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Use of Respirometry to Determine the Effect of Nutrient Enhancement on JP-8 Biodegradability

Christian T. Totten

USE OF RESPIROMETRY TO DETERMINE THE EFFECT OF NUTRIENT ENHANCEMENT ON JP - 8 BIODEGRADABILITY

THESIS

Christian T. Totten, Captain, USAF
AFIT\GEE\ENV\95D-17

DEPARTMENT OF THE AIR FORCE
AIR UNIVERSITY
AIR FORCE INSTITUTE OF TECHNOLOGY

Wright-Patterson Air Force Base, Ohio

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Thesis

Christian T. Totten, BS, MAS

Presented to the Faculty of the Graduate School of Engineering
of the Air Force Institute of Technology
In Partial Fulfillment of the
Requirements for the Degree of
Master of Science in Engineering and Environmental Management

Member

Member

Chairman

The views of this thesis are those of the author and do not necessarily reflect the views of the School of Engineering, the Air University, the United States Air Force, the Department of Defence, or any agency mentioned in the document.

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Abstract

The purpose of this study was to use respirometry to measure the impact of nutrient combinations or treatments on JP - 8 biodegradation under simulated fuel spill soil conditions. The study was designed observe one soil type at a constant fuel exposure level of 1% and a constant moisture of 60% of field capacity. Three experiments conducted were of two and three factor designs with potassium nitrate and sodium phosphate addition levels serving as two factors and fuel serving as a third factor in Experiments 1 and 2. Cumulative oxygen values obtained by the respirometer were assumed to be a direct indicator of JP-8 biodegradation. Therefore, cumulative oxygen values were used as the response variable for statistical analysis to determine the impact of nutrient enhancement on biodegradation of JP - 8. O_2 consumption and CO_2 production rate data were collected to ensure respiration activity levels were declining or stable to allow for cumulative oxygen data interpretation. Nutrient enhancement was found not to have a positive impact on biodegradation under these conditions. Additionally, losses due to degradation of the JP - 8 were found to be less than losses from volatilization.

USE OF RESPIROMETRY TO DETERMINE THE EFFECT OF NUTRIENT ENHANCEMENT ON THE BIODEGRADABILITY OF JP - 8 IN SOIL

1. INTRODUCTION

1.1 Origins of Jet Fuel (Martel, 1987)

The Heinkel He 178, which first flew on 27 August 1939, was propelled by the first successful turbine (jet) engine developed by Hans von Ohain. Because of its known performance and chemical characteristics, gasoline was the first fuel used. However, Ohain noted that designing a combuster that could utilize gasoline was a very difficult task. This led him to experiment with hydrogen because of its combustion qualities. Unfortunately, hydrogen is not a very stable fuel to store and handle, so it was obvious that other fuel alternatives would have to be pursued.

Sir Frank Whittle was another gas turbine engine pioneer working in England during the late 1930s and early 1940s. Whittle developed an engine which first took flight on a Gloster E28/32 aircraft on May 14, 1941. Gasoline was again considered, but because of its limiting characteristics as well as shortage during the war, other alternatives had to be pursued. Diesel fuel was the next option, however it was found that it could freeze during flight at extreme altitudes. The eventual fuel of choice was illuminating kerosene.

1.1.1 Air Force Jet Fuels (Martel, 1987)

Since these early days of gas turbine (jet) engine aviation, there has been a wide variety of fuels the Air Force has used to fuel its jet aircraft. During this infancy, jet engines were found to be more tolerant of fuels than their gasoline and diesel reciprocating engine cousins. However, the aircraft and engine fuel system components were found to be sensitive to chemical and physical properties of the fuel. This led the US Air Force and other North Atlantic Treaty Organization (NATO) air forces to develop strict specifications that fuels had to meet to insure proper aircraft performance for all stages of flight. In 1944, Jet Propellant (JP) - 1 was the first jet fuel developed according to military specification. Since then, seven additional JP class jet fuels have been developed through a better understanding of fuels technology and to meet the demand of changing aircraft and engine fuel system requirements.

As mentioned above, the first jet fuel used by the Air Force was JP-1 developed in 1944. The fuel was a Kerosene based fuel very similar to the illuminating Kerosene fuel first used by the British in their early jet engines. The similarity in fuel type was because the early US jet engine designs were based on the early British designs. However, JP-1 production efficiency was limited and as the US began to understand and develop its own jet engine technology, the evolution of Air Force jet fuels began.

JP-2 was developed in 1945 to help increase fuel availability due to limited JP-1 production efficiency. The fuel availability of JP-2 was increased because of its wide cut distillation process. A wide cut during the distillation of the crude oil used to create JP-2 was taken to capture both the naphtha (gasoline) and Kerosene portions of the distillate.

Unfortunately it did not meet viscosity and flammability specifications so it was limited to experimental use.

In 1947 the Air Force developed its second operation fuel. JP-3 was a wide-cut distillate fuel with vapor pressure characteristics similar to aviation gasoline. The high vapor pressure improved low temperature starting and high altitude relight. However, vapor lock and boil off problems were encountered because of high altitude flight.

JP-4 (NATO F-40) was the next operational fuel that entered service in 1951 and became the primary Air Force Jet fuel until recent replacement by JP-8. JP-4 was created using the wide-cut distillation process used in JP-2 and JP-3 production. JP-4 is typically broken down into 50 to 60 percent gasoline with the remainder being Kerosene. It retained similar low temperature starting characteristics, high altitude relight performance and availability as its predecessor. However it significantly improved over JP-3's boil off loss limitations.

JP-5 (F-44) was developed in 1952 for the Navy as a replacement for aviation gasoline that was being used at the time. The lead in the aviation gasoline was adhering to hot engine components resulting in performance problems. JP-5 was originally developed as a high flash point Kerosene that was to be blended with the aviation gasoline forming a mix similar to JP-4. However, the Navy found straight JP-5 to be an adequate fuel that met the unusual safety requirements of shipboard storage and use as well as met performance requirements for the unique aircraft missions.

JP-6 was developed specifically for the XB-70 program. It was very similar to JP-5 but with a lower freezing point and improved thermal oxidative stability. Fuel atomizer

nozzle fouling was found to occur, so fuels were developed to resist this tendency. This quality of the fuel to resist fouling is called thermal oxidative stability. The XB-70 program was short lived resulting in the discontinuation of JP-6 production.

In 1970, JP-7 was developed to replace the experimental fuel PF-1 that was used during the development of the SR-71. The SR-71's very unique role with mach 3+ cruising and high altitude surveillance missions called for an equally unique fuel. The result was a fuel with very low vapor pressure and excellent thermal oxidative stability. JP-7 was not produced by crude oil distillation as many of its predecessors. It was produced by blending special stocks that have had great care in aromatics removal. The result is a very low sulfur, nitrogen, and oxygen impurities but with poor lubricating abilities. Additives were used to improve fuel lubricity.

A characteristic of wide-cut fuels such as JP-4 is high volatility. This high volatility characteristic contributed to higher USAF aircraft losses during the Southeast Asian Conflict when compared to NAVY aircraft losses that used lower volatility JP-5 fuel. Post crash fires for aircraft using JP-4 was considerably higher than aircraft using Kerosene based fuels. Additionally, ground handling and storage is safer with low volatility Kerosene fuels. JP-8 was developed as an alternative to the primary jet fuel JP-4 to overcome the safety problems associated with high volatility while trading off slightly on aircraft performance and fuel availability . Since 1979 JP-8 has been the primary Air Force fuel used in the European theater. In the early 1990s Air Force bases in the continental United States began converting from JP-4 to JP-8 in a phased approach by region. It is currently both the NATO and USAF primary jet fuel.

The decision to replace JP-4 with JP-8 was primarily based on safety and NATO/US fuel standardization improvements. Environmental impact was considered on a broad scale basis addressing the three major areas of impact, air, water and land pollution. This broad brush impact analysis does not thoroughly investigate specific fate and potential remediation possibilities associated with accidental releases of JP-8.

1.2 Biodegradation and Bioremediation

An important aspect in understanding the fate of organic materials in the environment such as jet fuel, in particular JP-8, is their ability to be biodegraded. Biodegradation is simply a microbially mediated process of chemical breakdown of a substance to smaller products caused by microorganisms or their enzymes (Atlas and Bartha, 1993). It is greatly influenced by the nature and amount of the target compound present, environmental conditions, and certainly indigenous microbial communities present (Atlas and Bartha, 1993). In general, biodegradation can be divided into two broad categories, primary and partial biodegradation. Primary biodegradation is the substance going through single transformation while partial biodegradation can fall any where between two or more transformations to complete mineralization. Mineralization is the conversion of the organic material into carbon dioxide, water and other inorganic materials. Photooxidation and other abiotic mechanisms also play a role in such a transformations, however, mineralization gained through microbial activity is more significant (Grady, 1985). Biodegradation does not always lead to a benign outcome such as mineralization or some intermediate end point. In fact, a substance can be converted to a material more

toxic or less degradable than its original form (Grady, 1985). However, biodegradation will be considered as a process with a positive outcome in this thesis.

If an organic material is not susceptible to biodegradation, it can be classified as either recalcitrant or persistent. A chemical is considered recalcitrant if it is totally resistant to microbial attack. A persistent chemical fails to biodegrade under a specific set of conditions. It is not considered completely recalcitrant, however, certain conditions must exist in order for microbial attack to be successful.

As mentioned above, influences on successful biodegradability are nature and amount of the target substance, environmental conditions and the indigenous microbial communities. Of these factors, the most influential is nature of the substance and its relation to the microbial communities. The ability of the microbial population to break down a target material greatly depends on the population's familiarity with the substance. If the material has existed in the biosphere for millions of years through the evolution of many types of microbial communities, then it is likely there exists a microbial population that has developed the ability to break the material down. Fortunately many of the materials developed and used in industry are similar to compounds that are found naturally in the biosphere allowing microbes to be adapted to the substance to support successful biodegradation. However, there are many manmade substances that are not susceptible to microbial attack, so it is important to determine a material's biodegradive properties before a costly bioremediation project is started (Grady, 1985).

In order to increase the understanding and success of a target substance being biodegradable, studies using bioremediation technologies can be performed.

Bioremediation uses biodegradation as the primary tool for pollutant removal. Bioremediation techniques can be broken down into two large categories, nutrient enhancement or aeration, and introduction of xenobiotic microorganisms. Either techniques or a combination can possibly enhance the biodegradability of a target substance or pollutant. Bioremediation is popular because of the limited cost, manpower and equipment required to make it happen. With a large pollutant spill, cleanup could require manpower and equipment that a company or government agency might not be able to afford. Some drawbacks are that biodegradation is a slow process that is greatly influenced by environmental conditions and is limited to certain types of substances. However, for contaminated areas that have limited access, and if time is not critical in the cleanup process, bioremediation is a viable alternative (Atlas and Bartha, 1993). In order to apply them effectively, it is important to understand bioremediation techniques and their influences on biodegradation.

1.3 Respirometry

One of the oldest means of assessing and studying biodegradation of materials in an aerobic system is the measurement of oxygen consumption involved in the process (Tabak et al, 1989). Degradability of materials can be studied under conditions which avoid lack of nutrients, lack of oxygen, inadequate moisture levels, and imbalanced pH (Haug and Ellsworth, 1991). The temporal pattern of oxygen consumption can be used to determine rate constants for substrate decomposition. Metabolic respiration is measured

in these types of study. Any equipment used to study metabolic respiration is commonly referred to as a "respirometer".

The simplest form of respirometry is the Biochemical Oxygen Demand (BOD) bottle. An oxygen saturated solution of sample and water are placed in the bottle. Oxygen concentrations are measured over time to determine oxygen consumption. The draw back to this technique is that the oxygen resource is limited to that which can be dissolved in the solution water. Because of this, the sample quantity assessed is limited. In addition, solid samples are difficult to observe using this technique (Haug and Ellsworth, 1991).

Otto Warburg of Germany developed the first respirometer using manometry as the primary principle of its operation. He developed a 1-vessel manometric device to study the quantum requirement of leaves (Warburg, 1964). Since this development, respirometers have been modified to be used for a variety of purposes.

Respirometry has become a preferred method of bio-activity measurement because of its clear advantages. One example is the continuous and constant measurement of oxygen uptake that is achieved. Secondly, automation of newer apparatus allows for direct data output that can be readily recorded and processed for interpretation (i.e. tables or graphs). Dilution is generally not required, allowing measurement of activity in a more typical or natural state. Also, more samples and larger sample volumes can be observed. This improves statistical validity of the observed data and reduces data variability. Homogenous mixing can be achieved, providing uniform contact between microorganisms, substrates and oxygen. No chemical titrating is required. Respirometry is convenient for determining the effect different factors have on oxygen uptake, such as

substrate or nutrient types and concentrations, pH level, temperature, and a variety of other chemical and physical parameters. The time required to obtain useful information can be less than 5 days, the length of a typical BOD test. The test can be terminated at any recognizable point on the oxygen curve, saving resources and time. The bottom line is that respirometry is ideal for small scale bioremediation research because of the ease and control one has over the experiment parameters (Tabak et al, 1989).

1.3.1 The Micro-Oxymax Computerized O_2/CO_2 Respirometer

The Micro-Oxymax respirometer, the system used in this study, measures very low levels of O_2 consumption and CO_2 production. It is used to measure respiration in a wide variety of applications from microbial to small animal or fish activity. The Micro-Oxymax samples air in single or multiple measuring chambers in which the organism of interest is placed. The air is dehumidified and passed by highly sensitive O_2 and CO_2 sensors for measurement and is then returned back to the measuring chamber. Therefore the system is completely closed. However, the operator has the option to "refresh" the subject chamber air by configuring the system to refresh after a set number of samples taken or by O_2 or CO_2 percentage levels. The measured gas concentrations, sampling time, and measured volume of the measuring chamber are used to determine O_2 consumption and CO_2 production rates. If multiple chambers are used, each sample is taken individually. The system is automated by computer to allow continuous sampling to be performed as well as allow the operator to set sample intervals or length of time

between samples. The automation feature and ability to measure very low gas concentrations makes this an ideal device to use for bioremediation studies.

1.4 Specific Problem

The purpose of this thesis is to study the biodegradability characteristics of the Air Force's newest operational jet fuel, JP-8, using the nutrient enhancement bioremediation technique on contaminated soil under simulated spill conditions. Very little is understood or documented about JP-8's biodegradability characteristics and nutrient enhancement impact on these characteristics. The Micro-Oxymax respirometer will be used to measure microbial respiration of contaminated and non contaminated soil with varying levels of nutrient enhancement. A relative comparison of microbial respiration rates will be performed to determine the impact various levels of nutrients have on microbial activity. These respiration rates will be assumed to be correlated to microbial attack and biodegradation of the JP-8.

1.5 Research Objectives

1. Determine Nitrate and Phosphate enhancement interaction and individual impact on soil microbial respiration and JP-8 biodegradation under simulated spill conditions.
2. Estimate Percentage of JP-8 biodegraded using cumulative oxygen values as an indicator.
3. Account for losses of JP-8 due to volatilization.

4. Mass balance nitrate and phosphate changes and compare to cumulative oxygen values.

5. Estimate the amount of carbon utilized in biomass construction and other chemical reactions not recovered in CO_2 production.

6. Understand operating principles of the MicroOxymax® respirometer.

1.6 Scope of Study Limitations

1. Single soil type to be used throughout investigation.

2. Single soil moisture field capacity level used throughout investigation.

3. Single fuel level exposure will be studied.

4. Assume limiting nitrate and phosphate soil conditions.

5. Assume aerobic conditions exist for all processes

6. Assume uniform soil conditions for samples.

1.7 Summary and Overview

JP - 8 is the Air Force's newest operational jet fuel. During usual operations there is always a potential for an accidental spill. A simple technology of bioremediation, utilizing nutrient enhancement, can be an effective clean up tool under the right conditions with the right target compound. Chapter I discusses the history of JP-8 and what little is understood about its biodegradation characteristics. Microbial respiration can be an effective and simple method to study these characteristics. Chapter I concludes

with tying these elements together in a proposal to study nutrient impact on JP - 8 biodegradation by measuring microbial respiration.

Chapter II discusses research related to bioremediation of hydrocarbons and its advantages. Also, major factors impacting biodegradation are discussed. And finally, examples of how measurement of microbial respiration can be effective in bioremediation studies are presented.

Chapter III presents the methods and materials used throughout this study. It provides details on respirometer operating principles and describes specific experimental set ups. It also discusses the statistical design of each experiment and hypotheses tested.

Chapter IV presents the results of each experiment. It displays respirometer generated cumulative O_2 and CO_2 curves and associated consumption and production rate curves. The statistical results and analysis are presented. Correlational studies, vapor recovery information, and estimated biodegradation levels of JP - 8 are presented.

Chapter V summarizes the research and discusses findings and recommendations.

2. Literature Review

2.1 Introduction

Several technologies exist for cleaning up hydrocarbon contaminated soils. These technologies can be divided into two groups, in situ or ex situ. Ex situ or off site techniques include excavation of the contaminated soil and its disposal or incineration. In situ or on site techniques include low temperature desorption, in situ vapor recovery, containment using slurry walls and caps and of course, bioremediation. Bioremediation, in particular, has clear advantages over other in situ remediation techniques as well as ex situ options (NRC, 1993). Table 2-1 lists these advantages. Probably the most attractive

Table 2-1 Bioremediation Advantages (Baker, 1994; King, 1992)

Onsite Application
Undisturbed Environment
Eliminates transportation Costs and Liabilities
Eliminates Waste Permanently, Does Not Transfer to Another Medium
Eliminates Potential Long Term Liability
Attractive Economics
Can Be Coupled with other Treatment Technologies
Minimal Exposure to On-Site Workers
Long Term Protection of Public Health

advantage is cost. Ex situ soil remediation costs are estimated by the EPA to be as high as $100,000/acre (Hardy, 1994). This does not include excavation and other costs associated with site cleanup.

Hardy and Mabbula (1994) report that Hardy Environmental Services used In Situ bioremediation techniques to successfully close two sites in Delaware, one contaminated with gasoline and the other with diesel fuel. The gasoline contaminated site in Wilmington, Delaware was remediated in 17 weeks from 362 ppm TPH and 641 ppm BTEX to non detect of either contaminant. State groundwater gasoline protection limits of 100 ppm for TPH and 10 ppm for BTEX were used as a goal because of the impact contaminated soil can have on groundwater quality. The Fenwick Island site remediated 3,492 ppm TPH diesel contaminated soil to 580 ppm TPH. This was below the state diesel groundwater requirement of 1,000 ppm. BTEX is not required for diesel fuels. Each site was estimated to cost $3,060 and $2,750 respectively. These costs were well below the EPA estimate, making bioremediation appear to be a very attractive and effective remediation technique.

Bioremediation and environmental fate qualities of complex jet fuels must be understood in order to take advantage of the benefits discussed above. An understanding of complex jet fuels starts with their physical properties. The fuel may degrade, sorb to soil, or chemically or biologically degrade (Dean-Ross et al, 1992). JP-8 is currently the Air Force's primary jet fuel. Its characteristics in the environment and how they can be influenced are not widely documented and not well understood. The biodegradability of

JP-8 is of interest as a viable and inexpensive method for remediation of potential future spills.

2.2 Regulatory Influences

In 1976, the Resource Conservation and Recovery Act (RCRA) and the Toxic Substances Control Act (TSCA) established regulatory requirements that focused on soil and water resources and their environmental management. In 1980, the Comprehensive Environmental Response, Compensation and Liability Act (CERCLA) with the Superfund Amendments and Reauthorization Acts dealt with costs and associated clean up measures for existing contaminated sites. Additionally, the passage of the National Environmental Policy Act (NEPA) reflected further concern for our environmental future (King, 1992).

One of the primary concerns of spills is the potential contamination of ground water that may be used as or connected to drinking water sources. Because of this possibility, Maximum Contaminant Levels established for specific contaminants in the Safe Drinking Water Act are often used for remediation goals (Rittmann et al, 1994).

Each of these acts increased responsibility on the parts of previous waste generators as well as increased the awareness of the public. Long-term liability and cleaning up past problems were becoming a reality, especially to protect public health. With these changes in the environmental arena, remediation technologies to solve clean-up problems were needed. Cost effective methods that eliminate the problem while minimizing worker and public exposure were developed to meet the requirements of these regulations.

Bioremediation has become one of the most cost effective and viable alternatives for remediation.

2.3 Microorganisms

Autotrophic organisms such as plants and algae need sunlight for growth and energy. They will not metabolize in the dark, rendering them relatively useless in subsurface soil environments. Protozoans or single celled animals require high amounts of oxygen and water while grazing on other microbes, hence, they also provide little help. Heterotrophs such as bacteria and fungi derive energy from oxidation-reduction reactions making them ideal for hydrocarbon degradation in subsurface environments (King, 1992)

Bacteria are thought to have been in existence for 3 billion years with 85 percent of the types that exist today being developed before the separation of the continental plates (NRC, 1993). These microscopic organisms have been breaking down plant and animal wastes throughout this existence that would otherwise be trapped in detritus. The microbes have developed enzymes specifically suited for the breakdown of such wastes. Without these biodegradive capabilities, waste would build up and nutrients contained in these wastes would be trapped, unable to cycle usefully (NRC, 1993).

Sterile naturally occurring environments are not known to exist (King, 1992). With the exception of active volcanoes, microbes are virtually found everywhere in the environment and as Beijerinck stated, "everything is everywhere, the environment selects." Microbial populations are mixed and very diverse. Diversity is extremely important to allow a more robust and stable biodegradation process (NRC, 1993).

Diverse species will have microbial specialists that can work with high and low contaminant concentrations, pH extremes, toxic metals and solvents (NRC, 1993). Because of this ubiquity and diversity of microbes, they can work in concert to make biodegradation possible at just about any clean-up site.

Of the types of microbes that can exist in soil, bacteria and fungi account for practically all hydrocarbon degradation (King, 1992). The problem then becomes getting the bacteria and fungi to do what is require to the extent required.

The key to biodegradation is influencing the indigenous microbes to breakdown the target pollutant. Microbes can be influenced by aeration, nutrient addition, or addition of other chemicals required to stimulate metabolism. The limiting factor in biodegradation is the microbe's ability to use available resources to attack and metabolize the target pollutant. Additionally, the measurement of microbial activity or microbial by-products are good indicators of successful bioremediation (NRC, 1993).

2.3.1 Microorganism Acclimation (Lag Period)

Often microorganisms are not prepared to begin immediate degradation of a target compound. A contaminant introduced to the soil is an abrupt disruption of the microbial environment. Microorganisms are very sensitive to these disruptions and require time to adjust to the contaminant, assuming they are able to biodegrade the material. This adjustment time is known as an "acclimation" period or lag time during which the microbial growth and metabolism is temporarily interrupted (King, 1992). Depending on

the contaminant, concentration, and environmental conditions, this lag period could last from 1 hour to several months (Alexander, 1994).

If a hydrocarbon fuel is spilled on uncontaminated soil, the indigenous microbial community has likely not been exposed to this type of chemical before. Hydrocarbons provide a potential carbon substrate source to the indigenous microorganisms, causing them to make internal enzymatic changes to allow use of the new food source. The population makes these enzymatic changes to either attempt to transform the hydrocarbon perceived as a toxic threat or mineralize the hydrocarbon for energy and growth (King, 1992).

When detectable biodegradation begins, the acclimation period is considered over. The rate of metabolism at this point may be rapid or slow, but a second addition of the contaminant during this period will require little to no acclimation (Alexander, 1994). The population will maintain raised activity for some time, relative to similar soils that have not been contaminated and adjusted to the hydrocarbon contaminant source (Alexander, 1994).

2.3.2 Enzymes

Bacteria have developed the genetic capability to degrade materials through billions of years of exposure and evolution. Despite this inherent capability, the environment has a tremendous impact on whether biodegradation occurs. Several situations must exist to ensure biodegradation of a material: 1) a capable organism must be present, 2) an opportunity must exist for requisite enzymes to be synthesized, and 3) environmental

conditions must be sufficient for enzymatically catalyzed reaction(s) to proceed at a significant rate, 4) the molecule must be in a form that is available for microbial utilization, 5) and if the enzyme is inducible, conditions must allow induction to happen (Grady, 1985: Alexander, 1994). Additionally, to understand xenobiotic biodegradation, it must be known if bacteria can synthesize enzymes in the presence of a recognizable substrate and if their genetic capability responsible for enzyme synthesis has evolved over time to adjust to its environment (Grady, 1985). Fortunately enzymes are only specific in their catalytic function.

Enzymes are temperature sensitive, organic catalysts produced by living cells to catalyze highly specific reactions. Enzymes can function inside or outside cells. Enzymes secreted by the cell are extracellular enzymes and those associated with the protoplasm of the cell and function within the cell are intracellular enzymes. Enzymes are further categorized as being continuously produced (constitutive) and produced on demand (inducible) (Sawyer et al, 1994). Inducible enzymes are particularly of interest in bioremediation because these are the enzymes produced when a cell is exposed to an unfamiliar organic material. Many of the enzymes involved in the early stages of the breakdown of synthetic materials are inducible enzymes (Alexander, 1994).

As stated above, an enzyme is produced for specific catalytic function. However, depending on the enzyme, they are much less specific with respect to the type of substrate used for a specific function (Grady, 1985). The enzyme may bind to a natural substrate that has xenobiotic materials contained in it. If the charge makeup of the active site is not greatly altered by the presence of the xenobiotic material, the enzyme may use its specific

reaction to catalyze the xenobiotic substrate in addition to the natural substrate. This quality, known as gratuitous biodegradation, is important to xenobiotic biodegradation (Grady, 1985).

The ability of the xenobiotic compound to induce the production of the requisite enzyme for catalyzation is very important. In some instances the substrate is required in some threshold amount in order to induce enzyme production (Alexander, 1994). Similarity between natural and xenobiotic structures is critical for this induction. As structures become less similar, the ability of the xenobiotic to induce the requisite enzyme decreases, thus lowering the chances of biodegradability. In general, if the xenobiotic material does not induce the required enzymes, then biodegradation is reliant on the presence of a natural substrate for induction (Grady, 1985).

The effect an enzyme has on a xenobiotic substrate may not always be positive. Potentially there is a chance an intermediate material may be formed by the enzyme that is toxic to the organism or recalcitrant to further breakdown. This leads to the possibility of the newly formed material building up in the environment. However, xenobiotic materials generally are converted into intermediate substrates that induce the production of other enzymes that will further break down the xenobiotic. Additionally, some xenobiotic materials will induce the production of several enzymes that will be used throughout the breakdown process. In fact, because the set of enzymes were induced by the original xenobiotic material, it is likely that several of the created enzymes will aid in the breakdown of the intermediates created from the original material. This demonstrates the importance of several enzymes working in concert with each other to break down a

material and its intermediates. Biodegradation is not a one step process. It is easy to see why having many types of organisms is beneficial to the biodegradation of a material. Many organisms in concert with each other can create the needed enzymes to break down a xenobiotic and its intermediates. One organism can create enzyme A to initiate the transformation, and another organism may create enzyme B to further breakdown the product of the first organism and so on (Grady, 1985). If an intermediate compound accumulates it is because there is not an enzyme present to biodegrade it leading to the possibility of a particular organism or group of organisms being absent (King, 1992). If the intermediate material is hazardous enough, introduction of the missing organism or organisms may be worth the effort.

2.4 Metabolic Pathways (Aerobic)

Non-photosynthetic microorganisms such as bacteria and fungi require energy yielding oxidation-reduction reactions to provide them with energy . The carbon source and metabolic pathway used determines the amount of energy the microorganism will gain from such reactions (King, 1992). Aerobic degradation, anaerobic degradation, and fermentation are the three metabolic pathways microorganisms use to reduce or transform hydrocarbons to gain energy.

The aerobic pathway is the most efficient of the three pathways and when O_2 is available it is used above the other pathways (King, 1992). Oxygen is used in this pathway as an electron acceptor. A suitable substrate is oxidized by the microorganisms releasing the electron to the oxygen. Two reactions describing this process are as follows (See Figure 2-1):

$$C_nH_{n+2} + 3n+1/2O_2 \longrightarrow nCO_2 + n+1H_2O$$

OR

$$\text{Substrate} + O_2 \longrightarrow \text{Biomass} + CO_2 + H_2O + \text{Other Organics (King, 1992)}$$

In general, these reactions demonstrate the primary products to be CO_2 and H_2O. The conversion of a substrate to these compounds is called *mineralization.* This would be the ideal outcome of any bioremediation project because the final products are completely benign. However, when dealing with complex organic compounds, mineralization may not always occur. Incomplete degradation called *transformation* may be the final result (Baker, 1994). This may be advantageous because the final product may be more benign than the original product and hence may not be of environmental concern. On the other hand, the possibility exists that the material is more toxic than the original. This is generally not a problem when dealing with petroleum hydrocarbons. However it is important to note that disappearance of the target compound may not be giving the complete picture during a remediation project.

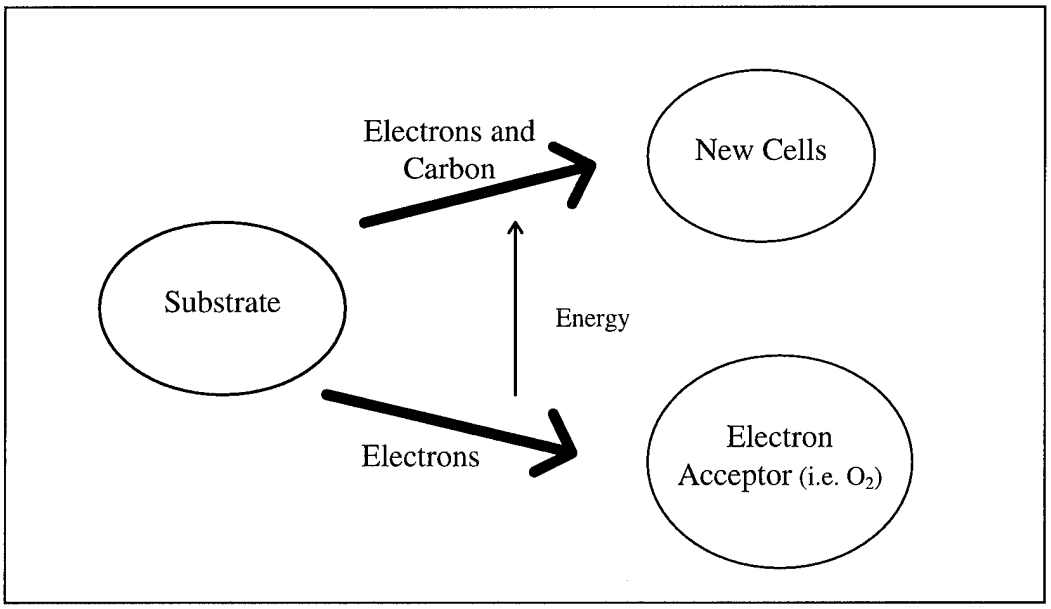


Figure 2-1 (NRC, 1993) Aerobic Biodegradation. Microbes gain energy from breaking chemical bonds. The energy is used to convert other electrons and carbon into cell biomass.

This process of using O_2 to aid in the destruction of an organic compound is called *aerobic respiration* (NRC, 1993). In the reaction described above, the contaminant loses electrons and is therefore oxidized while the oxygen gaining the electron is reduced. The contaminant is the electron donor while the oxygen is the electron acceptor. As electrons are released, energy is released which is used by the microorganism to convert some of the available carbon to biomass. The rest of the carbon not converted to biomass is oxidized to CO_2. Additionally, the remaining oxygen is reduced to H_2O (NRC, 1993).

2.4.1 Metabolism of Petroleum Hydrocarbons and JP-8

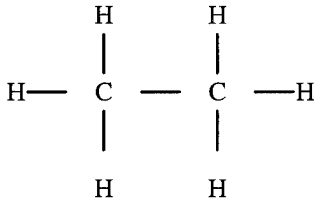
JP-8 (MIL-T-83133B) is a 99% kerosene based jet fuel which is very similar to commercial jet A-1 fuel but with corrosion inhibitor, lubricity improver, and fuel system icing inhibitor additives making up the other 1%. JP-8 has a minimum flash point of 37.8

°C, a maximum aromatics content from 20 - 25%, and a maximum freeze point of -47 °C (Martel, 1987). With JP-8 primarily comprised of kerosene, the physical and biodegradability characteristics of kerosene must be understood. The degradability of jet fuels similar to JP-8 have been demonstrated (Song and others, 1990; Turney and others, 1991). Jet fuels in general are relatively less persistent than other hydrocarbon contaminants such as Bunker C, diesel oil, and heating oil, but more so than gasoline (Autry, 1992)

Kerosene is a complex petroleum hydrocarbon. Petroleum hydrocarbons are divided into three major categories: aliphatic, alicyclic, and aromatic hydrocarbons. Each of these categories can be further divided into sub-categories depending on a variety of properties that will be discussed.

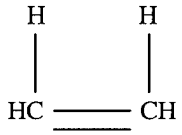
Aliphatic hydrocarbons are straight or branched chains of carbon atoms with the carbon valency requirements being met by hydrogen. They are identified with the empirical formula C_nH_m. Depending on the number of carbon-carbon bonds (saturation), aliphatics can be categorized as alkanes, alkenes, and alkynes (Baker, 1994). Figure 2-2 illustrates examples of aliphatic hydrocarbons.

(1) Alkanes C_nH_{2n+2}



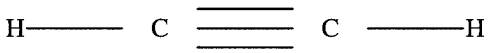
C_2H_6

(2) Alkenes C_nH_{2n}



C_2H_4

(3) Alkynes C_nH_{2n-2}

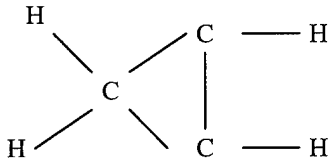


C_2H_2

Figure 2 - 2 (Baker, 1994) Aliphatic hydrocarbons. They are categorized by single, double or triple carbon bonds.

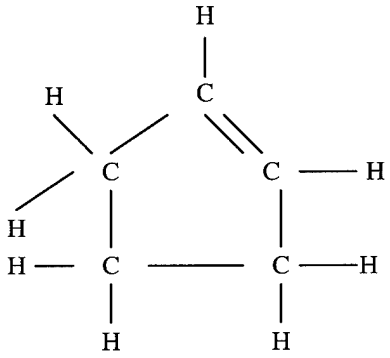
Alicyclic hydrocarbons are characterized by the presence of a carbon ring structure. They can be divided into three subcategories including cycloalkanes, cycloalkenes, and cycloalkynes. The presence of single, double and triple bonds distinguish three subcategories similarly to the aliphatic subcategories (See Figure 2-3).

(1) Cycloalkanes C_nH_{2n}



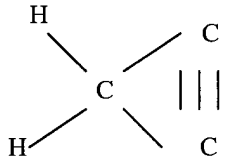
C_3H_6

(2) Cycloalkenes C_nH_{2n-2}



C_5H_8

(3) Cycloalkynes C_nH_{2n-4}



C_3H_2

Figure 2 - 3 (Baker, 1994) Examples of alicyclic hydrocarbons.

While aliphatic and alicyclic hydrocarbons are somewhat similar in structure, aromatic hydrocarbons are a completely separate category of hydrocarbons. The aromatics are based on the C_6H_6 benzene ring structure. Aromatics are divided into unsubstituted and substituted categories (Baker, 1994). Unsubstituted structures like anthracene are simply

combinations of the benzene ring. Substituted structures may have additional compounds (i.e. OH, CH_3) attached to the ring.

In general, kerosene is primarily comprised of aliphatic alkane hydrocarbons. Both normal and branched chains predominantly 11 to 12 carbons in length exist, however, there are usually more normal chains. Some alicyclic hydrocarbons exist in the form of cycloalkanes, aromatic and mixed aromatic cycloalkanes. Aromatics are represented in trace amounts by unsubstituted benzene and polyaromatic hydrocarbons (Baker, 1994). This complex mixture of hydrocarbons does not lend itself to any easy explanation of the metabolic pathways involved in kerosene biodegradation. A single pathway cannot describe the whole process. The following pathway descriptions only describe the process in general terms for the major components of kerosene discussed above. Before studying these pathways, the following "rules of thumb" are important to note (Baker, 1994)

1. Aliphatics generally degrade easier then aromatics.
2. Straight chained (normal) aliphatics degrade easier than branched chains.
3. Double and triple carbon-carbon bonds hinder degradation relative to single bonds.
4. Optimal chain length is between 10 and 20 carbons. Generally 9 carbons or less are difficult to degrade because of toxicity.

2.4.1.1 Alkanes (Atlas, 1993; Baker, 1994; Rittmann, 1994)

For alkanes with four or more carbon atoms, structure can begin to vary greatly. Structurally different molecules with identical composition are isomers. As the number of carbon atoms increase, so do the number of possible isomers. Fortunately the number of isomers that can occur only do so in small quantities. Hence, as previously discussed, alkanes can be broadly categorized as normal (*n* - alkanes) or branched alkanes. Normal alkanes are more readily degradable than branched alkanes and are degraded primarily by terminal methyl group oxidation. Subterminal oxidation is a secondary minor side reaction catalyzed by bacteria that also contribute to the biodegradation of the *n* - alkane.

Terminal methyl group oxidation biodegradation is initiated by monooxygenases or dioxygenases enzymes attacking the terminal methyl group (CH_3). A three step reaction performs the initial oxidation beginning with one atom of O_2 oxidizing the hydrocarbon forming an alcohol and the other forming H_2O with the reduced form of nicotinamide dinucleotide phosphate ($NADPH_2$) serving as an electron donor. The second step is further oxidation of the alcohol to an aldehyde and thirdly the aldehyde to a fatty acid.

The fatty acid is further catabolized via the metabolic pathway known as β - oxidation since the second carbon is modified in the process. The fatty acid is converted to the acyl coenzyme A form from which an acetyl CoA group is cleaved off, shortening the fatty acid chain by two carbon units. The cleaved acetyl CoA group is finally converted to CO_2 by the tricarboxylic acid (TCA) or Krebs cycle. The remaining fatty acyl-CoA molecule serves as the substrate for subsequent β - oxidation reactions. The following equations illustrate the initial and β - oxidation reactions.

Initial Oxidation

$$R - CH_2 - CH_3 + O_2 + NADPH_2 \longrightarrow R - CH_2 - CH_2 - OH + NADP + H_2O$$

$$R - CH_2 - CH_3 + O_2 \longrightarrow R - CH_2 - CH_2 - OOH$$

$$R - CH_2 - CH_2 - OOH + NADPH_2 \longrightarrow R - CH_2 - CH_2 - OH + NADP + H_2O$$

β - Oxidation

$$R - CH2 - CH2 - CH2 - COOH \xrightarrow{+CoH}$$

$$R - CH_2 - CH_2 - CH_2 - \overset{\overset{O}{\|}}{C} - CoA \xrightarrow{-2H+}$$

$$R - CH_2 - CH = CH - \overset{\overset{O}{\|}}{C} - CoA \xrightarrow{+H2O}$$

$$R - CH_2 - \underset{\underset{O}{|}}{CH} - CH_2 - \overset{\overset{O}{\|}}{C} - CoA \xrightarrow{-2H+}$$

$$R - CH_2 - \underset{\underset{O}{\|}}{C} - CH_2 - \overset{\overset{O}{\|}}{C} - CoA \xrightarrow{+CoA}$$

$$R - CH_2 - \underset{\underset{O}{\|}}{C} - CoA + CH_3 - \overset{\overset{O}{\|}}{C} - CoA$$

Acetyl CoA

↓

Tricarboxylic Acid Cycle

In subterminal oxidation, hydrocarbons do not have a terminal methyl group and must be attacked subterminally. Oxygen attaches to an internal carbon atom as opposed to a carbon atom at the end of the chain. First, a secondary alcohol is formed from this reaction which oxidizes to a ketone and then finally an ester. The ester bond is cleaved leaving a primary alcohol and a fatty acid. The alcohol portion is oxidized to an aldehyde and finally to a fatty acid. The original fatty acid and the alcohol derived fatty acid are both β - oxidized similarly to the alkanes and finally converted into CO_2.

Biodegradation of branched alkanes are initiated in the same manner as *n* - alkanes. However, the result of this initial oxidation is branched alkanoic acid. Even though it is a poor substrate for the enzymes of β - oxidation, β - oxidation is the metabolic pathway used. Additionally, branching reduces pathway efficiency.

2.4.1.2 Alicyclic Hydrocarbons (Rittmann, 1994)

Alicyclic hydrocarbons are similar to aromatic hydrocarbons in that they can either be substituted or unsubstituted. Each type is degraded in a separate manner from the other.

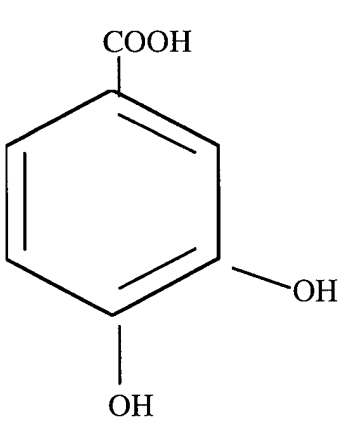
Unsubstituted alicyclic hydrocarbons do not have a terminal methyl group and must be degraded subterminally. Substituted alicyclics do have a terminal methyl group. For these hydrocarbons biodegradation can occur subterminally, similar to the unsubstituted alicyclics, or by terminal methyl group oxidation similar to *n* - alkane degradation.

2.4.1.3 Aromatic Hydrocarbons (Atlas 1993, Baker 1994)

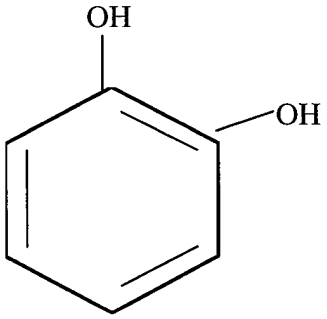
The nucleus of the aromatic compounds are opened either by *ortho-cleavage* or the *meta-cleavage* pathway. Initially the aromatic rings are changed to catechol and protocatechuate (See Figure 2-4) which are the starting substrates for cleavage leading to subsequent metabolic pathways.

The catechol and protocatechuate are aromatic nuclei with two adjacent carbons bearing hydroxyl groups. *Ortho-cleavage* introduces oxygen between these two hydroxyl groups to open the ring structure. The ring goes through a series of metabolites eventually forming acetyl-CoA and succinate. These two products can be converted to CO_2 through the TCA cycle.

Meta-cleavage introduces the oxygen into the ring adjacent to the hydroxyl groups opening the ring between a hydroxylated carbon and an adjacent unsubstituted carbon. The process produces acetaldehyde and pyruvate via a series of metabolites. These final products are further converted to CO_2 via the TCA cycle similarly to *ortho-cleavage*.



Protocatechuate



Catechol

Figure 2 - 4 The initial substrates for oxidative ring cleavage and subsequent pathways.

2.5 Environmental Factors Influencing Biodegradation

To this point, microorganisms and related microbiological functions and metabolic pathways associated with biodegradation have been discussed. Of course these functions and pathways are critical to understanding and predicting the success of biodegradation. However, these factors do not provide the complete picture of the biodegradation process. Environmental factors play a critical role in the success of biodegradation. Unfavorable environmental factors can greatly influence the numbers and activity of microorganisms. Some factors that can be modified relatively easily are soil moisture, soil pH, temperature, levels of inorganic nutrients, levels of electron acceptors, and types and levels of present carbon substrates (Baker 1994). The following is a discussion of each of these factors. Since oxygen is not considered to be limiting and the JP-8 is the present

carbon source, electron acceptors and carbon substrates will not be discussed in this section.

2.5.1 Soil Moisture

Soil moisture influences qualitatively and quantitatively all physical, chemical, and biochemical processes in soil (Harder, 1991). Water is necessary for the physiological requirements of microorganisms as well as metabolic by-product transport to and from microorganisms (Baker, 1994). Microbes lack mouths, therefore they depend on the solubility of the substrate so the material can be transported across cell membranes into the intercellular fluid where digestion takes place (King, 1992). The water insolubility of hydrocarbons make this process difficult. Microorganisms live in water, hence the hydrocarbon biodegradation must occur at the oil/water interface. Yeast and bacteria develop thick glycolipid rich cell walls when growing on hydrocarbons, and the hydrocarbons are soluble on these walls (Bleckmann, 1995). In general, water is a good thing, but like all good things, too much or too little can cause problems. To much water can saturate the soil and cause an anaerobic environment to develop. Biodegradation can still occur in the environment using nitrate as an electron acceptor, however, this process is much less efficient than the aerobic process.

R.M. Atlas suggests an optimal soil moisture range of 50 - 80% field capacity. Others have suggested more liberal ranges of 30 - 80% (Baker, 1994) and 30 - 90% (Dibble, 1979). Additionally, The EPA suggests 25 - 85% field capacity (Sims, 1989). All of these ranges are similar. It is important to note that field capacities usually range from 5 -

40% of the total soil weight. Hence, 50 percent field capacity would be 2.5 to 20% total moisture by weight. Field capacity is the amount of water retained by capillary action after gravitational water has drained out (Baker, 1994).

As the moisture content increases it adversely affects oxygen availability. Since both are critical, an optimal combination must be found (Harder, 1991). Oxygen uptake of oil contaminated and uncontaminated soil was found by Stegmann et al (1991) to be optimal at 60% field capacity. Moisture was found to be limiting if it dropped below a certain threshold. This is important due to bacterial mobility reduction and potential microorganism desiccation. This is less critical to fungi who can bridge air filled pores with hyphal growth (Harder, 1991). In general, moisture is an important factor that must be considered and, if at all possible, modified for optimal conditions.

2.5.2 Soil pH (Baker, 1994)

Microorganisms are sensitive to soil pH levels, so it is not surprising that bioremediation is impacted by pH. Fungi are usually less sensitive to pH levels than bacteria (Sims, 1989). Neutral or near neutral pH values typically support the fastest biodegradation of contaminants.

Soils world wide are acidic, therefore soil pH adjustment might be prudent for biodegradation improvement. A typical pH treatment method is liming. Soil lime requirements to raise pH are influenced by soil properties such as clay level, soil texture, etc. Existing soil pH and soil buffering capacity are critical influences and must be understood when modifying pH.

2.5.3 Soil Temperature (Baker, 1994)

Temperature affects biodegradation directly and indirectly. Changes in temperature directly affect microbial activity. It is generally observed that microbial activity increases with increasing temperature. Indirectly, temperature affects the soil matrix and the physiochemical state of the contaminants. It can influence soil volumes, oxidation-reduction reaction rates and water structure. Additionally, temperature changes occur both from seasonal changes as well as positional changes in the soil. EPA suggests 15 - 45 ^{o}C as an optimal range.

2.5.4 Inorganic Nutrients

Regardless of how a microbe breaks down a material, its cellular components are relatively fixed. Typically, 50% carbon, 14% nitrogen, 3% phosphorus, 2% potassium, 1% sulfur, 0.2% iron, and 0.5% of magnesium, calcium and chloride make up a bacterial cell. All are required and if any are in short supply or not available, competition will occur for the limited nutrients (hindering biodegradation of a carbon source) or degradation will not occur at all (NRC, 1993). While each of these elements are required for cell building, N and P are usually limiting with the other elements existing in sufficient quantities (King, 1992; Fredrickson et al, 1993; Tabak et al, 1990a; Dibble and Bartha, 1979). Rarely do the addition of elements other then P, N and O_2 stimulate biodegradation in naturally polluted environments (Alexander, 1994).

Biodegradation continues at a slower rate in the absence of added N and P. Because of its limited availability, biodegradation of oil and individual hydrocarbons are usually stimulated by N and P addition. Increased stimulation can occur immediately or may take time to become obvious. However, in some cases where N and P are sufficiently available and carbon is limiting, additions may be without benefit (Alexander, 1994).

Both nitrogen and phosphorus are important because of the critical roles they play in cell building. Nitrogen is required for synthesis of cellular proteins and cell wall components while phosphorus is needed for nucleic acids, cell membranes and ATP (Baker, 1994).

2.5.4.1 Liebeg's Law and Nitrogen/Phosphorus Availability

Liebeg's law of the minimum states that the yield or biomass of any organism will be determined by the nutrient present in the lowest (minimum) concentration in relation to the requirements of that organism (Atlas, 1993). In relation to nitrogen and phosphorus availability, either or both can be limiting in a soil ecosystem. Depending on which element is limiting, background soil conditions greatly impact whether the addition of N or P or both is required. For example, if N is limited and P is added, there will probably be no benefit. If P is limiting and P is added, it could be added to the point that N becomes limiting and so on. Hence, it is prudent to investigate combinations of N and P in order to determine at which point the target contaminant is the limiting component. A C:N:P ratio of 25:1:0.5 is cited by Baker, a C:N 10:1 and C:P 50:1 is cited by Alexander, and the EPA recommends 120:10:1 (Sims, 1989). These ratios are estimates that can be

used for predicting total amount of N and P required to destroy the target compound. However, maximum degradation rates may or may not be affected by nutrient levels, and therefore these ratios do not predict a maximum rate.

Nitrogen exists in soil in both organic and inorganic forms. Ammonia, nitrate, nitrite, nitrous oxide and nitric oxides are the inorganic forms with amino acids (proteins), free amino acids, and amino sugars making up the organic forms. Microorganisms prefer a reduced form of ammonia (ammonium ions) for assimilation and therefore tend to convert other organic and inorganic forms of nitrogen into ammonia (Baker, 1994). It is important to note that though ammonia is the preferred form of nitrogen, it is not very mobile and can become trapped in clay lattices. Fortunately, specific bacteria can gain energy from converting ammonia to nitrite and finally nitrate, increasing its mobility and hence, its availability. This also increases the possibility of leaching out of the soil and possibly reducing availability (Bleckmann, 1995). Additionally, moisture can affect the form of nitrogen because if anaerobic conditions occur, denitrification can occur.

Organic forms of phosphorus are generally found in humus while inorganic forms are combined with Fe, Al, Ca, F and other elements. These inorganic forms are not very soluble and tend to precipitate out. Additionally, they tend to react with clay and become bound into insoluble clay-phosphate forms (Baker, 1994). For these reasons phosphates tend to be limiting in natural soil, making addition a prudent alternative.

2.5.4.2 Nutrient Case Studies

Many studies have been performed to understand the impact nutrient supplementation has on soil recovery following an introduced stress such as a hydrocarbon spill. Tabak et al (1990b) demonstrated almost complete alkane utilization and significant polycyclic aromatic hydrocarbon biodegradation using Inipol EAP 22 fertilizer on Alaskan weathered crude oil constituents from the Exxon Valdez spill. Mueller studied various nutrient combination impact on the same Alaskan crude oil using a MicroOxymax® respirometer. Nutrient additions were found to enhance respiratory activity (BP Technologies, 1991). Harder et al, found that the addition of a nitrate and phosphate combination displayed significantly more respiratory activity than either nutrient added alone as well as with no nutrients at all. He concluded that hydrocarbon degradation is nutrient dependent (Harder et al, 1991). Thornton-Manning et al determined no impact of nutrient addition to phenols in top soil. However, it was found that nutrient (N & P) addition in two different subsurface soils had a significant impact with phosphorus impacting the most suggesting that phosphorus is limiting in a variety of soils (Thornton-Manning et al, 1987). Uziel found that the addition of nutrients further reduced soil contamination in an area of a former Chevron oil field. Reductions achieved by moisture addition stagnated at 75% reduction. This reduction was improved by the addition of nitrogen and phosphorus (Uziel, 1994).

Inorganic nutrients are vital components of hydrocarbon biodegradation. Their relationships are complicated and impact is significant. More research is required to get a better grasp of their place in bioremediation.

2.6 Methods of Measurement (Respirometry)

Methods of measurement can be divided into two categories: 1) contaminant specific monitoring 2) and, non specific monitoring. Contaminant specific methods such as gas chromatography (GC), GC and mass spectroscopy or other methods such as radiolabling are effective and relatively accurate. However, they can be complicated to perform and interpret as well as be very costly and time consuming. For this reason indirect methods of measure are more attractive, especially if they can be statistically supported by fewer direct measurements.

As previously discussed, aerobic biodegradation uses O_2 as the final electron acceptor. Hence, metabolic microbial respiration increases due to aerobic biodegradation processes. Additionally, due to mineralization of the target compound, CO_2 production increases. Measuring O_2 consumption and CO_2 production are affordable and relatively simple methods of indirect measurement that are gaining popularity.

Respirometry has been used as an affordable, easy, and relatively fast indirect method to study biodegradation. Tabak et al have used electrolytic respirometry for a wide variety of studies including biodegradation of Alaskan crude oil (Tabak, 1990b). Stegmann et al studied nutrient and moisture impact on hydrocarbon contaminated soil and demonstrated good correspondence between respirometer, bioreactor, and jar tests, supporting respirometry as a viable indirect method of measurement (Stegmann et al, 1991). As previously mentioned, Mueller studied nutrient supplementation of oil degrading microorganisms associated with oiled beach material using the MicroOxymax®. The nutrients were supplemented at four different levels of nitrogen and

phosphorus combinations and compared to a sterile sample. The result was increased activity for each increasing level of N and P combination with all combinations exceeding the untreated sample (BP Technologies, 1991). MacDonald measured O_2 consumption in microcosms constructed from core samples from a contaminated site. They determined that the oxygen consumption rate was highest at the layer nearest the trapped contaminants. This demonstrated respiration stimulation through hydrocarbon exposure (MacDonald, 1993).

Measuring respiration is an affordable, relatively simple method for determining microbial activity in soil. In aerobic biodegradation, O_2 consumption and CO_2 production are the fundamental activities. Measuring these activities can provide an interesting picture of the world of biodegradation.

2.7 Summary

In summary, JP-8 biodegradation has not been widely studied. It is a complex jet fuel comprised primarily of kerosene. The microbial activity and pathways used to degrade such a complex hydrocarbon in soil are complicated. Measuring respiration simplifies biodegradation studies by measuring the most basic fundamentals of aerobic degradation. Much can be learned from such studies with minimal associated costs and complications.

3. Methods and Materials

3.1 Measuring Microbial Activity using Respirometry

3.1.1 Set-up

A MicroOxymax® respirometer was used throughout this study. The set-up consisted of a sample pump, an Andros 5000 series CO_2 gas analyzer, a Citicel oxygen sensor and two expansion units that accommodate 10 subject chambers (sampling jars) each for a total of 20 chambers (See figure 3 - 1). The 20 (250 ml glass jars) were placed in an

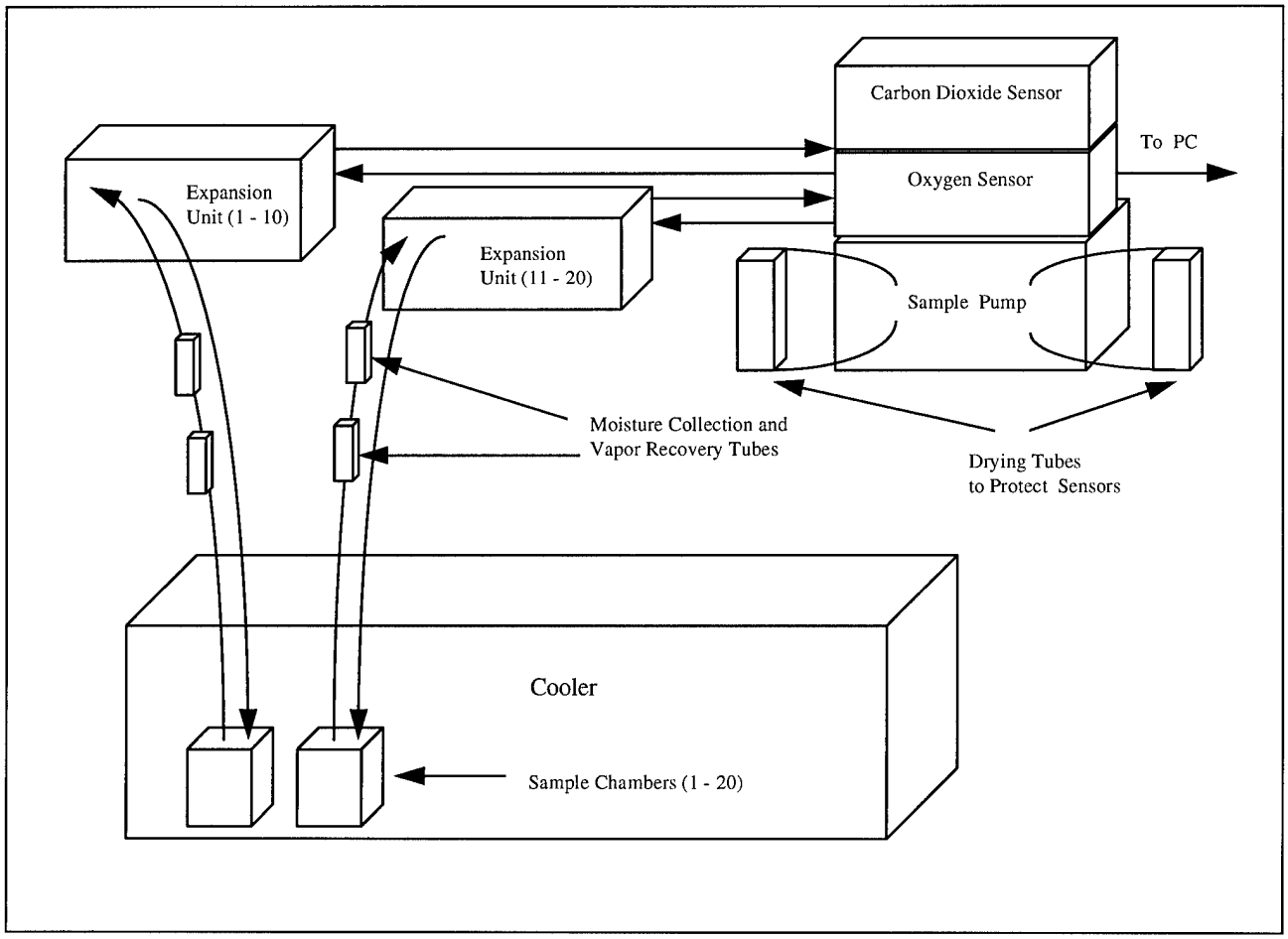


Figure 3 - 1 MicroOxymax® component set-up

insulated chamber to help stabilize the temperature fluctuations. The respirometer system is completely automated and controlled by a personal computer. The sensitivity of the sensors made this set-up effective for measuring low levels of O_2 consumption and CO_2 production from soil microbial activity.

3.1.2 CO_2 Sensor (Andros Inc.)

The CO_2 sensor is a stable, single beam, non-dispersive, infrared sensor operating over a range of zero to one percent. The sensor works on the principle that the gas of interest (CO_2) absorbs infrared light at a specific wavelength, proportional to the concentration of that gas. Different gases absorb light at characteristic wavelengths with each absorption pattern unique to a specific gas.

3.1.3 O_2 Sensor (City Technology LTD)

The oxygen sensor is an electrochemical device that measures oxygen concentration without being affected by pressure variations throughout the course of the test (See Figure 3 - 2). The Sensor is a self powered, diffusion limited, metal air battery type comprised of an anode, electrolyte and an air cathode. The cathode reduces the oxygen to hydroxyl ions by the following:

$$O_2 + 2H_2O + 4e^- \longrightarrow 4OH^-$$

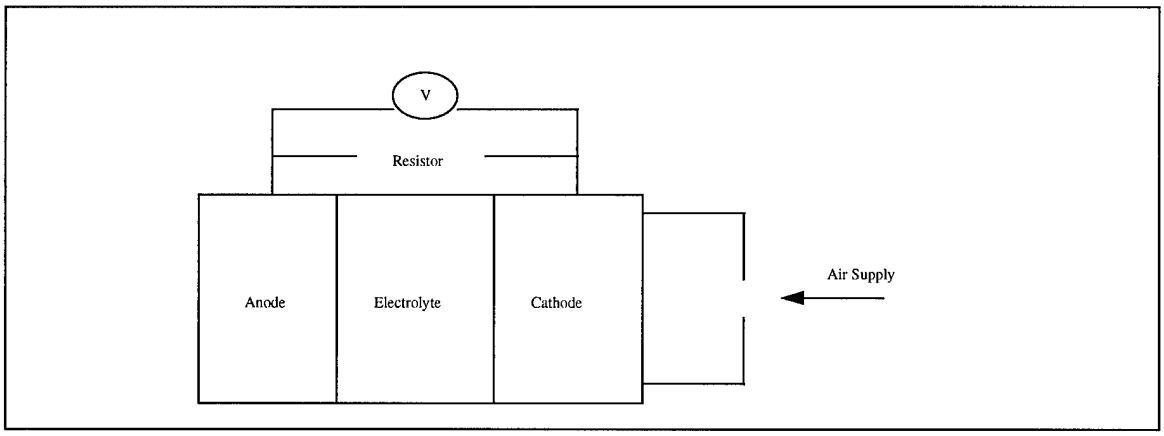


Figure 3 - 2 O_2 Sensor

The hydroxyl ions are then oxidized in the metal anode:

$$2Pb + 4OH \longrightarrow 2PbO + 2H_2O + 4e^-$$

A current is produced which is passed through a low value load resistor which in turn produces a voltage signal. The O_2 rate of consumption is proportional to the voltage signal which is proportional to the generated current.

3.1.4 Temperature Measurement (Micro-Oxymax Instruction Manual, 1994)

Temperature was measured periodically in the container storing the subject chambers. The measurements were taken with each O_2 and CO_2 measurement so there are corresponding temperature readings for each chamber. The temperature was measured with a platinum probe encased in a stainless steel housing.

3.1.5 Principle of Operation (Czekajewski et al, 1990)

The MicroOxymax® is a closed system in which, during the course of a sample interval, air is not replenished. A measurement consists of an initial sample at the beginning of the sample interval and a final sample taken at the end of the interval. During each sample, test chamber air is circulated through the O_2 and CO_2 sensors and returned to the test chamber. This method avoids potential pressure problems found in more conventional manometric respirometers.

Sample interval lengths can be varied from a minimum of 6 minutes to 24 hours. Interval length is determined purely on the needs of the user and the constraints of the experiment. Each sample interval is independent of the previous and following sample interval. The samples within the interval are relative to each other for measuring a change in O_2 and CO_2 concentrations and less so for their absolute values. This allows the test chambers to be refreshed (if desired) in between sample intervals without interfering with the sample measurements. The chambers can be refreshed by either exceeding a user established CO_2 concentration and falling below an O_2 value or simply after a set number of sample intervals. For setups with multiple test chambers, sampling and refreshing are performed sequentially.

The change in O_2 and CO_2 concentrations over the time of the interval determines consumption and production rates, respectively, so the initial and final sample values during the interval are not as critical as the change between them. These changes are used to calculate the cumulative O_2 consumption and CO_2 production for the interval. These cumulative values are saved and added to the previous cumulative total of the

previous sample intervals. This gives a running cumulative total for the length of the entire experiment. To understand how the actual O_2 and CO_2 values are arrived at, it is critical to understand the system mechanics of volume, pressure and sensor drift.

Test Chamber head space volume is a critical value for calculating O_2 and CO_2 concentrations. For determining test chamber head space volumes and eventually O_2 and CO_2 concentrations, reference pressures and volumes must be known. The MicroOxymax® uses the sensor chamber and reference chamber as reference volumes for determining the test chamber head space volume. Figure 3-3 illustrates how the chambers are connected to each other. The size of the illustrated boxes are not meant to imply relationships between the size of each chamber.

The reference chamber and the sensors both have constant volumes. However, sensor volume varies from one respirometer to another so sensor volume for each unit must be determined at the factory by the following method and set as a permanent value for each particular respirometer.

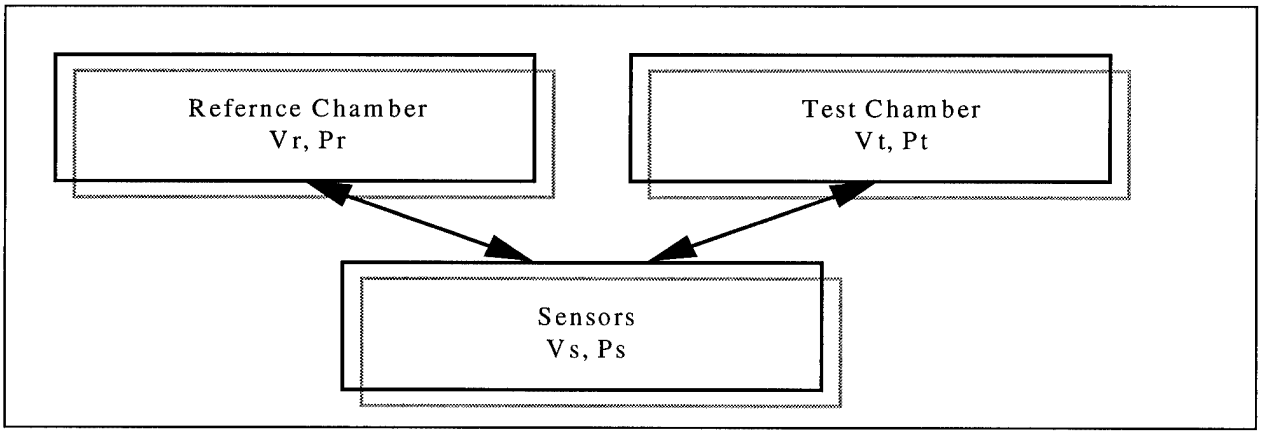


Figure 3 - 3 Test chamber, reference chamber, and sensor set-up - Vr, Vt, and Vs are reference chamber, test chamber and sensor volume respectively. Pr, Pt, and Ps are reference chamber, test chamber, and sensor pressure respectively.

The sensor volume (Vs) is determined by equalizing the test chambers, reference chamber and sensors to atmospheric pressure (Pa) and then each chamber is closed and isolated from each other. The sensor pressure (Ps) is then raised to a known value higher than atmospheric pressure. The reference chamber and sensors are then opened to each other resulting in a third pressure (Prs) created from the mixed Pr and Ps pressures. Knowing the initial sensor pressure, initial reference chamber pressure, reference chamber volume, and the resulting pressure, the volume of the sensors is determined by the following equation:

$$Vs = Vr/[(Ps - Pa/Prs - Pa) - 1]$$

With both the Vs and Vr values, the test chamber volume (Vt) can be solved for. Again the sensors, test chambers, and reference chamber are allowed to equalize to atmospheric pressure (Pa). The reference chamber and sensors are then pressurized to a preset level establishing a new Prs. Next, the test chamber is opened to mix Pt (Atmospheric pressure) with Prs establishing a new pressure (Pc). Vt is then determined by the following equation:

$$Vt = (Vs + Vr)[(Prs - Pa)/Pc - Pa) - 1]$$

This process is repeated for each test chamber.

The test chamber head space volumes above are used to determine O_2 and CO_2 concentrations in the following simplified manner. Initially, the reference chamber and sensors are opened to dehumidified atmospheric air. With both the sensor and reference chamber volumes known, O_2 and CO_2 concentrations can be measured in each and stored. The reference chamber is then closed off with stored reference air that will be important later. The sensors are then opened to the test chamber to be measured, allowing the test chamber air and sensor air to mix. The newly mixed air O_2 and CO_2 concentrations are measured by the sensors (after stabilization). The test chamber O_2 and CO_2 concentrations are then calculated using the original sensor concentrations, the mixed air concentrations, and the previously determined sensor and test chamber volumes. The sensors and test chamber are again isolated with the remaining sensor air being measured. The new sensor air serves as a reference and with the known sensor volume and test chamber volume of each of the subsequent test chambers, O_2 and CO_2 concentrations can be calculated by repeating the above process for each test chamber. Purging the sensor air in between each test chamber measurement can be performed to minimize test chamber cross talk.

Measurements and calculations are again performed at the end of the test interval (See Figure 3 - 4). When the second set of measurements are completed, the sensors are opened to the reference chamber air. Finally, with the known sensor air concentrations, the newly measured sensor/reference chamber air concentrations, and the sensor and reference chamber volumes, the reference chamber O_2 and CO_2 concentrations can be calculated. The new reference chamber O_2 and CO_2 concentrations are compared to the

initial reference concentrations stored at the beginning of the interval. Assuming the reference chamber air has not changed since the beginning of the interval, any changes/discrepancies between initial and final reference chamber O_2 and CO_2 concentrations is assumed to be caused by sensor drift. The sensor drift is then accounted for and incorporated appropriately into the second set of measured test chamber concentrations (See Figure 3 - 4).

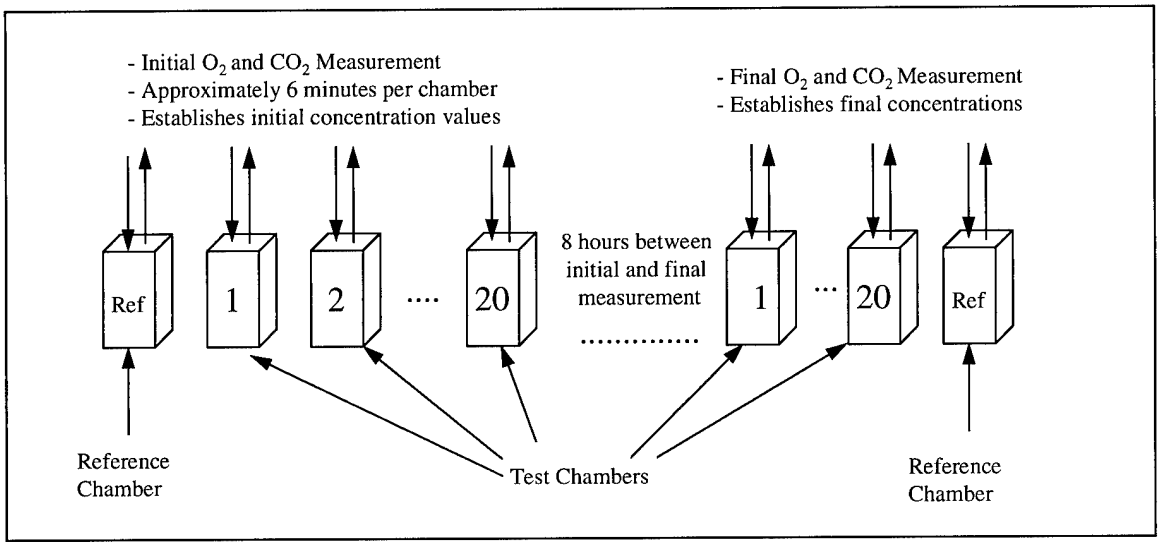


Figure 3 - 4 Sample Interval Illustration - Each of the twenty test chambers and the reference chamber is sampled twice during an interval. The final concentrations are compared to initial concentrations to determine concentration changes in each chamber. The change in concentration over the interval in between samples establishes consumption and production rates. The final reference concentration is compared to the initial reference concentration in order to account for sensor drift during the sample interval. Sensor drift is then applied accordingly to the final O_2 and CO_2 concentration measurements and finally, previously recorded rate data is adjusted accordingly.

3.2 Soil Characteristics

The soil used in this study was obtained from the wooded area adjacent to building # 470, Area B, Wright-Patterson Air Force Base, OH. Approximately two inches of top soil were removed before actual soil collection began. The soil was collected from an area

approximately one square foot in area. The collected soil was sifted to remove rocks, roots, and large clay pieces and then thoroughly mixed. CTL Engineering Inc. performed the soil analysis which is summarized in Table 3 - 1. Background phosphate and nitrate levels were determined using a Hach 2000 water kit, which will be discussed later. Table 3 -1 summarizes soil characteristic information.

Table 3 - 1 Soil Summary: Sandy Silt

Gravel	Coarse Sand	Medium Sand	Fine Sand	Silt	Clay	pH	%Field Capacity
3%	5%	13%	24%	39%	16%	7.92	57%

Background moisture was determined to be 20.21% or 57% field capacity. This was determined by taking a 50 gram sample of the soil, heating it to 105 °C for 24 hours. The soil was re-weighed and found to be 39.89 grams or 20.21% moisture by weight. 100% field capacity was determined by taking the 39.89 grams of dry soil and placing it into a plastic tube approximately six inches long and 1 1/2" in diameter with a filter placed on one end to hold the soil in place. The filtered end of the tube (approximately one inch) was suspended in a 500 ml beaker of water to allow capillary action to draw the water up the length of the tube. This took several days. The final weight was measured to be 61.75 grams with 21.88 grams of this as water resulting in 35.43% moisture by weight. This is considered to be 100% field capacity. The natural percent field capacity was determined by the following:

$$20.21/35.43 = 57.04\%$$

Moisture additions will discussed in detail later.

This soil was also used by James A. Baker in a concurrent thesis effort. He labeled this particular soil as soil A in that effort. He exposed the soil to varying levels of JP-8 concentrations without inorganic nutrient additions. The one gram (1% of soil weight) JP-8 exposure studied in his effort is similar to treatment one with one gram (1% of soil weight) JP-8 exposure (discussed in detail later) of this effort.

Soil was stored in a conventional refrigerator prior to use, at approximately 40 ^{o}F. This was done to slow background microbial activity and help preserve the state of the soil. Additionally, this protected the soil from evaporating moisture and becoming dry, potentially harming the indigenous microbes.

3.3 Experimental Design and Statistical Analysis of Measured Cumulative Oxygen

Three separate experiments were designed to study the impact nutrient addition has on JP-8 biodegradation under specific soil conditions. Nitrate and phosphate were assumed to be limiting in the soil used for this study. Therefore, their addition under simulated spill conditions may positively impact JP-8 biodegradation. For this reason, each of these nutrients were added individually and in various combinations to determine if positive individual and combinational impacts exist for JP-8 biodegradation.

Cumulative oxygen, because of its direct relationship to hydrocarbon degradation, was used to measure JP-8 biodegradation. If nutrient addition positively impacts biodegradation there will be an increase in microbial respiration leading to higher cumulative oxygen values. For this reason, the cumulative oxygen values measured

during the course of each experiment were statistically analyzed by analysis of variance (ANOVA) to determine main effects for each factor and interaction between factors. The results will be used to assess the impact the treatments have on JP-8 biodegradation.

Experiment 1 was originally designed similarly to Experiment 2 (discussed in the following paragraph). However, mechanical problems limited the amount of valid data, which prevented a statistical analysis. The data were simply displayed graphically and used as a qualitative comparison to data gained from Experiment 2.

Experiment 2 was designed to determine and compare the impact of nutrient additions with and without the presence of fuel (JP-8) on hydrocarbon biodegradation. Cumulative oxygen values measured during each experiment were assumed to be directly related to hydrocarbon biodegradation, therefore these values were used as the response variable in the statistical analysis. Figure 3-5 illustrates the nutrient and fuel combinations or treatments used for experiments 1 & 2. The experiment was a three factor design and modeled as follows:

$$y_{ijkl} = \mu + \alpha_i + \beta_j + \gamma_k + (\alpha\beta)_{ij} + (\alpha\gamma)_{ik} + (\beta\gamma)_{jk} + \varepsilon_{ijkl}$$

Table 3-2 - Variables for three factor ANOVA

y	Oxygen (Response Variable)
μ	Overall Mean of the Experiment
α	Nitrate (Main Effect)
β	Phosphate (Main Effect)
γ	Fuel (Main Effect)
$\alpha\beta$	Nitrate*Phosphate (Interaction)
$\alpha\gamma$	Nitrate*Fuel (Interaction)
$\beta\gamma$	Phosphate*Fuel (Interaction)
ε	error term

Because replicates were not collected, three way interaction between nitrate, phosphate and fuel was assumed to be 0. This allows the three way interaction to be used as the error term for this analysis.

The data from Experiment 2 data were analyzed using a three factor analysis of variance (ANOVA) using Statistix® (Statistix Users Manual, 1985) and SAS software. Significant two-way interaction and main effects were tested using the ANOVA. See Appendix A for output and data and Chapter 4 for discussion of results.

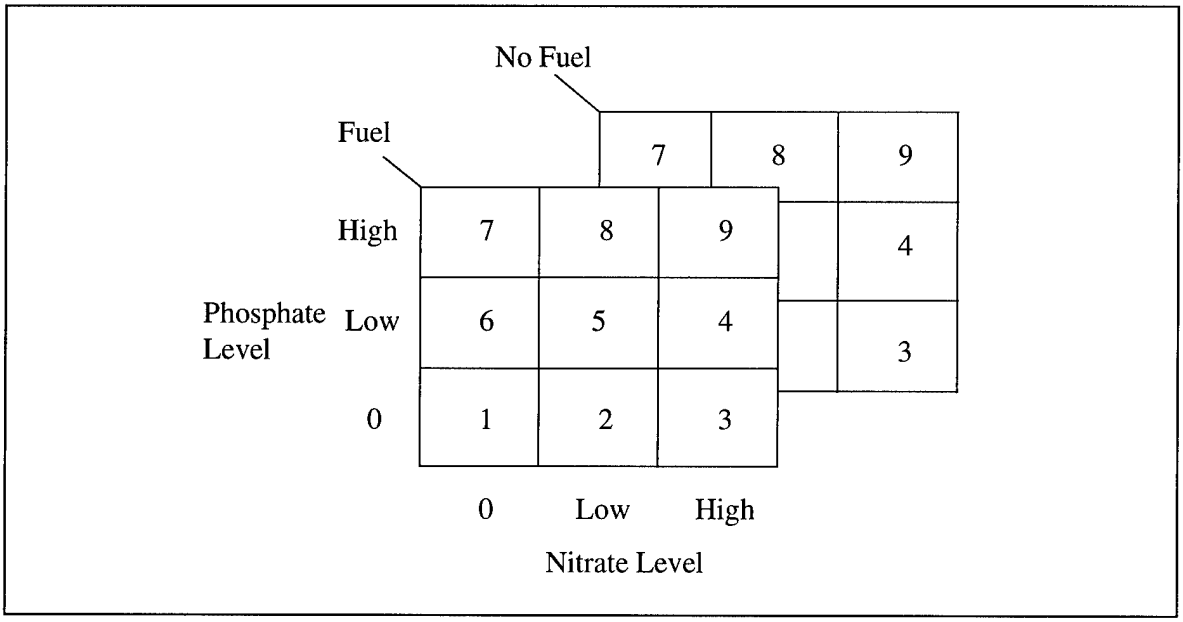


Figure 3-5 Inorganic nutrient & fuel combinations (Treatments) for experiments 1 & 2. This design only allowed one sample per cell to be measured.

Experiment 3 was designed with each expansion unit containing a complete set of nine treatments. This was done to determine if there was a statistical difference between measurements taken through each unit. A Paired T Test was performed to determine if there was a statistical difference between the measurements obtained from each

expansion unit (See Appendix B for data and output). The variable tested was cumulative oxygen values at the end of the experiment. Results will be discussed in Chapter 4.

Experiment 3 was designed with one fuel level resulting in a two factor design in which cumulative oxygen was again used as the response variable. (See Figure 3-6). This design allowed two cumulative oxygen samples per treatment to be measured. Details of each experimental setup are discussed in the following three sections (See Appendix C for treatment and fuel placement with respect to microcosm). The model for Experiment 3 is mathematically represented as follows with Table 3 - 3 defining the variables:

$$y_{ij} = \mu + \alpha_i + \beta_j + (\alpha\beta)_{ij} + \varepsilon_{ij}$$

Table 3-3 - Variables for two factor ANOVA

y	Oxygen (Response Variable)
μ	Overall Mean of the Experiment
α	Nitrate (Main Effect)
β	Phosphate (Main Effect)
$\alpha\beta$	Nitrate*Phosphate (Interaction)
ε	error term

Since replicates of cumulative oxygen values were collected for each treatment, the error term for this analysis is pure error. A two factor ANOVA calculating main effects by nitrate and phosphate and nitrate/phosphate interaction on cumulative oxygen values was performed using Statistix® software (See Appendix D for data and output). The results of this analysis will be discussed in Chapter 4.

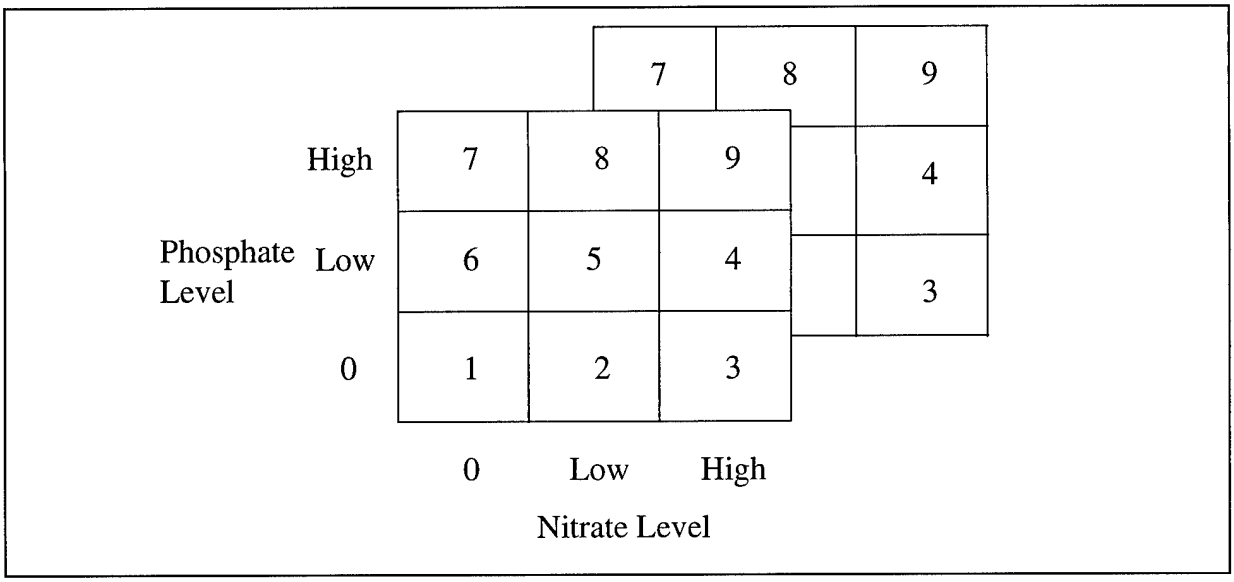


Figure 3-6 Experiment 3 two factor design allowing two measurements per treatment.

3.3.1 Experiment 1

18 of the 20 test chambers were filled with 100 grams each of soil in its natural state. Two test chambers were left empty as indicators of background noise readings and to ensure no unusual measurements trends occurred. Nine of the 18 test chambers were randomly spiked with 0.65 grams of JP-8 (See Table 3-4 for experimental set up summary). Each of these nine chambers were enhanced with one of the nine nutrient treatments described. The chambers were randomly placed throughout the 20 available positions. The other nine chambers were not spiked with JP-8, but were still treated with one of the nine treatment combinations. This was done to determine the impact, if any, of inorganic nutrient addition alone and to measure background activity of the soil without the addition of nutrients (i.e. treatment 1).

The inorganic nutrients added were potassium nitrate and sodium phosphate. See Table 3-5 for an inorganic nutrient addition summary. One ml of the appropriate stock nutrient solution was added to each test chamber requiring the addition of phosphate or nitrate, according to the matrix. For chambers not requiring any phosphate or nitrate (i.e. treatment one), 2 ml of distilled water was added. Treatment two required a low level nitrate and no phosphate, therefore 1 ml of 0.001 gm/ml solution was added with 1 ml of distilled water. Treatment nine required high concentrations of both so 1 ml of 0.01 gm/ml KNO_3 and 1 ml of $NaPO_4$ were each added to the appropriate test chamber. Distilled water was used in place of the stock solutions to ensure consistent moisture addition to each test chamber. Each addition of solution, water, and fuel was performed by pipeting.

The addition of 2 ml of water/stock solution to the existing soil yields a 62.7% field capacity.

$$22.21/35.43 = 62.7\%$$

This field capacity is well within the ideal ranges described in Chapter 2.

The respirometer was configured to measure chamber activity over an eight hour interval. The sensors were purged between samples to prevent test chamber crosstalk. The test chambers were refreshed if the CO_2 level reached or exceeded 0.5%.

Due to equipment complications only the test chambers attached to the first expansion unit (Chambers 1 - 10) displayed reliable data. The second expansion unit (chamber 11 - 10) was ineffective for most of the test period yielding sporadic O_2 and CO_2 rate information. The test was run for only one week due to these complications.

Table 3-4 Experiment 1 set up summary

Fuel Added (grams) per Chamber	Soil Added (grams) per Chamber	Experiment Length (weeks)	Moisture Added (ml) per Chamber
0.65	100	1	2

3.3.2 Experiment 2

Experiment 2 was set up in the same fashion as experiment 1 except for two differences (See Table 3-5 and 3-6). 1.25 ml or 1 gram of JP-8 was used in the fuel spiked test chambers and the test period lasted 45 intervals or approximately three weeks.

Table 3-5 Experiment 1 & 2 nutrient treatments (See Appendix E for calculations)

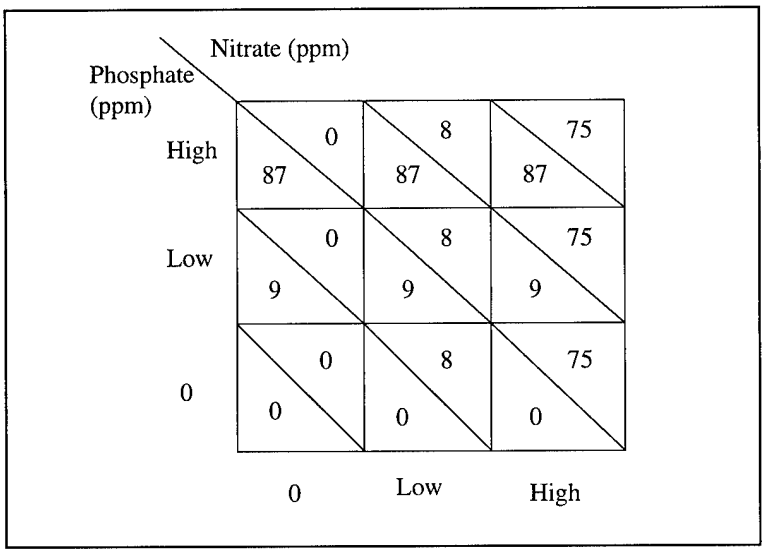


Table 3-6 Experiment 2 set up summary

Fuel Added (grams) per Chamber	Soil Added (grams) per Chamber	Experiment Length (weeks)	Moisture Added (ml) per Chamber
1	100	3	2

3.3.3 Experiment 3

Experiments 1 and 2 demonstrated that nutrient addition without the presence of JP-8 did not increase respiratory activity in the soil above background respiration rates. This prompted the elimination of the nine treatment combinations without JP-8 addition. JP-8 was added to all 18 chambers allowing duplicates of treatment combinations. One of the two remaining chambers was left empty while the other was filled with 100 grams of soil with no JP-8 or nutrient enhancement.

The original stock solutions used in the first two experiments were determined to be lower in the levels of nitrate and phosphate than originally calculated. New, stronger solutions were mixed. Tables 3-7 and 3-8 summarize the experiment set-up and nutrient treatment details. 100 grams of soil was again used with 1 gm of JP-8 spiking. Treatment combinations were again randomly placed throughout the test chambers. However, each expansion unit contained a complete set of the nine treatments, unlike the previous two experiments which allowed random placement throughout all twenty chambers. Stock solutions, water, a fuel were all pipetted into the soil containing test chambers similarly to experiments one and two. Again, one ml of the appropriate stock solution or distilled water was added to give the correct inorganic nutrient concentration to dry soil ratio similarly to Experiments 1 & 2. After appropriate additions all soil was thoroughly mixed inside each test chamber. Additionally, there were two free microcosm positions available (one in each expansion unit). One of the positions was set up with an empty chamber to account for background noise and ensure the equipment was functioning properly. The other available chamber was filled with untreated soil. This

was done to provide a reference for the treated chambers and to support the finding from Experiments 1 & 2 that fuel addition greatly impacts the respiratory activity of the microcosm. However, this reference chamber was not included in the statistical analysis of the experiment.

Table 3-7 - Experiment 3 nutrient treatments (See Appendix E for calculations)

Phosphate (ppm) \ Nitrate (ppm)	0	Low	High
High	172 / 0	172 / 15	172 / 153
Low	17 / 0	17 / 15	17 / 153
0	0 / 0	0 / 15	0 / 153

The sampling configuration was modified to sample over four hour intervals. This was done over the first twelve days and the last twelve days of the experiment. The intermediate days were measured in eight hour intervals. The shortened interval was used because of the possibility of depleting enough oxygen during the eight hour interval to inhibit microbial activity. It was used during the first twelve days because the highest oxygen consumption rates are expected early in the experiment. It was reintroduced during the final twelve days of the experiment because high O_2 depletion was experienced during the eight hour interval test days. The test period lasted four weeks.

Table 3-8 - Experiment 3 set up summary

Fuel Added (grams) per Chamber	Soil Added (grams) per Chamber	Experiment Length (weeks)	Moisture Added (ml) per Chamber
1	100	4	2

3.4 Inorganic Nutrient Soil Analysis (Hach Water Analysis Handbook, 1992)

Upon completion of the second and third experiment, each test chamber soil sample was analyzed for nitrate and phosphate concentrations and compared to initial concentration levels. Duplicates of each sample constructed for experiments one and two were made at the end of each experiment to represent time zero or initial conditions. The Hach DR/2000 spectrophotometer water analysis kit was used to analyze the soil. The following describes the extraction and extractant analysis methods used to analyze the soil.

Nitrate soil extraction was performed using the aqueous method. This method uses a nitrate extraction reagent or flocculating agent that settles out the soil. Nitrate ions are very soluble in water and require no other chemical extractants. This method leaves a clear extractant which is analyzed as an indicator of soil nitrate levels. The extractant is analyzed by Hach Method 8152, Nitrate soil analysis (0 to 55 ppm NO_3^- - N). This method is a modification of the cadmium reduction method. The cadmium reduces the nitrate present in the sample to nitrite. The nitrite ion reacts in an acidic medium with sulfanilic acid to form an intermediate diazonium salt. This salt couples to chromotropic

acid to a form a pink colored product. The pink color concentration is proportional to the nitrate concentration in the extract.

Phosphate was extracted from the soil using the Acid/Fluoride method. Similar to the nitrate extraction method, a flocculating agent is used to cause the soil particles to coagulate and fall out leaving a clear extractant solution that can be analyzed. The extractant is analyzed using Hach Method 8181 (0 to 225 ppm). In this method the indicator combines with an ascorbic acid reducing agent in a single powder formulation called PhosVer 3 reagent. The indicator consists of sodium molybdate which forms a complex with the phosphate ion. This complex is reduced by the ascorbic acid to form a blue species. The blue color intensity is proportional to phosphate concentration.

Soil samples to be analyzed from Experiment 2 were stored in a conventional refrigerator at approximately 40 ^{o}F. Samples stored containing JP-8 demonstrated microbial activity even when stored in the cooler environment. As a precaution, all samples to be analyzed from Experiment 3 were stored in a freezer at approximately 20 ^{o}F. This was done to further reduce (but not eliminate) microbial activity during storage.

The nitrate and phosphate data collected using the above methods were used to establish time = 0 and time = N concentration levels in the soil for each particular test chamber (and treatment). Over the course of the experiment, it was assumed there would be a decrease or total change in nitrate and phosphate levels due to microbial activity associated with hydrocarbon mineralization. The changes in nutrient levels should correspond to cumulative oxygen levels measured in the same test chamber. A correlation analysis using Experiment 2 and 3 data was performed comparing nutrient

changes for each test chamber to the corresponding cumulative oxygen value for that chamber. Additionally, changes in nitrate concentrations should correlate to changes in nitrate concentrations. A correlation analysis was performed using these two factors. The analyses were performed using Microsoft Excel™ software. These values were then compared to significance tables to quantify the statistical significance of these correlation values.

3.5 Moisture and Organic Vapor Trapping

To account for JP-8 reduction due to volatilization, activated charcoal traps were constructed and placed in the inlet line leading from the test chamber to the expansion unit. Each chamber filled with soil had a charcoal trap in line regardless of JP-8 presence. This was to determine differences, if any, in weight changes of JP-8 exposed charcoal filters and unexposed traps. The charcoal content in each trap was pre-weighed prior to experiment start. A final weight measurement was taken at the completion of the experiments two and three to determine volatile uptake into the charcoal. Unfortunately weight increases are caused both by volatile organics uptake as well as moisture uptake. Moisture uptake was reduced by placing traps of magnesium perchlorate upstream of the charcoal traps. However, this method is not 100% efficient. Therefore, for traps not exposed to JP-8, weight increases were assumed to be caused by residual moisture uptake which in turn could be subtracted out of the weight increases observed for traps exposed to JP-8.

Additional moisture measurements were performed using a thermogravimetric analyzer (TGA). This method has the advantage of measuring percent weight loss over a given temperature range. A small sample of charcoal was heated from 30 °C to 600 °C. Any weight loss observed below 100 °C was assumed to be moisture loss (See Figure 3-7 for example TGA results and moisture percent weight reduction curve). The percent reduction in weight indicates percent moisture for the entire charcoal content of the tube. Several TGA samples were performed on a blank tube and a JP-8 exposed tube to increase statistical confidence. This method was performed as a comparison to the values measured by simply weighing the entire sample. Disadvantages to this method are the sample size is very small relative to the entire trap content, and the test periods are lengthy (approximately one hour per sample). This is why gross weighing of the charcoal in each tube was the preferred method.

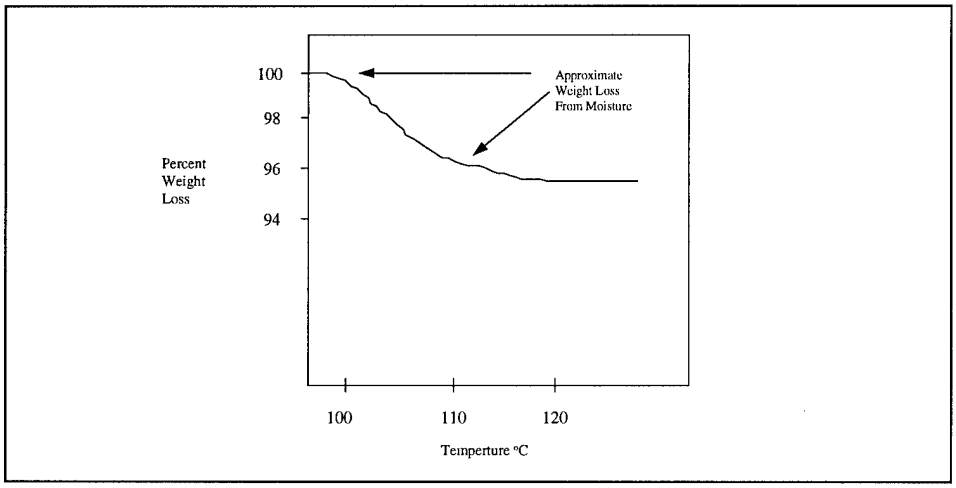


Figure 3-7 Example TGA curve. Decrease @ 100 °C from moisture loss.

Both the charcoal and moisture traps were constructed of 3 - 4 inch long glass tubes 1/2" in diameter. Rubber stoppers were placed in each that could accommodate the tubing leading from the test chamber to the expansion unit. Filters were place between the rubber stopper and the charcoal/magnesium perchlorate. Figure 3-8 illustrates the trap setup.

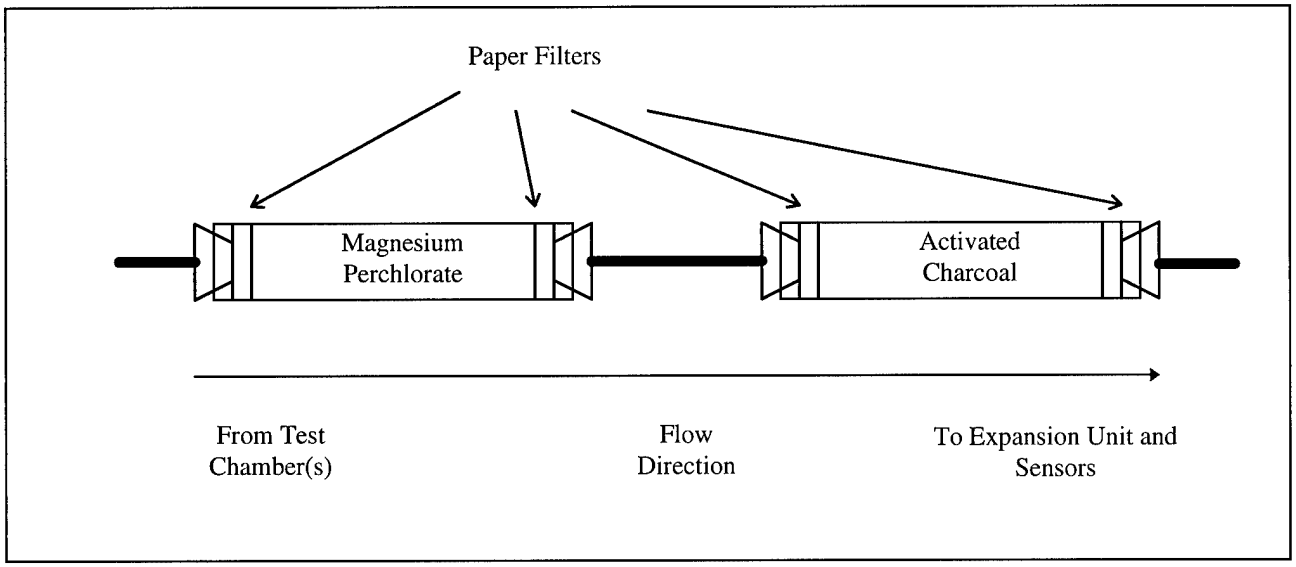


Figure 3-8 Moisture and organic vapor recovery trap.

3.6 Estimation of Hydrocarbon Mineralization

The amount of hydrocarbons mineralized were estimated using the final cumulative oxygen values from Experiments 2 and 3. The following reaction was used as an estimate of the JP-8 mineralization process.

$$C_{11}H_{24} + 17O_2 ======> 11CO_2 + 12H_2O$$

Each final cumulative oxygen value measured was used to estimate the O_2 value in the above equation. Cumulative oxygen was used instead of cumulative CO_2 values because not all biodegraded carbon is converted to CO_2. Approximately 40% of the carbon is used in producing microbial biomass. However, it is assumed that all O_2 consumed is used in the breakdown of the hydrocarbon. $C_{11}H_{24}$ was used as an estimate because it is a common chain found in JP-8 (Wright Fuels Lab Chromatograph Results of JP-8). Standard pressure and temperature were assumed for converting the cumulative oxygen volume to mass. Once the oxygen mass was determined it was a simple conversion to $C_{11}H_{24}$ through the balanced equation ratios. It is important to note that this conversion of O_2 to $C_{11}H_{24}$ assumes complete mineralization of the hydrocarbon. This assumption may be an over simplification of the actual degradation process and underestimate the percentage of the hydrocarbon degraded to some intermediate compound, rather than complete mineralization. For example, if the hydrocarbon is degraded to an intermediate compound, the same amount of oxygen required for complete mineralization can actually degrade more of the hydrocarbon to some intermediate point. Hence, using complete mineralization as an estimate underestimates what may have actually been degraded to some intermediate level. See Appendix F for all calculations related to hydrocarbon mineralization estimation.

4. Results and Discussion

4.1 Introduction

This chapter presents results of three experiments and associated analysis performed throughout the course of this study. Figures displaying cumulative oxygen consumption, cumulative carbon dioxide production, oxygen consumption rate and carbon dioxide production rates are presented and discussed. Two and three factor ANOVA results derived from respirometer generated cumulative oxygen values are presented and discussed. Inorganic nutrient analyses performed during Experiments 2 and 3 are presented and compared to associated cumulative oxygen values. Estimated hydrocarbon degradation calculated from cumulative oxygen values are presented and compared to estimated volatile vapor recovery.

4.2 Experiment 1 Discussion of Results

4.2.1 Respirometer Data and Analysis

Figures 4 - 1a through 4 - 1f display respirometry measured data. Figure 4 - 1a and 4 - 1b show the cumulative oxygen consumption and oxygen consumption rate curves respectively for test chambers 1 - 10 over 140+ hour experiment. Due to technical problems with the respirometer, cumulative oxygen and carbon dioxide values for chambers 11 - 20 could not be obtained. Associated cumulative CO_2 and CO_2 rate curves for chambers 1 - 10 are displayed in figures 4 - 1d and 4 - 1e respectively. Figures 4 - 1e and 4 - 1f display O_2 and CO_2 rate data for test chambers 11 - 20. These two figures display valid data between 80 and 100 hours. It is important to note that even during this

short period there is a distinguishable difference between rate measured in test chambers treated with fuel and those without. This will be discussed in detail later. Table 4 - 1 summarizes available cumulative oxygen and carbon dioxide values and for each treatment and test chamber.

Table 4 - 1 Experiment 1 Summary of Respirometry Results

N/P Fuel			Carbon Dioxide (uL)	Carbon Dioxide (uL)	
Treatment	Chamber	Oxygen(uL)	Actual	Theoretical	Actual/Theoretical
0/0 F	9	37642	11711	24357	0.48
L/0 F	N/A	N/A	N/A	N/A	N/A
H/0 F	4	47865	15823	30971	0.51
H/L F	N/A	N/A	N/A	N/A	N/A
L/L F	2	54272	16698	35117	0.48
0/L F	8	57514	17494	37215	0.47
0/H F	N/A	N/A	N/A	N/A	N/A
L/H F	7	57995	15832	37526	0.42
H/H F	6	81603	24319	52802	0.46
0/0 NF	3	11961	6280	7739	0.81
L/0 NF	N/A	N/A	N/A	N/A	N/A
H/0 NF	5	11737	5979	7595	0.79
H/L NF	N/A	N/A	N/A	N/A	N/A
L/L NF	N/A	N/A	N/A	N/A	N/A
0/L NF	N/A	N/A	N/A	N/A	N/A
0/H NF	10	11373	6273	7359	0.85
L/H NF	N/A	N/A	N/A	N/A	N/A
H/H NF	N/A	N/A	N/A	N/A	N/A

Note: Test Chambers 11 - 20 experienced technical problems throughout the course of experiment 1. The data collected from those test chambers were considered to be incomplete.

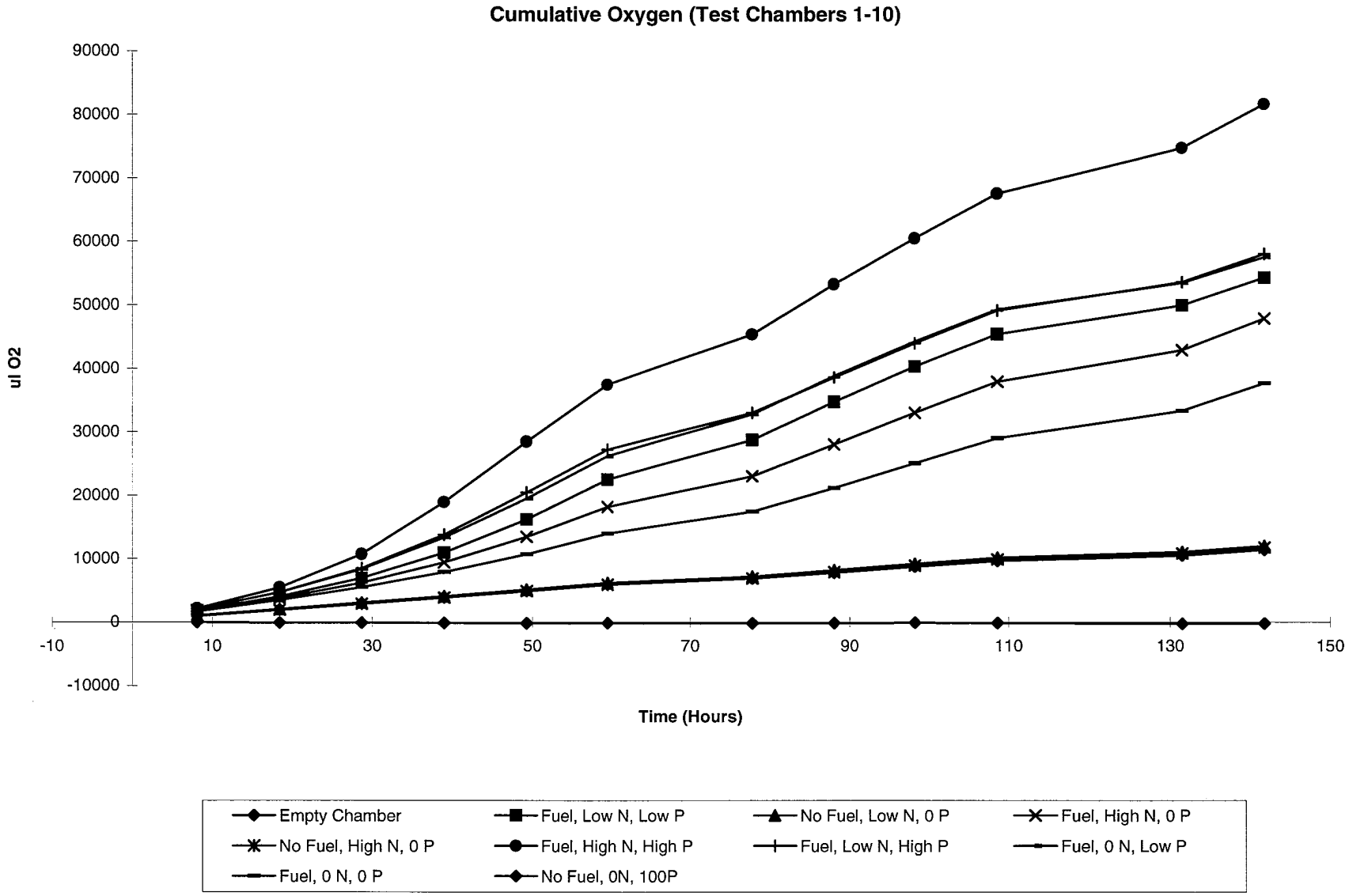
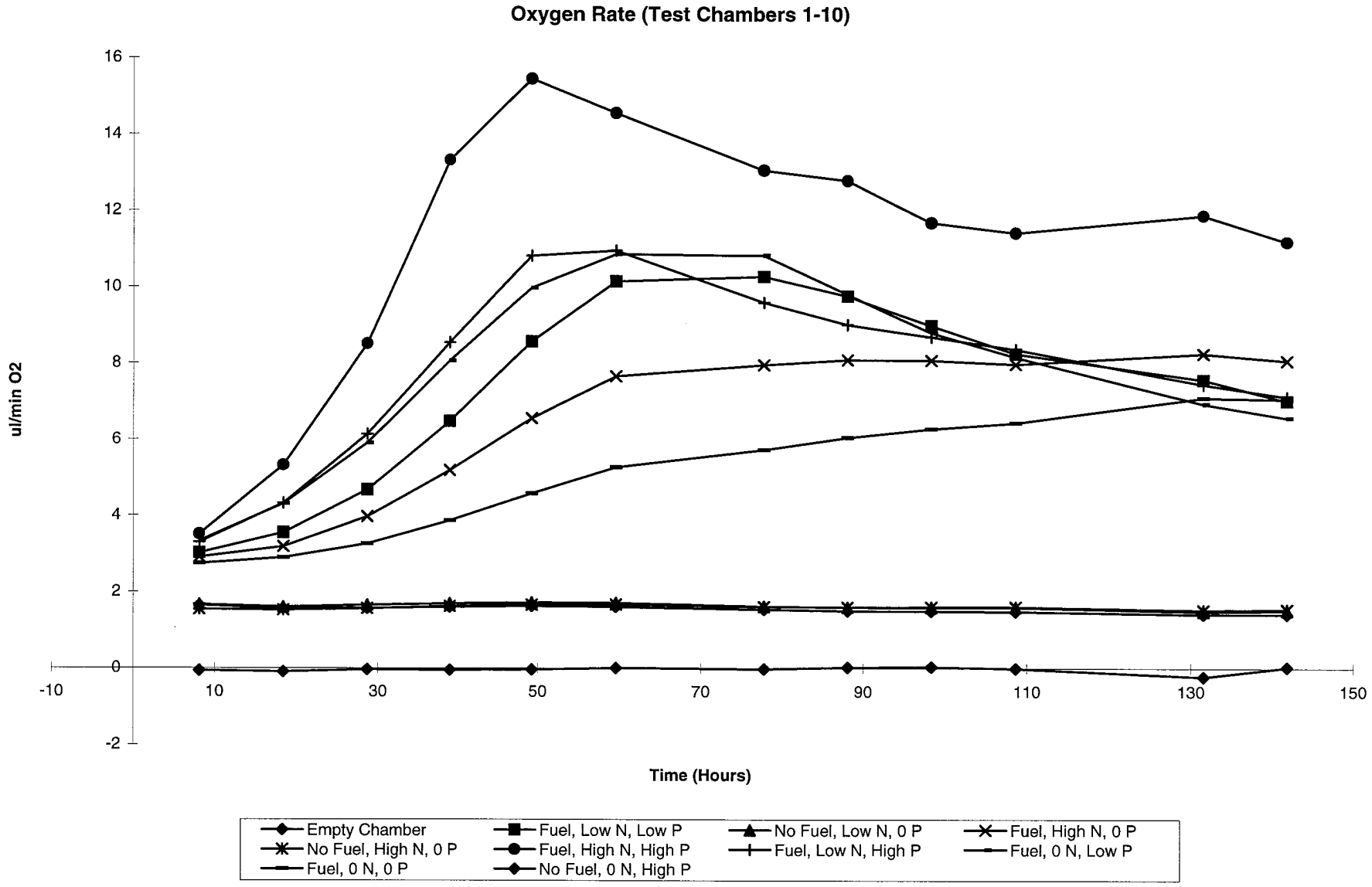


Figure 4 - 1a Experiment 1 Cumulative Oxygen Consumption (Test Chambers 1 - 10) vs Time



Fugure 4 - 1b Experiment 1 Oxygen Consumption Rate (Test Chambers 1 - 10) vs Time

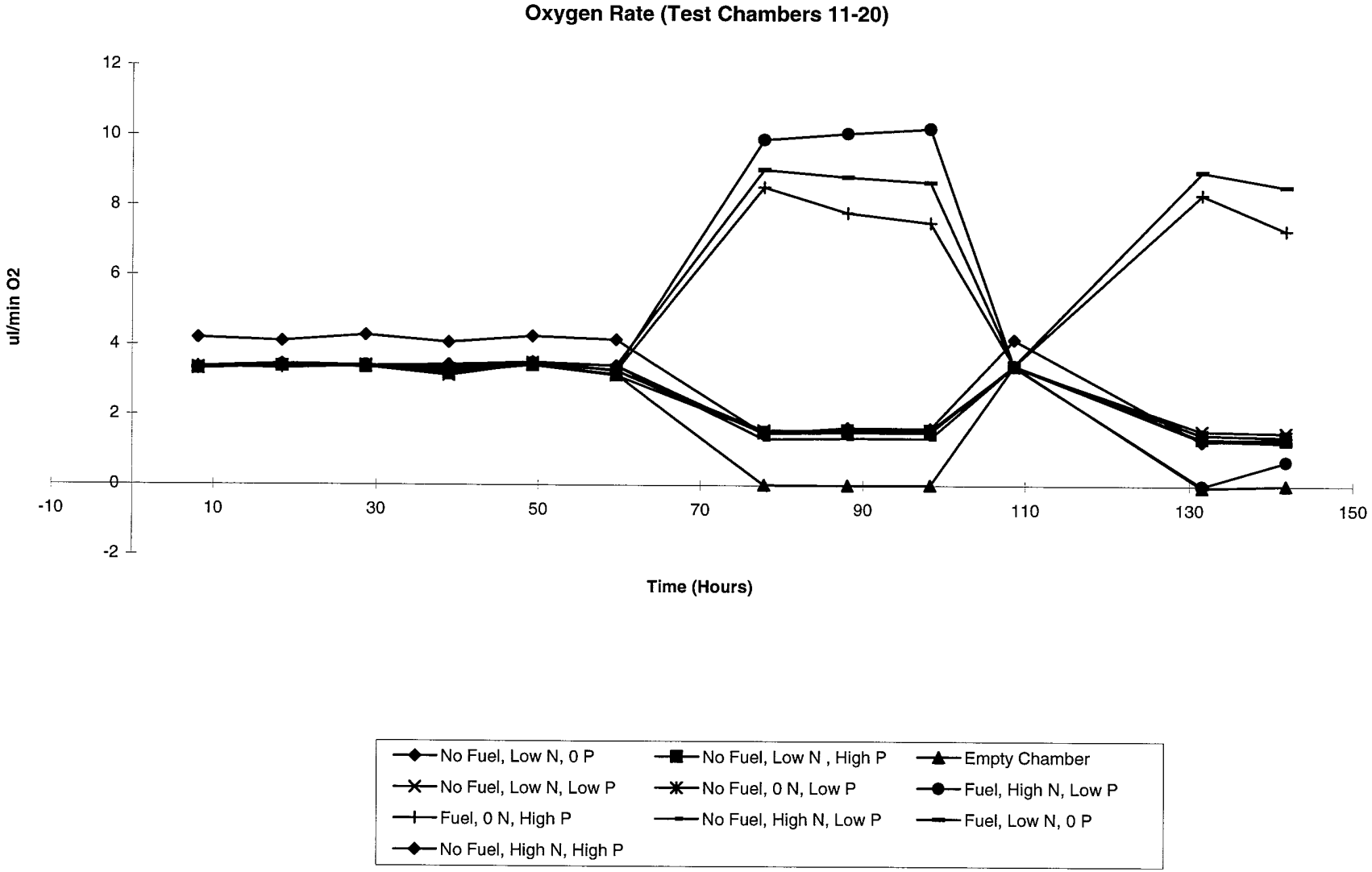


Figure 4 - 1c Experiment 1 Oxygen Consumption Rate (Test Chambers 11 - 20) vs Time

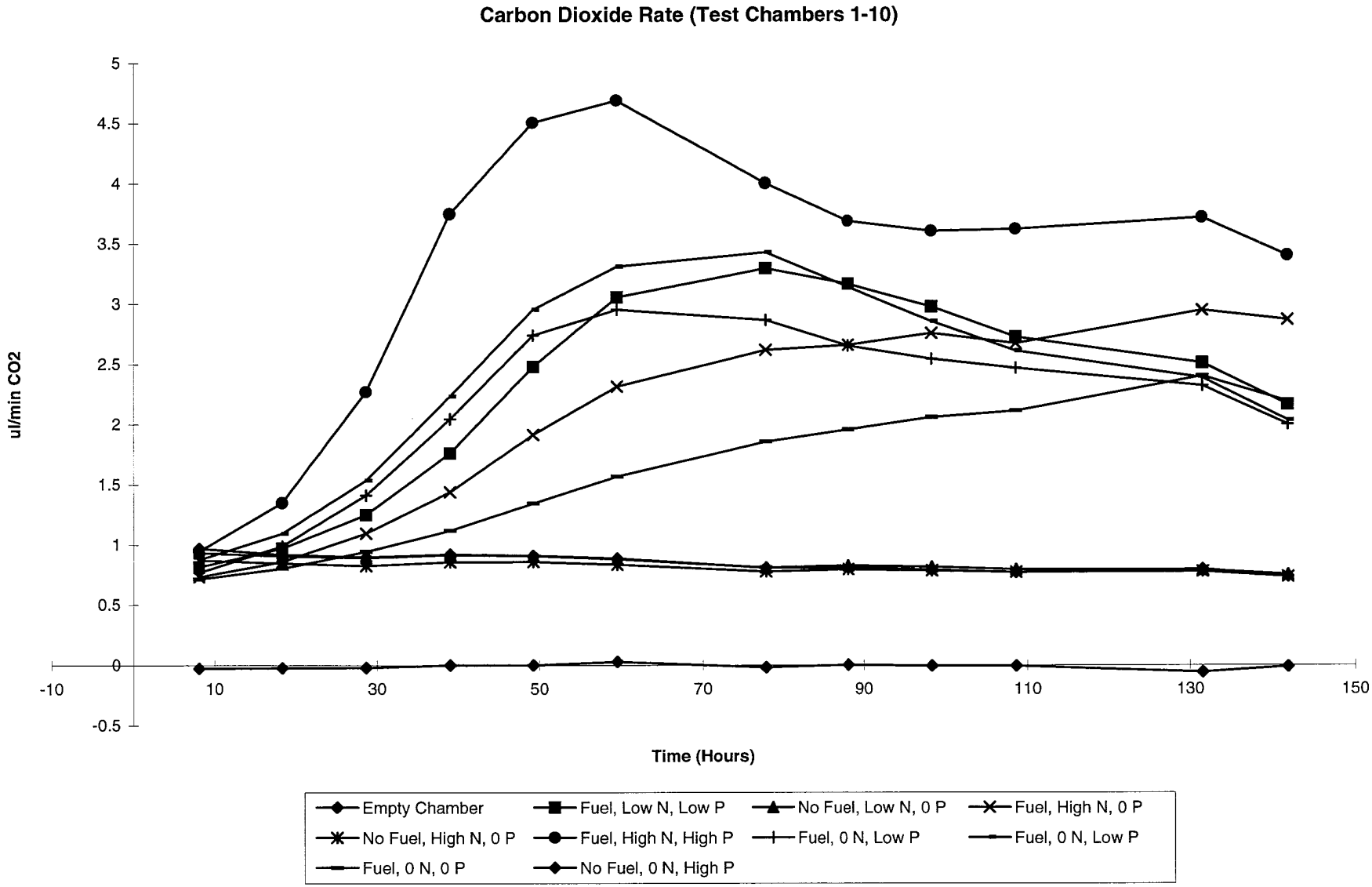


Figure 4 - 1e Experiment 1 Carbon Dioxide Production Rate (Test Chambers 1 - 10) vs Time

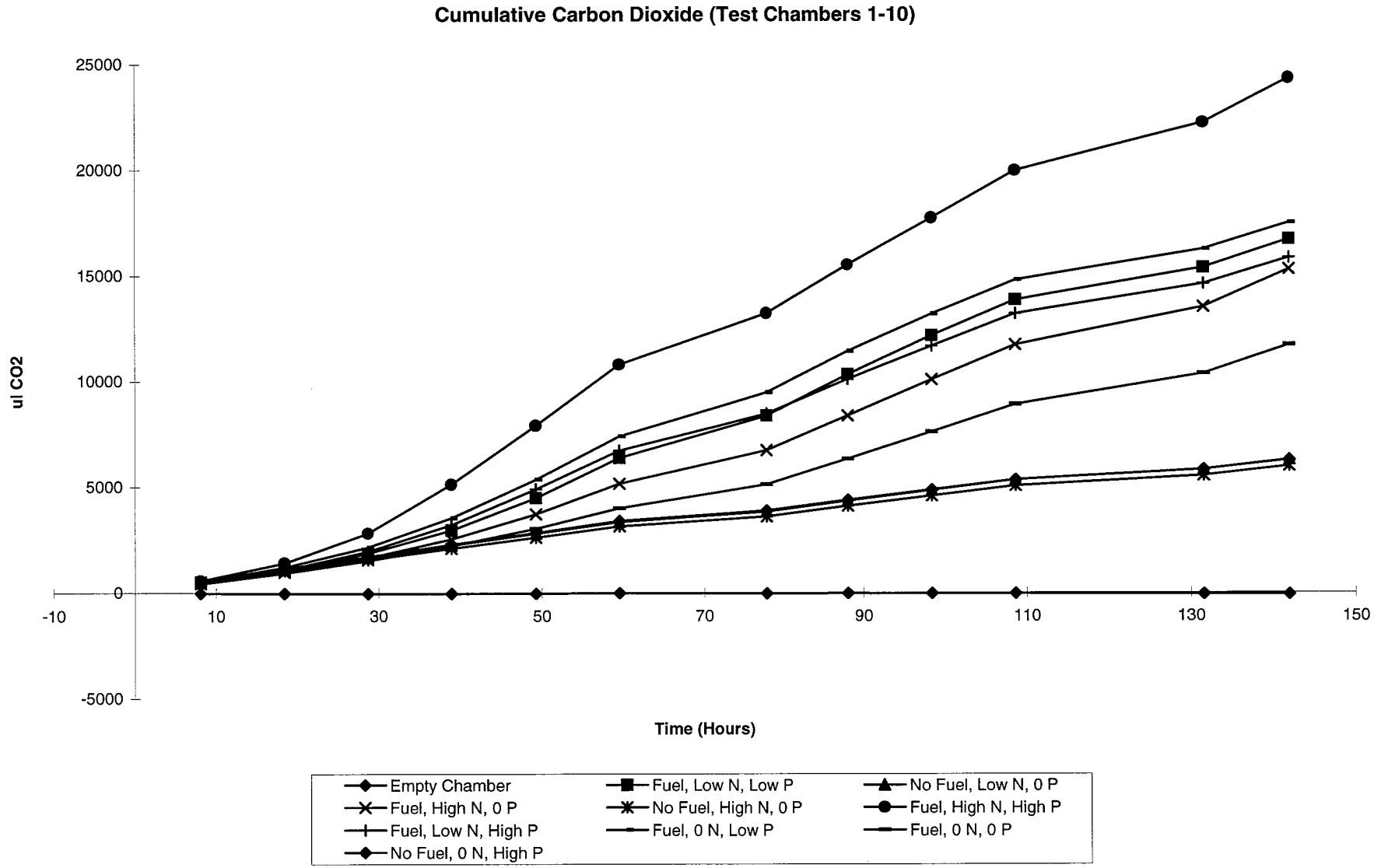


Figure 4 - 1d Experiment 1 Cumulative Carbon Dioxide Production (Test Chambers 1 - 10) vs Time

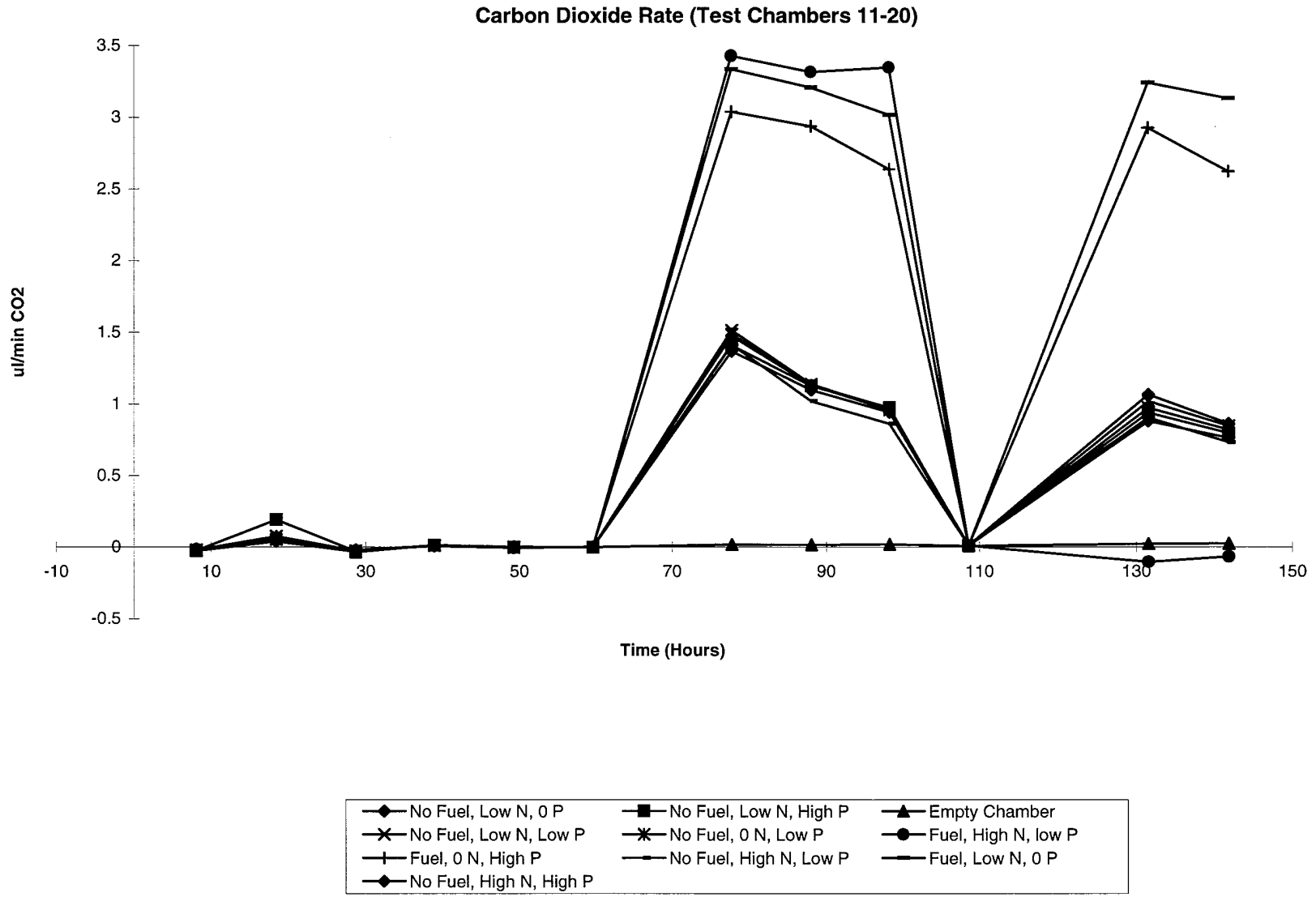


Figure 4 - 1f Experiment 1 Carbon Dioxide Production Rate (Test Chambers 11 - 20) vs Time

For this particular experiment statistical analyses were not performed on the respirometer generated data. However, a qualitative analysis provides some relevant information. From Figures 4 - 1a,b and 4 - 1d,e it is important to note that the O_2 and CO_2 cumulative and rate curves mimic each other but on different scales. This is to be expected. However, upon further investigation it was found that measured CO_2 values are approximately 42 - 51% of the values calculated from the following equation (See Table 4 - 1).

$$C_{11}H_{24} + 17O_2 ========> 11CO_2 + 12H_2O + \text{Biomass}$$

The values of CO_2 production for the test chambers not treated with jet fuel are somewhat closer to expected values. Hinchee and Ong assumed for every 3 carbons removed 1 carbon is used in biomass production. It is interesting to note that the recoverability of CO_2 for fuel treated test chambers is somewhat less than for untreated chambers. This might be because that there is more carbon available in the fuel treated chambers which can be made available for biomass production.

The most obvious piece of information gained from this experiment is the impact JP - 8 addition has on the O_2 consumption and CO_2 production rates. Test chambers without fuel were at much lower consumption and production rates than those measured in the fuel treated chambers. Figures 4 - 1c and 4 - 1f for chambers 11 - 20 support this with a distinguishable difference between fuel and non fuel treated chambers. Chambers treated with fuel and the various inorganic combinations (treatments) displayed qualitative differences. However, these differences were not proven statistically. The no-fuel treated test chambers displayed no differences what so ever for each nutrient combination.

Additionally, and initial lag was not seen on the rate curves because the test chambers were stored for the initial 24 hours of the experiment, allowing microbial acclimation prior to the initial measurement. These lines overlapped and were practically identical. As expected, the empty test chamber did not show changes of O_2 or CO_2.

4.3 Experiment 2 Discussion of Results

4.3.1 Respirometry Data and Analysis

Figures 4 - 2 a,b and 4 - 2e,f display O_2 consumption and CO_2 production rates for all twenty test chambers. Again, the chambers without fuel did not display any visible impacts between treatments. In light of this, the test chamber O_2 consumption and CO_2 production rates associated with JP - 8 addition are all displayed together against the untreated soil reference (treatment 1) in Figures 4 - 2c and 4 - 2g. Based on these rates, Cumulative O_2 and CO_2 values for these same treatments and test chambers are displayed in figures 4 - 2d and 4 - 2h. Table 4 - 2 summarizes Experiment 2 respirometry results.

The first initial dip seen on the O_2 and CO_2 rate curves within the first 25 hours is microbial acclimation or lag period. After the initial lag, most of the rate curves are smooth and rounded throughout the course of the experiment. However, the high nitrate and high phosphate (treatment 9) O_2 rate curve (See Figure 4 - 2b) displayed a rapid climb between 50 and 60 hours followed by a sharp decrease in consumption rate. This is possible due to the O_2 consumption rate exceeding the available oxygen in the test chamber, resulting in a peak followed by a sharp decrease in microbial activity. This can be solved by shorter sampling intervals which allow a more frequent test chamber refresh.

Another interesting curve is the high nitrate, 0 phosphate (treatment 3) curve. The O_2 consumption and CO_2 production rate activity is slow throughout the experiment until the 300 hour point, at which it overtakes all other test chamber activity rates. This increase in rates is further observed in the resulting O_2 and CO_2 figures. This test chamber was the lowest cumulative producer/consumer until the 300 hour point, at which its cumulative values begin to overtake several of the other test chamber cumulative value curves. Unfortunately the experiment was not long enough to observe the final outcome of this activity.

Table 4 - 2 Experiment 2 summary of respirometry results

N/P Fuel Treatment	Chamber	O2 (uL)	CO2 (uL) Actual	CO2 (uL) Theoretical	Actual/ Theoretical
0/0 F	15	204061	78086	132039	0.59
L/0 F	20	217770	79012	140910	0.56
H/0 F	19	239831	88265	155185	0.57
H/L F	7	282209	96049	182606	0.53
L/L F	11	247672	87095	160258	0.54
0/L F	6	223851	84313	144845	0.58
0/H F	9	230945	81692	149435	0.55
L/H F	10	235096	84522	152121	0.56
H/H F	12	284320	94703	183972	0.51
0/0 NF	18	64447	24803	41701	0.59
L/0 NF	14	64459	24869	41709	0.60
H/0 NF	2	45069	17562	29162	0.60
H/L NF	1	51892	18420	33577	0.55
L/L NF	3	55511	22649	35919	0.63
0/L NF	16	78084	29637	50525	0.59
0/H NF	13	54510	20476	35271	0.58
L/H NF	5	49729	20229	32178	0.63
H/H NF	4	43840	18486	28367	0.65

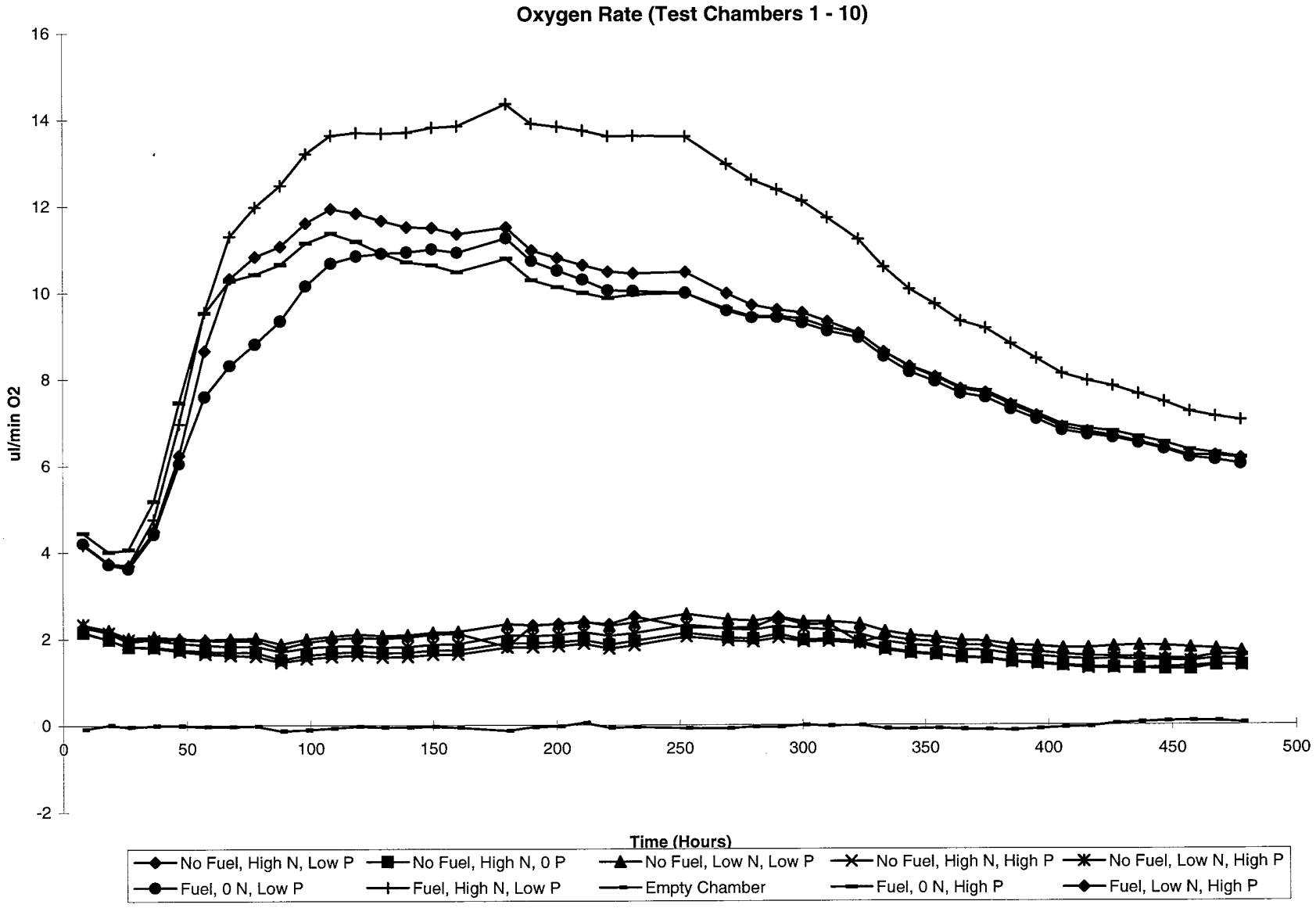


Figure 4 - 2a Experiment 2 Oxygen Consumption Rate (Test Chambers 1 - 10) vs Time

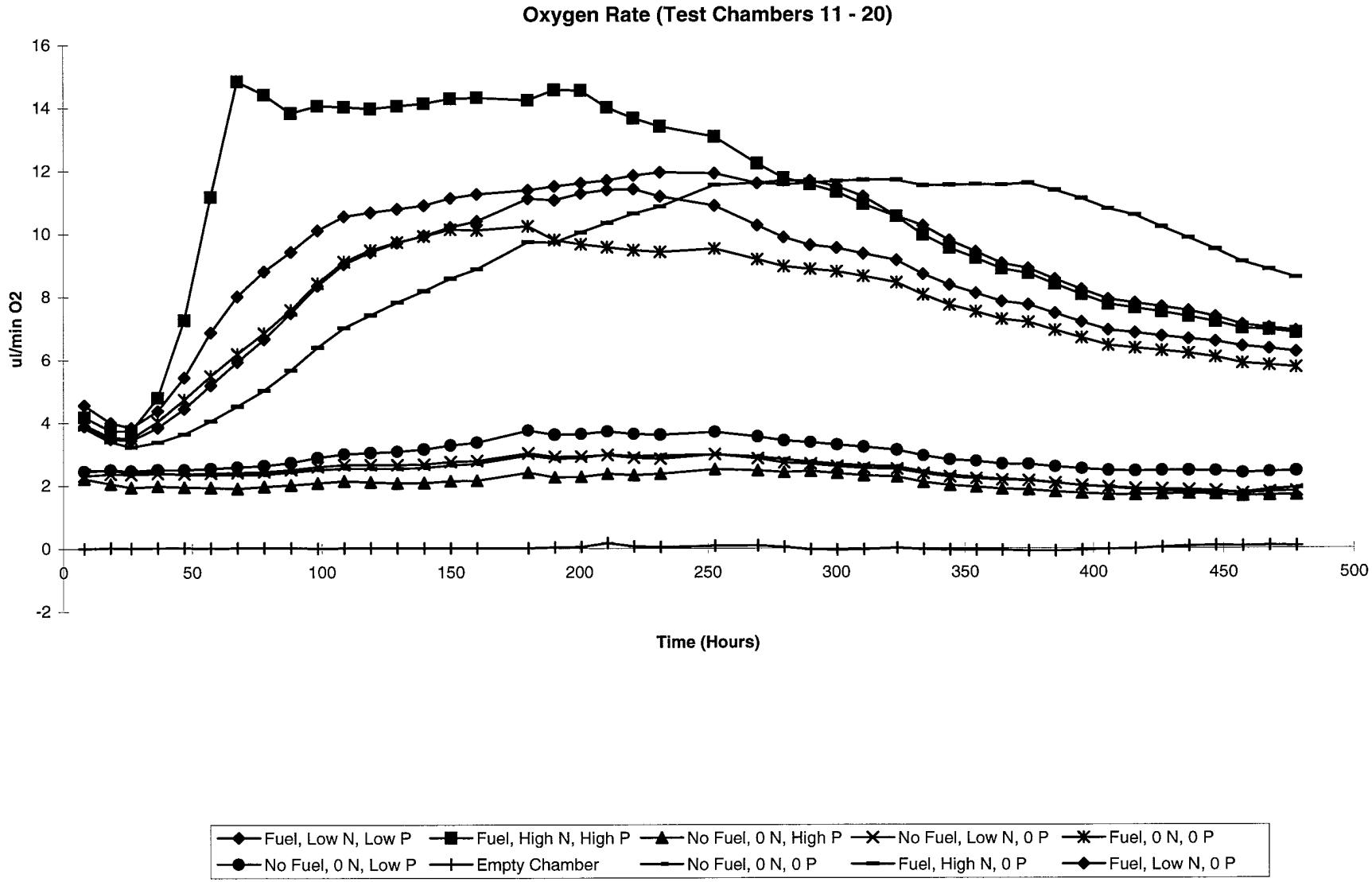


Figure 4 - 2b Experiment 2 Oxygen Consumption Rate (Test Chambers 11 - 20) vs Time

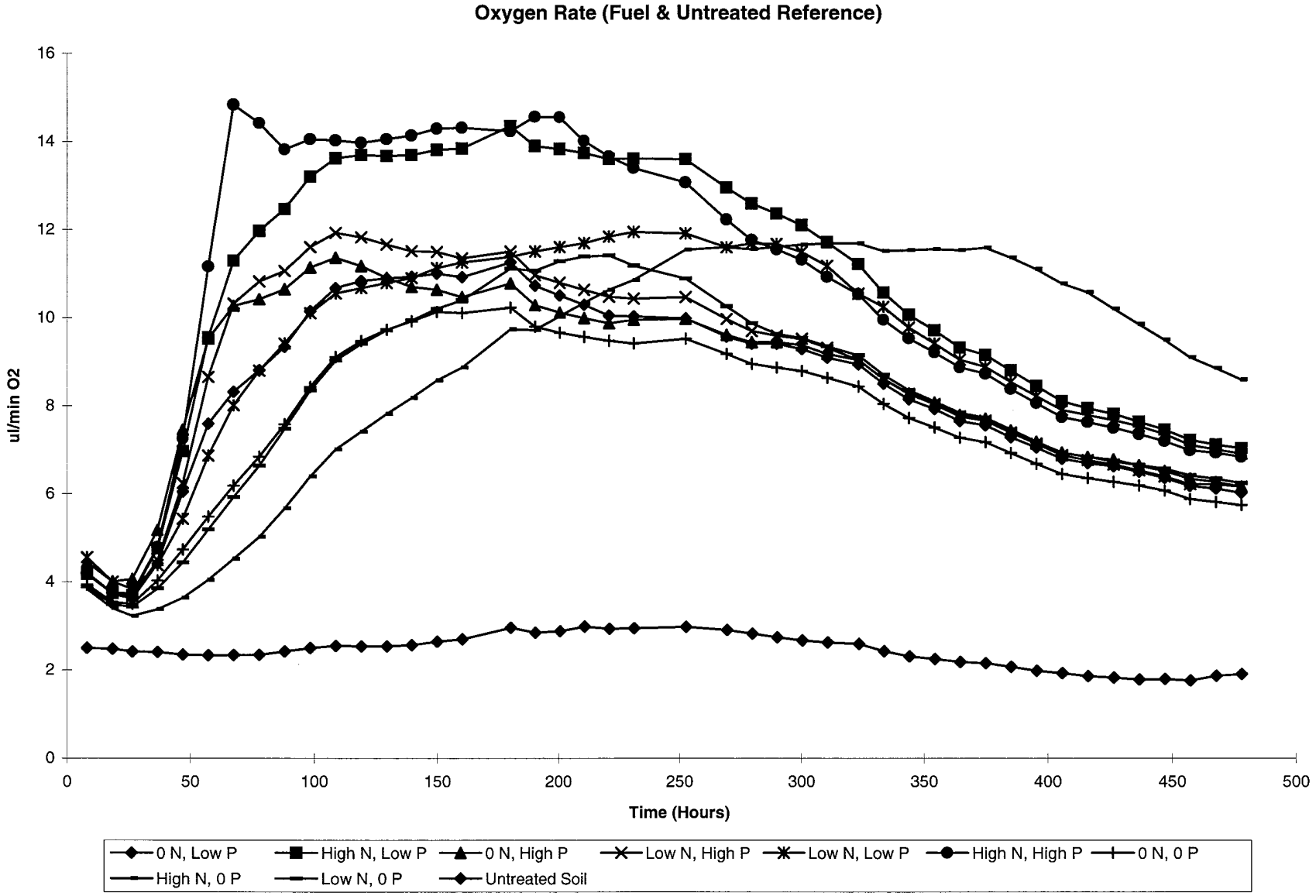


Figure 4 - 2c Experiment 2 Oxygen Consumption Rate (Fuel and Untreated Reference) vs Time

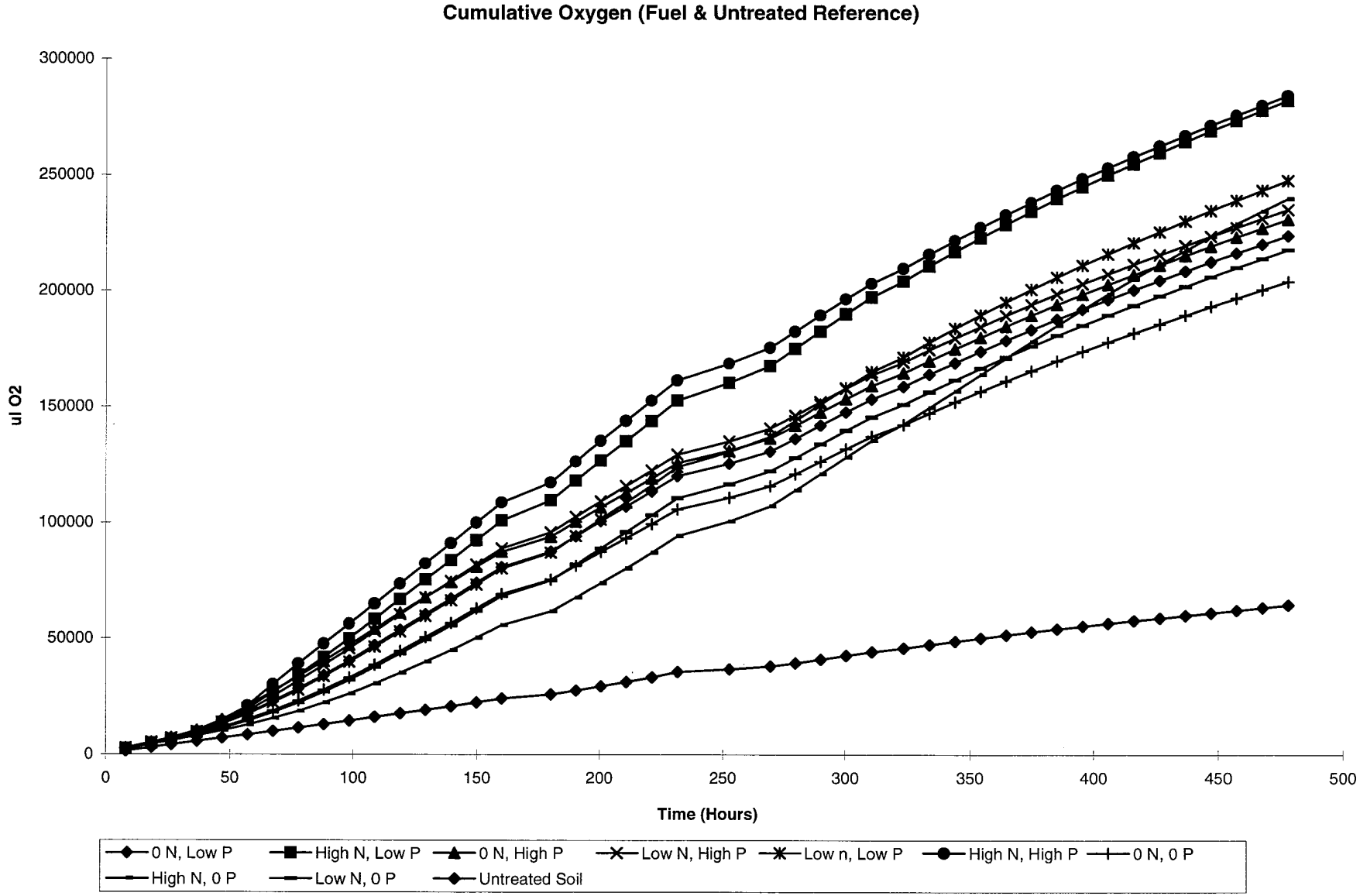


Figure 4 - 2d Experiment 2 Cumulative Oxygen Consumption (Fuel Untreated Reference) vs Time

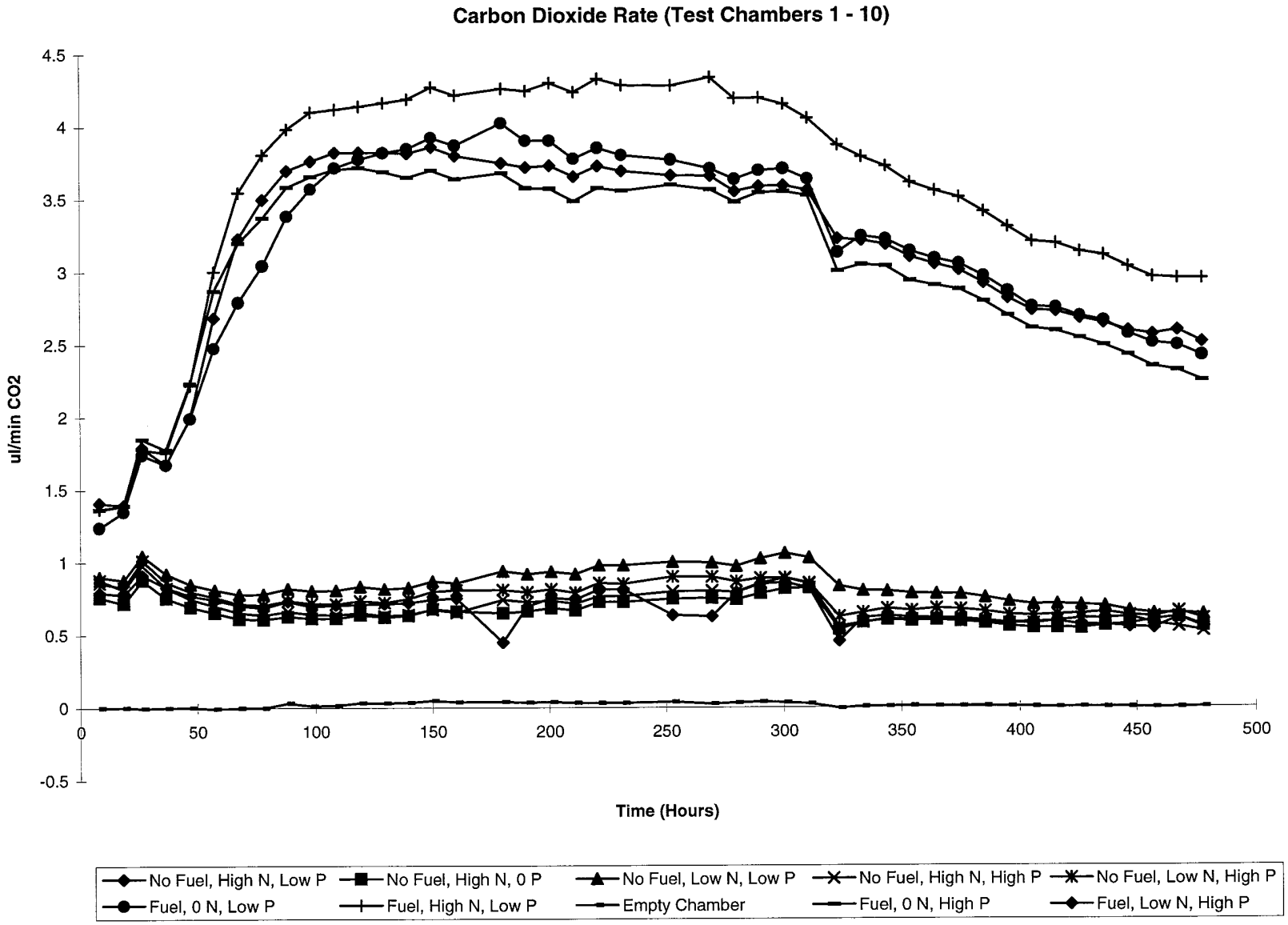


Figure 4 - 2e Experiment 2 Carbon Dioxide Production Rate (Test Chambers 1 - 10) vs Time

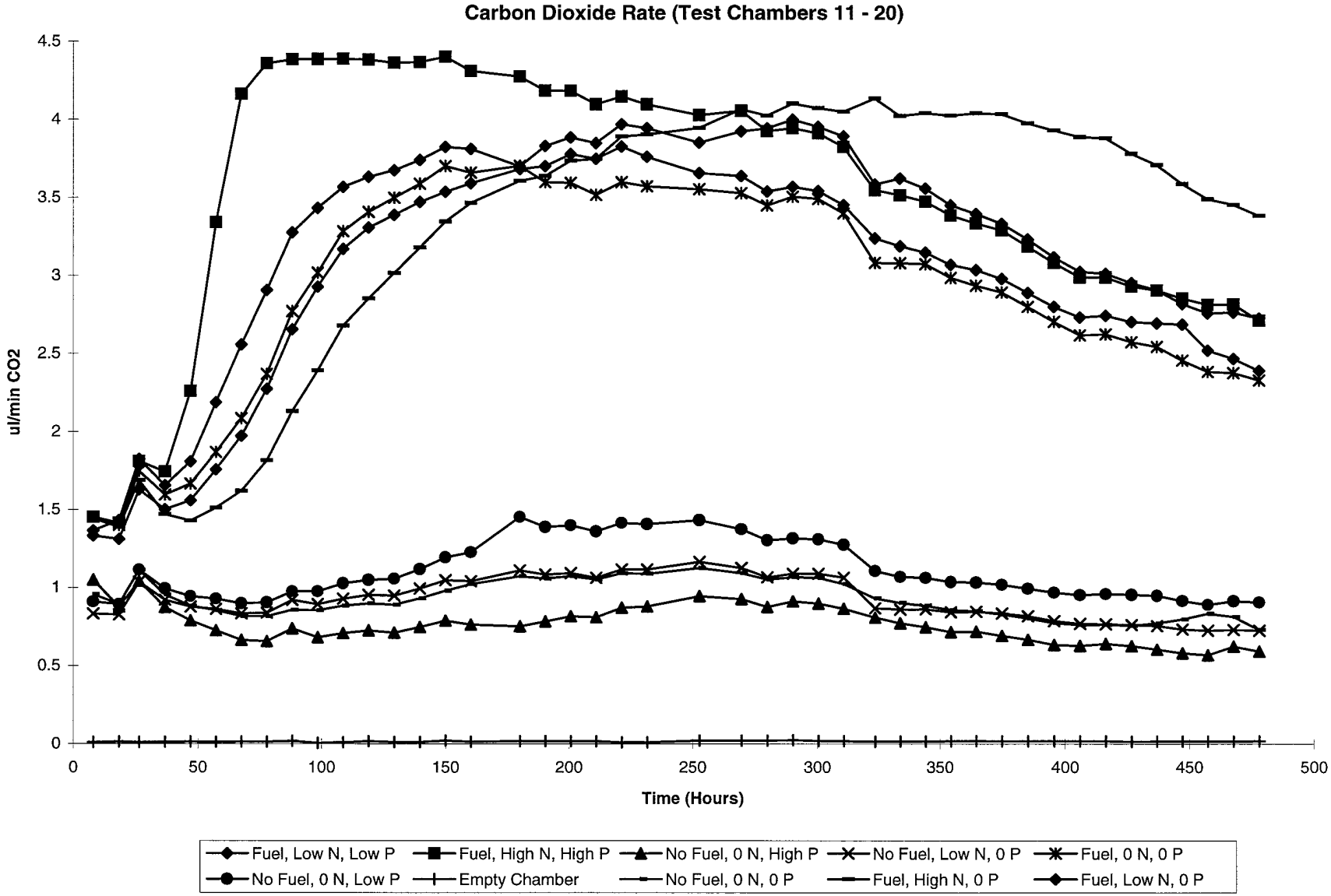


Figure 4 - 2f Experiment 2 Carbon Dioxide Production Rate (Test Chambers 11 - 20) vs Time

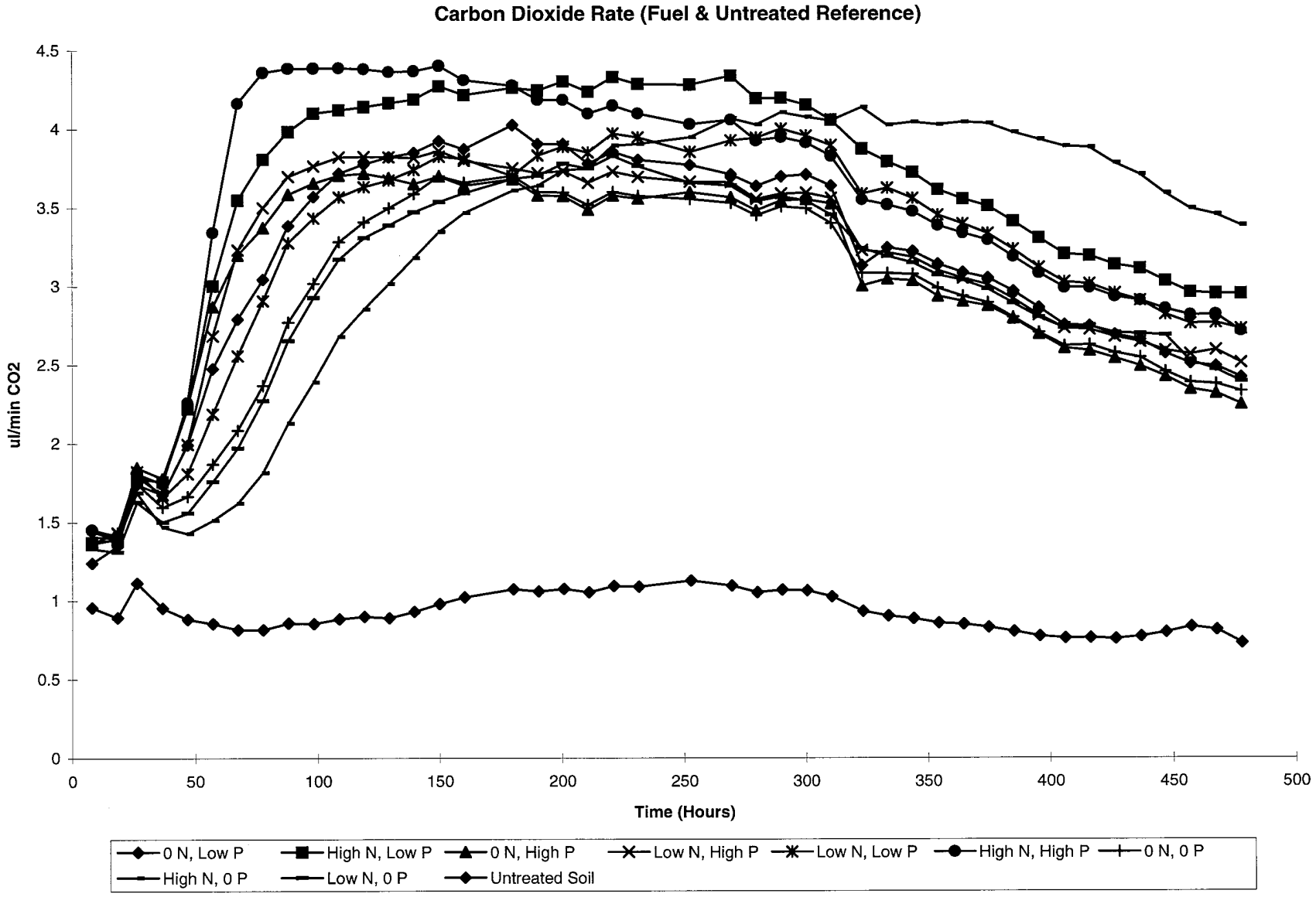


Figure 4 - 2g Experiment 2 Carbon Dioxide Production Rate (Fuel Untreated Reference) vs Time

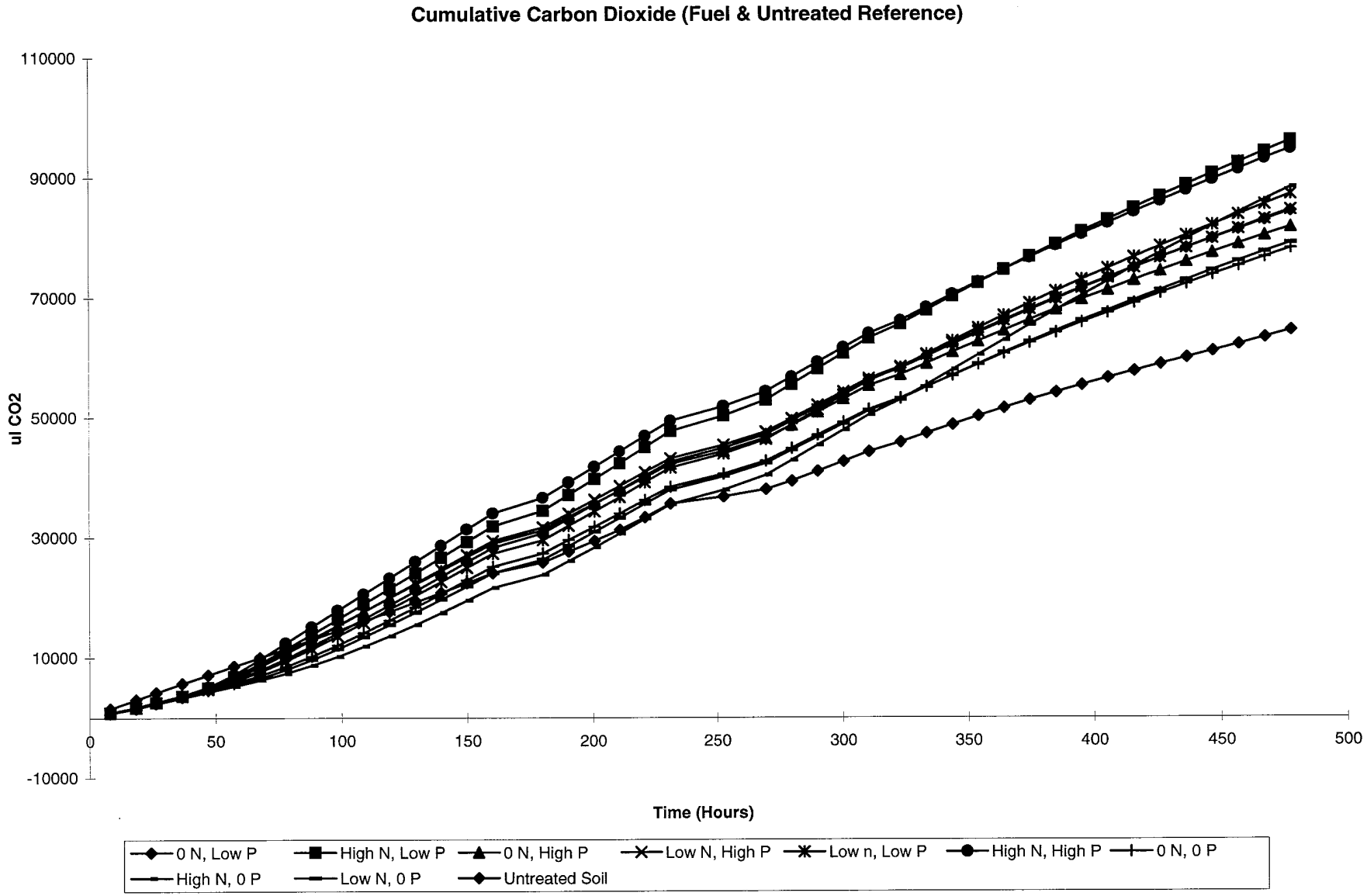


Figure 4 - 2h Experiment 2 Cumulative Carbon Dioxide Production (Fuel and Untreated Reference) vs Time

4.3.2 Experiment 2 - Analysis of Variance Results (See Appendix A for Computer Output)

A three factor ANOVA was performed using the cumulative oxygen values as the response variable. Table 4 - 3 is a summary of these findings.

Table 4 - 3 Experiment 2 ANOVA Results

Rejection Region	Nitrate (α)	Phosphate (β)	Fuel (γ)	$\alpha*\beta$	$\alpha*\gamma$	$\beta*\chi$
$p < 0.05$	0.0532	0.044	0.0000	0.5278	0.0040	0.0298

With a rejection value of $p < 0.05$, phosphate and fuel each have an impact on JP-8 biodegradation as indicated by the response variable. Phosphate and nitrate do not interact so there is not a synergistic or antagonistic effect caused by their combination on JP-8 biodegradation. However, the interactions of nitrate/fuel and phosphate/fuel have an impact on the response variable. These interactions indicate that nitrate/fuel and phosphate/fuel interact in combination different from the sum of there separate effects. Tukey's multiple comparison was performed for these interaction effects. This analysis is used to identify significant differences in cumulative oxygen values between levels of variables that interact. From the results of the Tukey analysis it can be determined if nutrient addition has a positive impact on biodegradation as indicated by the cumulative oxygen values with or without the presence of fuel. Table 4-4 summarizes theses results with respect to phosphate addition.

Table 4 - 4 Tukey Analysis of Oxygen by Phosphate
Note: Means with the same letter are not significantly different

Phosphate Level	Fuel Level	Tukey Grouping	Mean
Low	Yes	A	0.25122
High	Yes	AB	0.25012
Zero	Yes	B	0.22055
Low	No	C	0.06182
Zero	No	C	0.05799
High	No	C	0.04936

The analysis indicates a significant difference between fuel groups. However, phosphate enhancement impacts the response variable only when exposed to JP-8. There appears to be a statistical difference between the low and zero groups with the high group not being statistically different from either when exposed to JP-8. Additionally, within the group not exposed to JP-8, phosphate addition does not impact the response variable. Table 4-5 summarizes the results with respect to nitrate addition.

Table 4 - 5 Tukey Analysis of Oxygen by Nitrate

Nitrate Level	Fuel Level	Tukey Grouping	Mean
High	Yes	A	0.26878
Low	Yes	B	0.23349
Zero	Yes	B	0.21962
Zero	No	C	0.06568
Low	No	C	0.05656
High	No	C	0.04693

Similarly to phosphate addition, there is a significant difference between fuel groups. Additionally, the nitrate addition only impacts cumulative oxygen when exposed to JP-8. The high nitrate addition clearly separates itself from the low and zero groups.

4.3.3 Inorganic Recovery Analysis (See Appendix F for Data)

Figures 4 - 3a through 4 - 3f display relationships between cumulative oxygen, cumulative carbon dioxide, nitrate change, and phosphate change. Table 4 - 6 summarizes inorganic changes.

Table 4 - 6 Experiment 2 Nitrate and Phosphate Changes

N/P Fuel Treatment	Chamber	Nitrate Δ (mg)	Nitrate mg/day	Phosphate Δ (mg)	Phosphate mg/day
0/0 F	15	0.68	0.034	0.16	0.008
L/0 F	20	0.92	0.046	0.12	0.006
H/0 F	19	2.28	0.114	-0.12	-0.006
H/L F	7	1.28	0.064	0.6	0.03
L/L F	11	0.8	0.04	1.24	0.062
0/L F	6	0.56	0.028	0.28	0.014
0/H F	9	0.28	0.014	1.6	0.080
L/H F	10	0.84	0.042	1.28	0.064
H/H F	12	1.92	0.096	1.56	0.078
0/0 NF	18	-0.08	-0.004	-0.12	-0.006
L/0 NF	14	0.12	0.006	-0.04	-0.002
H/0 NF	2	0.84	0.042	0.08	0.004
H/L NF	1	-0.24	-0.012	-0.2	-0.01
L/L NF	3	-0.04	-0.002	1.12	0.056
0/L NF	16	-0.08	-0.004	-0.04	-0.002
0/H NF	13	-0.24	-0.012	1.04	0.052
L/H NF	5	-0.08	-0.004	-0.84	-0.042
H/H NF	4	-0.28	-0.014	0.6	0.03

A correlational study was performed with the following results (See Table 4 - 7).

Table 4 - 7 Experiment 2 Inorganic Correlational Analysis Results - If the correlation value exceeds the rejection value, the null hypothesis can be rejected and hence a correlational relationship exists between the tested variables.

Relationship	Correlation (Linear Strength)	Rejection Region (95% for N=18)
Cumulative O_2 (L) vs CO_2 (L)	0.99	Corr > 0.400
Cumulative O_2 (L) vs Nitrate Δ (mg)	0.78	Corr > 0.400
Cumulative O_2 (L) vs Phosphate Δ (mg)	0.44	Corr > 0.400
Cumulative CO_2 (L) vs Nitrate Δ (mg)	0.77	Corr > 0.400
Cumulative CO_2 (L) vs Phosphate Δ (mg)	0.43	Corr > 0.400
Phosphate Δ (mg) vs Nitrate Δ (mg)	0.19	Corr > 0.400

These results indicate a strong linear relationship between O_2 and CO_2 (See Figure 4 - 3a). The nitrate and cumulative O_2 were fairly well correlated with the other test relationships being correlated considerably weaker. Phosphate vs nitrate was the only test values not correlated. Additionally, with cumulative oxygen closely correlated with cumulative carbon dioxide, similar correlational relationships are seen between CO_2 values and nitrate Δ and phosphate Δ. It is also important to note that because the time = 0 samples used for initial nitrate and phosphate measurement were not taken from the actual test chamber analyzed for time = N, large error could be associated with the Δ values for each inorganic.

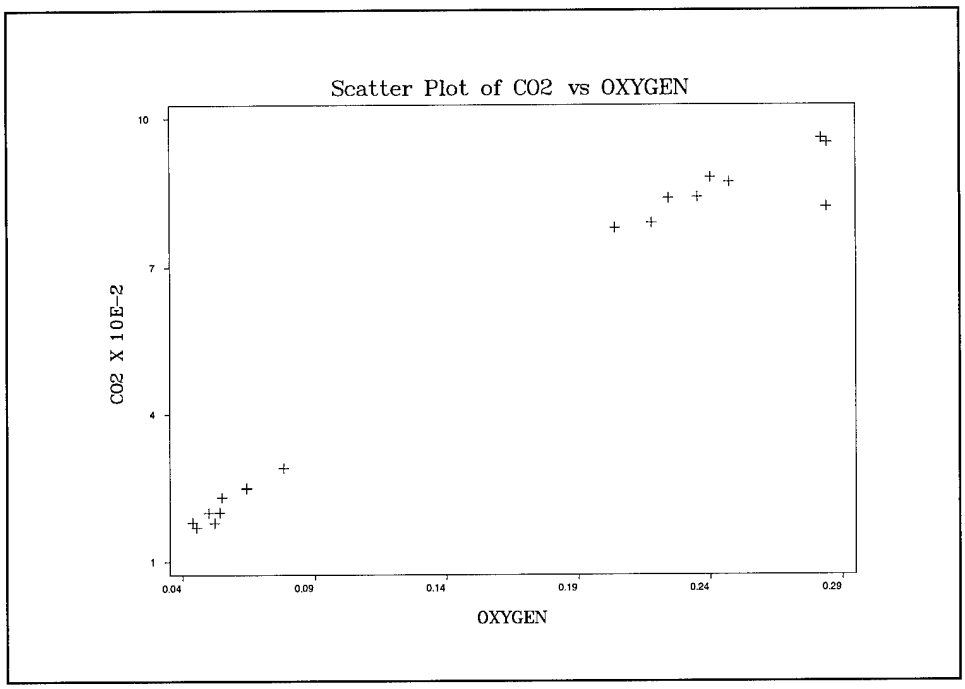


Figure 4 - 3a Experiment 2 Cumulative Oxygen(L) vs Cumulative Carbon Dioxide(L)

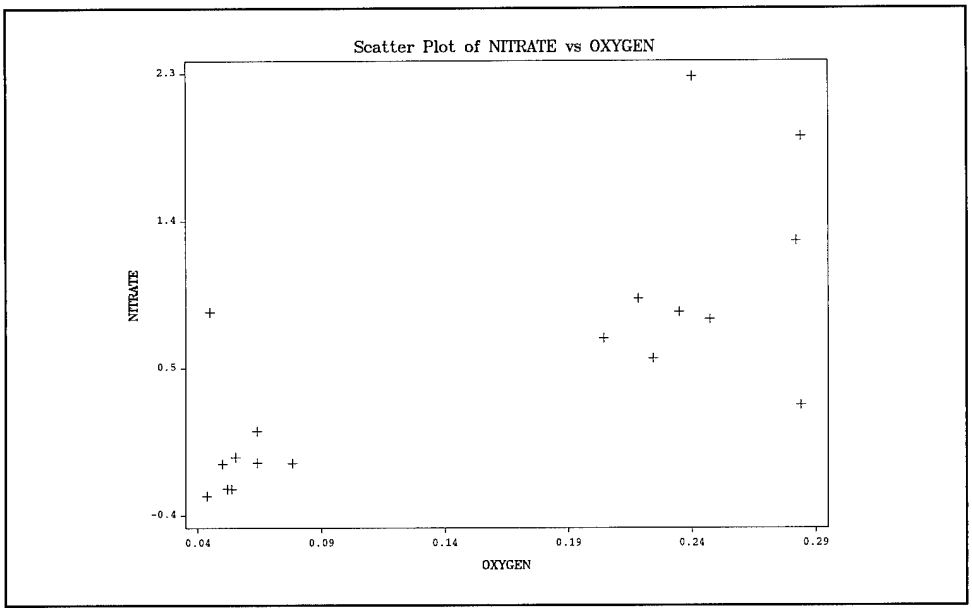


Figure 4 - 3b Experiment 2 Cumulative Oxygen(L) vs Nitrate Δ(mg)

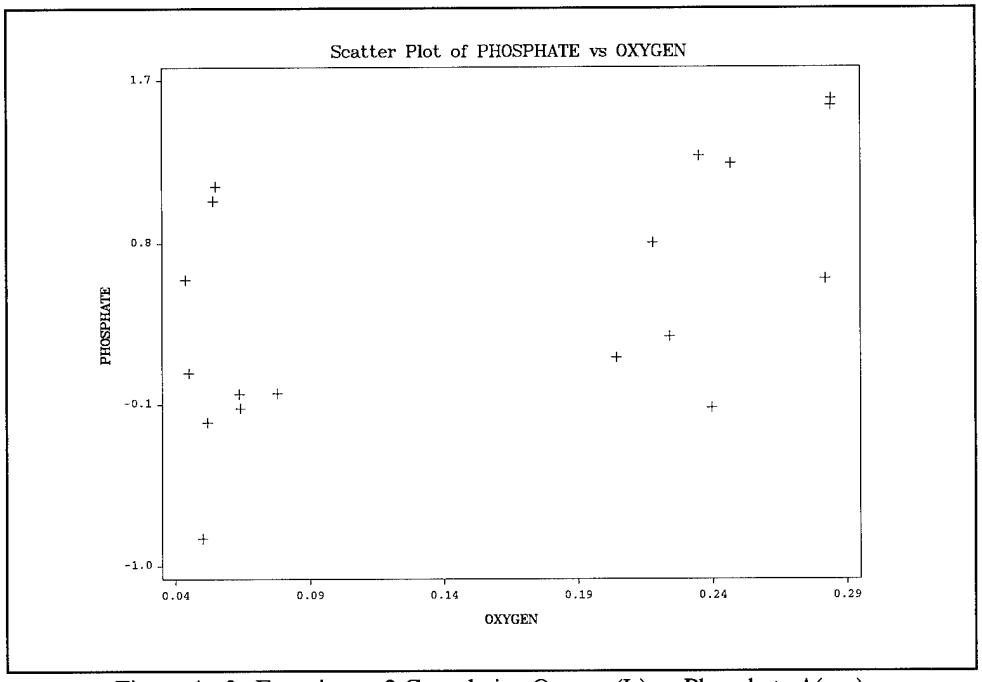


Figure 4 - 3c Experiment 2 Cumulative Oxygen(L) vs Phosphate Δ(mg)

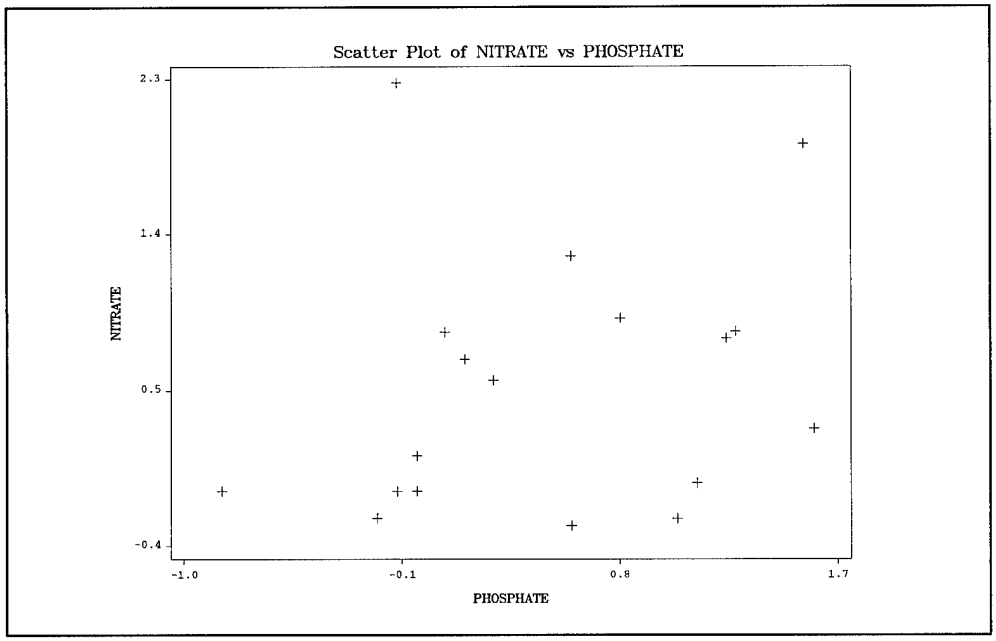


Figure 4 - 3d Experiment 2 Nitrate Δ(mg) vs Phosphate Δ(mg)

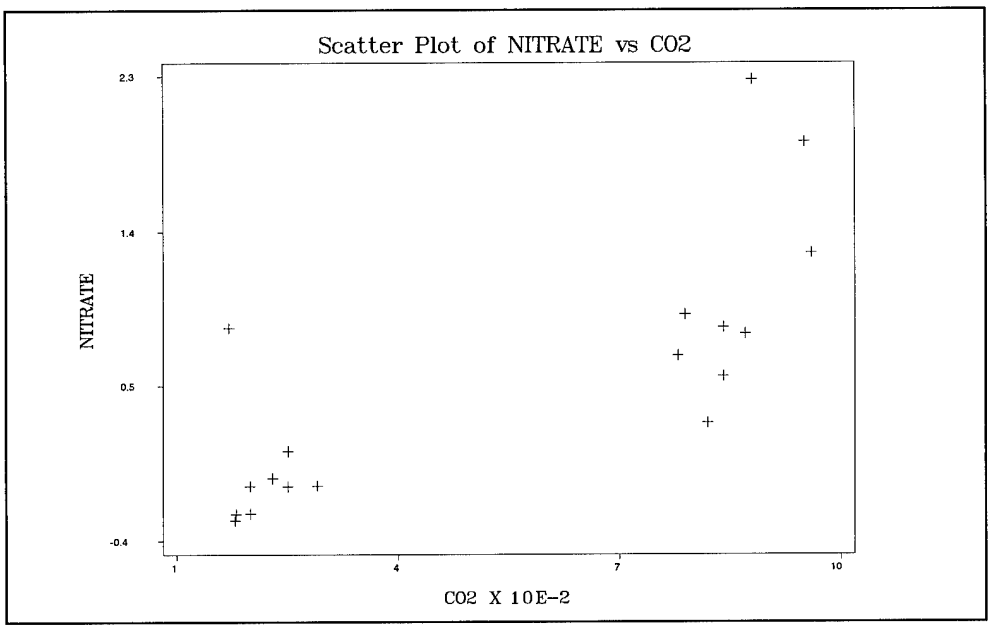


Figure 4 - 3e Experiment 2 Cumulative Carbon Dioxide (L) vs Nitrate Δ(mg)

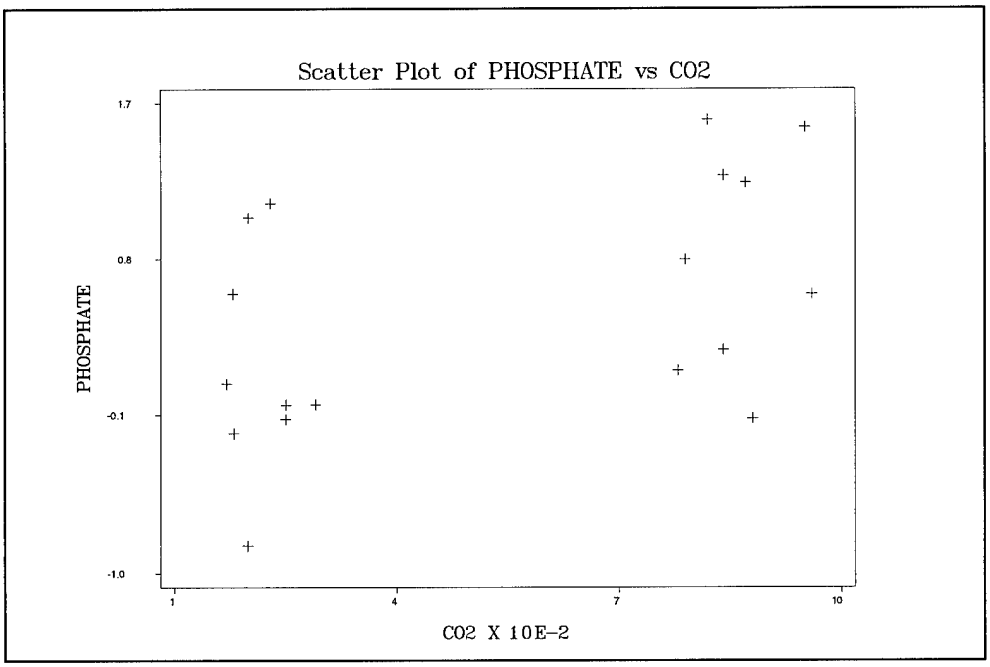


Figure 4 - 3f Experiment 2 Cumulative Carbon Dioxide(L) vs Phosphate Δ(mg)

4.3.4 Estimated % Hydrocarbon Mineralized (See Appendix F for Calculations)

For test chambers containing fuel, degradation values ranged from 6.28% to 9.7% of the original 1 gram of fuel added to each test chamber. Table 4 - 8 summarizes the values associated with each test chamber and treatment.

Table 4 - 8 Experiment 2 Percent Hydrocarbon Degradation

N/P Fuel Treatment	Chamber	Percent Hydrocarbon Δ	Degradation Rate mg/day
0/0 F	15	6.28	3.14
L/0 F	20	6.87	3.43
H/0 F	19	7.81	3.90
H/L F	7	9.61	4.80
L/L F	11	8.14	4.07
0/L F	6	7.13	3.56
0/H F	9	7.43	3.71
L/H F	10	7.60	3.80
H/H F	12	9.70	4.85

4.3.5 Vapor Recovery Analysis

Table 4 - 9 summarizes weight changes associated with charcoal traps exposed and not exposed to JP - 8 during testing. The traps unexposed to JP - 8 were assumed to have an increase in weight as a result of moisture uptake. The weight change of the traps exposed to JP - 8 were averaged and the average weight change of the unexposed traps were subtracted. The resultant 0.162 gram increase was assumed to be charcoal uptake of JP - 8 vapors. This accounts for approximately 16% of the original 1 gram of JP - 8 added to each chamber. Ross et al found that the major removal process of JP - 8 in the aquatic environment is evaporation. This seems to be the case in the soil environment as well.

The Thermogravimetric Analyzer estimation of moisture content was found to average 4.6% weight loss at the 100 °C temperature (Appendix H displays TGA curve) . The samples were taken from a blank with an original weight of 2.328 grams. Assuming 4.6% of that is moisture, this is a result of approximately 0.115 grams of moisture. This result is somewhat higher than the moisture estimation discussed above. The variability from sample to sample when using the TGA might explain this difference. Ideally it would be best to heat the entire charcoal sample and measure weight loss at the 100 °C point for a more accurate estimation of moisture content and uptake.

Table 4 - 9 Vapor Recovery Summary Experiment 2

Treatment N/P Fuel	Chamber	Initial Carbon Weight (grams)	Final Carbon Weight (grams)	Change (grams)	grams/day
0/0 F	15	2.242	2.450	0.207	0.0103
L/0 F	20	2.433	2.664	0.230	0.0115
H/0 F	19	2.199	2.411	0.211	0.0105
H/L F	7	2.315	2.493	0.177	0.0088
L/L F	11	2.096	2.290	0.194	0.0097
0/L F	6	2.139	2.348	0.209	0.0104
0/H F	9	2.772	2.990	0.217	0.0108
L/H F	10	2.305	2.504	0.198	0.0099
H/H F	12	2.274	2.481	0.206	0.0103
0/0 NF	18	2.617	2.670	0.052	0.0026
L/0 NF	14	2.407	2.441	0.034	0.0017
H/0 NF	2	2.580	2.620	0.040	0.0020
H/L NF	1	2.657	2.701	0.044	0.0022
L/L NF	3	2.213	2.260	0.046	0.0023
0/L NF	16	2.175	2.213	0.038	0.0019
0/H NF	13	2.288	2.328	0.040	0.0020
L/H NF	5	2.407	2.449	0.042	0.0021
H/H NF	4	2.308	2.354	0.046	0.0023
Blank		2.260	2.306	0.047	0.0023
Blank		2.328	N/A	N/A	N/A

Average Change With Fuel	Average Change W/O Fuel	Average Difference (grams)
0.205	0.043	0.162

4.4 Experiment 3 Discussion of Results

4.4.1 Respirometry Data and Analysis

Figure 4 - 4a and 4 - 4b display cumulative oxygen consumption for all treatments, including an untreated soil reference and empty test chamber control. Associated O_2 consumption rates are displayed in Figures 4 - 4c and 4 - 4d. Similarly cumulative carbon dioxide production values are displayed in Figure 4 - 4e,f with production rate curves displayed in Figure 4 - 4g,h. The CO_2 rate curves display an unusual saw tooth pattern from approximately the 500 hour point to experiment completion. This pattern was not observed in the O_2 rate curves. This pattern may have been caused by alternating driers from one sample interval to another. The driers are in place as a final moisture trap prior to sample air reaching the O_2 and CO_2 sensors. As these driers absorb moisture they become depleted. If the driers are moist enough, the H_2O may interfere with the CO_2 in the air prior to reaching the sensors and cause discrepancies in CO_2 measurement. However, when the equipment was broken down and cleaned at experiment completion, neither drier was completely consumed. This pattern remains a mystery. Table 4 - 10 summarizes Experiment 3 respirometry results.

Table 4 - 10 Experiment 3 Summary of Respirometry Results

N/P Fuel Treatment	Chamber	O2 (uL)	CO2 (uL) Actual	CO2 (uL) Theoretical	Actual/ Theoretical
0/0 F	2	257752	112064	166781	0.67
L/0 F	9	273366	116581	176884	0.66
H/0 F	10	269501	121638	174383	0.70
H/L F	5	284727	128125	184235	0.70
L/L F	1	281805	128655	182344	0.71
0/L F	4	267636	118865	173176	0.69
0/H F	3	285145	130374	184506	0.71
L/H F	8	287787	128568	186215	0.69
H/H F	6	298901	130388	193407	0.67
0/0 F	11	285190	128065	184535	0.69
L/0 F	18	270379	118090	174951	0.67
H/0 F	12	304025	133109	196722	0.68
H/L F	20	276200	132490	178718	0.74
L/L F	15	268020	123515	173425	0.71
0/L F	14	305128	133576	197436	0.68
0/H F	13	272203	122842	176131	0.70
L/H F	17	287436	129232	185988	0.69
H/H F	16	306317	135913	198205	0.69
Untreated	7	87329	35334	56507	0.63

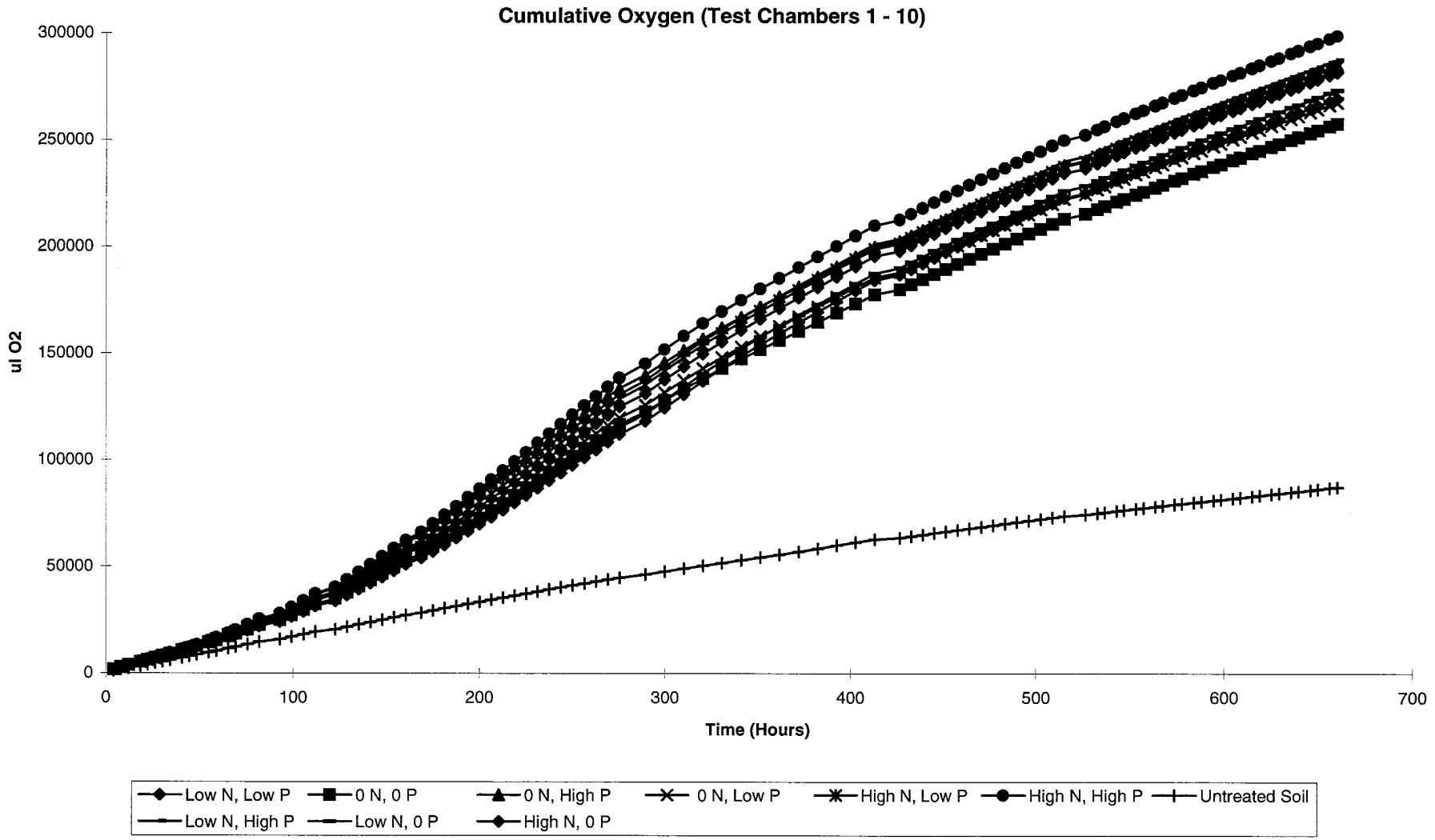


Figure 4 - 4a Experiment 3 Cumulative Oxygen Consumption (Test Chambers 1 - 10) vs Time

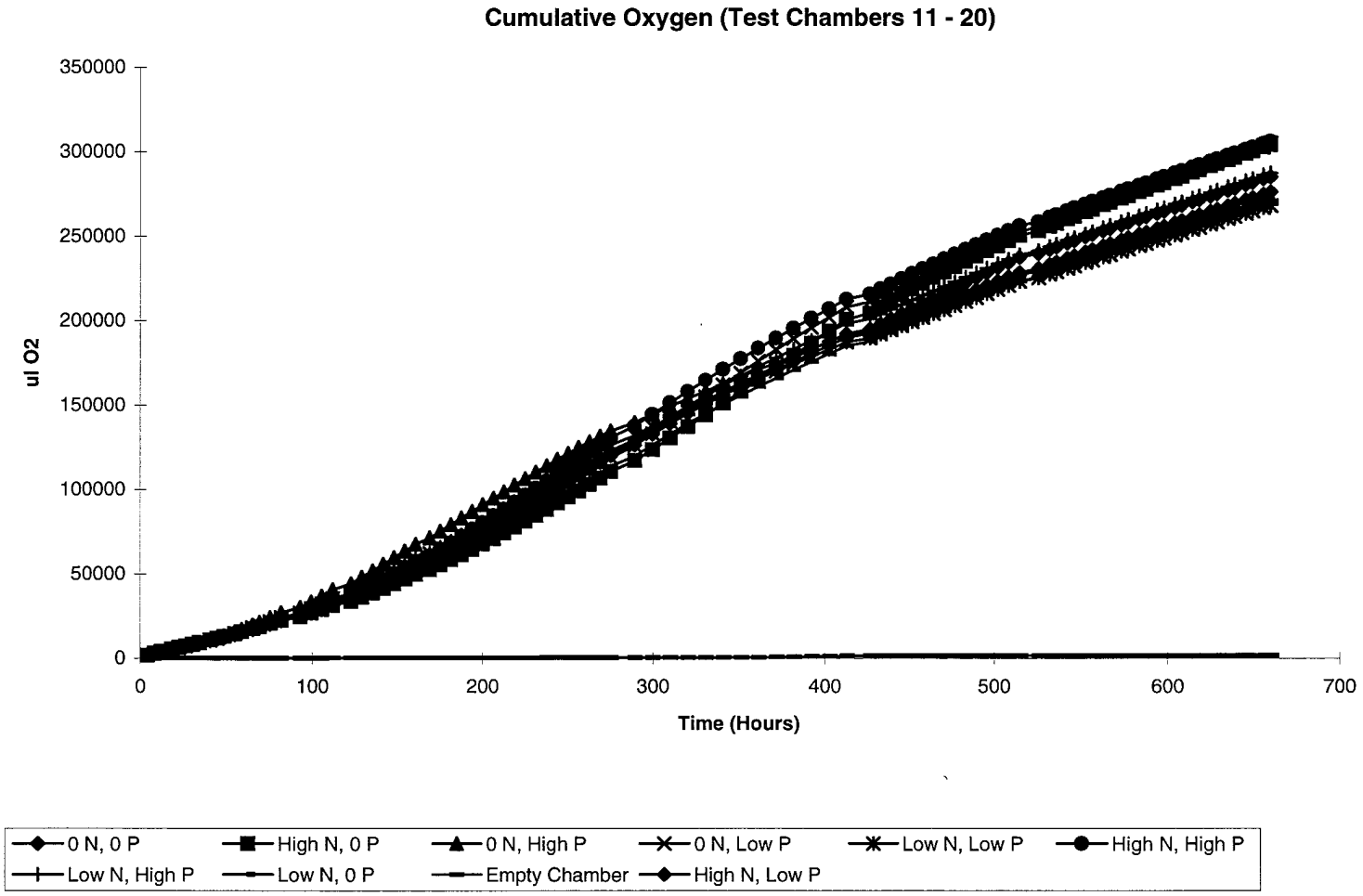


Figure 4 - 4b Experiment 3 Cumulative Oxygen Consumption (Test Chambers 11 - 20) vs Time

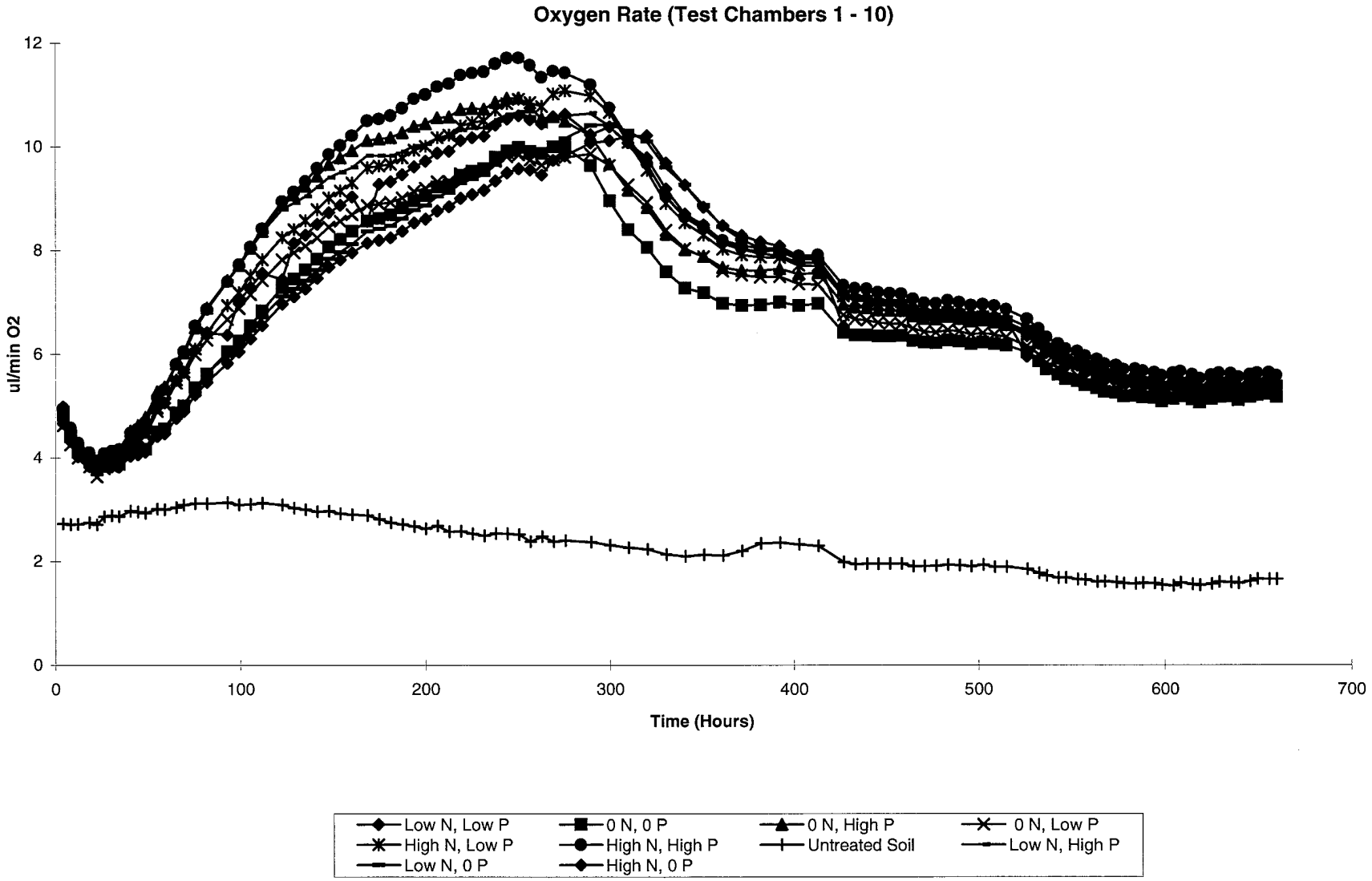


Figure 4 - 4c Experiment 3 Oxygen Consumption Rate (Test Chambers 1 - 10) vs Time

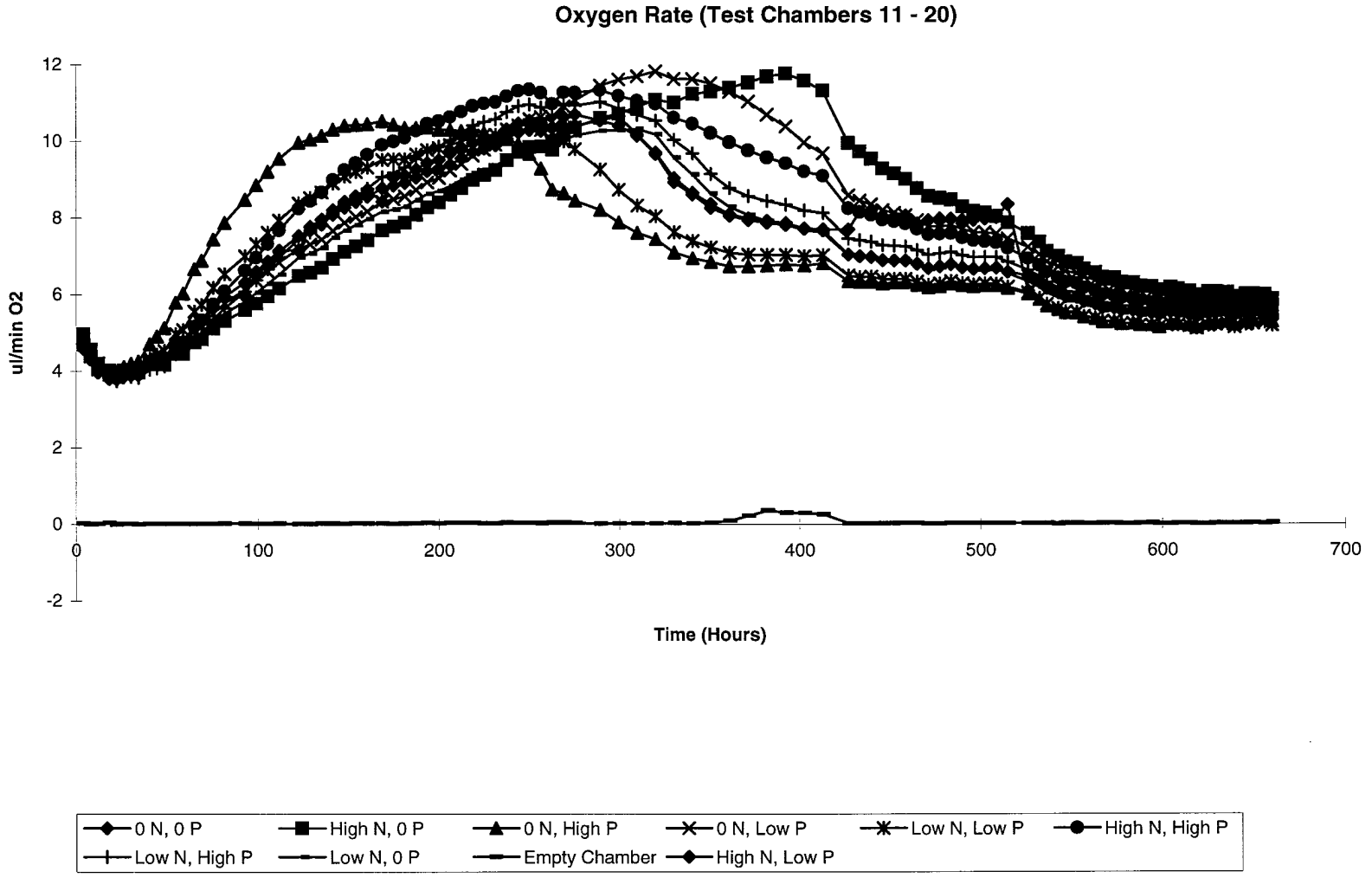


Figure 4 - 4d Experiment 3 Oxygen Consumption Rate (Test Chambers 11 - 20) vs Time

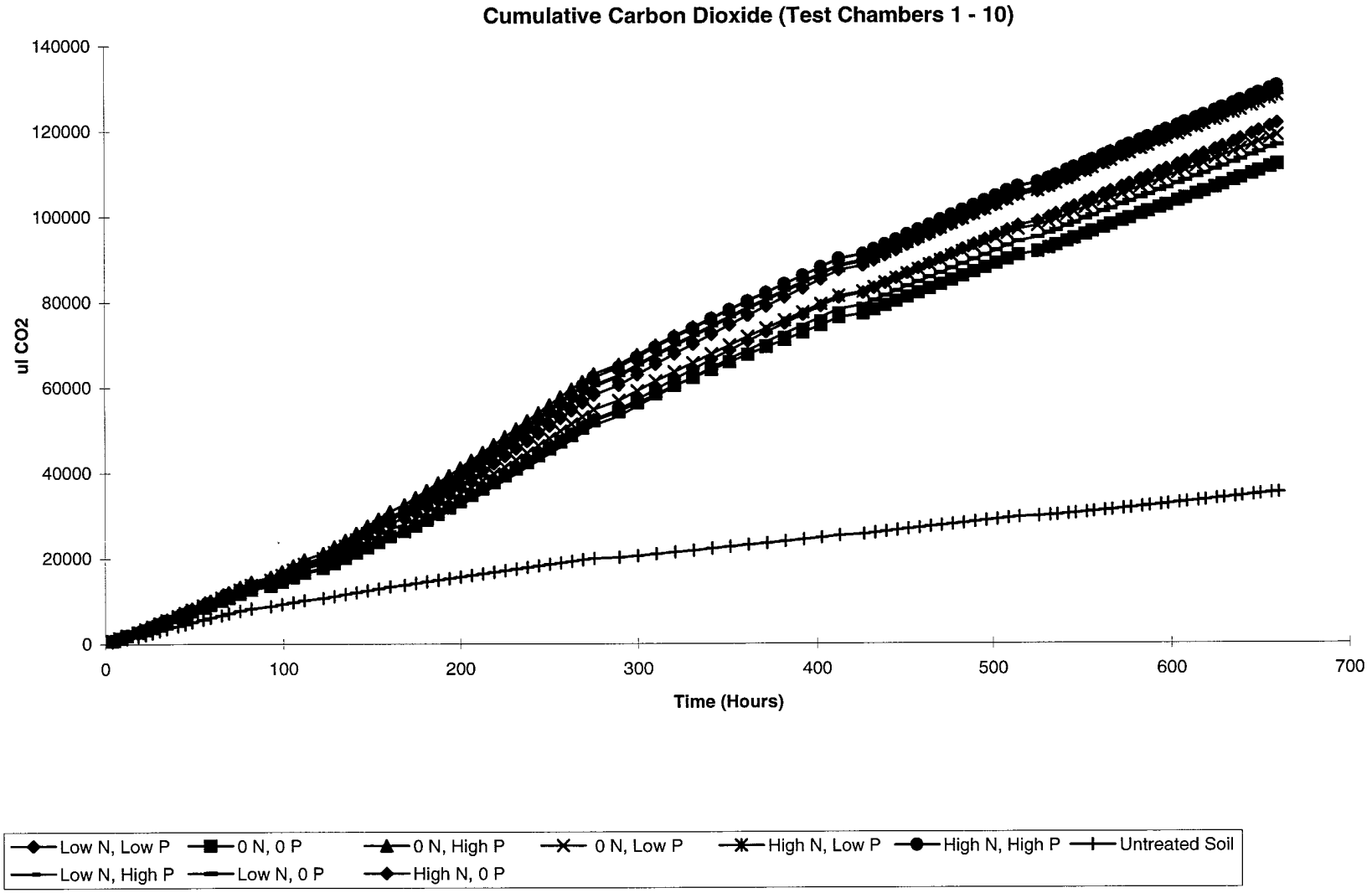


Figure 4 - 4e Experiment 3 Cumulative Carbon Dioxide Production (Test Chambers 1- 10) vs Time

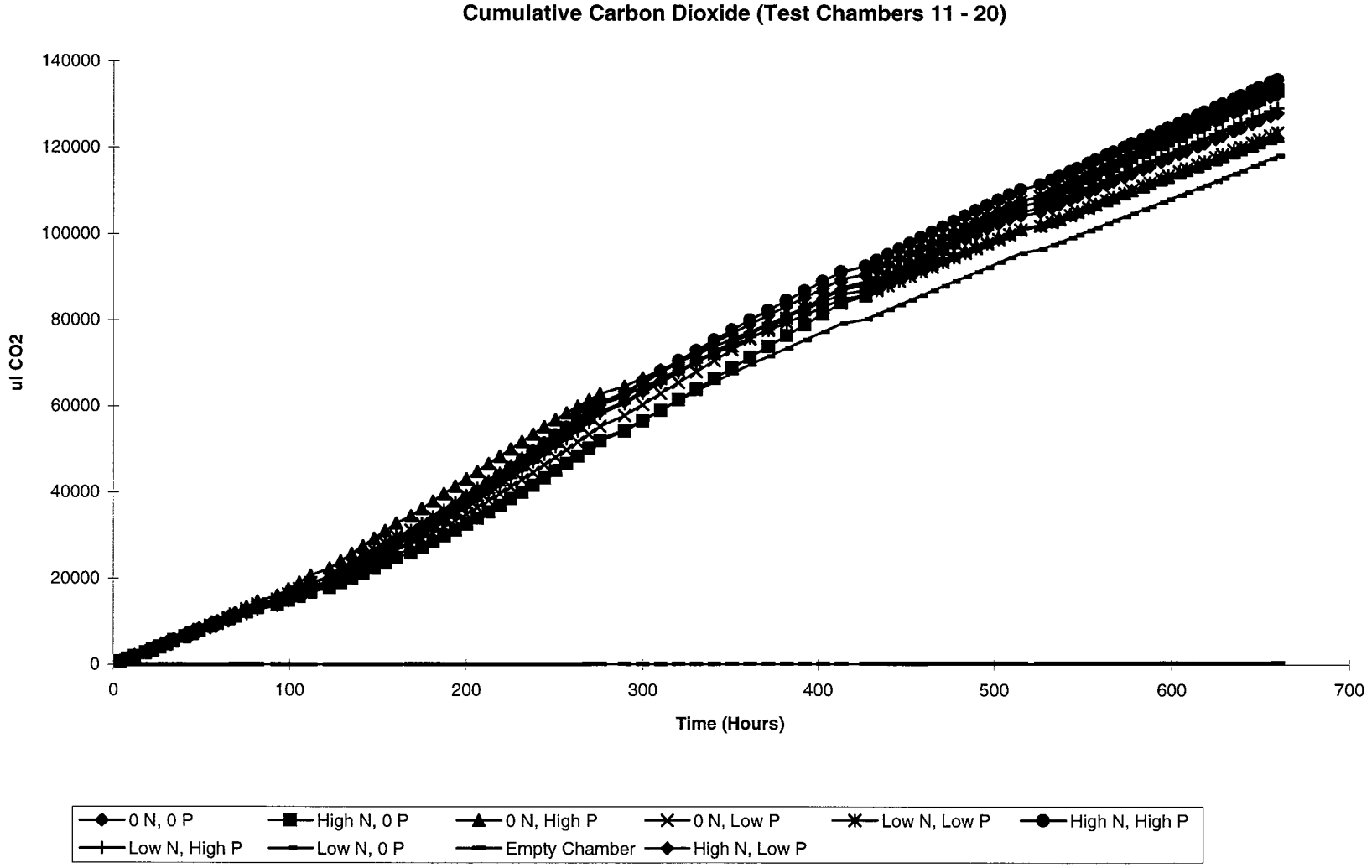


Figure 4 - 4f Experiment 3 Cumulative Carbon Dioxide Production (Test Chambers 11 - 20) vs Time

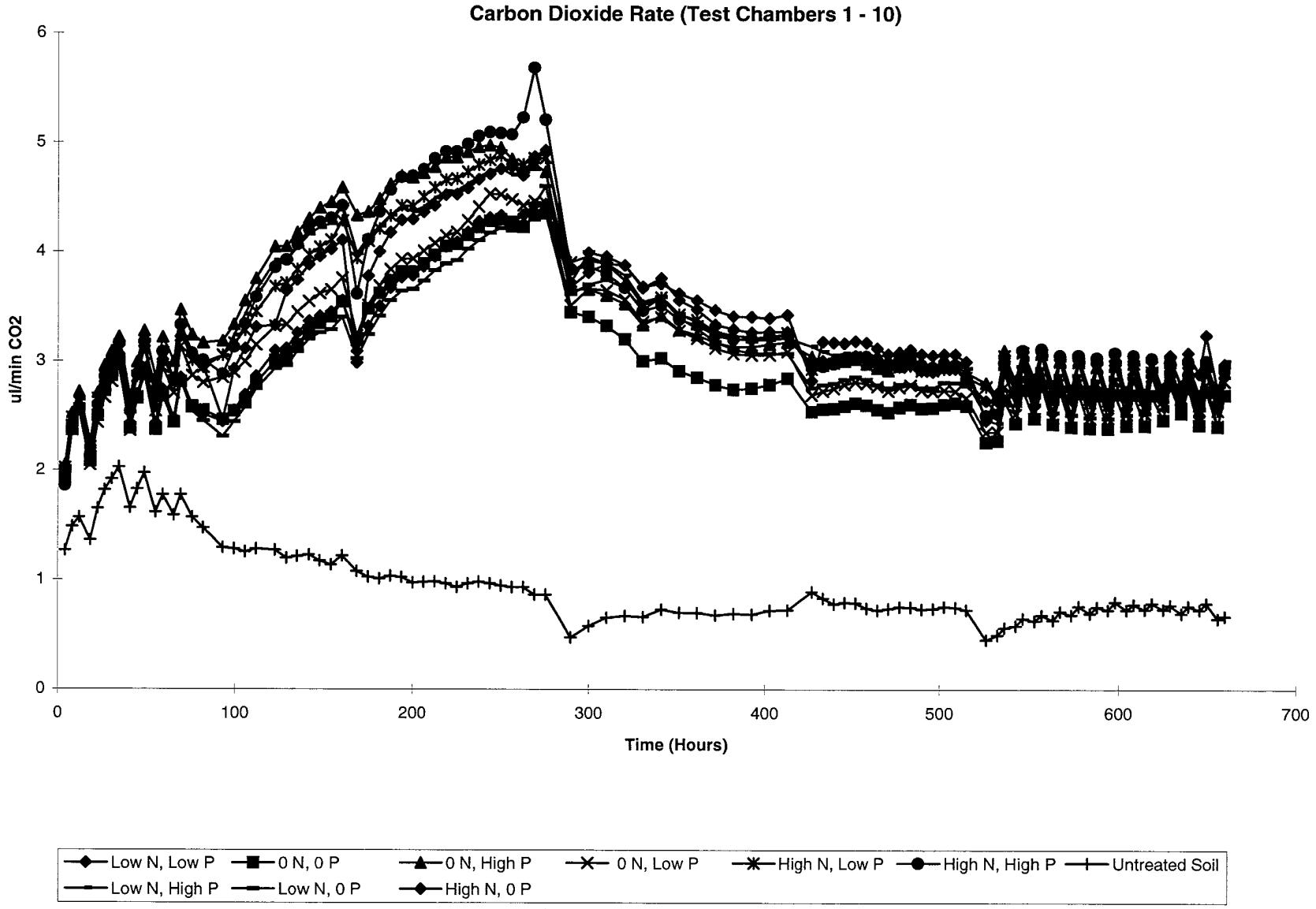


Figure 4 - 4g Experiment 3 Carbon Dioxide Production Rate (Test Chambers 1 - 10) vs Time

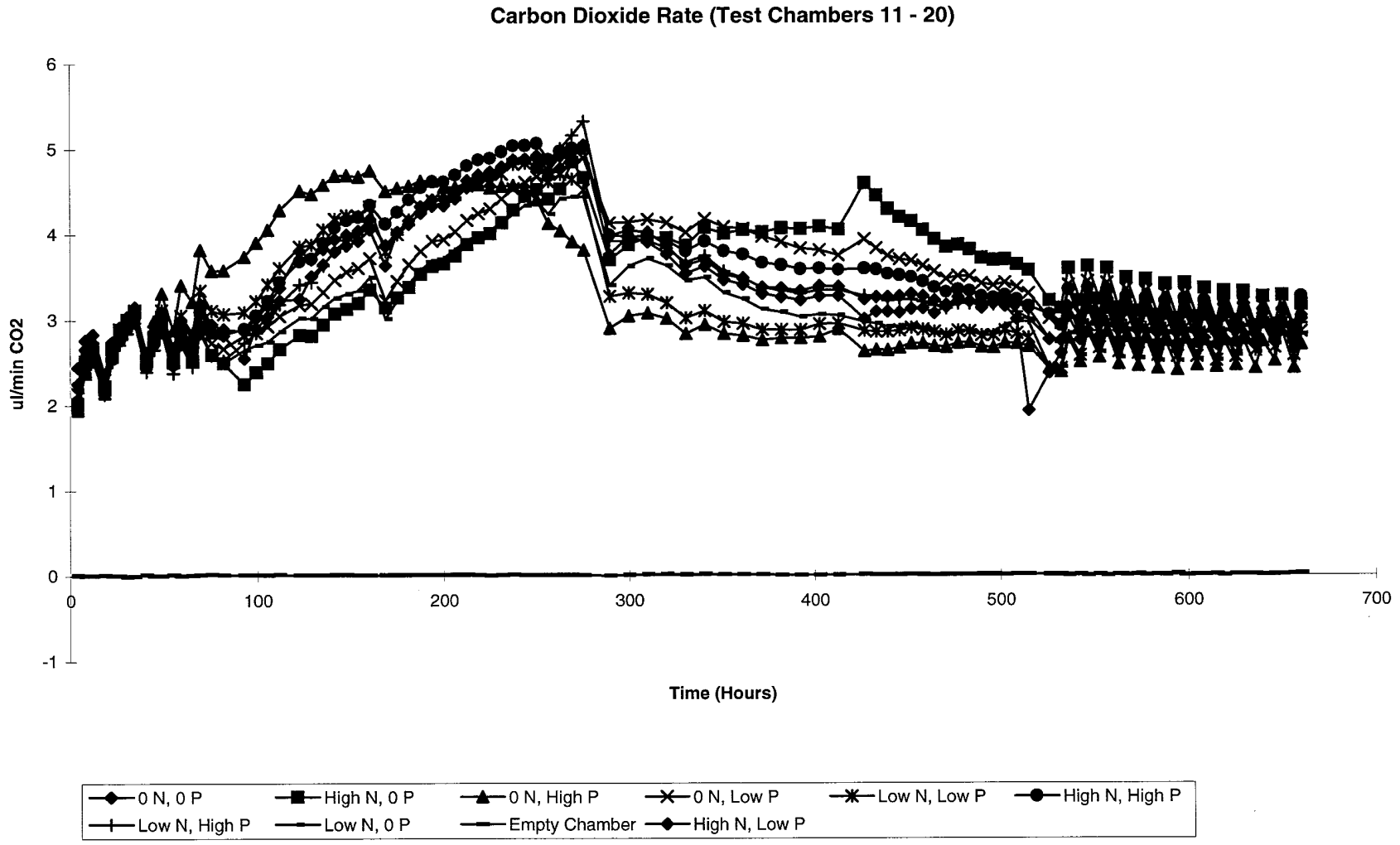


Figure 4 - 4h Experiment 3 Carbon Dioxide Production Rate (Test Chambers 11 - 20) vs Time

4.4.2 Experiment 3 - Analysis of Variance Results (See Appendix F for Statistix Data)

The results of the two factor ANOVA performed using cumulative O_2 as the response variable are as follows. Initially, each expansion unit (set of ten microcosms) was compared using a Paired t Test to determine if there is a significant difference between values measured in expansion unit 1 and unit 2 (See Table 4 - 11).

Table 4 - 11 Paired T Test Results

Rejection Region	P Value
$P < 0.05$	0.3774

Cannot reject the null, therefore there is no significant difference between mean values in each expansion unit. The following two factor ANOVA yielded the following results (See Table 4 - 12).

Table 4 - 12 Experiment 3 Two Factor ANOVA Results

Rejection Region	Nitrate (α) P Value	Phosphate (β) P Value	$\alpha * \beta$ P Value
$P < 0.05$	0.3061	0.4157	0.5346

No Significant impact by either nitrate or phosphate on the response variable. Additionally, there is no interaction between nitrate and phosphate.

4.4.3 Inorganic Recovery Analysis (See Appendix F)

Figures 4 - 5a through 4 - 5f display relationships between cumulative oxygen, cumulative carbon dioxide, nitrate change, and phosphate change. Nitrate and phosphate

changes are summarized in Table 4-13. A correlational study was performed with the following results (See Table 4-14).

Table 4 - 13 Experiment 3 Nitrate and Phosphate Changes

N/P Fuel Treatment	Chamber	Nitrate Δ (mg)	Nitrate mg/day	Phosphate Δ (mg)	Phosphate mg/day
0/0 F	2	0.32	0.012	-0.39	-0.014
L/0 F	9	0.6	0.022	-0.17	-0.006
H/0 F	10	1.8	0.066	0.01	0.002
H/L F	5	0.36	0.013	1.59	0.059
L/L F	1	0.6	0.022	2.25	0.083
0/L F	4	1.8	0.066	2.44	0.090
0/H F	3	0.32	0.011	4.01	0.148
L/H F	8	0.6	0.022	4.73	0.175
H/H F	6	1.84	0.068	4.60	0.170
0/0 F	11	0.36	0.013	0.07	0.002
L/0 F	18	0.56	0.021	0.33	0.012
H/0 F	12	1.8	0.066	0.38	0.014
H/L F	20	0.4	0.015	2.68	0.099
L/L F	15	0.12	0.004	2.41	0.089
0/L F	14	1.48	0.055	2.20	0.081
0/H F	13	0.48	0.018	4.70	0.174
L/H F	17	0.48	0.018	5.21	0.193
H/H F	16	1.76	0.065	4.60	0.170
Untreated	7	-0.36	-0.013	-0.07	-0.002

Table 4 - 14 Experiment 3 Inorganic Correlational Analysis Results - If the correlation value exceeds the rejection value, the null hypothesis can be rejected and hence a correlational relationship exists between the tested variables.

Relationship	Correlation (Corr) (Linear Strength)	Rejection Region (95% for N=18)
Cumulative O_2 (L) vs CO_2 (L)	0.87	Corr > 0.400
Cumulative O_2 (L) vs Nitrate Δ (mg)	0.48	Corr > 0.400
Cumulative O_2 (L) vs Phosphate Δ (mg)	0.39	Corr > 0.400
Cumulative CO_2 (L) vs Nitrate Δ (mg)	0.26	Corr > 0.400
Cumulative CO_2 (L) vs Phosphate Δ (mg)	0.52	Corr > 0.400
Phosphate Δ (mg) vs Nitrate Δ (mg)	0.04	Corr > 0.400

Again, these results indicate a strong linear relationship between O_2 and CO_2 (See Figure 4 - 5a). O_2 vs nitrate and CO_2 vs phosphate indicate a weak correlation. Additionally, O2 vs phosphate borders the rejection region, so a weak correlation may exist. CO_2 vs nitrate was not correlated, which was a surprise since O_2 vs nitrate was correlated. Phosphate vs nitrate was not correlated at all.

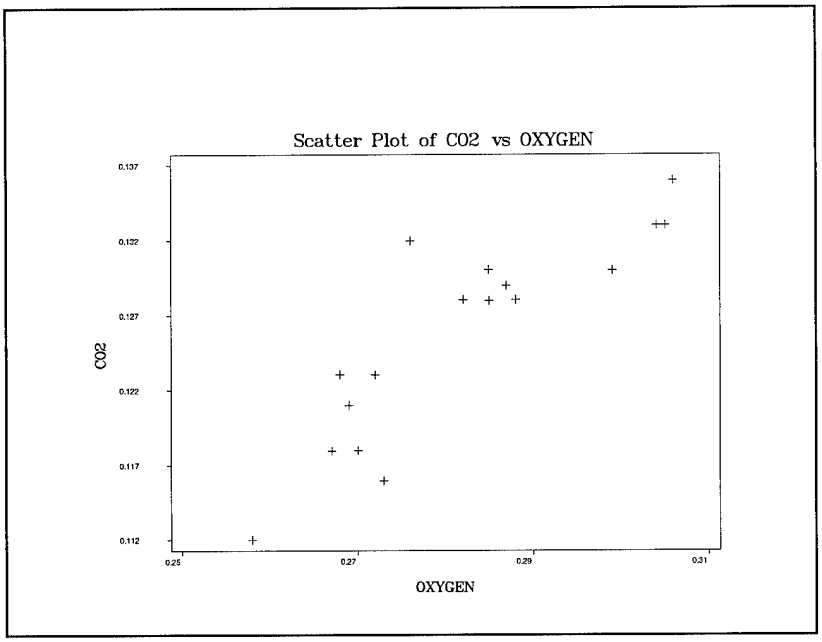


Figure 4 - 5a Experiment 3 Cumulative Oxygen(L) vs Cumulative Carbon Dioxide(L)

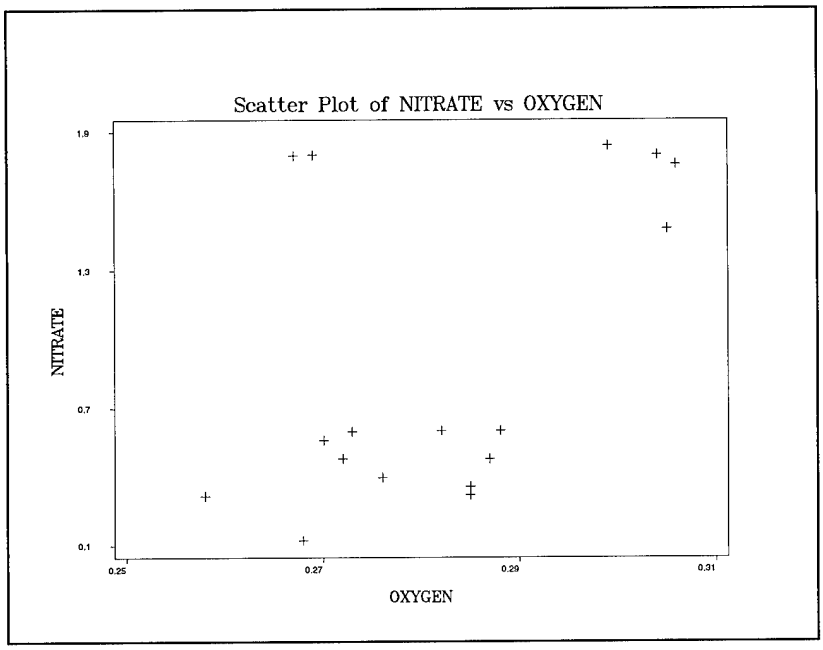


Figure 4 - 5b Experiment 3 Cumulative Oxygen(L) vs Nitrate Δ(mg)

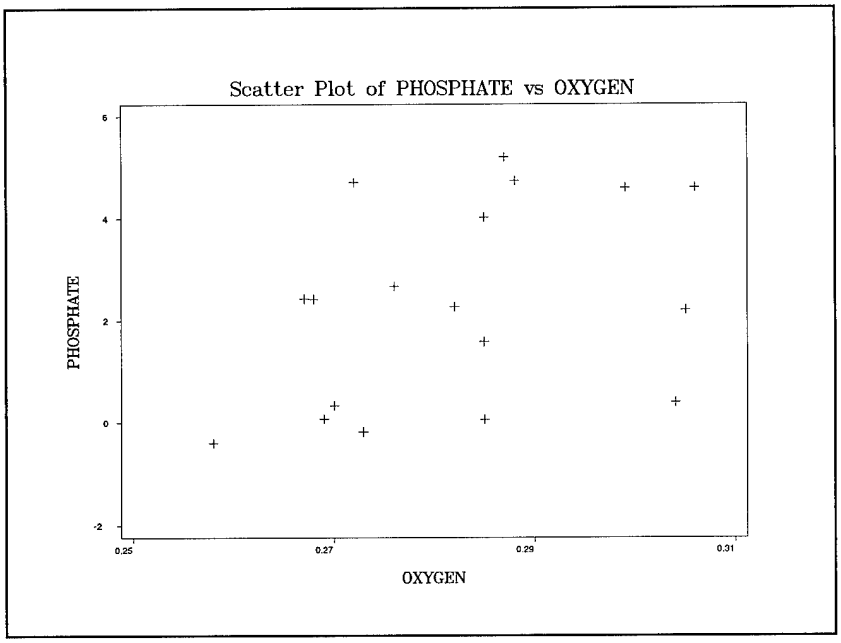


Figure 4 - 5c Experiment 3 Cumulative Oxygen(L) vs Phosphate Δ(mg)

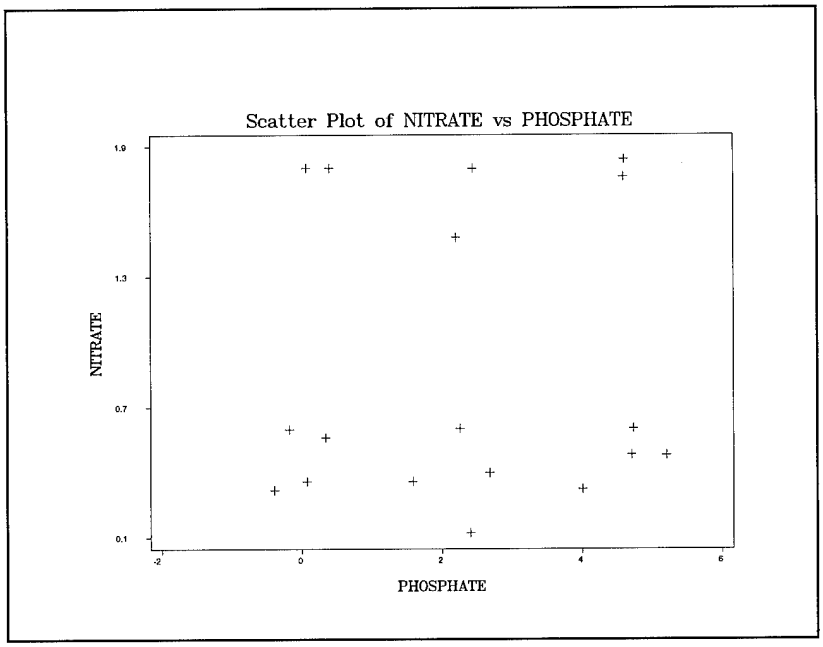


Figure 4 - 5d Experiment 3 Nitrate Δ(mg) vs Phosphate Δ(mg)

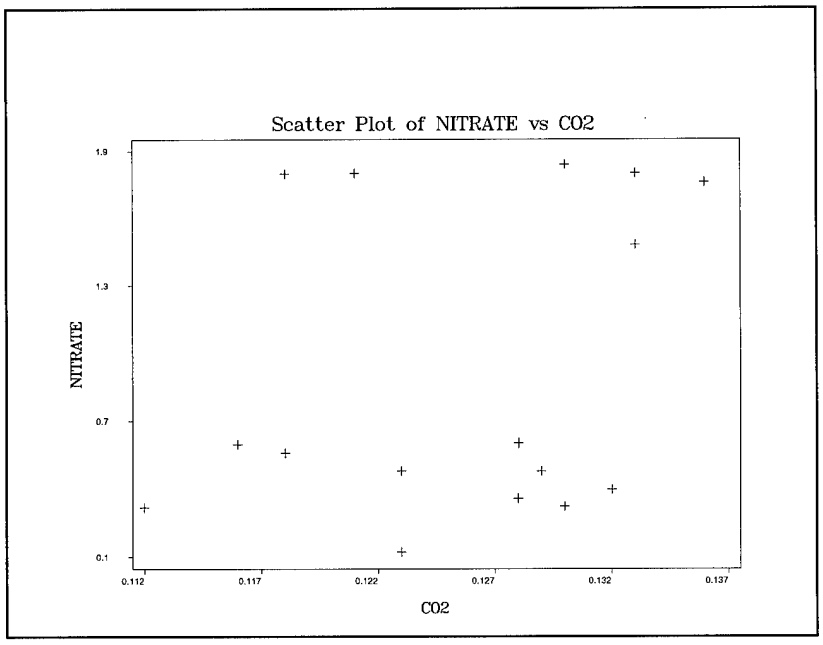


Figure 4 - 5e Experiment 3 Cumulative Carbon Dioxide(L) vs Nitrate Δ(mg)

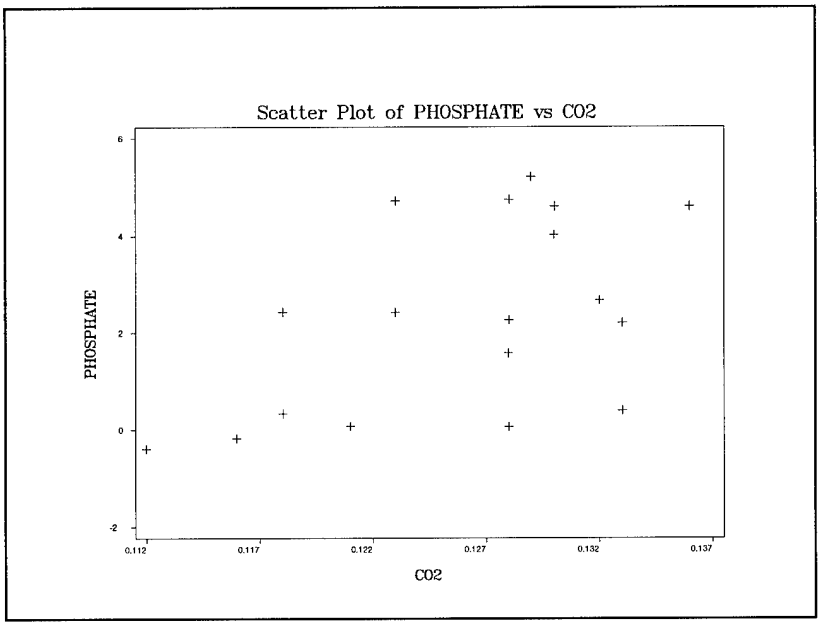


Figure 4 - 5f Experiment 3 Cumulative Carbon Dioxide(L) vs Phosphate Δ(mg)

4.4.4 Estimated % Hydrocarbon Degradation (See Appendix F for Calculations)

Table 4 - 15 summarizes the estimated percentage of JP-8 degraded over the course of the experiment. The values range from a low of 7.26 % to a high of 9.32 %. During the period of this experiment, JP-8 did not yield very high biodegradation percentages. This is probably because of the complexity of kerosene. The results of the inorganic analysis discussed above indicate there is a residual of nitrate and phosphate remaining in the soil samples. This means that the inorganics are not limiting. The slowed consumption and production rates of O_2 and CO_2 during the last week of the experiment could be a result of the easily consumed carbons chains being depleted, while the more recalcitrant chains remain.

Table 4 - 15 Experiment 3 Percent Hydrocarbon Degradation

N/P Fuel Treatment	Chamber	Percent Hydrocarbon Δ	Degradation Rate mg/day
0/0 F	2	7.26	2.65
L/0 F	9	7.92	2.93
H/0 F	10	7.76	2.87
H/L F	5	8.41	3.11
L/L F	1	8.28	3.06
0/L F	4	7.68	2.84
0/H F	3	8.42	3.12
L/H F	8	8.54	3.16
H/H F	6	9.01	3.34
0/0 F	11	8.43	3.12
L/0 F	18	7.80	2.89
H/0 F	12	9.23	3.42
H/L F	20	8.04	2.98
L/L F	15	7.70	2.85
0/L F	14	9.27	3.43
0/H F	13	7.87	2.91
L/H F	17	8.52	3.15
H/H F	16	9.32	3.45

4.4.5 Vapor Recovery Analysis

The results of the vapor recovery analysis are summarized in Table 4 - 16. The volatilization measured during this experiment was estimated to be 0.249 grams trapped by the charcoal. This indicates that nearly 25% of the original 1.0 gram of JP- 8 volatilized during the course of the experiment. The measured moisture and trapped vapor values are somewhat higher than those values recorded in experiment 2. This was probably due in part to the longer experiment time, allowing more time for moisture and volatile trapping.

Table 4 - 16 Experiment 3 Vapor Recovery Summary

Treatment N/P Fuel	Chamber	Initial Carbon Weight (grams)	Final Carbon Weight (grams)	Change (grams)	grams/day
0/0 F	2	2.359	2.674	0.317	0.0117
L/0 F	9	2.502	2.854	0.352	0.013
H/0 F	10	2.163	2.463	0.327	0.0121
H/L F	5	2.071	2.402	0.330	0.0122
L/L F	1	1.979	2.310	0.330	0.0122
0/L F	4	2.145	2.505	0.360	0.0133
0/H F	3	2.235	2.539	0.304	0.0112
L/H F	8	2.342	2.715	0.372	0.0137
H/H F	6	2.365	2.708	0.343	0.0127
0/0 F	11	2.148	2.482	0.333	0.0123
L/0 F	18	2.573	2.892	0.318	0.0117
H/0 F	12	2.183	2.508	0.325	0.0120
H/L F	20	2.033	2.393	0.360	0.0133
L/L F	15	2.356	2.674	0.317	0.0117
0/L F	14	2.328	2.643	0.315	0.0116
0/H F	13	2.358	2.707	0.348	0.0128
L/H F	17	2.309	2.655	0.345	0.0127
H/H F	16	2.313	2.624	0.310	0.0115
Untreated	7	2.2142	2.299	0.085	0.0031

Average Change With Fuel	Average Change W/O Fuel	Average Difference (grams)
0.334	0.085	0.249

5. Conclusions and Recommendations

5.1 Introduction

This concluding chapter draws together the research presented in the previous chapters. It will discuss an overview of the research, summarize the findings, and provide recommendations.

5.2 Overview

The purpose of this study was to use respirometry to measure the impact of nutrient combinations or treatments on microbial respiration under simulated fuel spill soil conditions. The experiment was designed to study one soil type at a constant fuel exposure level of 1% and a constant moisture level of 60% of field capacity. Oxygen was assumed to be readily available, allowing all degradation processes to be aerobic. The experiments were of two and three factor designs with potassium nitrate and sodium phosphate levels serving as two factors and fuel serving as a third factor in experiments 1 and 2. Cumulative oxygen values obtained by the respirometer are used as the response variable in each of the ANOVAs performed. O_2 consumption and CO_2 production rate data were collected to ensure respiration activity levels were on the decline or stable to allow for cumulative oxygen data interpretation.

The literature review revealed that biodegradation is a growing and effective remedial action, under the right conditions. The complexity of the target compound, nutrient availability, soil pH, and moisture content are some of the factors that affect

biodegradation. Of these factors, nutrient availability can be influenced relatively easily. It is generally assumed that nutrient availability, especially nitrates and phosphates, are limiting in usual soil conditions. The factor that can be controlled the least, if at all, is the target compound. Not much is understood about JP - 8 biodegradability in soil conditions. This study combined both of these factors of concern to get a better understanding of JP - 8's biodegradive characteristics while studying nutrient impact under very specific conditions. Much of the literature supports enhanced biodegradation when nutrient enhancement techniques are applied, while few note no effect by such treatment.

5.3 Summary of Findings

a. In Experiment 2, nitrate and phosphate addition were each found to have a main effect on microbial respiration in the presence of JP-8. This is assumed to be an indication that each had a main effect on the biodegradation of JP-8. The cumulative oxygen values measured at the highest level of nitrate addition in the presence of JP-8 were found to be significantly higher than values measured at low or zero nitrate addition. The cumulative oxygen values associated with the low level was significantly higher than values measured at the zero level. However, both were not significantly different from the values measured at the high phosphate addition level. Additionally, nutrient enhancement did not impact background respiration in those chambers without JP-8.

Experiment 3 did not demonstrate any main effect or interaction caused by nitrate or phosphate addition on JP-8 biodegradation. Cumulative oxygen values measured for

each treatment were not significantly different from each other under these specific conditions.

b. More loss of JP - 8 was due to evaporation than biodegradation. Evaporation loss was estimated to be as high as 25% while reduction due to biodegradation was estimated to be 9.3 % as a best result.

c. Nitrate and phosphate consumption values correlated weakly to cumulative oxygen consumption values. However, they did not correlate at all to each other.

d. A large percentage of the degraded carbon was not recovered as CO_2. It was estimated that as much as 50% was not recovered. The carbon was assumed to be contributing to biomass construction and other soil chemical reactions.

e. Despite early operational problems, the MicroOxymax® respirometer proved to be a convenient and easy to use piece of equipment, ideal for this type of research. However, it is critical to ensure moisture, organic vapors, and dust are filtered from the sample air leaving each test chamber prior to entry into the system components.

5.4 Recommendations

a. Intermediate nitrate and phosphate measurements performed throughout the experiment period may provide more information on nutrient utilization.

b. A better method for applying nutrient stock solutions might improve nutrient distribution.

c. Obtain actual time = 0 nitrate and phosphate measurements from the actual chamber being analyzed.

d. Allow the experiment to continue for longer periods to study long term microbial activity. Additional, intermediate nutrient enhancement throughout the course of an experiment, should also be investigated.

e. Minimize soil storage periods. Try to ensure soil freshness for each experiment.

f. Analysis of time = 0 hydrocarbon concentration and time = n concentration to determine actual change of concentration over the course of an experiment.

g. Study the physical changes of JP - 8 (by gas chromatography/mass spectroscopy or equivalent) throughout the course of an experiment to determine which chains of the JP - 8 were successfully degraded and which ones were persistent.

5.5 Closing Comments

In general, under the specific conditions of this these study, JP-8 was found to biodegradable. However, nutrient enhancement did not conclusively demonstrate positive impact on JP-8 biodegradation. Additionally, under these conditions, JP-8 demonstrated a higher volatilization rate than biodegradation rate.

Appendix A - Experiment 2 Statistical Data

STUDENT EDITION OF STATISTIX 4.0 EXPER2, 09/28/95, 18:02

VIEW DATA

CASE	OXYGEN	NITRATE	PHOSPHATE	FUEL
1	0.2041	0.0000	0.0000	1.0000
2	0.2178	1.0000	0.0000	1.0000
3	0.2398	2.0000	0.0000	1.0000
4	0.2239	0.0000	1.0000	1.0000
5	0.2476	1.0000	1.0000	1.0000
6	0.2822	2.0000	1.0000	1.0000
7	0.2309	0.0000	2.0000	1.0000
8	0.2351	1.0000	2.0000	1.0000
9	0.2843	2.0000	2.0000	1.0000
10	0.0644	0.0000	0.0000	0.0000
11	0.0645	1.0000	0.0000	0.0000
12	0.0451	2.0000	0.0000	0.0000
13	0.0781	0.0000	1.0000	0.0000
14	0.0555	1.0000	1.0000	0.0000
15	0.0519	2.0000	1.0000	0.0000
16	0.0545	0.0000	2.0000	0.0000
17	0.0497	1.0000	2.0000	0.0000
18	0.0438	2.0000	2.0000	0.0000

STUDENT EDITION OF STATISTIX 4.0 EXPER2, 09/28/95, 18:02

ANALYSIS OF VARIANCE TABLE FOR OXYGEN

SOURCE	DF	SS	MS	F	P
NITRATE (A)	2	8.032E-04	4.016E-04	6.67	0.0532
PHOSPHATE (B)	2	9.068E-04	4.534E-04	7.53	0.0440
FUEL (C)	1	0.15275	0.15275	2536.85	0.0000
A*B	4	2.236E-04	5.591E-05	0.93	0.5278
A*C	2	0.00358	0.00179	29.72	0.0040
B*C	2	0.00115	5.771E-04	9.58	0.0298
A*B*C	4	2.409E-04	6.021E-05		
TOTAL	17	0.15966			
GRAND AVERAGE	1	0.39701			

14:38 Tuesday, October 17, 1995 1

General Linear Models Procedure

Class Level Information

Class	Levels	Values
FUEL	2	0 1
NITRATE	3	0 1 2
PHOSPHATE	3	0 1 2
FN	6	0 1 2 10 11 12
FP	6	0 1 2 10 11 12

Number of observations in data set = 18

The SAS System 14:38 Tuesday, October 17,

General Linear Models Procedure

Dependent Variable: OXYGEN

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	13	0.15941885	0.01226299	203.70	0.0001
Error	4	0.00024080	0.00006020		
Corrected Total	17	0.15965965			

R-Square	C.V.	Root MSE	OXYGEN Mean
0.998492	5.224328	0.00775885	0.14851389

Source	DF	Type I SS	Mean Square	F Value	Pr > F
FUEL	1	0.15275154	0.15275154	2537.41	0.0001
NITRATE	2	0.00080317	0.00040158	6.67	0.0532
PHOSPHATE	2	0.00090660	0.00045330	7.53	0.0440
FN	2	0.00357943	0.00178972	29.73	0.0040
FP	2	0.00115450	0.00057725	9.59	0.0298
NITRATE*PHOSPHATE	4	0.00022361	0.00005590	0.93	0.5277

Source	DF	Type III SS	Mean Square	F Value	Pr > F
FUEL	0	0.00000000	.	.	.
NITRATE	0	0.00000000	.	.	.
PHOSPHATE	0	0.00000000	.	.	
FN	2	0.00357943	0.00178972	29.73	0.0040
FP	2	0.00115450	0.00057725	9.59	0.0298
NITRATE*PHOSPHATE	4	0.00022361	0.00005590	0.93	0.5277

General Linear Models Procedure

Tukey's Studentized Range (HSD) Test for variable: OXYGEN
NOTE: This test controls the type I experimentwise error rate, but generally has a higher typeII error rate than REGWQ.

Alpha= 0.05 df= 4 MSE= 0.00006
Critical Value of Studentized Range= 6.706
Minimum Significant Difference= 0.03

Means with the same letter are not significantly different.

Tukey Grouping	Mean	N	FN
A	0.268787	3	12
B	0.233497	3	11
B			
B	0.219620	3	10
C	0.065680	3	0
C			
C	0.056567	3	1
C			
C	0.046933	3	2

General Linear Models Procedure

Tukey's Studentized Range (HSD) Test for variable: OXYGEN
NOTE: This test controls the type I experimentwise error rate, but generally has a higher type II error rate than REGWQ.

Alpha= 0.05 df= 4 MSE= 0.00006
Critical Value of Studentized Range= 6.706
Minimum Significant Difference= 0.03
Means with the same letter are not significantly different.

Tukey Grouping	Mean	N	FP
A	0.251227	3	11
A			
B A	0.250123	3	12
B			
B	0.220553	3	10
C	0.061827	3	1
C			
C	0.057993	3	0
C			
C	0.049360	3	2

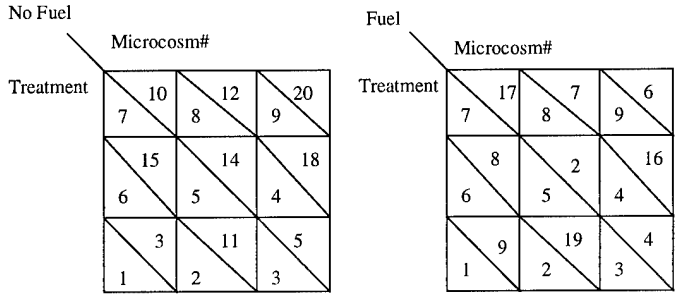
Appendix B - Experiment 3 Paired T Test

```
STUDENT EDITION OF STATISTIX 4.0                                EXPAN,
09/28/95, 18:15
VIEW DATA
 CASE     EXPAN1     EXPAN2
    1     0.2778     0.2852
    2     0.2734     0.2704
    3     0.2685     0.3040
    4     0.2676     0.3051
    5     0.2818     0.2680
    6     0.2848     0.2762
    7     0.2815     0.2722
    8     0.2878     0.2874
    9     0.2989     0.3063
```

```
STUDENT EDITION OF STATISTIX 4.0                                EXPAN,
09/28/95, 18:15
PAIRED T TEST FOR EXPAN1 - EXPAN2
MEAN       -5.86E-03
STD ERROR   6.27E-03
T               -0.93
DF                  8
P               0.3774
CASES INCLUDED 9    MISSING CASES 0
```

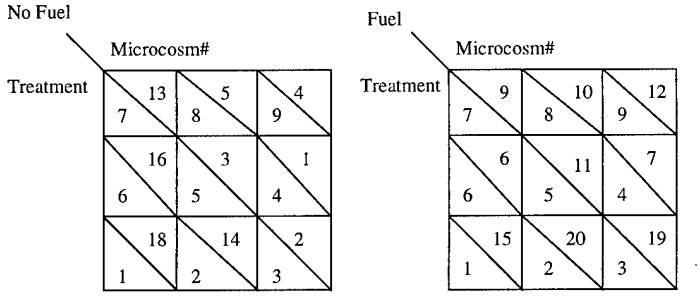
Appendix C - Experimental Set-Up Summary

Microcosm Locations by Treatment
Experiment 1



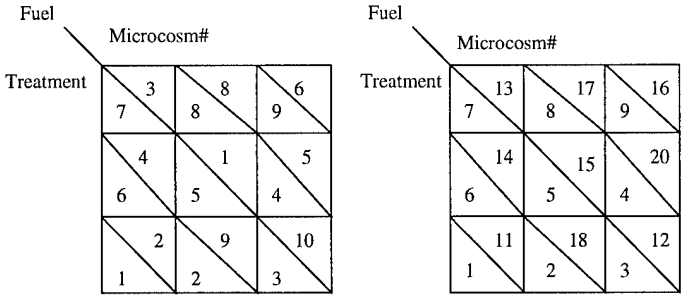
Empty Microcosms: 1, 13

Microcosm Locations by Treatment
Experiment 2



Empty Microcosms: 8, 17

Microcosm Locations by Treatment
Experiment 3



Untreated Soil: 7 Empty Microcosm: 19

Appendix D - Experiment 3 Statistix Data

STUDENT EDITION OF STATISTIX 4.0 EXPER3,
09/28/95, 18:18
VIEW DATA

CASE	NITRATE	OXYGEN	OXYGEN2	PHOSPHATE	WITHIN
1	1.0000	277752.0	0.2778	1.0000	1.0000
2	1.0000	285190.0	0.2852	1.0000	2.0000
3	2.0000	273366.0	0.2734	1.0000	1.0000
4	2.0000	270379.0	0.2704	1.0000	2.0000
5	3.0000	268501.0	0.2685	1.0000	1.0000
6	3.0000	304025.0	0.3040	1.0000	2.0000
7	1.0000	267621.0	0.2676	2.0000	1.0000
8	1.0000	305128.0	0.3051	2.0000	2.0000
9	2.0000	281805.0	0.2818	2.0000	1.0000
10	2.0000	268020.0	0.2680	2.0000	2.0000
11	3.0000	284777.0	0.2848	2.0000	1.0000
12	3.0000	276200.0	0.2762	2.0000	2.0000
13	1.0000	281545.0	0.2815	3.0000	1.0000
14	1.0000	272203.0	0.2722	3.0000	2.0000
15	2.0000	287787.0	0.2878	3.0000	1.0000
16	2.0000	287436.0	0.2874	3.0000	2.0000
17	3.0000	298901.0	0.2989	3.0000	1.0000
18	3.0000	306317.0	0.3063	3.0000	2.0000

STUDENT EDITION OF STATISTIX 4.0 EXPER3,
10/06/95, 14:17
ANALYSIS OF VARIANCE TABLE FOR OXYGEN2

SOURCE	DF	SS	MS	F	P
NITRATE (A)	2	4.303E-04	2.151E-04	1.23	0.3361
PHOSPHATE (B)	2	3.114E-04	1.557E-04	0.89	0.4429
A*B	4	5.843E-04	1.461E-04	0.84	0.5346
WITHIN (C)					
A*B*C	9	0.00157	1.744E-04		
TOTAL	17	0.00290			
GRAND AVERAGE	1	1.44327			

Appendix E - Inorganic Nutrient Addition Calculations

Inorganic Nutrient Addition Calculations - Experiments 1 & 2

Potassium Nitrate (KNO_3)

High Concentration Addition

$KNO_3 := 2.01$ grams $\quad$ $Water := 200$ ml

$HighKNO_3 := \frac{KNO_3}{Water}$ $\quad$ $HighKNO_3 = 0.01$ gms/ml stock solution

Percentage of nitrate in solution

Molecular weight of nitrate = $MWNO := 14 + 3 \cdot (15.99)$ $\quad$ $MWNO = 61.97$

Molecular weight of potassium nitrate = $MWPN := MWNO + 39.102$ $\quad$ $MWPN = 101.072$

$\frac{MWNO}{MWPN} \cdot HighKNO_3 = 0.006$ grams/ml of nitrate in stock solution

Percentage of nitrogen in solution

Molecular weight of nitrogen = $MWN := 14$

$\frac{MWN}{MWPN} \cdot HighKNO_3 = 0.001$ grams/ml of nitrogen in stock solution

dry weight of soil = $DWS := 80$ grams

amount of stock solution added = $ASSA := 1$ ml

Relation of nitrate added per dry weight of soil used in test chamber

$$\frac{ASSA \cdot 0.006}{DWS} \cdot 10^6 = 75$$ ppm of nitrate added

Relation of nitrogen added per dry weight of soil used in test chamber

$$\frac{ASSA \cdot 0.001}{DWS} \cdot 10^6 = 12.5$$ ppm of nitrogen added

Inorganic Nutrient Addition Calculation - Experiment 1 & 2

Potassium Nitrate (KNO_3)

Low Concentration Addition

$KNO_3 := .204$ grams $\quad$ $Water := 200$ ml

$LowKNO_3 := \frac{KNO_3}{Water}$ $\quad$ $LowKNO_3 = 0.001$ gms/ml stock solution

Percentage of nitrate in solution

Molecular weight of nitrate = $\quad MWNO := 14 + 3 \cdot (15.99)$ $\quad MWNO = 61.97$

Molecular weight of potassium nitrate = $\quad MWPN := MWNO + 39.102$ $\quad MWPN = 101.072$

$\frac{MWNO}{MWPN} \cdot LowKNO_3 = 0.000625$ grams/ml of nitrate in stock solution

Percentage of nitrogen in solution

Molecular weight of nitrogen = $\quad MWN := 14$

$\frac{MWN}{MWPN} \cdot LowKNO_3 = 0.00014$ grams/ml nitrogen in stock solution

dry weight of soil = $\quad DWS := 80$ grams

amount of stock solution added = $\quad ASSA := 1$ ml

Relation of nitrate added per dry weight of soil used in test chamber

$$\frac{ASSA \cdot 0.000625}{DWS} \cdot 10^6 = 7.813 \quad \text{ppm of nitrate added}$$

Relation of nitrogen added per dry weight of soil used in test chamber

$$\frac{ASSA \cdot .00014}{DWS} \cdot 10^6 = 1.75 \quad \text{ppm of nitrogen added}$$

Inorganic Nutrient Addition Calculations - Experiments - 1 & 2

Sodium Phosphate, Monobasic ($NaH_2PO_4H_2O$)

High Concentration Addition

$SP := 2.02$ grams $Water := 200$ ml

$HighSP := \frac{SP}{Water}$ $HighSP = 0.01$ gms/ml stock solution

Percentage of phosphate in solution

Molecular weight of phosphate = $MWPO := 30.97 + 4 \cdot 15.99$ $MWPO = 94.93$

Molecular weight of sodium phosphate = $MWSP := MWPO + 4 \cdot 1 + 15.99 + 22.9$ $MWSP = 137.9$

$\frac{MWPO}{MWSP} \cdot HighSP = 0.00695$ grams/ml of phosphate in stock solution

Percentage of phosphorus in solution

Molecular weight of phosphorus = $MWP := 30.97$

$\frac{MWP}{MWSP} \cdot HighSP = 0.00227$ grams/ml of phosphorus in stock solution

dry weight of soil = $DWS := 80$ grams

amount of stock solution added = $ASSA := 1$ ml

Relation of phosphate added per dry weight of soil used in test chamber

$$\frac{ASSA \cdot 0.00695}{DWS} \cdot 10^6 = 86.875$$ ppm of phosphate added

Relation of phosphorus added per dry weight of soil in test chamber

$$\frac{ASSA \cdot 0.00227}{DWS} \cdot 10^6 = 28.375$$ ppm of phosphorus added

Inorganic Nutrient Addition Calculations - Experiments 1 & 2

Sodium Phosphate, Monobasic ($NaH_2PO_4H_2O$)

Low Concentration Addition

$SP := .202$ grams $Water := 200$ ml

$LowSP := \frac{SP}{Water}$ $LowSP = 0.001$ gms/ml stock solution

Percentage of phosphate in solution

Molecular weight of phosphate = $MWPO := 30.97 + 4 \cdot 15.99$ $MWPO = 94.93$

Molecular weight of sodium phosphate = $MWSP := MWPO + 4 \cdot 1 + 15.99 + 22.98$ $MWSP = 137.9$

$\frac{MWPO}{MWSP} \cdot LowSP = 0.0006953$ grams/ml of phosphate in stock solution

Percentage of phosphorus in solution

Molecular weight of phosphorus = $MWP := 30.97$

$\frac{MWP}{MWSP} \cdot LowSP = 0.00023$ grams/ml of phosphorus in stock solution

dry weight of soil = $DWS := 80$ grams

amount of stock solution added = $ASSA := 1$ ml

Relation of phosphate added per dry weight of soil used in test chamber

$$\frac{ASSA \cdot 0.000695}{DWS} \cdot 10^6 = 8.688$$ ppm of phosphate added

Relation of phosphorus added per dry weight of soil used in test chamber

$$\frac{ASSA \cdot 0.00023}{DWS} \cdot 10^6 = 2.875$$ ppm of phosphorus added

Inorganic Nutrient Addition Calculations - Experiment 3

Potassium Nitrate (KNO_3)

High Concentration Addition

$KNO_3 := 10.003$ grams $Water := 500$ ml

$HighKNO_3 := \frac{KNO_3}{Water}$ $HighKNO_3 = 0.02$ gms/ml stock solution

Percentage of nitrate in solution

Molecular weight of nitrate = $MWNO := 14 + 3 \cdot (15.99)$ $MWNO = 61.97$

Molecular weight of potassium nitrate = $MWPN := MWNO + 39.102$ $MWPN = 101.072$

$\frac{MWNO}{MWPN} \cdot HighKNO_3 = 0.01227$ grams/ml of nitrate in stock solution

Percentage of nitrogen in solution

Molecular weight of nitrogen = $MWN := 14$

$\frac{MWN}{MWPN} \cdot HighKNO_3 = 0.00277$ grams/ml of nitrogen in stock solution

dry weight of soil = $DWS := 80$ grams

amount of stock solution added = $ASSA := 1$ ml

Relation of nitrate added per dry weight of soil used in test chamber

$$\frac{ASSA \cdot 0.01227}{DWS} \cdot 10^6 = 153.375 \quad \text{ppm of nitrate added}$$

Relation of nitrogen added per dry weight of soil used in test chamber

$$\frac{ASSA \cdot 0.00227}{DWS} \cdot 10^6 = 28.375 \quad \text{ppm of nitrogen added}$$

Inorganic Nutrient Addition Calculations - Experiment 3

Potassium Nitrate (KNO_3)

Low Concentration Addition

$KNO_3 := 1.003$ grams $\quad$ $Water := 500$ ml

$LowKNO_3 := \frac{KNO_3}{Water}$ $\quad$ $LowKNO_3 = 0.002$ gms/ml stock solution

Percentage of nitrate in solution

Molecular weight of nitrate = $MWNO := 14 + 3 \cdot (15.99)$ $\quad$ $MWNO = 61.97$

Molecular weight of potassium nitrate = $MWPN := MWNO + 39.102$ $\quad$ $MWPN = 101.072$

$\frac{MWNO}{MWPN} \cdot LowKNO_3 = 0.00123$ grams/ml of nitrate in stock solution

Percentage of nitrogen in solution = $MWN := 14$

$\frac{MWN}{MWPN} \cdot LowKNO_3 = 0.00028$ grams.ml of nitrogen in stock solution

dry weight of soil = $DWS := 80$ grams

amount of stock solution added = $ASSA := 1$ ml

Relation of nitrate added per dry weight of soil used in test chamber

$$\frac{ASSA \cdot 0.00123}{DWS} \cdot 10^6 = 15.375 \quad \text{ppm added}$$

Relation of nitrogen added per dry weight of soil used in test chamber

$$\frac{ASSA \cdot 0.00028}{DWS} \cdot 10^6 = 3.5 \quad \text{ppm of nitrogen added}$$

Inorganic Nutrient Addition Calculations - Experiment 3

Sodium Phosphate, Monobasic ($NaH_2PO_4H_2O$)

High Concentration Addition

$SP := 10.0122$ grams $Water := 500$ ml

$HighSP := \frac{SP}{Water}$ $HighSP = 0.02$ gms/ml stock solution

Percentage of phosphate in solution

Molecular weight of phosphate = $MWPO := 30.97 + 4 \cdot 15.99$ $MWPO = 94.93$

Molecular weight of sodium phosphate = $MWSP := MWPO + 4 \cdot 1 + 15.99 + 22.98$ $MWSP = 137.9$

$\frac{MWPO}{MWSP} \cdot HighSP = 0.0138$ grams/ml of phosphate in stock solution

Percentage of phosphorus in solution

Molecular weight of phosphorus = $MWP := 30.97$

$\frac{MWP}{MWSP} \cdot HighSP = 0.0045$ grams/ml of phosphorus in stock solution

dry weight of soil = $DWS := 80$ grams

amount of stock solution added = $ASSA := 1$ ml

Relation of phosphate added per dry weight of soil used in test chamber

$$\frac{ASSA \cdot 0.0138}{DWS} \cdot 10^6 = 172.5$$ ppm of phosphate added

Relation of phosphorus added per dry weight of soil used

$$\frac{ASSA \cdot 0.0045}{DWS} \cdot 10^6 = 56.25$$ ppm of phosphorus added

Inorganic Nutrient Addition Calculations - Experiment 3

Sodium Phosphate, Monobasic ($NaH_2PO_4H_2O$)

Low Concentration Addition

$SP := 1.00$ grams $Water := 500$ ml

$LowSP := \frac{SP}{Water}$ $LowSP = 0.002$ gms/ml stock solution

Percentage of phosphate in solution

Molecular weight of phosphate = $MWPO := 30.97 + 4 \cdot 15.99$ $MWPO = 94.93$

Molecular weight of sodium phosphate = $MWSP := MWPO + 4 \cdot 1 + 15.99 + 22.98$ $MWSP = 137.9$

$\frac{MWPO}{MWSP} \cdot LowSP = 0.00138$ grams/ml of phosphate in stock solution

Percentage of phosphorus is solution

Molecular weight of phosphorus = $MWP := 30.97$

$\frac{MWP}{MWSP} \cdot LowSP = 0.00045$ grams/ml of phosphorus in stock solution

dry weight of soil = $DWS := 80$ grams

amount of stock solution added = $ASSA := 1$ ml

Relation of phosphate added per dry weight of soil used in test chamber

$$\frac{ASSA \cdot 0.00138}{DWS} \cdot 10^6 = 17.25$$ ppm of phosphate added

Relation of phosphorus to per dry weight of soil used in test chamber

$$\frac{ASSA \cdot 0.00045}{DWS} \cdot 10^6 = 5.625$$ ppm of phosphorus added

Appendix F - Mineralization Calculations

Hydrocarbon Mineralization Estimates - Experiment #2

Assumptions: - JP - 8 predominantly $C_{11}H_{24}$

- Standard temperature and pressure
- Measured consumed oxygen from each test chamber minus background oxygen consumption is completely used for hydrocarbon mineralization

The following reaction equation was used to estimate mineralization:

$$C_{11}H_{24} + 17O_2 ======> 11CO_2 + 12\ H_2O$$

PV=nRT

ORIGIN≡1

R := .0821

P := 1 atm

T := 300 K

Background Respiration Values

$$BG := \begin{bmatrix} .0644 \\ .0645 \\ .0451 \\ .0781 \\ .0555 \\ .0519 \\ .0545 \\ .0497 \\ .0438 \end{bmatrix}$$

Respiration Values to be Substituted for O_2

$$RESP := \begin{bmatrix} .2042 \\ .2178 \\ .2398 \\ .2239 \\ .2476 \\ .2822 \\ .2309 \\ .2351 \\ .2843 \end{bmatrix}$$

n := 9

$$AVGBG := \frac{\sum_{i=1}^{n} BG_i}{n}$$

AVGBG = 0.056

Converting the oxygen from volume (liters) to moles

$i := 1..9 \qquad n_i := \frac{P \cdot (RESP_i - AVGBG)}{R \cdot T}$ # of moles of oxygen consumed for each test chamber with fuel

n_i
0.006
0.00655
0.00745
0.0068
0.00776
0.00917
0.00709
0.00726
0.00925

converting the moles of oxygen to moles of $C_{11}H_{24}$

$n_i := \frac{n_i}{17}$ number of moles of $C_{11}H_{24}$

n_i
$3.53 \cdot 10^{-4}$
$3.855 \cdot 10^{-4}$
$4.38 \cdot 10^{-4}$
$4.001 \cdot 10^{-4}$
$4.567 \cdot 10^{-4}$
$5.393 \cdot 10^{-4}$
$4.168 \cdot 10^{-4}$
$4.268 \cdot 10^{-4}$
$5.443 \cdot 10^{-4}$

$mw := 178$ molecular weight of $C_{11}H_{24}$

converting moles to mass (grams) $mass_i := n_i \cdot mw$

$mass_i$
0.063
0.069
0.078
0.071
0.081
0.096
0.074
0.076
0.097

original mass of 1 gram was input into each test chamber

converting to percent $\frac{mass_i}{1} \cdot 100$

6.284
6.862
7.797
7.121
8.129
9.6
7.419
7.597
9.689

Hydrocarbon Mineralization Estimates - Experiment #3

$$RESP := \begin{bmatrix} .2577 \\ .2733 \\ .2695 \\ .2847 \\ .2818 \\ .2676 \\ .2851 \\ .2877 \\ .2989 \\ .2851 \\ .2703 \\ .3040 \\ .2762 \\ .2680 \\ .3051 \\ .2722 \\ .2874 \\ .3063 \end{bmatrix}$$

$BKGRD := .087$ liters

$i := 1 .. 18$

$$n_i := \frac{P \cdot (RESP_i - BKGRD)}{R \cdot T}$$

number of moles of oxygen

$n =$

	1
1	0.0069
2	0.0076
3	0.0074
4	0.008
5	0.0079
6	0.0073
7	0.008
8	0.0081
9	0.0086
10	0.008
11	0.0074
12	0.0088
13	0.0077
14	0.0073
15	0.0089

number of moles of $C_{11}H_{24}$

$\frac{n}{17} =$

	1
1	$4.077 \cdot 10^{-4}$
2	$4.449 \cdot 10^{-4}$
3	$4.359 \cdot 10^{-4}$
4	$4.722 \cdot 10^{-4}$
5	$4.652 \cdot 10^{-4}$
6	$4.313 \cdot 10^{-4}$
7	$4.731 \cdot 10^{-4}$
8	$4.793 \cdot 10^{-4}$
9	$5.061 \cdot 10^{-4}$
10	$4.731 \cdot 10^{-4}$
11	$4.378 \cdot 10^{-4}$

converting moles of $C_{11}H_{24}$ to mass (grams)

$$mass_i := \frac{n_i}{17} \cdot 178$$

Converting to percent with 1 gram originally input into test chamber

mass =

	1
1	0.073
2	0.079
3	0.078
4	0.084
5	0.083
6	0.077
7	0.084
8	0.085
9	0.09
10	0.084
11	0.078
12	0.092
13	0.08
14	0.077
15	0.093

mass·100 =

	1
1	7.257
2	7.92
3	7.758
4	8.405
5	8.281
6	7.678
7	8.422
8	8.532
9	9.008
10	8.422
11	7.792
12	9.225
13	8.043
14	7.695
15	9.272

Appendix G - HACH SOIL EXTRACTION ANALYSIS

Table G - 1 Experiment 2 Nitrate Results

N/F Fuel												
Treatmen	Chamber	T = 0		Mean	Std Dev	grams	T = N		Mean	Std Dev	grams	Δ(grams)
0/0 F	15	16	16	16	0	0.00128	8	7	7.5	0.71	0.0006	0.00068
L/0 F	20	19	19	19	0	0.00152	7	8	7.5	0.71	0.0006	0.00092
H/0 F	19	37	36	36.5	0.71	0.00292	8	8	8	0.00	0.00064	0.00228
H/L F	7	25	23	24	1.41	0.00192	8	8	8	0.00	0.00064	0.00128
L/L F	11	17	19	18	1.41	0.00144	9	7	8	1.41	0.00064	0.0008
0/L F	6	15	14	14.5	0.71	0.00116	8	7	7.5	0.71	0.0006	0.00056
0/H F	9	12	12	12	0.00	0.00096	9	8	8.5	0.71	0.00068	0.00028
L/H F	10	19	18	18.5	0.71	0.00148	8	8	8	0.00	0.00064	0.00084
H/H F	12	32	30	31	1.41	0.00248	8	6	7	1.41	0.00056	0.00192
0/0 NF	18	16	16	16	0.00	0.00128	16	18	17	1.41	0.00136	-
L/0 NF	14	19	19	19	0.00	0.00152	19	16	17.5	2.12	0.0014	0.00012
H/0 NF	2	37	36	36.5	0.71	0.00292	24	28	26	2.83	0.00208	0.00084
H/L NF	1	25	23	24	1.41	0.00192	27	27	27	0.00	0.00216	-0.00024
L/L NF	3	17	19	18	1.41	0.00144	19	18	18.5	0.71	0.00148	-
0/L NF	16	15	14	14.5	0.71	0.00116	15	16	15.5	0.71	0.00124	-0.00008
0/H NF	13	12	12	12	0.00	0.00096	15	15	15	0.00	0.0012	-0.00024
L/H NF	5	19	18	18.5	0.71	0.00148	19	20	19.5	0.71	0.00156	-0.00008
H/H NF	4	32	30	31	1.41	0.00248	34	35	34.5	0.71	0.00276	-0.00028

Table G - 2 Experiment 2 Phosphate Results

N/F Fuel Treatmen	Chamber	T = 0		Mean	Std Dev	grams	T = N		Mean	Std Dev	grams	Δ(grams)
0/0 F	15	42	41	41.5	0.71	0.00332	38	41	39.5	2.12	0.00316	0.00016
L/0 F	20	32	34	33	1.41	0.00264	30	33	31.5	2.12	0.00252	0.00012
H/0 F	19	22	29	25.5	4.95	0.00204	24	30	27	4.24	0.00216	-0.00012
H/L F	7	30	37	33.5	4.95	0.00268	27	25	26	1.41	0.00208	0.0006
L/L F	11	32	40	36	5.66	0.00288	19	22	20.5	2.12	0.00164	0.00124
0/L F	6	27	28	27.5	0.71	0.0022	23	25	24	1.41	0.00192	0.00028
0/H F	9	52	50	51	1.41	0.00408	28	34	31	4.24	0.00248	0.0016
L/H F	10	32	52	42	14.14	0.00336	27	25	26	1.41	0.00208	0.00128
H/H F	12	43	55	49	8.49	0.00392	25	34	29.5	6.36	0.00236	0.00156
0/0 NF	18	42	41	41.5	0.71	0.00332	36	50	43	9.90	0.00344	-0.00012
L/0 NF	14	32	34	33	1.41	0.00264	32	35	33.5	2.12	0.00268	-
H/0 NF	2	22	29	25.5	4.95	0.00204	27	22	24.5	3.54	0.00196	8.00E-4
H/L NF	1	30	37	33.5	4.95	0.00268	42	30	36	8.49	0.00288	-0.0002
L/L NF	3	32	40	36	5.66	0.00288	22	22	22	0.00	0.00176	0.00112
0/L NF	16	27	28	27.5	0.71	0.0022	28	28	28	0.00	0.00224	-
0/H NF	13	52	50	51	1.41	0.00408	36	40	38	2.83	0.00304	0.00104
L/H NF	5	32	52	42	14.14	0.00336	55	50	52.5	3.54	0.0042	-0.00084
H/H NF	4	43	55	49	8.49	0.00392	43	40	41.5	2.12	0.00332	0.0006

Table G - 3 Experiment 3 Nitrate Results

N/F Fuel												
Treatmen	Chamber	T = 0		Mean	Std Dev	grams	T = N		Mean	Std Dev	grams	Δ(grams)
0/0 F	2	12	10	11	1.41	0.00088	7	7	7	0.00	0.00056	0.00032
L/0 F	9	13	14	13.5	0.71	0.00108	6	6	6	0.00	0.00048	0.0006
H/0 F	10	30	28	29	1.41	0.00232	6	7	6.5	0.71	0.00052	0.0018
H/L F	5	12	10	11	1.41	0.00088	5	8	6.5	2.12	0.00052	0.00036
L/L F	1	13	14	13.5	0.71	0.00108	6	6	6	0.00	0.00048	0.0006
0/L F	4	30	28	29	1.41	0.00232	6	7	6.5	0.71	0.00052	0.0018
0/H F	3	12	10	11	1.41	0.00088	7	7	7	0.00	0.00056	0.00032
L/H F	8	13	14	13.5	0.71	0.00108	5	7	6	1.41	0.00048	0.0006
H/H F	6	30	28	29	1.41	0.00232	6	6	6	0.00	0.00048	0.00184
0/0 F	11	12	10	11	1.41	0.00088	7	6	6.5	0.71	0.00052	0.00036
L/0 F	18	13	14	13.5	0.71	0.00108	6	7	6.5	0.71	0.00052	0.00056
H/0 F	12	30	28	29	1.41	0.00232	7	6	6.5	0.71	0.00052	0.0018
H/L F	20	12	10	11	1.41	0.00088	7	5	6	1.41	0.00048	0.0004
L/L F	15	13	14	13.5	0.71	0.00108	6	18	12	8.49	0.00096	0.00012
0/L F	14	30	28	29	1.41	0.00232	5	16	10.5	7.78	0.00084	0.00148
0/H F	13	12	10	11	1.41	0.00088	5	5	5	0.00	0.0004	0.00048
L/H F	17	13	14	13.5	0.71	0.00108	8	7	7.5	0.71	0.0006	0.00048
H/H F	16	30	28	29	1.41	0.00232	7	7	7	0.00	0.00056	0.00176
Untreated	7	12	10	11	1.41	0.00088	16	15	15.5	0.71	0.00124	-0.00036

Table G - 4 Experiment 3 Phosphate Results

N/F Fuel													
Treatmen	Chamber	T = 0		Mean	Std Dev	grams	T = N			Mean	Std Dev	grams	Δ(grams)
0/0 F	2	32	25	28.5	4.95	0.00228	28	38	34	33.33	5.03	0.0026666	-0.000386
L/0 F	9	32	25	28.5	4.95	0.00228	32	34	26	30.67	4.16	0.0024533	-0.000173
H/0 F	10	32	25	28.5	4.95	0.00228	25	33	25	27.67	4.62	0.0022133	6.66E-4
H/L F	5	55	48	51.5	4.95	0.00412	29	35	31	31.67	3.06	0.0025333	0.001586
L/L F	1	55	48	51.5	4.95	0.00412	20	29	21	23.33	4.93	0.0018666	0.002253
0/L F	4	55	48	51.5	4.95	0.00412	19	24	20	21.00	2.65	0.00168	0.00244
0/H F	3	85	92	88.5	4.95	0.00708	34	48	33	38.33	8.39	0.0030666	0.004013
L/H F	8	85	92	88.5	4.95	0.00708	25	40	23	29.33	9.29	0.0023466	0.004733
H/H F	6	85	92	88.5	4.95	0.00708	28	36	29	31.00	4.36	0.00248	0.0046
0/0 F	11	32	25	28.5	4.95	0.00228	26	35	22	27.67	6.66	0.0022133	6.66E-4
L/0 F	18	32	25	28.5	4.95	0.00228	22	30	21	24.33	4.93	0.0019466	0.000333
H/0 F	12	32	25	28.5	4.95	0.00228	20	29	22	23.67	4.73	0.0018933	0.000386
H/L F	20	55	48	51.5	4.95	0.00412	16	21	17	18.00	2.65	0.00144	0.00268
L/L F	15	55	48	51.5	4.95	0.00412	19	24	21	21.33	2.52	0.0017066	0.002413
0/L F	14	55	48	51.5	4.95	0.00412	21	23	28	24.00	3.61	0.00192	0.0022
0/H F	13	85	92	88.5	4.95	0.00708	27	34	28	29.67	3.79	0.0023733	0.004706
L/H F	17	85	92	88.5	4.95	0.00708	29	21	20	23.33	4.93	0.0018666	0.005213
H/H F	16	85	92	88.5	4.95	0.00708	28	37	28	31.00	5.20	0.00248	0.0046
Untreated	7	32	25	28.5	4.95	0.00228	25	40	23	29.33	9.29	0.0023466	-6.66E-4

Appendix H - Thermogravimetric Analyzer Curve

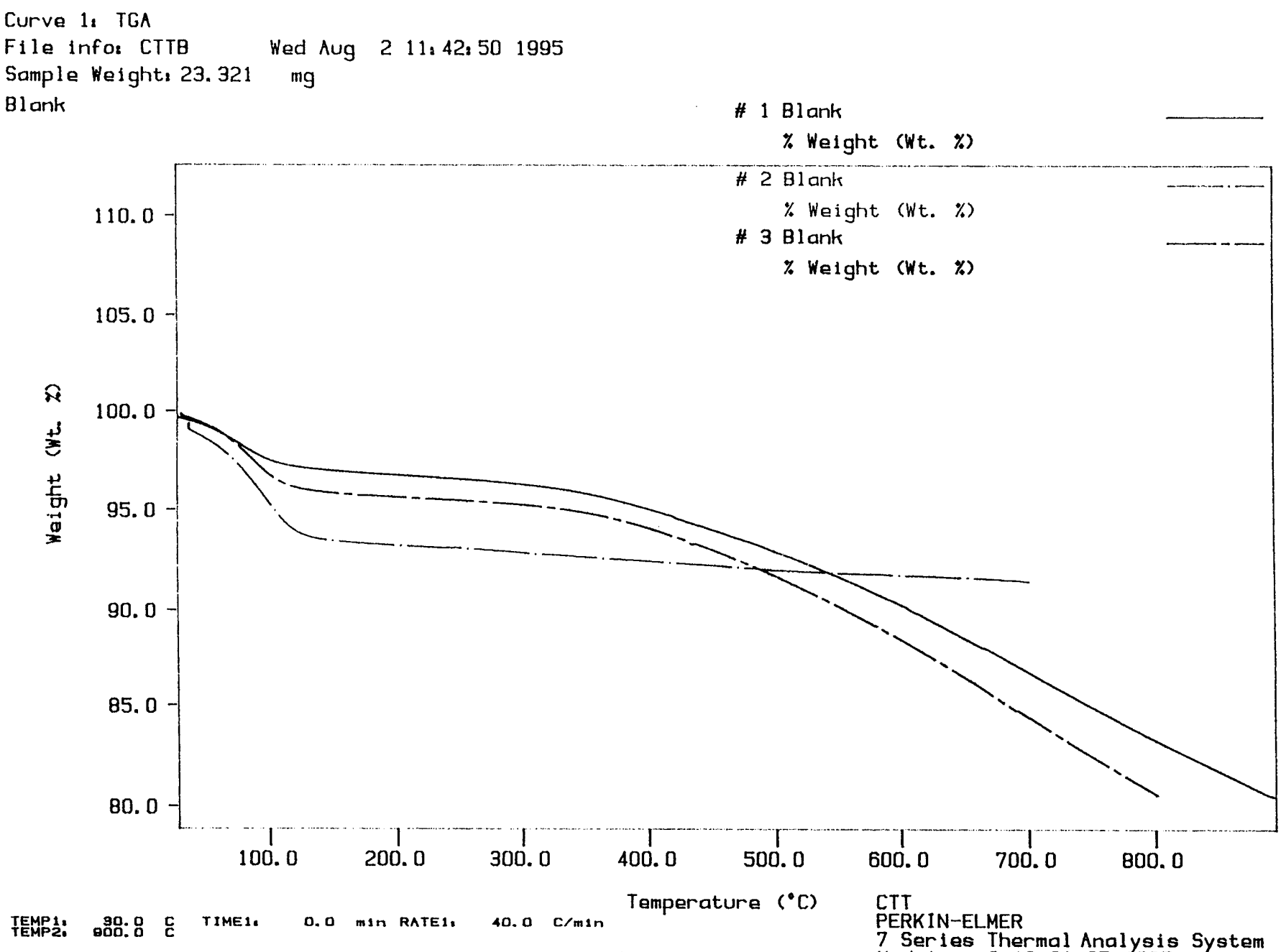


Figure H-1 TGA Curve #1

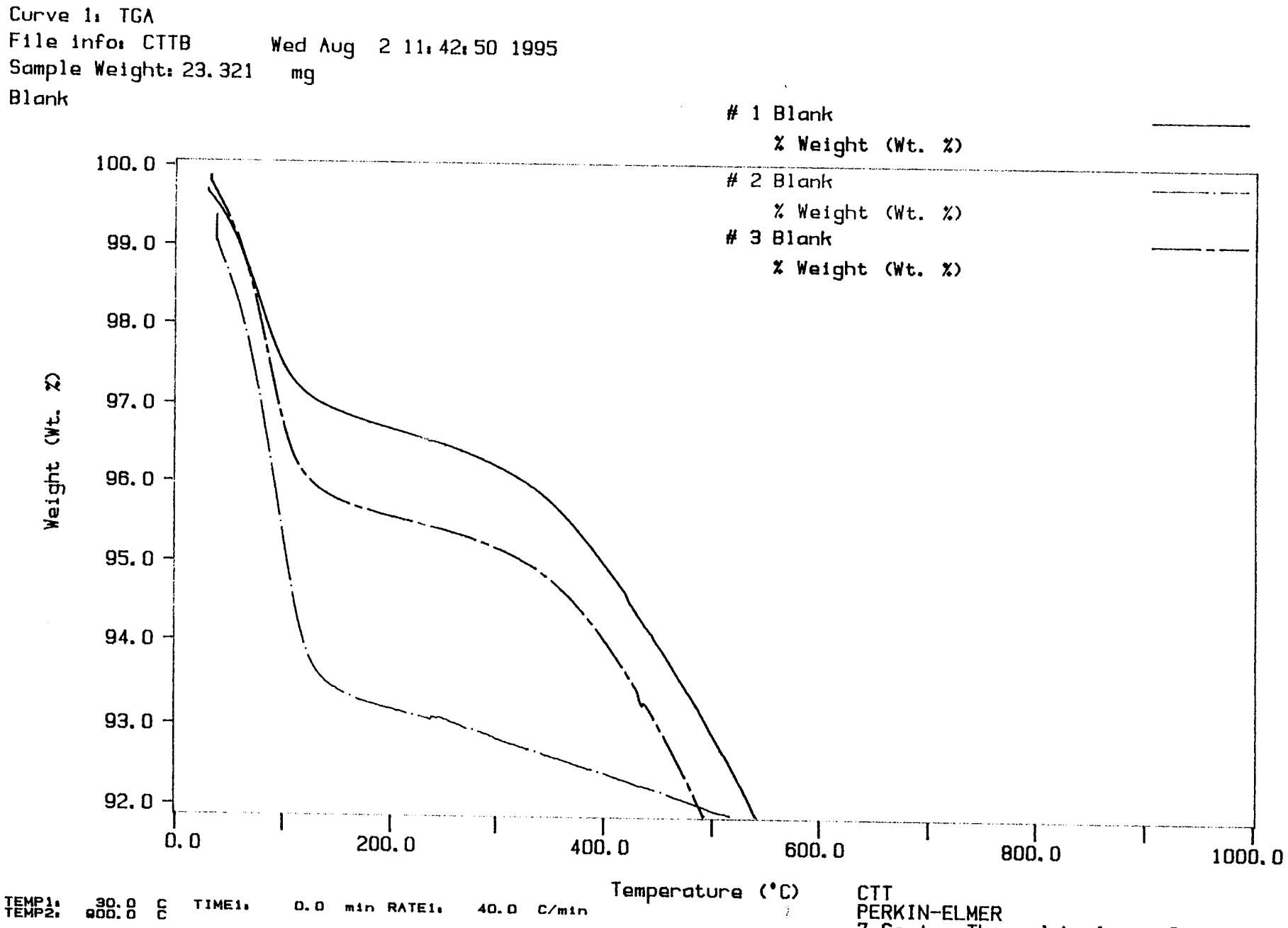


Figure H-2 TGA Curve #2

Bibliography

Alexander, Martin. Biodegradation and Bioremediation. San Diego: Academic Press, 1994.

Andros Incorporated. 5000 Series Gas Analyzers. Berkely, California: No Date.

Atlas, Ronald M, and Richard Bartha. Microbial Ecology: Fundementals and Applications. Redwood City, CA: The Benjamin Cummings Publishing Company, Inc, 1993.

Autry, Andrew R. and Gary M. Ellis. "Bioremediation: And Effective Remedial Alternative for Petroleum Hydrocarbon Contaminate Soil," Environmental Progress, 11: 318 - 323 (Nov 1992)

Baker, Katherine H. and Diane S. Herson. Bioremediation. New York: McGraw - Hill, Inc, 1994.

Bleckmann, Charles A. Class Notes, ENVR 525, Microbiology. School of Engineering, Air Force Institute of Technology, Wright-Patterson AFB OH, Winter Quarter 1995.

BP Technologies, Inc. Excerpt From a Letter by Dr. J. Mueller, Environmental Microbiologist, USEPA. July 17, 1991.

City Technology LTD. Oxygen Citicels. No Date.

Czekajewski et al. Method and Apparatus For Measuring Respiration, Oxidation and Similar Interacting Between a Sample and a Selected Component of a Fluid Medium. United States Patent No. 4,947,339. August 7, 1990.

Dean-Ross, Deborah, Howard Mayfield, and Jim Spain. "Enviromental Fate and Effects of JP-8," Chemosphere, 24: 219 - 228 (1992).

Devore, Jay L. Probability and Statistics for Engineering and the Sciences. Pacific Grove, California. Brooks/Cole Publishing Company, 1991.

Dibble, J. T. and R. Bartha. "Effect of Environmental Parameters on the Biodegradation of Oil Sludge," Applied and Environmental Microbiology, 37: 729 - 739 (Apr 1979)

Fredrickson, James K., Harvey Bolton Jr., and Fred J. Brockman. "In Situ and On Site Bioreclamation," Environmental Science & Technology, 27: 1711 - 1716 (1993).

Grady, C. P. Leslie, Jr. "Biodegradation: Its Measurement and Microbiological Basis," Biotechnology & Bioengineering, 27: 660 - 674 (1985).

Hach Water Analysis Handbook 2nd Edition. Loveland, Colorado: Hach Company. 1992.

Harder, H., B. Korzel-Seidel, and Th. Hopner. "Hydrocarbon Biodegradation in Sediments and Soils: A Systematic Examination of Physical and Chemical Conditions - Part IV Special Aspects of Nutrient Demands," Science and Technology, 44: 59 - 62 (Feb 1991).

Harder, H. and Th. Hopner. "Hydrocarbon Biodegradation in Sediments and Soils: A Systematic Examination of Physical and Chemical Conditions - Part V Moisture," Science and Technology, 44: 59 - 62 (Sep 1991).

Hardy, John J., and Sunand Mabbula. "Close a Site for $3,000," Soils: 30 - 35 (Mar 1994).

Haug, Roger T. and William F. Ellsworth. 'Measuring Compost Substrate Degradability," Biocycle, 32: 56 - 62 (Jan 1991).

Hinchee, Robert E. and Say Kee Ong. "A Rapid In Situ Test for Measuring Aerobic Biodegradation Rates of Hydrocarbons in Soil". Journal of Air Waste Management Association, 42: Oct 1994.

King, R. Barry, Gilbert M. Long, and John K. Sheldon. Practical Environmental Bioremediation. Boca Raton: Lewis Publishers, 1992.

MacDonald, Jacqueline A. "Performance Standards for In Situ Bioremediation," Environmental Science & Technology, 27: (1993).

Martel, Charles R. "Military Jet Fuels, 1944 - 1987," Report No. AFWA-TR-87-2062, Aero Propulsion Laboratory, Air Force Wright Aeronautical Laboratories, AFSC, Wright-Patterson AFB OH. Nov 1987.

Micro-oxymax v5.12 Instruction Manual. Columbus, OH: Columbus Instruments International Corporation, 1994.

National Research Council. In Situ Bioremediation, When Does It Work? Washington D.C.: National Academy Press, 1994.

Rittmann, Bruce E. and Others. In Situ Bioremediation. Park Ridge, NJ: Noyles Publications, 1994.

Sawyer, Clair N., Perry C. McCarty, and Gene F. Parkin. Chemistry for Environmental Engineering. New York: McGraw Hill, Inc, 1994.

Sims, J. L., R. C. Sims and J. E. Mathews. "Bioremediation of Contaminated Surface Soils," EPA 160019-89/073,: 1 - 23 (August 1989).

Song, Hung-Gyu, Xiaping Wang, and Richard Bartha. "Bioremediation Potential of Terrestrial Fuel Spills," Applied and Environmental Microbiology, 56: 652 - 656 (Mar 1990).

Statistix Version 4.0 User's Manual. Tallahassee, FL. Analytical Software, 1992.

Stegmann, R., S. Lotter, and J. Heerenklage. Biological Treatment of Oil Contaminated Soils in Bioreactors In R.E. Hinchee and R. F. Olfenbuttle, On Site Bioreclamation: Processes for Xenobiotic and Hydrocarbon Treatment. Stoneham MA: Butterworth and Heinemann, 1991.

Tabak, Henry H. and Others. "Laboratory Studies Evaluating The Enhanced Biodegradation of Weathered Crude Oil Components Through The Application of Nutrients," 83rd Annual Meeting & Exhibition, Pittsburgh, PA: June 24 - 29, 1990a.

Tabak, Henry H. and Others "Biodegradation of The Alaskan Weathered Crude Oil Constituents by The Indigenous Microbial Consortium," Proceedings from The 63rd Annual Water Pollution Control Federation Conference Research Symposium on "Toxic Contaminant Control". Washington D.C. (Oct 7 - 11, 1990b).

Tabak, Henry H. and Others. "Respirometric Methods for Determination of Biodegradation Kinetics for Hazardous Organic Compounds," ACS Environmental Chemistry Division Symposium on Chemical and Biochemical Detoxification of Hazardous Wastes II, 1 - 35 Miami Beach, FL: Sep 10 - 15, 1989.

Thornton-Manning, Janice R., Danial D. Jones and Thomas W. Federle. "Effects of Experimental Manipulation of Environmetal Factors on Phenol Mineralization in Soil," Environmental Toxicology and Chemistry,6: 615 - 621 (1987).

Turney, Glen R., Timothy P. Alen and John N. Zikopoulos. "Multiphase Biological Remediation," IGT et al Gas & Oil Environmental Biotechnology 4th International Symposium. 30 - 39. Colorado Springs, CO: Dec 9 - 11, 1991.

Uziel, Michael. "Cleaning up Weathered Crude: Research Points to Bioremediation," National Environmental Journal. 41 - 42 (March/April 1994).

Warburg, Otto, "Prefatory Chapter," Annual Review of Biochemistry, 33: 1 - 14 (1964).

Vita

Capt Christian T. Totten [redacted].

He graduated from Fitch Senior High School in 1984 and entered undergraduate studies at Western New England College Springfield, Massachusetts. He graduated with a Bachelor of Science degree in Mechanical Engineering in May 1988. He received his commission on 1 September 1989. His first assignment was at Pope AFB as Chief of Bioenvironmental Engineering. While at Pope, he earned a Master of Aeronautical Science degree in Aeronautics from Embry-Riddle Aeronautical University. In May 1994, he entered the School of Engineering, Air Force Institute of Technology.

Permanent Address: [redacted]

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13. ABSTRACT *(maximum 200 words)*

The purpose of this study was to use respirometry to measure the impact of nutrient combinations or treatments on JP - 8 biodegradation under simulated fuel spill soil conditions. The study was designed observe one soil type at a constant fuel exposure level of 1% and a constant moisture of 60% of field capacity. Three experiments conducted were of two and three factor designs with potassium nitrate and sodium phosphate addition levels serving as two factors and fuel serving as a third factor in Experiments 1 and 2. Cumulative oxygen values obtained by the respirometer were assumed to be a direct indicator of JP-8 biodegradation. Therefore, cumulative oxygen values were used as the response variable for statistical analysis to determine the impact of nutrient enhancement on biodegradation of JP - 8. O_2 consumption and CO_2 production rate data were collected to ensure respiration activity levels were declining or stable to allow for cumulative oxygen data interpretation. Nutrient enhancement was found not to have a positive impact on biodegradation under these conditions. Additionally, losses due to degradation of the JP - 8 were found to be less than losses from volatilization.

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