9602			
Initial	550.1201	523.6420	142.0383	48.39
	682.8458			
	522.1006			
10-day	445.8194	467.0584	39.2572	40.74
	433.2553			
	550.9621			
20-day	346.3586	425.3634	89.7919	35.10
	378.7696			
	381.0095			
30-day	350.1653	362.4007	13.3743	26.59
	356.0272			
	338.5996			
40-day	301.5787	312.1356	18.8399	19.79
	296.2286			
	322.8417			
50-day	308.5494	314.2522	6.1815	20.07
	311.3656			
	308.2261			
Final	279.1225	291.2144	12.3806	16.96
	286.2945			

Note:
Slope of Standard curve = 0.7394
Intercept of Standard Curve = 165.8362
Weight of Sample = 10 g

TPH = ((Mean-165.8362)/(0.7394x10))

APPENDIX G

Data for Characterization of Gas Chromatograms from TPH Analysis of Reactor Samples

Table 13. TPH Gas Chromatogram Characterization Data for Distillate Tank-Bottom Reactor

Sample	GC Area	TPH (mg/g)	GC Area C8 to C10	TPH C8 to C10 (mg/g)	GC Area C10 to C16	TPH C10 to C16 (mg/g)	GC Area > C16	TPH > C16 (mg/g)
Initial	1044.652	278.97	203.24	54.27	721.03	192.54	120.40	32.15
10-day	897.4514	226.38	102.23	25.78	593.36	149.67	201.84	50.91
20-day	829.5443	202.11	104.30	25.41	504.38	122.88	220.86	53.81
30-day	822.2887	199.52	52.94	12.84	473.51	114.89	295.84	71.78
40-day	937.7256	240.77	33.89	8.70	588.54	151.11	315.30	80.95
50-day	778.8408	183.99	47.73	11.27	414.89	98.01	316.24	74.70
Final	651.6780	138.56	37.28	7.92	312.79	66.50	301.59	64.12

Table 14. TPH Gas Chromatogram Characterization Data for Gasoline Tank-Bottom Reactor

Sample	GC Area	TPH (mg/g)	GC Area C8 to C10	TPH C8 to C10 (mg/g)	GC Area C10 to C16	TPH C10 to C16 (mg/g)	GC Area > C16	TPH > C16 (mg/g)
Initial	459.4256	13.88	169.892	5.13	121.868	3.68	167.653	5.06
10-day	327.6928	9.09	111.26	3.08	41.89	1.19	174.61	4.84
20-day	210.8142	4.85	74.01	1.70	9.56	0.22	127.10	2.92
30-day	188.5958	4.04	75.94	1.62	12.95	0.27	99.69	2.13
40-day	246.0484	6.13	155.34	3.87	2.65	0.06	87.91	2.19
50-day	142.1072	2.35	90.24	1.49	0	0	51.93	0.85
Final	81.0555	0.13	51.38	0.08	0	0	31.99	0.05

Table 15. TPH Gas Chromatogram Characterization Data for Mixed Tank-Bottom Reactor

Sample	GC Area	TPH (mg/g)	GC Area C8 to C10	TPH C8 to C10 (mg/g)	GC Area C10 to C16	TPH C10 to C16 (mg/g)	GC Area > C16	TPH > C16 (mg/g)
Initial	523.6420	48.39	189.79	17.53	280.81	25.95	53.05	4.90
10-day	467.0584	40.74	76.73	6.69	284.05	24.77	106.27	9.27
20-day	425.3634	35.10	79.27	6.54	220.45	18.19	125.65	10.36
30-day	362.4007	26.58	65.14	4.77	183.42	13.45	113.84	8.35
40-day	312.1356	19.78	48.32	3.06	147.03	9.32	116.78	7.40
50-day	314.2522	20.07	54.02	3.45	144.19	9.21	116.04	7.41
Final	291.2144	16.95	64.08	3.73	119.28	6.94	107.85	6.28

APPENDIX H

Data from GC Analysis of Extract from Activated Carbons

Table 16. Data from GC Analysis of Extract from Activated Carbon

Sample	GC Area	Mean	Standard Deviation	TPH (mg)
Distillate Tank-Bottom Reactor	267.5638 187.0560 196.1640 428.2421	269.756	96.6666	301.89
Gasoline Tank-Bottom Reactor	261.2825 466.7565 350.9478 356.3772	358.841	72.8557	1363.07
Mixed Tank- Bottom Reactor	543.2058 545.5814 579.9009 464.4157	533.276	42.3251	6626.20

Note:

Weight of activated carbon in column = 40 g
Weight of carbon used for extraction = 3 g

For Distillate Tank-Bottom Reactor :-
Slope of standard curve = 0.2799 Intercept of standard curve = 263.9201
TPH = ((Mean - 263.9201) x 40/(.2799 x 3)

For Gasoline Tank-Bottom Reactor :-
Slope of standard curve = 2.7533 Intercept of standard curve = 77.3736
TPH = ((Mean - 77.3736) x 40/(2.7533 x 3)

For Mixed Tank-Bottom Reactor :-
Slope of standard curve = 0.7394 Intercept of standard curve = 165.8362
TPH = ((Mean - 165.8362) x 40/(.7394 x 3)

APPENDIX I

Mass Balance Calculations on the 20th Day TPH of the Reactors

Mass Balance on 20th Day TPH of Distillate Tank-Bottom Reactor:

Density of Distillate tank-bottom sludge = 1143.27 g/L

Assume density of seed & nutrient solution = 1000 g/L

∴ Initial mass of reactor contents = (1143.27 x 1 + 1000 x 1.1) = 2243.27 g

Density of reactor contents = 2243.27/2100 = 1.068 g/ml

Assume that two 60 ml samples were removed and replaced with tap water of density 1 g/ml

Mass lost up to the 20th day due to sampling = 2 x 60 x (1.068 - 1.000) = 8.16 g

∴ Mass of reactor contents at the 20th day = 2243.27 - 8.16 = 2235.11 g

∴ Initial TPH content of the reactor = 2243.27 x 278.97 = 625,800 mg

∴ 20th day TPH content of the Reactor = 2235.11 x 202.11 = 451,700 mg

Total reduction in TPH = 625,800 - 451,700 = 174,100 mg

Total volatile organics captured = 300g

∴ % of TPH removed that was biologically degraded = (174,100 - 300) x 100/174,100 = 99.8

Mass Balance on 20th Day TPH of Gasoline Tank-Bottom Reactor:

Density of Gasoline tank-bottom sludge = 1267.38 g/L

Assume density of seed & nutrient solution = 1000 g/L

∴ Initial mass of reactor contents = (1267.38 x 1 + 1000 x 1.1) = 2367.38 g

Density of reactor contents = 2367.38/2100 = 1.127 g/ml

Assume that two 60 ml samples were removed and replaced with tap water of density 1 g/ml

Mass lost up to the 20th day due to sampling = 2 x 60 x (1.127 - 1.000) = 15.24 g

Mass of reactor contents at the 20th day = 2367.38 - 15.24 = 2352.14 g

∴ Initial TPH content of the reactor = 2367.38 x 13.88 = 32,850 mg

∴ Final TPH content of the reactor = 2352.14 x4.85 = 11,400 mg

Total reduction in TPH = 32,850 - 11,400 = 21,450 mg

Total volatile organics captured = 1350 mg

∴ % of TPH removed that was biologically degraded = (21,450- 1350) x 100/21,450= 93.7

Mass Balance on 20th Day TPH of Mixed Tank-Bottom Reactor:

Density of Distillate tank-bottom sludge = 1143.27 g/L

Density of Gasoline tank-bottom sludge = 1267.38 g/L

Assume density of seed & nutrient solution = 1000 g/L

$\therefore$ Initial mass of reactor contents = (1143.27 x 0.5 + 1267.38 x 0.5 + 1000 x 1.1) = 2305.33 g

Density of reactor contents = 2305.33/2100 = 1.098 g/ml

Assume that two 60 ml samples were removed and replaced with tap water of density 1 g/ml

Mass lost up to the 20th day due to sampling = 2 x 60 x (1.098 - 1.000) = 11.76 g

Mass of reactor contents at the 20th day = 2305.33 - 11.76 = 2293.57 g

Initial TPH content of the reactor = 2305.33 x 48.39 = 111,550 mg

Final TPH content of the reactor = 2293.57 x 35.10 = 80,500 mg

Total reduction in TPH = 111,550 - 80,500 = 31,050 mg

Total volatile organics captured = 6650 mg

$\therefore$ % of TPH removed that was biologically degraded = (31,050 - 6650) x 100/31,050 = 78.6

APPENDIX J

Mass Balance Calculations on the Final TPH of the Reactors

Mass Balance on Final TPH of Distillate Tank-Bottom Reactor:

Density of Distillate tank-bottom sludge = 1143.27 g/L

Assume density of seed & nutrient solution = 1000 g/L

$\therefore$ Initial mass of reactor contents = (1143.27 x 1 + 1000 x 1.1) = 2243.27 g

Assume that six 60 ml samples were removed and replaced with tap water of density 1 g/ml

$\therefore$ Mass lost up to the 60th day due to sampling = 6 x 60 x (1.068 - 1.000) = 24.48 g

$\therefore$ Final mass of reactor contents = 2243.27 -24.48 = 2218.79 g

(Note: Final sample density = 1.037 g/ml; $\therefore$ Actual final mass of reactor contents = 2177.7 g)

$\therefore$ Initial TPH content of the reactor = 2243.27 x 278.97 = 625,800 mg

$\therefore$ 20th day TPH content of the Reactor = 2218.79 x 138.56 = 307,400 mg

Total reduction in TPH = 625,800 - 307,400 = 318,400 mg

Total volatile organics captured = 300g

$\therefore$ % of TPH biologically degraded = (318,400 - 300) x 100/625,800 = 50.8

Mass Balance on Final TPH of Gasoline Tank-Bottom Reactor:

Density of Gasoline tank-bottom sludge = 1267.38 g/L

Assume density of seed & nutrient solution = 1000 g/L

$\therefore$ Initial mass of reactor contents = (1267.38 x 1 + 1000 x 1.1) = 2367.38 g

Assume that six 60 ml samples were removed and replaced with tap water of density 1 g/ml

$\therefore$ Mass lost up to the 60th day due to sampling = 6 x 60 (1.127 - 1.000) = 45.72 g

$\therefore$ Final mass of reactor contents = 2367.38 - 45.72 = 2321.66 g

(Note: Final sample density = 1.060 g/ml; $\therefore$ Actual final mass of reactor contents = 2226 g)

$\therefore$ Initial TPH content of the reactor = 2367.38 x 13.88 = 32,850 mg

$\therefore$ Final TPH content of the reactor = 2321.66 x0.13 = 300 mg

Total reduction in TPH = 32,850 - 310 = 32,550 mg

Total volatile organics captured = 1350 mg

$\therefore$ % of TPH biologically degraded = (32,550 - 1350) x 100/32,850= 95.0

Mass Balance on Final TPH of Mixed Tank-Bottom Reactor:

Density of Distillate tank-bottom sludge = 1143.27 g/L

Density of Gasoline tank-bottom sludge = 1267.38 g/L

Assume density of seed & nutrient solution = 1000 g/L

∴ Initial mass of reactor contents = (1143.27 x 0.5 + 1267.38 x 0.5 + 1000 x 1.1) = 2305.33 g

Assume that six 60 ml samples were removed and replaced with tap water of density 1 g/ml

∴Mass lost up to the 60th day due to sampling = 6 x 60 x (1.098 - 1.000) = 35.28 g

∴Final mass of reactor contents = 2305.33 - 35.28 = 2270.05 g

(Note: Final sample density = 1.055 g/ml; ∴Actual final mass of reactor contents = 2215.5 g)

Initial TPH content of the reactor = 2305.33 x 48.39 = 111,550 mg

Final TPH content of the reactor = 2270.05 x 16.96 = 38,500 mg

Total reduction in TPH = 111,550 - 38,500 = 73,050 mg

Total volatile organics captured = 6650 mg

∴ % of TPH biologically degraded = (73,050 - 6650) x 100/111,550 = 59.5

Appendix K

Data from the Solids Analysis of the Samples from the Reactors.

Table 17. Data from Solids Analysis of Distillate Tank-Bottom Reactor Samples

Sample No.	Sample Wt. (g)	Sample Wt. after Heating at 103°C (g)	Sample Wt. after Heating at 550°C (g)	Total Solids Content (%)	Mean	Volatile Solids Content (%)	Mean
Initial	2.5533	0.8786	0.4731	34.41	34.98	15.88	16.98
	3.3622	1.1596	0.6173	34.49		16.13	
	4.0985	1.4775	0.7012	36.05		18.94	
10-day	4.5351	1.4576	0.7234	32.14	33.40	16.19	17.12
	3.6897	1.1988	0.5756	32.49		16.89	
	2.3781	0.8461	0.4114	35.58		18.28	
20-day	3.1228	1.1226	0.6155	35.95	34.18	16.24	14.70
	2.1445	0.6751	0.4012	31.48		12.77	
	4.3517	1.5288	0.8717	35.13		15.10	
30-day	2.7875	0.9045	0.5530	32.45	32.57	12.61	12.70
	2.3334	0.7490	0.4566	32.10		12.53	
	3.4057	1.1286	0.6869	33.14		12.97	
40-day	2.3256	0.6991	0.4254	30.06	30.77	11.77	13.12
	3.7638	1.2040	0.6590	31.99		14.48	
	2.5717	0.7777	0.4406	30.24		13.11	
50-day	2.6678	0.7464	0.4628	27.98	30.05	10.63	12.69
	4.2071	1.3320	0.7199	31.66		14.55	
	3.5248	1.0758	0.6215	30.52		12.89	
Final	1.6165	0.4018	0.2345	24.81	27.50	10.35	12.25
	2.8308	0.7952	0.4538	28.09		12.06	
	3.4444	1.0195	0.5256	29.60		14.34	

Note:

% Total solid content = (Sample wt. after heating to 103°C) x 100/(Sample wt.)

% Volatile solids content =(Sample wt. after heating to 105°C - sample wt. after heating to 550°C) x 100/(sample weight)

Table 18. Data from Solids Analysis of Gasoline Tank-Bottom Reactor Samples

Sample No.	Sample Wt. (g)	Sample Wt. after Heating at 103°C (g)	Sample Wt. after Heating at 550°C (g)	Total Solids Content (%)	Mean	Volatile Solids Content (%)	Mean
Initial	2.7495	0.7710	0.4683	28.04	27.31	11.01	11.32
	4.3500	1.1514	0.6781	26.47		10.88	
	3.4140	0.9365	0.5237	27.43		12.09	
10-day	1.3750	0.3425	0.1869	24.91	25.58	11.32	11.17
	2.3587	0.6328	0.3531	26.83		11.86	
	2.8260	0.7065	0.4146	25.00		10.33	
20-day	3.7140	0.8821	0.5545	23.75	23.85	8.82	9.12
	3.9254	0.9421	0.5719	24.00		9.43	
	4.0629	0.9670	0.5973	23.80		9.10	
30-day	2.2464	0.5104	0.3222	22.72	22.87	8.38	8.42
	3.6978	0.8446	0.5344	22.84		8.39	
	3.2072	0.7393	0.4670	23.05		8.49	
40-day	3.0405	0.5841	0.3250	19.21	23.69	8.52	8.51
	2.3956	0.4377	0.2336	18.27		8.52	
	3.8320	1.2872	0.9615	33.59		8.50	
50-day	1.8371	0.4176	0.2896	22.73	22.38	6.97	6.38
	2.9658	0.7417	0.5424	25.01		6.72	
	5.3789	1.0435	0.7503	19.40		5.45	
Final	3.6712	0.6425	0.4153	17.50	21.71	6.19	6.24
	2.1604	0.4960	0.3627	22.96		6.17	
	4.2018	1.0366	0.7698	24.67		6.35	

Note:

% Total solid content = (Sample wt. after heating to 103°C) x 100/(Sample wt.)

% Volatile solids content =(Sample wt. after heating to 105°C - sample wt. after heating to 550°C) x 100/(sample weight)

Table 19. Data from Solids Analysis of Mixed Tank-Bottom Reactor Samples

Sample No.	Sample Wt. (g)	Sample Wt. after Heating at 103°C (g)	Sample Wt. after Heating at 550°C (g)	Total Solids Content (%)	Mean	Volatile Solids Content (%)	Mean
Initial	3.5806	1.1522	0.6327	32.18	32.73	14.51	14.89
	5.6275	1.8616	1.0062	33.08		15.20	
	5.0466	1.6624	0.9077	32.94		14.96	
10-day	2.7882	0.832	0.4824	29.84	31.49	12.54	13.74
	4.3437	1.4204	0.7784	32.70		14.78	
	3.4858	1.1134	0.6292	31.94		13.89	
20-day	2.672	0.7925	0.4967	29.66	28.72	11.07	11.34
	4.1504	1.1302	0.6176	27.23		12.35	
	3.5742	1.0462	0.6673	29.27		10.60	
30-day	3.4481	0.9793	0.6576	28.40	29.11	9.33	10.11
	2.6323	0.7526	0.4933	28.59		9.85	
	4.3532	1.3203	0.8340	30.33		11.17	
40-day	4.6185	1.2040	0.7283	26.07	26.16	10.30	10.69
	4.6918	1.3076	0.7591	27.87		11.69	
	4.8776	1.1965	0.3042	24.53		10.09	
50-day	2.9054	0.7272	0.4756	25.03	25.27	8.66	9.38
	2.4465	0.6251	0.3763	25.55		10.17	
	3.5797	0.9032	0.5699	25.23		9.31	
Final	2.0632	0.5208	0.3231	25.24	25.54	9.58	9.84
	2.9115	0.7363	0.4513	25.29		9.79	
	5.2517	1.3702	0.8372	26.09		10.15	

Note:

% Total solid content = (Sample wt. after heating to 103°C) x 100/(Sample wt.)

% Volatile solids content =(Sample wt. after heating to 105°C - sample wt. after heating to 550°C) x 100/(sample weight)

APPENDIX L

Calculations for Losses in Total Solids Content of the Reactors due to Sampling

Losses in Total Solids Content of Distillate Tank-Bottom Reactor due to Sampling

Mass of reactor contents at start of operation = 2243.27 g

Initial total solids content of the reactor = 34.98%

∴Mass of solids initially in the reactor = 2243.27 x 0.3498 = 784.70 g

Final total solids content of the reactor = 27.5%

∴ % reduction in the total solids content of the reactor = $\frac{(34.98-27.50)}{34.98}$ **x100 = 21.38%**

Total volume of reactor contents removed as samples = 60 x 6 = 360 ml

Density of reactor contents = 2243.27/2100 = 1.068 g/ml

∴Mass of reactor contents removed as samples = 360 x 1.068 = 384.48 g

Average total solids content of the sample = (34.98 + 27.5)/2 = 31.24%

∴Mass of solids removed from the reactor by sampling = 384.48 x 0.3124 = 120.11 g

∴ % reduction in the total solids content of the reactor due to sampling = $\frac{120.11}{784.70}$ **x100 = 15.31%**

Losses in Total Solids Content of Gasoline Tank-Bottom Reactor due to Sampling

Mass of reactor contents at start of operation = 2367.38 g

Initial total solids content of the reactor = 27.31%

∴Mass of solids initially in the reactor = 2367.38 x 0.2731 = 646.53 g

Final total solids content of the reactor = 21.71%

∴ % reduction in the total solids content of the reactor = $\frac{(27.31-21.71)}{27.31}$ **x100 = 20.51%**

Total volume of reactor contents removed as samples = 60 x 6 = 360 ml

Density of reactor contents = 2367.38/2100 = 1.127 g/ml

∴Mass of reactor contents removed as samples = 360 x 1.127 = 405.72 g

Average total solids content of the sample = (27.31 + 21.71)/2 = 24.51%

∴Mass of solids removed from the reactor by sampling = 405.72 x 0.2451 = 99.44 g

∴ % reduction in the total solids content of the reactor due to sampling = $\frac{99.44}{646.53}$ **x100 = 15.38%**

Losses in Total Solids Content of Mixed Tank-Bottom Reactor due to Sampling

Mass of reactor contents at start of operation = 2305.33 g

Initial total solids content of the reactor = 32.73%

∴Mass of solids initially in the reactor = 2305.33 x 0.3273 = 754.53 g

Final total solids content of the reactor = 25.54%

∴ % reduction in the total solids content of the reactor = $\frac{(32.73 - 25.54)}{32.73} \text{x}100 = 21.97\%$

Total volume of reactor contents removed as samples = 60 x 6 = 360 ml

Density of reactor contents = 2305.33/2100 = 1.098 g/ml

∴Mass of reactor contents removed as samples = 360 x 1.098 = 395.28 g

Average total solids content of the sample = (32.73 + 25.54)/2 = 29.14%

∴Mass of solids removed from the reactor by sampling = 395.28 x 0.2914 = 115.18 g

∴ % reduction in the total solids content of the reactor due to sampling = $\frac{115.18}{754.53} \text{x}100 = 15.27\%$

APPENDIX M

Data from the COD Analysis of the Samples from the Reactors

Table 20. Data from the COD Analysis of Distillate Tank-Bottom Samples

Sample No.	Sample Weight (g)	Volume of Water Added (ml)	Absorbance	COD (mg/g)	Mean (mg/g)
Initial	0.1650	100	0.473	588.0941	562.56
	0.1650	100	0.462	573.9097	
	0.1971	100	0.531	554.9262	
	0.1971	100	0.511	533.3366	
10-day	0.2688	100	0.557	427.4846	400.86
	0.2688	100	0.561	430.6508	
	0.4328	100	0.774	372.1767	
	0.4328	100	0.776	373.1599	
20-day	0.2783	100	0.543	402.1888	380.91
	0.2783	100	0.536	396.8372	
	0.4619	100	0.801	361.1644	
	0.4619	100	0.806	363.4696	
30-day	0.2394	100	0.522	448.8766	391.58
	0.2394	100	0.494	423.9917	
	0.5382	100	0.899	348.7065	
	0.5382	100	0.889	344.7532	
40-day	0.2302	100	0.355	312.4637	333.55
	0.2302	100	0.391	345.7373	
	0.3667	100	0.595	335.4049	
	0.3667	100	0.604	340.6269	
50-day	0.2598	50	0.565	224.4226	213.03
	0.2598	50	0.571	226.8795	
	0.4775	50	0.918	200.7501	
	0.4775	50	0.915	200.0818	
Final	0.3157	50	0.827	272.9723	238.41
	0.3157	50	0.841	277.6899	
	0.4662	50	0.9	201.5086	
	0.4662	50	0.9	201.5086	

Note: COD = [{(Absorbance - 0.016933)/0.00047} x volume of water added/(1000 x weight of sample)]

Table 21. Data from the COD Analysis of Gasoline Tank-Bottom Samples

Sample No.	Sample Weight (g)	Volume of Water Added (ml)	Absorbance	COD (mg/g)	Mean (mg/g)
Initial	0.9001	50	0.309	34.5195	40.97
	0.9001	50	0.356	40.0743	
	0.8830	50	0.372	42.7781	
	0.8830	50	0.403	46.5130	
10-day	2.1228	50	0.637	31.0743	34.15
	2.1228	50	0.782	38.3409	
	2.0436	50	0.773	39.3583	
	2.0436	50	0.552	27.8538	
20-day	0.8546	50	0.153	16.9380	17.50
	0.8546	50	0.236	27.2701	
	1.2783	50	0.229	17.6487	
	1.2783	50	0.115	8.1614	
30-day	1.3023	50	0.135	9.6447	9.41
	1.3023	50	0.180	13.3207	
	1.0653	50	0.078	6.0983	
	1.0653	50	0.103	8.5948	
40-day	1.1285	25	0.108	4.2924	6.47
	1.1285	25	0.238	10.4199	
	1.3385	25	0.117	3.9766	
	1.3385	25	0.198	7.1955	
50-day	0.9308	25	0.089	4.1183	7.00
	0.9308	25	0.160	8.1757	
	0.8256	25	0.091	4.7720	
	0.8256	25	0.187	10.9570	
Final	1.1323	25	0.146	6.0631	5.32
	1.1323	25	0.186	7.9422	
	1.3860	25	0.071	2.0750	
	1.3860	25	0.153	5.2219	

Note: COD = [{(Absorbance - 0.016933)/0.00047} x volume of water added/(1000 x weight of sample)]

Table 22. Data from the COD Analysis of Mixed Tank-Bottom Samples

Sample No.	Sample Weight (g)	Volume of Water Added (ml)	Absorbance	COD (mg/g)	Mean (mg/g)
Initial	0.2270	100	0.517	468.7103	468.76
	0.2270	100	0.516	467.7730	
	0.1535	100	0.358	472.7521	
	0.1535	100	0.353	465.8216	
10-day	0.7578	100	0.718	196.8371	211.79
	0.7578	100	0.671	183.6411	
	0.6602	100	0.684	214.979	
	0.6602	100	0.798	251.7184	
20-day	0.1796	100	0.234	257.1518	189.73
	0.1796	100	0.158	167.1172	
	0.4044	100	0.325	162.0825	
	0.4044	100	0.345	172.6051	
30-day	0.3540	100	0.346	197.7804	195.01
	0.3540	100	0.355	203.1897	
	0.6506	100	0.577	183.1589	
	0.6506	100	0.616	195.9131	
40-day	0.2453	50	0.473	197.7895	144.87
	0.2453	50	0.491	205.5958	
	0.6138	50	0.522	87.5375	
	0.6138	50	0.522	88.5774	
50-day	0.0802	50	0.150	176.5095	159.61
	0.0802	50	0.151	177.836	
	0.2355	50	0.318	135.7423	
	0.2360	50	0.346	148.3667	
Final	0.2040	50	0.194	92.3378	88.53
	0.2040	50	0.197	93.9027	
	0.4107	50	0.331	81.3523	
	0.4107	50	0.351	86.5329	

Note: COD = [{(Absorbance - 0.016933)/0.00047} x volume of water added/(1000 x weight of sample)]

APPENDIX N

Mass Balance Calculations on the Metals Content of the Reactors

Mass Balance on the Chromium Content of Distillate Tank Bottom Reactor:

Density of distillate tank-bottom sludge = 1143.27g/L

Assume density of seed and nutrient solution = 1000 g/L

∴ Mass of reactor contents at start of operation = (1143.27 x 1 + 1000 x 1.1) = 2243.27 g

Density of reactor contents = 2243.27/2100 = 1.068 g/ml

Assume that six 60 ml samples were removed and replaced with tap water of density 1 g/ml

∴ Loss in mass due to sampling = 6 x 60 x (1.068 - 1.000) = 24.48 g

∴ Mass of reactor contents at the end of operation = 2243.27 - 24.48 = 2218.79 g

(Note: Final sample density = 1.037 g/ml; ∴ Actual final mass of reactor contents = 2177.7 g)

Initial chromium content of the reactor = 2243.27 x 0.086 = 192.92 mg

Final chromium content of the reactor = 2218.79 x 0.066 = 146.44 mg

% reduction in the chromium content of the reactor = (192.92 - 146.44) x 100/192.92 = 24.09

Mass Balance on the Lead Content of Distillate Tank Bottom Reactor:

Density of distillate tank-bottom sludge = 1143.27g/L

Assume density of seed and nutrient solution = 1000 g/L

∴ Mass of reactor contents at start of operation = (1143.27 x 1 + 1000 x 1.1) = 2243.27 g

Density of reactor contents = 2243.27/2100 = 1.068 g/ml

Assume that six 60 ml samples were removed and replaced with tap water of density 1 g/ml

∴ Loss in mass due to sampling = 6 x 60 x (1.068 - 1.000) = 24.48 g

∴ Mass of reactor contents at the end of operation = 2243.27 - 24.48 = 2218.79 g

(Note: Final sample density = 1.037 g/ml; ∴ Actual final mass of reactor contents = 2177.7 g)

Initial lead content of the reactor = 2243.27 x 0.152 = 340.98 mg

Final lead content of the reactor = 2218.79 x 0.132 = 292.88 mg

% reduction in the lead content of the reactor = (340.98 - 292.88) x 100/340.98 = 14.11

Mass Balance on Chromium Content of Gasoline Tank Bottom Reactor:

Density of gasoline tank-bottom sludge = 1267.38g/L

Assume density of seed and nutrient solution = 1000 g/L

∴ Mass of reactor contents at start of operation = (1267.38 x 1 + 1000 x 1.1) = 2367.38 g

Density of reactor contents = 2367.38/2100 = 1.127 g/ml

Assume that six 60 ml samples were removed and replaced with tap water of density 1 g/ml

∴Loss in mass due to sampling = 6 x 60 x (1.127 - 1.000) = 45.72 g

∴ Mass of reactor contents at the end of operation = 2367.38 - 45.72 = 2321.66 g

(Note: Final sample density = 1.06 g/ml; ∴Actual final mass of reactor contents = 2226.0 g)

Initial chromium content of the reactor = 2367.38 x 0.264 = 624.99 mg

Final chromium content of the reactor = 2321.66 x 0.225 = 522.37 mg

% reduction in the chromium content of the reactor = (624.99 - 522.37) x 100/624.99 = 16.42

Mass Balance on the Lead Content of Gasoline Tank Bottom Reactor:

Density of gasoline tank-bottom sludge = 1267.38g/L

Assume density of seed and nutrient solution = 1000 g/L

∴ Mass of reactor contents at start of operation = (1267.38 x 1 + 1000 x 1.1) = 2367.38 g

Density of reactor contents = 2367.38/2100 = 1.127 g/ml

Assume that six 60 ml samples were removed and replaced with tap water of density 1 g/ml

∴Loss in mass due to sampling = 6 x 60 x (1.127 - 1.000) = 45.72 g

∴ Mass of reactor contents at the end of operation = 2367.38 - 45.72 = 2321.66 g

(Note: Final sample density = 1.06 g/ml; ∴Actual final mass of reactor contents = 2226.0 g)

Initial lead content of the reactor = 2367.38 x 0.400 = 946.95 mg

Final lead content of the reactor = 2321.66 x 0.159 = 369.14 mg

% reduction in the lead content of the reactor = (946.95 - 369.14) x 100/946.95 = 61.02

Mass Balance on the Chromium Content of Mixed Tank Bottom Reactor:

Density of distillate tank-bottom sludge = 1143.27g/L

Density of gasoline tank-bottom sludge = 1267.38g/L

Assume density of seed and nutrient solution = 1000 g/L

Mass of reactor contents at start of operation = ((1143.27 + 1267.38) x 0.5 + 1000 x 1.1) = 2305.33 g

Density of reactor contents = 2305.33/2100 = 1.098 g/ml

Assume that six 60 ml samples were removed and replaced with tap water of density 1 g/ml

∴ Loss in mass due to sampling = 6 x 60 x (1.098 - 1.000) = 35.28 g

∴ Mass of reactor contents at the end of operation = 2305.33 - 35.28 = 2270.05 g

(Note: Final sample density = 1.055 g/ml; ∴ Actual final mass of reactor contents = 2215.5 g)

Initial chromium content of the reactor = 2305.33 x 0.230 = 530.23 mg

Final chromium content of the reactor = 2270.05 x 0.168 = 381.37 mg

% reduction in the chromium content of the reactor = (530.23 - 381.37) x 100/530.23 = 28.07

Mass Balance on the Lead Content of Mixed Tank Bottom Reactor:

Density of distillate tank-bottom sludge = 1143.27g/L

Density of gasoline tank-bottom sludge = 1267.38g/L

Assume density of seed and nutrient solution = 1000 g/L

Mass of reactor contents at start of operation = ((1143.27 + 1267.38) x 0.5 + 1000 x 1.1) = 2305.33 g

Density of reactor contents = 2305.33/2100 = 1.098 g/ml

Assume that six 60 ml samples were removed and replaced with tap water of density 1 g/ml

∴ Loss in mass due to sampling = 6 x 60 x (1.098 - 1.000) = 35.28 g

∴ Mass of reactor contents at the end of operation = 2305.33 - 35.28 = 2270.05 g

(Note: Final sample density = 1.055 g/ml; ∴ Actual final mass of reactor contents = 2215.5 g)

Initial lead content of the reactor = 2305.33 x 0.350 = 806.87 mg

Final lead content of the reactor = 2270.05 x 0.165 = 374.56 mg

% reduction in the chromium content of the reactor = (806.87 - 374.56) x 100/806.87 = 53.58

APPENDIX O

Data from the MPN Analysis of Samples from the Reactors

Table 23. Data from the MPN Analysis of the Samples from the Reactors

	Distillate Tank-Bottom		Gasoline Tank-Bottom		Mixed Tank-Bottom	
Sample	No of + ve tubes	MPN Index	No of + ve tubes	MPN Index	No of + ve tubes	MPN Index
Initial	5-0-0	2300	5-1-0	3000	5-0-0	2300
10-day	5-0-0	2300	5-2-0	5000	5-0-0	2300
20-day	5-3-0	8000	5-4-0	13000	5-1-0	3000
30-day	5-3-2	14000	5-4-4	35000	5-2-0	5000
40-day	5-2-0	5000	5-3-2	14000	5-4-0	13000
50-day	5-0-0	2300	5-2-0	5000	5-3-0	8000
Final	5-0-0	2300	5-0-0	2300	5-1-0	3000

APPENDIX P

Data from the Toxicity Analysis of the Samples from the Reactors

Initial Sample from Distillate Tank-Bottom Reactor

	Control	5.625%	11.25%	22.5%	45%	EC50
Start	90	85	91	88	83	
5 minutes	76	14	8	2	0	2.579
10 minutes	79	18	10	2	0	3.389

Initial Sample from Gasoline Tank-Bottom Reactor

	Control	5.625%	11.25%	22.5%	45%	EC50
Start	90	94	84	89	88	
5 minutes	72	59	39	24	12	14.174
10 minutes	75	61	41	25	8	13.277

Initial Sample from Mixed Tank-Bottom Reactor

	Control	5.625%	11.25%	22.5%	45%	EC50
Start	90	97	87	87	83	
5 minutes	79	25	14	4	1	3.568
10 minutes	83	29	15	5	0	3.6535

20-day Sample from Distillate Tank-Bottom Reactor

	Control	5.625%	11.25%	22.5%	45%	EC50
Start	90	93	79	104	85	
5 minutes	73	6	0	0	0	
10 minutes						

20-day Sample from Gasoline Tank-Bottom Reactor

	Control	5.625%	11.25%	22.5%	45%	EC50
Start	90	97	94	81	87	
5 minutes	64	59	47	28	17	21.588
10 minutes	62	55	42	23	11	16.760

20-day Sample from Mixed Tank-Bottom Reactor

	Control	5.625%	11.25%	22.5%	45%	EC50
Start	90	79	90	74	84	
5 minutes	78	24	19	8	4	3.581
10 minutes	79	26	20	8	3	4.398

40-day Sample from Distillate Tank-Bottom Reactor

	Control	5.625%	11.25%	22.5%	45%	EC50
Start	90	92	78	81	77	
5 minutes	73	14	6	2	0	2.183
10 minutes	71	14	6	1	0	

40-day Sample from Gasoline Tank-Bottom Reactor

	Control	5.625%	11.25%	22.5%	45%	EC50
Start	90	95	77	61	81	
5 minutes	75	72	52	32	30	36.36
10 minutes	75	70	47	26	22	24.722

40-day Sample from Mixed Tank-Bottom Reactor

	Control	5.625%	11.25%	22.5%	45%	EC50
Start	90	86	85	112	82	
5 minutes	74	22	14	11	3	3.057
10 minutes	74	23	14	10	1	2.729

Final Sample from Distillate Tank-Bottom Reactor

	Control	5.625%	11.25%	22.5%	45%	EC50
Start	90	74	85	105	125	
5 minutes	75	14	10	6	1	3.013
10 minutes	76	14	10	5	0	3.262

Final Sample from gasoline Tank-Bottom Reactor

	Control	5.625%	11.25%	22.5%	45%	EC50
Start	90	82	82	78	77	
5 minutes	72	56	49	34	22	27.020
10 minutes	76	58	49	33	20	22.827

Final Sample from Mixed Tank-Bottom Reactor

	Control	5.625%	11.25%	22.5%	45%	EC50
Start	90	86	91	104	78	
5 minutes	74	29	19	13	1	3.834
10 minutes	75	29	19	11	0	4.058

Phenol Standard

	Control	5.625%	11.25%	22.5%	45%	EC50
Start	90	68	59	58	63	
5 minutes	87	56	41	29	24	27.240
10 minutes	90	58	41	29	24	25.507

Phenol Standard

	Control	5.625%	11.25%	22.5%	45%	EC50
Start	90	89	94	83	79	
5 minutes	78	70	62	44	30	33.678
10 minutes	78	67	59	41	27	29.313

Neutralized Extraction Solvent

	Control	5.625%	11.25%	22.5%	45%	EC50
Start	90	93	102	102	95	
5 minutes	65	75	80	79	64	
10 minutes	58	65	70	71	60	

Neutralized Extraction Solvent

	Control	5.625%	11.25%	22.5%	45%	EC50
Start	90	95	94	98	100	
5 minutes	75	85	83	90	78	
10 minutes	71	78	77	81	73	

Sodium Acetate Solution

	Control	5.625%	11.25%	22.5%	45%	EC50
Start	90	87	94	88	88	
5 minutes	75	65	71	63	61	2420
10 minutes	70	59	66	59	56	864

Sodium Acetate Solution

	Control	5.625%	11.25%	22.5%	45%	EC50
Start	90	114	105	102	105	
5 minutes	72	89	79	784	70	216
10 minutes	71	89	79	73	69	115

VITA

Sasi Kumar Pullottupadath

Candidate for the Degree of

Master of Science

Thesis: BIOLOGICAL TREATMENT OF TANK-BOTTOM SLUDGES IN AEROBIC BATCH REACTORS

Major Field : Environmental Engineering

Biographical:

Personal Data: Born in Ernakulam, Kerala, India, On March 10, 1961, the son of Bhaskara Menon and Sarojini.

Education: Graduated from B.E.M High School, Palghat, Kerala, India, in May 1976; received Bachelor of Technology Degree in Civil Engineering from Indian Institute of Technology, Madras, India, May 1983. Completed the requirements for the Master of Science Degree at Oklahoma State University in July, 1995.

Experience: Worked as construction engineer and project engineer in different companies in Dubai, U.A.E, from 1983 to 1993.