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BIODEGRADATION OF DEICING AGENTS IN VARIOUS SOIL TYPES

AnnMarie Halterman-O'Malley, Second Lieutenant, USA

AFIT/GEE/ENV/97D-09

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Wright-Patterson Air Force Base, Ohio

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THESIS

AnnMarie Halterman-O'Malley, B.S.

Second Lieutenant, USAF

December 1997

Presented to the Faculty of the Graduate School of Engineering

of the Air Force Institute of Technology

Air Education and Training Command

In Partial Fulfillment of the

Requirements of the Degree of

Master of Science in Engineering and Environmental Management

Dr. Charles Bleckmann (Chairman)

Major Edward Heyse

Prof. Dan Reynolds

AFIT/GEE/ENV/97D-09

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Acknowledgments

First, I would like to thank my thesis advisor, Dr. Charles Bleckmann, for his guidance and support throughout this research project. Just as evolution has always been the answer, your assistance and tutelage cleared this enigmatic path to guide me.

I would also like to acknowledge the assistance and patience of the other members of my committee, Major Ed Heyse and Professor Dan Reynolds. Prof. Reynolds, your talent to decipher my coded statistical language and heavy "Canadian" accent are unparalleled. Thank you so much for the tools required to examine the data with such a high level of confidence. Now if we could just understand the theory of Chaos as well.

Major Heyse, thank you for standing up to your reputation and taking the time to do a complete and thorough review of this research. Your timely questions during the defense proved to nearly push my envelope of knowledge but what didn't kill me, only made me stronger. I was counting on your consistency.

The people working at Columbus Instruments International, Inc deserve to be mentioned. Their technical support during times of frustration and drought were always motivating and convincing.

Laura, I have gained something much greater than can be viewed here, an eternal friendship. I hope that the falling of the leaves have helped you learn the meaning of the ancient Chinese curse, "May you live in interesting times." As you know, these pages would have never existed without you.

And David, who was there from the start. To the man who is not just a marvelous and supportive husband but my valued editor, literary critic and adviser. Dave, everyday breathes dullness until we are finally together in the mountains.

Thank you all.

AnnMarie Halterman-O'Malley

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Abstract

Airports have found that spent deicing fluids eventually reach the airfield's stormwater system and soil surrounding the airfield. Due to toxic effects of spent deicing fluids, containing and collecting the fluid for treatment can be a very costly activity for airports. One alternative to dispose of the used deicing fluid is to institute a land treatment system if it can be proven that the deicing fluid is readily biodegradable in a soil system.

The primary concern of this research effort was to monitor the behavior of a simulated aircraft deicing fluid in various soil systems. An automated respirometer monitored the behavior of the microbial activity in soil that was contaminated with aircraft deicing fluid (ADF). Reapplication of the contaminant to the soil, one time soil loading variations and a lime additive analyzed in an attempt to maximize the biodegradation of the contaminant.

Analysis of the oxygen consumption rates of the deicing agents, provide biodegradation rates and the amount of time required for treatment of propylene glycol-based ADF. Since deicing agents do not sorb strongly to soil, determining the effects of the contaminant in different soil types helps optimize biodegradation. During the 660 hours of experimental time, the effectiveness of the high clay system was significantly higher than that of the sandy soil. The sandy soil results were increased greatly when a reapplication process was

implemented. The high-clays most effective system was a one time high concentration injection of PG. A summary of the results is shown below.

SOIL TYPE	CONCENTRATION	RESPIRATION RATE (ml/day/kg)	BIODEGRADATION RATE (mg/day/kg)	% DEGRADED
HC	3X300 mg PG	13.32	157.5	20
	1X900 mg PG	15.2	188	24.78
	1X600 mg PG	14.03	167	32.95
	1X300 mg PG	9.1	76	30.06
SS	3X300 mg PG	3.11	49.5	6.222
	1X900 mg PG	1.53	11	1.49
	1X600 mg PG	2.09	21	4.4
	1X300 mg PG	7.13	113	48.16

*Note: 3X indicates the number of injections of the given concentration of PG.

Reapplication of the contaminant, lime additions and varying concentrations of contaminant were the methods used to attempt to optimize the batch system. These methods were typically effective for one of the two soil types, but rarely worked on both soils.

BIODEGRADATION OF DEICING AGENTS IN VARIOUS SOIL MEDIA

1. INTRODUCTION

1.1 GENERAL ISSUE

Airports have found that spent deicing fluids eventually reach the airfield's stormwater system and soil surrounding the airfield. Due to the high biological oxygen demand (BOD) exerted by glycols, they are not readily treated either by surface waters or wastewater treatment plants. Therefore, if the spent deicing fluids can not be recycled easily or economically, they need to be contained and collected to allow treatment and disposal. In the age where there is a focus on environmental concern and safety, an economical and safe disposal of spent aircraft deicing fluid is the optimal solution. One alternative to dispose of the used deicing fluid may be to institute a land treatment system if it can be proven that the deicing fluid is amendable to treatment in such a system.

1.2 SPECIFIC PROBLEM

The principal concern regarding the environmental impacts of deicing activities relates to oxygen consumed during the decomposition of deicing materials contained in runoff. Oxygen consumption occurs when bacteria decompose organic materials (including deicing chemicals). This phenomenon can deplete all dissolved oxygen from water if the rate of decomposition is high enough. Therefore, the intent of this research is to determine if the actual impact of

oxygen consumption on a soil media is feasible for a land treatment system.

1.3 PURPOSE OF RESEARCH

The purpose of this experimental study is to monitor the biodegradation behavior of deicing agents in soils, which has been subjected to repeated applications of deicing agents. The Micro Oxymax Respirometer measures the microbial metabolism (oxygen uptake and carbon dioxide production of the microbial population). This work parallels the work done by Johnson (1997) but models the effects of repeated contamination on soil. This study is in support of Major Jeff Cornell's research at the University of Colorado-Boulder. His work focuses on developing a land treatment system for the Air Force to treat deicing agents.

The primary goals of this study will be to determine:

- The variation (if any) of the microbial metabolism from different soil types being repeatedly contaminated with propylene glycol.
- Nutrient concentrations which amplify and/or constrain microbial metabolism of deicing agents.
- The variation (if any) of the microbial metabolism for repeatedly contaminated versus one contamination for the different soil types.

1.4 TERMS USED IN THIS STUDY

Anti-icing- Spreading or spraying of a liquid deicing agent directly onto the aircraft before the snow or ice are present.

Biodegradation-The microbially mediated process of chemical breakdown of a substance to smaller products caused by microorganisms or their enzymes.

BOD- Biological oxygen demand (BOD) refers to the amount of dissolved oxygen required by aerobic and facultative microorganisms to stabilize organic matter in water (biochemical oxygen demand).

Deicing- Spreading or spraying of a liquid deicing agent on already formed ice and collected snow.

EG- Ethylene glycol

Land Treatment-Addition of degradable organics to soil systems and allowing indigenous microorganisms to degrade materials.

PG- Propylene glycol

Microorganism- Microscopic organisms that exist naturally in the environment (algae, bacteria, fungi, protozoa, and viruses).

RER- Respiratory exchange rate which is the ratio of carbon dioxide production to oxygen consumption.

Respirometry- Measurement of oxygen uptake and carbon dioxide production associated with biological systems.

1.5 ORGANIZATION OF STUDY

This chapter discussed the need to determine the actual oxygen consumption for the biodegradation of propylene glycol in two separate soil matrices. Chapter 2 consists of a literature review regarding the development and adjustments that have been made while using aircraft deicing fluids. Chapter 3 describes the methodology used in completing the laboratory work, obtaining the required materials and statistically validating the data. The standard methods used in this chapter made it possible to meet the objectives of the research and successfully

collect the required data. Chapter 4 examines the data collected and investigates the questions set forth in this research. The final summary of this research, limitations and follow-on suggestions can be found in Chapter 5.

2, LITERATURE REVIEW

2.1 INTRODUCTION

As the enforcement of the Clean Water Act of 1987 gets stricter, deicing fluids are among the chemicals that are being evaluated to find a more environmentally friendly substitute. Recognizing the need to comply with the regulations, Brigadier General James E. McCarthy, the Air Force Civil Engineer, directed an immediate USAF wide prohibition on the use of ethylene glycol on 31 March 1992 (Pro-Act 1995). Therefore, since that time, the Air Force has used only PG based ADFs. A favorable future development would be an environmentally safe land treatment method for spent glycol deicing fluids. This chapter outlines the history of aircraft deicing fluids (ADF), discusses the biodegradation process, and describes the chemical composition of glycols.

2.2 HISTORY OF ADF IN THE AIR FORCE

The Navy can be credited for the development of military deicing/anti-icing fluids (Cross Talk, 1996). The original design specifications were to create a solution that would remove the snow and ice build-up on the aircraft and minimize the effects of corrosion on the airframes. The commercial ADF's were developed using International Standards Organization (ISO) and Society of Automotive Engineers (SAE) performance specifications for Type I and Type II Freezing Point Depressants (FPD).

Since Commercial and Military ADF classifications are different, some confusion has arisen during discussions about ADF. All Air Force bases must use Mil-spec Type I ADF. Military Type I fluid is propylene glycol based (Fronapfel and Malinowski). The Commercial classifications are as follows: Type I fluid is "unthickened" and has low viscosity, and Type II fluid is "thickened", which makes it a better anti-icer than Type I fluid because it allows the aircraft to have extended holdover times after being sprayed. Aircraft treated with Type I ADF have very short holdover times and may have to be resprayed in the event of delays. Type I commercial fluid can be applied with existing Air Force equipment, but Type II commercial fluid cannot (Cross Talk, 1996).

Approximately 49-80 percent of deicing fluid applied to an aircraft is deposited on the pavement around the deicing area, either through overspray or drippage. The remaining ADF is eventually lost to drippage and sloughing during taxiing and take-off. This material is dispersed and deposited all across the airfield. The majority of the ADF pools and enters the storm water system serving the airfield. The remaining ADF makes its way to the soil surrounding the airfield.

The principal concern regarding the environmental impacts of deicing activities relates to oxygen consumed during the decomposition of the deicing materials contained in runoff. Oxygen consumption occurs when bacteria decompose

organic material. This phenomenon places heavier than normal oxygen demands on the surrounding environment. Both national and state requirements to obtain or renew storm water discharge (NPDES) permits are becoming more stringent every year (Dorn et al.). By modifying the present de-icing practices, the Air Force hopes to avoid adverse affects caused by new permit requirements enacted by each installation's State environmental agencies (Cross Talk, 1996).

The use of propylene glycol as the major constituent of ADF has not always been the case. Ethylene glycol ADF was the most popular type of Air Force deicing fluid prior to its elimination in 1992. Banning EG caused the Air Force to switch all de-icing operations over to the use of propylene glycol (Pro-Act Dec 1995). The next event in the studies of glycols focused on the impact to aquatic species caused by pure glycols (Majewski et al., 1978). Other studies followed Majewski with laboratory research including both Type I and II mixtures. The studies found both mixtures to be more acutely and chronically toxic than pure ethylene and propylene glycol (Hartwell et al. and Pillard, 1993 and 1994). The addition of corrosive inhibitors, wetting agents, and thickening agents have proven to cause increased toxicity. Determining which additives are causing the increase in toxicity has been difficult since the exact make-up of the mixtures are proprietary.

Currently the Air Force is evaluating the Commercial Type I FPD with the intent

of using it (Cross Talk, 1996). Regardless of which fluid type is used, propylene glycol is still the major component. The current ADF used by the Air Force, Mil-spec Type I, is designed to remove snow and ice (they do not use anti-icing solutions). If this change takes place, the SAE Type I Commercial Fluid will be used to anti-ice the aircraft just prior to flight. The same equipment will be able to be used, however, personal will need to be retrained as the different ADF characteristics involve different procedures.

2.3 RULES AND REGULATIONS

The Environmental Protection Agency (EPA) Storm Water Discharge regulations became effective on 17 December 1990. These regulations establish storm water regulations and standards under the National Pollution Discharge Elimination System (NPDES) permit program. Traditionally, NPDES programs have focused on reducing point source pollutants (Oakley et al., 1991). However, the "definition of point sources is now being expanded to include sources previously considered as nonpoint" (Novotny, 1988). The EPA has defined storm water discharges associated with industrial activity to include over 100,000 facilities, particularly airports (U.S. Environmental Protection Agency, 1991). Not only do airports fall into this category, but U.S. Air Force base activities also meet the regulatory definition of industrial activities and are included under NPDES provisions. Industrial activities that result in direct storm water discharge into waters of the United States and storm water discharge

through municipal storm sewers are required to obtain a NPDES permit from the EPA (Leiter et al., 1991). Airports that hold an existing storm water discharge permit are not required to take additional action under the new rule until the expiration of their permit (Leiter et al, 1991).

In most states, the EPA has delegated authority to the state-level environmental regulatory agencies to handle their respective NPDES programs. Therefore, most bases deal with either state or regional regulators (Boyd, 1991). Due to the state's control of the NPDES permits, each Air Force base may be held to different requirements and standards. Regardless of the requirements imposed on a base, the overall objective of this research is to help prove that a land treatment system can be an effective solution for disposal for spent ADF. By proving that land treatment is a plausible solution to treat used ADF, the Air Force could substantially decrease the financial burden of disposing the fluid.

2.4 CHEMICAL COMPOSITION OF GLYCOLS

Glycol environmental impacts have been studied extensively because of their wide commercial use. As pointed out by Klecka et al. (1993), biodegradation of glycols, in both anaerobic and aerobic conditions, have been studied in wastewater (Bridie et al., 1979; Lamb and Jenkins, 1952; Mills and Stack, 1954; Price et al., 1974), activated sludge units (Kaplan et al., 1982; Pitter, 1976), in various microbial cultures (Dwyer and Tiedje, 1983, 1986; Haines and

Alexander, 1975; Kawai et al., 1978), and more recently, in different soil matrices (Keckla et al., 1993; Bausmith and Neufeld, 1996). However, since the research effort for glycols in soil have been recent, there is relatively little information on the lifetime of glycols in soil (Kleckla et al., 1993). The purposes of most of the studies listed above were done to test direct discharge and treatment procedures for specific airports.

Municipal wastewater treatment facilities contain several unit processes that have proven to be successful at treating ADF wastes. Sabeh and Narisiah (1992) found that ADF is rapidly degraded in sequencing batch reactors. They confirmed that the chemical oxygen demand (COD) removal efficiencies were greater than 99 percent and first-order decay coefficients in excess of 1.5 $days^{-1}$. ADF has been successfully treated in activated sludge systems (Jank et al., 1974; Kilroy and Gray, 1992). These systems reduce the oxygen-depleting capabilities of ADF and make the waste more suitable for direct discharge.

Although these processes at wastewater treatment plants have been found to be successful treating used ADF, the fluid creates significant operational problems in these system. Without adequate pretreatment, ADF can impose BOD loads that may cause the system to exceed the design capacity. Treatment efficiencies tend to be reduced when the ADF fluids generate significant scum formation and "bulked" sludge in aerated biological reactors (Jank et al., 1974;

Kilroy and Gray, 1992). Studies have also show that the chemical additives used in many deicing fluids are toxic to microorganisms at high concentrations and may adversely affect biological treatment processes (McGahey and Bouwer, 1992; Sabeth and Narasiah, 1992). Municipal wastewater treatment facilities are reluctant to accept spent deicing fluids because of these operational problems and possible permit violations. The rejections of these wastes cause airports to either pretreat their ADF wastes or explore other alternative methods for disposal.

On-site biodegradation of ADF with aerated lagoons is one pretreatment option. However, this approach has several drawbacks. Aerated lagoons require huge storage capacities, long retention times and create unpleasant odors (Sills and Blakeslee, 1991). Biological treatment systems could be effective reducing the organic load of ADF wastes without compromising the available land space or aesthetic qualities of the airport. Pretreatment allows the waste to be disposed of through Publicly-Owned-Treatment-Works (POTW). The effluent quality required for POTW discharge is generally lower than the quality required for surface water discharge (NPDES) permits.

Literature provides evidence that although pure glycol compounds may be toxic to aquatic life at high concentrations, the chemical additives included in ADF other than glycol make ADF more toxic than the pure glycols (Hartwell et al.,

1993). In a study done by ENSR Consulting and Engineering (1993), the toxicity of formulated ADFs were tested against pure glycol, it was found that the formulations were more toxic. Table 2.1 displays the results of the study conducted on *Pimephales promelas* (fathead minnow).

TABLE 2.1

ACUTE TOXICITY TESTS USING FORMULATED

AND PURE GLYCOL COMPOUNDS

MATERIAL	COMPOSITION	48 HOUR LC_{50}	96 HOUR LC_{50}
Propylene Glycol	Formulated	791 mg/L	709 mg/L
Ethylene Glycol	Formulated	8,541 mg/L	8,045 mg/L
Propylene Glycol	Pure	61,200 mg/L	55,770 mg/L
Ethylene Glycol	Pure	81,950 mg/L	72,860 mg/L

*Note: LC_{50} = 50% mortality concentration

Soil has proven to be a favorable medium for biodegradation of glycol based fluids (Klecka et al., 1993; McGahey and Bouwer, 1992). The work done by Klecka et al. (1993) reported that the biodegradation kinetics of ADF in soil nearly approaches zero-order when oxygen is not a limiting factor. McGahey and Bouwer (1992) demonstrated that the first-order degradation constant of ethylene glycol in soil could reach 2.9 $days^{-1}$ at an initial concentration of 100 parts per million (ppm). The higher the concentration of ADF applied to the soil, the more likely the system will be zero order. The conclusions of all the studies

done in a soil medium indicate that the biodegradation of ADF is expected to be very rapid in surface soils where microorganisms are present and oxygen is readily available. These studies also showed an increase in biodegradation when there was an increase in soil temperature. The research done at this time suggests that a land treatment system appears to be a possibility, however there has not been enough research done to evaluate a full-scale approach.

2.4.1 Ethylene Glycol

Ethylene glycol (EG) is "a colorless, odorless, hygroscopic liquid, infinitely soluble in water and many organic liquids" (Aerospace, 1987). EG has the chemical formula $C_2H_6O_2$, a freezing point of 8.6 °F (-13 °C) for pure liquid, and a eutectic temperature in aqueous solution of –58 °F (-50 °C) (US Department of Transportation, 1991). Eutectic temperature is defined as the lowest possible melting temperature obtainable with specified mixtures of certain compounds (Dictionary of Geological Terms-Revised Edition, 1976).

Oral ingestion of EG can result in depression, respiratory and cardiac failure, kidney damage and brain damage (Aerospace, 1987). However, the acute and chronic oral toxicity of EG and propylene glycol (PG) to humans and other terrestrial life is generally perceived to be low (Sills and Blakeslee, 1991). None of the glycols used as de/anti-icing agents have been demonstrated to be either a carcinogenic or mutagenic hazard (Aerospace, 1987). One exception may be

associated with an animal carcinogen, 1,4 dioxane, which is present in some technical grades of EG. In fact, 1,4 dioxane has been known to induce tumors in laboratory animals and is thus classified and regulated as a potential carcinogen to humans (Sills and Blakeslee, 1991). Technical grades of EG are used to formulate automotive antifreeze. Some suppliers of aircraft deicers currently use technical grade EG to formulate aircraft deicers in certain areas of the United States (Sills and Blakeslee, 1991).

2.4.2 Propylene Glycol

Propylene glycol appears to be less toxic to humans than EG. However, it may cause skin rashes and irritation if held in contact with the skin for any extended period of time (Arco, 1990). PG is miscible with water and has a potential for significant mobility in ground water. It is also classified as readily biodegradable (Fetter, 1992). The following chart summarizes the given chemical characteristics for PG:

TABLE 2.2

CHEMICAL CHARACTERISTICS FOR PROPYLENE GLYCOL

Chemical Characteristics	Value	Reference
Boiling Point (°C) at 760 mm Hg	188.2 °C	Sax and Lewis (1989)
Freezing Point (°C) at 760 mm Hg	-59 °C	Sax and Lewis (1989)
Vapor Pressure (mm Hg) at 20°C	0.08	Sax and Lewis (1989)
Solubility in Water	hygroscopic	Sax and Lewis (1989)
Octanol/Water Partition Coefficient (K_{ow})	$3.89X10^{-2}$	Miller (1979)
Organic Carbon/Water Partition Coefficient (K_{oc})	$2.4X10^{-2}$	Miller (1979)

Propylene glycol is commonly used in moisturizers as a humectant. It is believed by cosmetic companies to hold moisture in the skin, keeping skin feeling soft and looking young. PG is used in cigar humidifiers to keep the cigars stored at the proper moisture. Also, it is used as a solvent in paints, inks and coatings. PG is a member of the aliphatic hydrocarbon group of alkanes. The chemical formula for PG is $C_3H_8O_2$. The structure has two OH (alcohol) groups attached to the 2 and 3 carbons.

2.4.3 Glycols in the Environment

From an environmental perspective, both EG and PG have high water solubility, weak sorption to soils, and are highly mobile in the soil/groundwater system (Aerospace, 1987). Studies using ethylene glycol in sandy soils show the constituent to follow closely to the overall movement of water with little or no

retardation (Aerospace, 1987). Sorption calculations for unsaturated topsoil models estimate "that only 0.4% of the glycol is expected to be sorbed by soil particles" (Aerospace, 1987). This information is important for this research since sorption could limit the amount of PG available for the microorganisms to biodegrade. Since sorption is negligible, we can disregard sorption as a possible pathway for removal of PG. Due to a low Henry's Constant of $1.2(10^{-8})$ m^3/mole, PG will tend to stay in water and not volatilize to the air. This could also pose a problem if the waste would easily leach from a land treatment facility into ground or surface water.

The acute and chronic aquatic toxicity of both EG and PG was found to be low in both freshwater and saltwater environments (Arco, 1990). Both EG and PG are not considered bioaccumulative in nature and exhibit a high degree of biodegradability under normal soil/water conditions. These chemicals are, therefore, classified as non-persistent agents in the environment (Sills and Blakeslee, 1991). The biodegradability can be a major problem, since both glycol deicing constituents exhibit very high Biochemical Oxygen Demand (BOD) and thus have the potential to deplete available dissolved oxygen in water receiving the runoff effluent (Sills and Blakeslee, 1991).

> The biodegradation of glycols is so rapid and extremely oxygen-demanding that it can deplete dissolved oxygen (DO) levels and threaten oxygen-dependent aquatic life in receiving waters. The 5-day biochemical oxygen demand (BOD_5) at 20 °C for pure EG has

> been reported to be in the range of 400,000 to 800,000 mg/L...The BOD_5 of pure PG is considerable higher (about 1,000,000 mg/L) (Sills and Blakeslee, 1991).

The theoretical ratio of oxygen consumption to carbon dioxide production can be determined using the equation for theoretical oxygen demand (ThOD). This equation calculates the amount of oxygen required to convert an organic material to carbon dioxide, water and ammonia (Sawyer et al., 1995). This calculation is shown in Table 2.3.

The software for the respirometer includes a calculation of the measured ratio of oxygen uptake to carbon dioxide production. Comparing the theoretical to actual ratios allows us to determine if complete mineralization has taken place. The ThOD equation also makes it possible to calculate the amount of oxygen required to degrade PG.

TABLE 2.3

THEORETICAL OXYGEN DEMAND CALCULATION

BASIC EQUATION FOR ThOD:

$$C_nH_aO_bN_c+(n+a/4-b/2-3/4c)O_2 \rightarrow nCO_2+(a/2-3/2c)H_2O+cNH_3$$

PROPYLENE GLYCOL ($C_3H_8O_2$) RATIO:

$$C_3H_8O_2 + 4O_2 \rightarrow 8CO_2 +4H_2O$$

MOLAR RATIO O_2:CO_2= 0.5

Molecular weight $C_3H_8O_2$=76.094 mg PG/mole

$$\therefore \frac{\textbf{128 mg } O_2}{\textbf{76.094 mg PG}}$$

$$= \textbf{1.68 mg } O_2\textbf{/mg PG}$$

2.5 BIODEGRADATION

In favorable environmental conditions, all natural organic compounds degrade (Atlas and Bartha, 1993). Biodegradation can be defined as the "biologically catalyzed reduction in the complexity of chemicals" (Alexander, 1994). This reduction in complexity of chemicals is the microbially mediated process of breaking down a substance to smaller products caused by microorganisms or their enzymes (Atlas and Bartha, 1993). In the case of propylene glycol, biodegradation frequently leads to the complete conversion of the original compound to the inorganic products CO_2 and H_2O (Alexander, 1994). This conversion of the organic substrate to inorganic products is known as

mineralization or ultimate biodegradation. Due to the microorganisms' ability to mineralize anthropogenic compounds, microorganisms play a large role in soils, waters, and sediments (Alexander, 1994). Since it is possible to monitor the O_2 uptake and CO_2 production of the microorganisms, the biodegradation of components of aircraft deicing fluid can be studied.

In the batch system used in this research, four components change with time; the amount of O_2 and CO_2 in the bottles, the concentration of propylene glycol and, finally, the biomass of the microbes. Since degradation products cannot always be anticipated, monitoring the conversion of oxygen to carbon dioxide is the most clear-cut proof of mineralization (Atlas and Bartha, 1993).

The ability of the microbial population to destroy synthetic chemicals depends on a variety of physical, chemical, and biological factors that affect the growth, activity and existence of the microorganisms. The availability of O_2, presence of toxins, or other environmental characteristics that may promote, restrict, or prevent biodegradation all have an impact on the rate of biodegradation in the soil (Alexander, 1994).

The final elements that may impact the biodegradation rate are the environmental characteristics. Most microorganisms have a range of tolerance for abiotic factors. Temperature, pH, and salinity are just a few examples of

these factors that affect the microorganisms growth and activity (Alexander, 1994). Depending on which organisms are present the tolerance range is different. In the event that a particular environment contains several species that are able to transform the contaminant, the tolerance range is much broader than if only a single microorganism is present. If abiotic factors are outside of the indigenous population's tolerance range, no biodegradation activity will occur.

Bausmith and Neufeld (1996), were able to show an increase in the base level biodegradation constant by more than a factor of 4 through the use of sludge-amendment, lime-amendment, and soil-aeration. "The effect of pH on biodegradation of polluting chemicals has received scant attention, although it is common practice to add lime to bioremediate acid soils or subsoil's materials containing harmful organic compounds" (Alexander, 1994). During research, the repeated application of PG-water solution slowly alters the pH of the batch system. With the use of lime-amendment, it should be possible to keep the pH range within the tolerance of the microorganisms and help maintain the optimum biodegradation rate.

3. METHODOLOGY

3.1 OVERVIEW

This chapter describes the methodology used to document the biodegradation of propylene glycol in various soil types. Biodegradation was monitored using a respirometer to measure the oxygen consumption and carbon dioxide production of the indigenous soil microbes. A high-clay soil (HC) and a sandy soil (SS) were used as matrices for a test of biodegradation of a propylene glycol-water solution simulation of aircraft deicing fluid in a land treatment system. Over a 12-day test period, soil samples were re-contaminated every 96 hours to monitor the biodegradation of the propylene glycol. The sample bottles were connected to the respirometer, which allowed for monitoring of the headspace gases over the 12-day period. The bottles were monitored every 6 hours for a total of 48 readings in RUN1 and 61 readings in RUN2. The rate of microbial metabolism could be calculated on the basis of the observed oxygen consumption and carbon dioxide production.

3.2 SOILS

In their evaluation of JP-8 fuel degradation, Baker (1995) and Totten (1995) used three different soil types. To focus the laboratory work, this research used two soil types, similar to those used by Baker and Totten. By analyzing the very different soil types, a range of reactions can be seen. For that reason, the microbial respiration rates were monitored for both sandy soil and high-clay soil.

3.2.1 Soil Collection

The high-clay soil was collected from an area adjacent to building 470 in area B of Wright-Patterson Air Force Base, Ohio. The sandy soil was collected from the a streambed located north of Highway 35 and east of north Fairfield road in Beavercreek, Ohio. Both samples were obtained by clearing the upper 1 to 3 inches of topsoil (including the vegetation) and collecting the next 10 inches of soil with a clean shovel. Enough soil was collected to complete all experiments required. This allowed for the same samples of soil to be used for all the experiments. The main advantage of using the same soil was to increase the level of consistency between experiments. Using only one soil sample made it possible to decrease variations of soil characteristics (i.e. surface area, size distribution, fraction of organic contents and soil type).

3.2.2 Soil Preparation

To ensure a consistent soil medium during testing, the soil was sieved to eliminate any remaining vegetation roots and rocks that could cause variation in the results. A plastic pool filter with 6 mm square openings on the sides and bottom was used as the sieve. After the soil was collected and sieved, it was refrigerated at 4° C to decrease the microbial activity. Before experiments were started, the soil was allowed to sit out until it reached room temperature. Then samples of 100 grams of soil were weighted out and placed in the 250 ml bottles.

Before each experiment, the soil was tested to ensure that the soil still maintained the same moisture content as it did for the first experiment. This kept the 100 grams of soil used in any microcosm consistent throughout the laboratory work.

3.2.3 Soil Characteristics

Physical characteristics of the (sieved) soils were determined by A&L Great Lakes Laboratories. Grain size analysis was conducted by method MSA Part 1 (1986). Soil type was determined according to MSA Part 1 (1986). The results of the analyses are summarized in Table 3.1. The complete report, from A&L Great Lakes Laboratories, is included in Appendix A.

TABLE 3.1

PHYSICAL ANALYSIS OF SOILS

	PARTICLE SIZE ANALYSIS* (%)				
SOIL	Sand	Silt	Clay	GROUP NAME	MOISTURE CONTENT (%)
HC	42	34	24	Loamy	21
SS	86	7	7	Loamy Sand	15

***Method: MSA Part 1 (1986)**

Source: A&L Great Lake Laboratories. Report, Project No. PR#F61TNV71690100, 8 Aug 97

3.2.4 Soil Chemistry

To accurately evaluate the biodegradation of PG the chemical make up of the soil was also important. Although a complete analysis was not needed to complete this research, the amount of total organic carbon, phosphates, potassium, magnesium and calcium levels were determine. Table 3.3 summarizes gives a brief look at the soil chemistry found in each soil. To examine the complete lab report, please reference Appendix A.

TABLE 3.2

PHYSICAL ANALYSIS OF SOILS

SOIL	pH	ORGANIC MATTER (%)	PHOSPHORUS (ppm-P)	POTASSIUM (K) (ppm)	MAGNESIUM Mg (ppm)	CALCIUM Ca (ppm)
HC	7.3	5.3	10 (L)	105 (M)	298 (H)	2150 (H)
SS	8.0	0.7	2 (VL)	25 (VL)	133 (VL)	5175 (VH)

* VL=very low L=low M=Medium H=High VH=very high

3.2.5 Soil Moisture

Depending on when soil samples are retrieved from the environment, the moisture content of the soil may vary. Since soil moisture has a significant affect on biodegradation, it was appropriate to monitor and adjust the soil moisture to achieve an optimum moisture level. Finding the field capacity of a soil is the accepted means for measuring soil moisture. Defined by Lyon et al. (1952), field capacity is the percentage of water that can be held in a soil matrix by capillary

forces when adequate drainage is provided. It is generally believed that by maintaining the soil moisture between 25% and 85% field capacity provides an optimal range for microbial activity (Sims et al., 1989). Monitoring the moisture content in each experiment also made it possible to maintain the necessary moisture content to encourage biodegradation. In order for microorganisms to carry out a metabolic transformation, they need adequate moisture for their growth and activity. If there is an inadequate supply of water in the soil, it may severely restrict the biodegradation (Alexander, 1994). Excess water displaces air from the pore space in the soil and "waterlogs" the soil. The excess water allows the soil to become anaerobic and unfavorable for aerobic processes. To find the field capacity of the two soils used, the following processes were performed prior to running any experiments.

Grab samples of each the high-clay and sandy soils were weighed and dried in a Thelco laboratory oven for 48 hours at 104°C to determine the amount of water in the soil during collection. This was an important process because it ensured that each experiment contained the same amount of moisture before contamination. By refrigerating the samples, there was only a slight variation between experiments. Attempting to keep all experimental runs consistent, the moisture content of the samples was corrected to meet the starting field capacity level for each soil type (high-clay 21% and sandy soil 15%).

Field capacity was determined using a process similar process to Thomas (1996). The test determined the amount of water that must be added to the soil to achieve 100% field capacity. A 70% field capacity level was picked as the standard soil moisture for these experiments. The moisture content of the soils was below 70% field capacity. Therefore, distilled water was added to each of the soil samples to bring the moisture content to 70% of field capacity. Running all experiments at consistent water contents minimized the variations in biological activity.

3.4 RESPIROMETER

The Micro-Oxymax respirometer was used in the closed loop mode to measure the oxygen uptake and carbon dioxide production of soil microorganisms. Totten (1995), Baker (1995) and Thomas (1996) used the same machine and process. More details on the respirometer can be found in those theses. The respirometer's sample pump draws the air contained in each bottle into the Andros 5000 CO_2 gas analyzer and Citicel O_2 sensor. The computer records the CO_2 and O_2 concentrations and the air is pumped back into the bottle that it was drawn from.

The machine schedule was set up to take these readings for each of the 20 bottles every 6 hours. Immediately following the readings, each bottle was refreshed with ambient air for 60 seconds. Because we know the amount of PG

placed in each bottle and the amount of oxygen it consumes, we can be sure that oxygen is always present and that it is not a limiting factor for biodegradation. Since the bottles are refreshed with ambient air every 6 hours, there is little concern about the process going anaerobic due to depletion of oxygen in the bottles. Each test was performed using 250-ml bottles containing 100 grams of fresh, uncontaminated soil. During the test, the bottles were re-injected with simulated deicing fluid after every 96 hours. The refresh function on the respirometer ensures that the bottles in the experiment will have O_2 at all times during the experiments. With the repeated application of PG-water solution, there is always contaminant present.

Since microorganisms can be sensitive to environmental temperature changes, the sample bottles were incubated at 30° $\pm$ 1°C. Since the sample bottles were held in an incubator, the bottles were only exposed to light during re-injection of the contaminate solution. Studies have shown that glycols may degrade in the presence of ultraviolet light (i.e., photodegradation), though photodegradation is generally considered to be a minor fate process affecting glycols in water (Syracuse Research Corporation, 1989). Because of this, photodegradation was expected to be negligible fate process for propylene glycol during these experiments.

The respirometer used in this research was able to collect data on 20 different bottles during a run. Two experiments, each run for 12 days, were completed. The following Table outlines the format of the two experiments.

TABLE 3.3

BOTTLE BREAKOUT OF EXPERIMENTS

EXPERIMENT	SOIL TYPE	REPL	VOLUMETRIC CONCENTRATION (ppm)	# OF INJECT OF PG	PERCENT OF DRY WEIGHT OF SOIL	TOTAL AMT SOLUTION ADDED (ml)
AMRUN1	High-clay	9	100,000	3	1.14	0.9
AMRUN1	Sandy	9	100,000	3	1.06	0.9
AMRUN1	Control	2	0	0	0.0	0.0
AMRUN2	High-clay	4	100,000 w/lime	3	1.14	0.9
AMRUN2	Sandy	4	100,000 w/lime	3	1.06	0.9
AMRUN2	Control	2	0	0	0.0	0.0
AMRUN2	High-clay	2	100,000	1	0.38	0.3
AMRUN2	Sandy	2	100,000	1	0.35	0.3
AMRUN2	High-clay	2	100,000	1	0.76	0.6
AMRUN2	Sandy	2	100,000	1	0.706	0.6
AMRUN2	High-clay	1	100,000	1	1.14	0.9
AMRUN2	Sandy	1	100,000	1	1.06	0.9

Although there are only two experiments shown in the above Table, other trial runs were completed. Uncontaminated soil was run in the bottles to gain an understanding of the microbial activity present in the soil and provide background soil data. Range-finding tests were completed to determine possible concentrations for use in the full-scale experiments. After finding an appropriate concentration of PG-water solution (10% PG) to test, experiment 1 was started.

3.5 EXPERIMENT SET-UP

All experiments were setup identically to avoid any discrepancies between runs. The schematic diagram seen in Figure 3.1 represents the experimental setup. Each experiment used 20, 250 ml bottles. An AMBI-HI-LOW incubator, manufactured by Lab Line, allowed for temperature control and eliminated exposure to light. Each bottle was connected to the expansion unit with 1/8" outside diameter nylon tubing.

Before each experiment, several adjustments and checks were done on the equipment to ensure that the machine was running properly. The gas sensors were first calibrated to ensure a standard starting point for all experiments. A zero reading for the O_2 and CO_2 sensors was obtained by circulating nitrogen gas through the sensors. Then the calibration gas was circulated through the sensors and the machine was adjusted to the known concentrations of oxygen and carbon dioxide in the calibration gas. Once the contaminant was placed in the bottles, the final check was run on the system. Each bottle was checked for restrictive errors and leaks with the respirometer software.

3.5.1 Moisture Removal

Throughout the experiment, the air pumped out of the bottles and into the O_2 and CO_2 sensors were first filtered through a drier containing magnesium perchlorate.

The amount of water collected by these driers depended on the temperature of the room during testing. The larger the difference in temperature between the incubator, where the bottles are stored, and room temperature, the more moisture collected in the driers. The other location of moisture removal is in the inlet valve where the ambient air is pumped into the machine to refresh the bottles between samples. The compound used to extract the moisture in the air is Drierite®. The compounds were monitored by regular observation and changed periodically to protect the sensitive respirometer.

3.5.2 Temperature Control

The room where the respirometer is set up had fluctuations in ambient temperature. The incubator held all the bottles in the experiment at the same constant temperature throughout the 12-day test period. Temperature readings were taken as the bottled air reaches the sensors of the respirometer. It is not possible to show a relationship between temperature and biodegradation rate since the temperature readings taken by the machine do not accurately reflect the temperature of the soil in the incubator. There are two probes connected to the machine which are extended into the incubator through the back side where the 1/8" tubing for the bottles exits the incubator. Although the probes provide for a general temperature in the incubator, they do not reflect the temperature of the soil in the bottles. It is not possible to stick a probe in the soil to get exact measurements because the bottles are sealed to eliminate gas leaks.

Depending on the room temperature during the sample, the temperature of the air reported by the probes is slightly altered. As the two ports in the back of the incubator provide enough space for all 20 bottles to have 2 1/8" tubes connected to the expansion units, they also provide a place for heat to either enter or exit the incubator. Typically the probes, due to their placement in the incubator, report a slightly cooler temperature.

3.6 LABORATORY PROCEDURES

3.6.1 Solution Preparation

Using pure propylene glycol from Mallinckrodt Baker Lab, a 100,000 ppm (10% PG) mixture of PG and distilled water was created. To eliminate the possibility of the solution biodegrading between experiments, a new solution was mixed for each experiment. Literature values for the average biodegradation rate for propylene glycol are between 78.9 and 88 mg PG/kg soil/day (Klecka et al., 1993). Using these findings, the solution was prepared to follow similar concentrations. With 4 days between injections of contaminant, approximately 300 mg PG was added during each injection. Since each bottle contained 100 grams of wet soil, the mg PG/Kg soil concentration was different for the two soils (see Table 3.4 below). Also shown in Table 3.4 is the concentration of PG for the bottles that were not reinjected. The concentration of the bottles reinjected 3 times with 300 mg PG is the same as the concentration for the bottles injected

with 900 mg PG. From the research of Klecka et al. (1993), this mixture would be able to biodegrade most of the solution added to the bottle during each 96-hour period.

TABLE 3.4

CONCENTRATION (mg PG/kg soil)

SOIL	DRY WEIGHT (g)	AMOUNT PG ADDED (mg)	CONCENTRATION (mg PG/kg soil)
HC	79	300	3800
SS	85	300	3530
HC	79	600	7600
SS	85	600	7060
HC	79	900	11400
SS	85	900	10600

3.6.2 Soil Controls

Each of the experiments included a control bottle that provided a measurement of background respiration for virgin soil. These controls used the same soils at 70% field capacity but did not contain any PG-water solution.

3.6.3 Soil Contamination

Once the batch system bottles held the soil samples at 70% field capacity, they were ready to be contaminated with the premixed PG-water solution. Both soil

types were injected with 3 grams of the 100,000-ppm solution previously described. To ensure an even distribution of the solution, the bottles were mixed using a sterile spatula. In an effort to enhance the soil's ability to use the oxygen in the bottle, approximately 10 holes were placed in the soil going all the way down to the bottom of the bottle. This was done to help eliminate anaerobic zones in the soil system.

3.6.4 Re-injection of Contaminant

In an attempt to show the soils ability to continually degrade PG, the sample bottles were re-injected with 3 grams of the 100,000-ppm solution every 96 hours. In field situations, the time intervals between contaminant application will vary. By reintroducing contaminants at a steady interval, the results should show a linear biodegradation rate of PG. The reapplication of PG to the soil will help to determine if acclimated soil bacteria would reduce the time required for biodegradation, and therefore increase the permissible ADF application rates.

3.6.5 pH Balance Addition

During AMRUN2, hydrated lime was added to each of the soil types to attempt to improve the pH to enhance the microbial environment. Bausmith and Neufeld (1996) applied hydrated lime to their system and were able to show an increased biodegradation rate by more than a factor of 4 by implementing this strategy.

For both soils, the pH was determined using a Hach hand held pH meter. Soil slurry composed of approximately 50 grams of soil and 50 ml distilled water was mixed with a magnetic stir bar to ensure a completely mixed solution. Table 3.5 displays the results found with the Hach meter.

TABLE 3.5

SOIL pH VALUES

SOIL	HACH METER READING	A&L LAB RESULTS	AMOUNT LIME ADDED (mg)	ENDING pH READING
HC	7.0	7.3	50	8.2
SS	7.6	8.0	50	8.5

The goal of adding the correct amount of lime to the soil was to achieve an increase in soil pH and keep the soil between 5.5 and 8.5 pH. This tolerance range is considered most suitable for microbial activity (Sims, Sims and Matthews, 1989). The process used to spike the bottles was to dissolve 50 mg hydrated lime into the 3 ml PG-water solution added to the bottles during the first injection. The effects of this one time pH adjustment will be compared to the biodegradation rates found in AMRUN1. Table 3.6 breaks down the time sequence of AMRUN1 and Table 3.7 shows the sequence of AMRUN2.

TABLE 3.6

AMRUN1 TIMETABLE

	3 JULY 1997	7 JULY 1997	11 JULY 1997
WATER ADDITION	HC: 13 ml SS: ----	HC: ---- SS: ----	HC: ---- SS: ----
PG-WATER SOLUTION	HC: 3 ml SS: 3 ml	HC: 3 ml SS: 3 ml	HC: 3 ml SS: 3 ml

Note: PG injection 1 took place at hour 0, injection 2 at 96 hours and injection 3 at 192 hours.

TABLE 3.7

AMRUN2 TIMETABLE

	11 AUGUST 1997	15 AUGUST 1997	19 AUGUST 1997
WATER ADDITION	HC: 7 ml SS: ----	HC: ---- SS: ----	HC: ---- SS: ----
PG-WATER SOLUTION	HC: 3 ml SS: 3 ml	HC: 3 ml SS: 3 ml	HC: 3 ml SS: 3 ml
LIME ADDITION	----	HC: 50 mg SS: 50 mg	----

*This Table documents the timetable for bottles 1-4 and 11-14. The results of these bottles are compared to AMRUN1 in chapter 4. PG injection 1 took place at hour 0, injection 2 at 96 hours and injection 3 at 192 hours.

3.7 DATA COLLECTION

The software records or calculates the following parameters during sampling: time of sample, temperature, percentage of O_2 in the sample, uptake of O_2 in uL/hr, cumulative O_2 uptake in uL, percentage of CO_2 in the sample, production of CO_2 in uL/hr, cumulative CO_2 production in uL and the respiratory exchange rate (RER), the rates between CO_2 production and O_2 uptake. The experiments

were designed to take these readings every 6 hours for the 12 day run period.

3.8 DATA ANALYSIS

Using the Micro-Oxymax 6.05 software and conversion function, a graphical comparison of the data collected was completed on Office 97 Excel. The statistical approach used to analyze the data was both descriptive and analytical. Data was collected for empty bottles, uncontaminated soil, PG contaminated soil and PG contaminated soil with lime-amendment.

3.8.1 Reproducibility and Repeatability

Whenever completing a laboratory experiment, it is critical to prove that the methods instituted during the design and completion of the lab work can be repeated within an experiment and results can be reproduced between experiments. Limiting experimental errors and proving statistically that the work completed could be redone provides confidence for follow-on researchers. Controlling variables like temperature, moisture content and fraction of organic carbon help eliminate some variations. Using replicate bottles of the same contaminant concentrations and using the same soil sample provided a way to confirm that the results are consistent. A more in-depth discussion of reproducibility and repeatability can be found in Chapter 4.

3.9 STATISTICAL DESIGN

Multiple bottles went through the same treatment process in each experiment to allow for sample means to be used to test the statistical hypotheses. Table 3.8 summarizes the statistical analyses. For more details on how each test was completed, reference Appendix C.

TABLE 3.8

STATISTICAL DESIGN SUMMARY

RESEARCH ELEMENT	STATISTICAL APPROACH
TEST1: Level of PG in soil and type of soil significantly affects total oxygen uptake.	Two-factor ANOVA (w/interaction) Analysis of Means (Tukey)
TEST2: Type of soil significantly affects total oxygen uptake.	Two-factor ANOVA (w/interaction) Analysis of Means (Tukey)
TEST3: The addition of lime significantly affects total oxygen uptake.	Two-factor ANOVA (w/interaction) Analysis of Means (Tukey)
TEST4: The reapplication process of contaminant significantly affects total oxygen uptake.	T-test

4. DATA ANALYSIS

4.1 INTRODUCTION

During the laboratory research, Captain Johnson and I both conducted two experiments on the respirometer. Between experiments maintenance on the machine and correction of errors found during startup of the next experiment were completed. Experiments AMRUN1 and AMRUN2 were successfully completed with a total of 660 hours of respirometer data.

In each experiment, when the bottles were re-injected with contaminant, the machine had to be turned off. Once the experiment was shut down, to restart the experiment, each bottle had to be rerun through the restrictive errors and leak checks. As the experiment was turned back on, the machine does an initial reading of oxygen concentration and then does the first posted reading eight hours later. Since the first reading after the reinjection is eight hours after start-up, it is not consistent with all the other six hour readings. It is possible to do a simple extrapolation of those two readings among the experimental data. Without extracting those two data points, the data appears to be in three phases. The primary reason for this problem was opening the bottle to atmospheric air. Diagrams D-1 and D-2 in Appendix D show exactly where the phase breaks were put in each experiment.

4.2 DIFFERENCES BETWEEN SOIL TYPES

The first goal of this study was to determine if variation of microbial metabolism occurred between the two separate soil types. A graphical comparison of the two soils can be viewed in Appendix D pages D-1 (RUN1) and D-2 (RUN2). Although the graphs appear to show a significant difference between soil types (high clay showing more than triple the amount of O_2 uptake of sandy soil), some statistical work was done to verify the results shown on the graphs. To demonstrate a significant difference between the soil types it is important to first determine if biodegradation occurred.

4.2.1 Soil Respiration Rates

To prove this statistically, a simple ANOVA test was done to show that the amount of PG added to the soil and the soil interact to effect cumulative O_2 uptake. The factors being tested here were the level of PG and the type of soil. For more specific data on all statistical work done in this chapter please reference Appendix C. The test was conducted for each experiment separately and since both results indicated that the PG and soil do interact, there was no need to combine the results of the two experiments and redo the test. Knowing that the addition of PG into the soil affected the respiration rate of the microcosms present, a comparison between respiration rates in the high clay soil and sandy soil could be made. Table 4.1 summaries the results of these statistical tests.

Significant differences in respiration rates between soil types were identified by a Tukey pairwise comparison of means. With a 95 % confidence interval (CI), the mean of square results from test 1 was used to determine the standard deviation of D_{hat}. The difference between the mean cumulative O_2 uptake numbers for each given soil was compared to the 95% CI number calculated. In both RUN1 and RUN2, the difference of means was greater than half the CI. The results of the Tukey comparison for both RUN1 and RUN2 support the assumption that there is a significant difference between respiration rates for the two soil types (see Table 4.1).

TABLE 4.1

STATISTICAL SUMMARY OF ANOVA TEST AND TUKEY PAIRWISE TEST FOR LEVEL OF PG AND SOIL TYPE

TEST CONDUCTED	FINDINGS OF TEST	MEASURE
TEST1: Level of PG in soil and type of soil significantly affects total oxygen uptake.	RUN1: **Interaction** between factors RUN2: **Interaction** between factors	F-Value: 51.25>4.75 F-Value: 46.43>4.75
TEST2A: Type of soil significantly affects total oxygen uptake (without contaminant present).	RUN1: **Significant** difference between soil types RUN2: **Significant** difference between soil types	PG,SOILS D ½ CI 0,HC*SS 80537>74191 0,HC*SS 100259>87829
TEST2B: Type of soil significantly affects total oxygen uptake (with contaminant present).	RUN1: **Significant** difference between soil types RUN2: **Significant** difference between soil types	PG,SOILS D ½ CI 900,HC*SS 333427>74191 900,HC*SS 333427>74191

Having proven that the respiration rates between sandy soil and high clay were significantly different, quantifying the difference helps understand the ability of the soils to biodegrade PG. Using the Mathcad template in Appendix E, calculations of the respiration rate (ul/min/kg soil), total PG consumed and biodegradation rate (ml/day/kg) were done for each soil type at various PG concentrations. This section is concerned with the respiration rates between the two soil types. Appendix E page E-3 shows the significant difference between the respiration rates of the two soils broken down into phases. The results for RUN1 and RUN2 for each phase and soil were averaged together to complete the chart. The results of Table 4.2 show that the respiration rate for HC was greater than four times higher in all cases than SS.

TABLE 4.2

SOIL RESPIRATION AND BIODEGRADATION RATES

SOIL	PHASE	RESPIRATION (ml/day/kg)	BIODEGRADATION (mg/day/kg)
HC RUN 1	1	12.05	159
	2	12.67	167
	3	13.32	176
HC RUN2	1	15.46	119
	2	**11.18**	88
	3	15.25	236
SS RUN1	1	3.35	54
	2	2.77	41
	3	3.11	46
SS RUN2	1	2.7	36
	2	2.89	46
	3	3.84	74

Notice how the HC phase 2 number is smaller than the phase 1 number (Table 4.1). For some unexplained reason, this phase had a much lower O_2 uptake than would have been expected. After doing a through check on the machine, there appeared to be no mechanical errors causing the uncharacteristic decrease. These abnormal results of the HC phase 2 in RUN2 can be viewed on page D-8. In past, others have experienced these blips in data and were unable to explain any possible sources of error (Baker 1995 and Johnson 1997). There appears to be a trend with this type of problem appearing in the first expansion unit (bottles 1-10). The data attained for the sandy soil bottles (11-20) did not seem to be affected by these blips.

4.2.2 Soil Biodegradation Rates

From this experiment, it was possible to show the differences between the biodegradation of PG on the two soil types. On pages D-1 and D-2, the averages of the two separate runs for the two soils are plotted against each other. Notice that the control bottle for the high clay soil had a higher respiration rate than the spiked sandy soil. Of course this is to be expected since there is more nutrients available for the microcosms in the high clay soil than in the sandy soil. After plotting the results and finding a significant difference between the respiration rates of the two control bottles, it is obvious that there is a larger indigenous microcosm population in the HC soil.

By statistically proving there was a significant difference between the respiration rates of the two soils, the data plots show an increased lag time with the sandy soil. This lag time can be seen on page D-12 where the mean of the bottles for RUN1 is graphed. The two humps in the graph represent the lag phase experienced. The HC results plotted on page D-11 show a pretty stable oxygen uptake for RUN1. There appeared to be no overload or inhibition of PG in the HC graphs. As mentioned in section 4.2.1, the respiration rates of the two soils varied greatly. Another parameter calculated for each of the soils was biodegradation rates in mg PG/day/kg soil. The program used to determine these rates can be seen in Appendix E. Table 4.2 summarizes the results of these calculations.

The difference between the biodegradation rates with the two soils, as shown in Table 4.2, varies with the HC soil being 1.9 to 3.3 times higher than SS. When considering where to landtreat ADF, researchers will want to know what type of soil would be ideal. With the research done in this report, the use of a HC soil will biodegrade PG much quicker than SS. For a graphical presentation of the difference, see page E-4. Notice how the biodegradation rates between HC phase one and two are very similar, however, phase three is greatly increased. That is a result of the decrease in lag time during that phase.

4.2.3 Affects of Lime Additive

The second primary goal of this research was to determine if adding a soil nutrient/buffer would amplify and/or constrain microbial metabolism of the contaminant. As discussed in section 3.6.5, the pH balance additive was effective for Bausmith and Nuefeld (1996) where they used a pan system to monitor the biodegradation of ADF. A Tukey pairwise comparison of means was completed on the cumulative O_2 consumption results for RUN1 and RUN2 (see pages C-7, 8). This was the same process used for the statistical test 2 discussed in section 4.2.1. Four random bottles in RUN1 were compared to the four bottles injected with the lime additive from RUN2. The results of that test indicate that there was no significant difference between bottles with lime additive and bottles without the lime present. The graphical comparison of the

biodegradation rates for HC and SS RUN1 and RUN2 data can be viewed on pages E-5 and E-6. There are no trends apparent between the two experimental runs. Therefore, the use of the lime additive did not prove to increase the microbial metabolism. Table 4.3 summarizes the results from the statistical tests.

TABLE 4.3

STATISTICAL SUMMARY OF ANOVA TEST AND TUKEY PAIRWISE TEST FOR LIME ADDITION

TEST CONDUCTED	FINDINGS OF TEST
TEST3: The addition of lime significantly affects total oxygen uptake.	HC SOIL: **No significant** difference between lime/no lime runs SS SOIL: **No significant** difference between lime/no lime runs

4.2.4 Variation of Biodegradation Rates Depending on Soil Loading

The primary difference between RUN1 data and RUN2 data is the adjustments made to the number of replicates used to observe the behavior of respiration in the bottles reinjected with 300 mg of PG. By injecting different concentrations of contaminant into the left over bottles, the behavior of the soil systems with varied loading schemes can be analyzed. Sections 4.4.1.2 and 4.4.2.2 specifically discuss the results of RUN2 for the two soils. Below, Table 4.4 summarizes the

respiration rates, percent degraded and biodegradation rates for the soil loading variations.

TABLE 4.4

RESULTS OF SOIL LOADING RATE VARIATIONS

SOIL TYPE	CONCENTRATION	RESPIRATION RATE (ml/day/kg)	BIODEGRADATION RATE (mg/day/kg)	% DEGRADED
HC	3X300 mg PG	13.32	157.5	20
	900 mg PG	15.2	188	24.78
	600 mg PG	14.03	167	32.95
	300 mg PG	9.1	76	30.06
SS	3X300 mg PG	3.11	49.5	6.222
	900 mg PG	1.53	11	1.49
	600 mg PG	2.09	21	4.4
	300 mg PG	7.13	113	48.16

4.2.5 Variation in Biodegradation Depending on Reinjection

The final objective for this research was to determine if the reinjection of contaminant to the soil media would reduce the lag time and increase the biodegradation rate of PG. By reinjecting the soil with contaminant it was possible to determine if acclimated soil bacteria would reduce the time required to biodegrade PG, and therefore increase the permissible ADF application rates. To determine if a significant increase or decrease in respiration occurred for the two soil types a single sided T-test was preformed on the data. By utilizing the single injection bottle in RUN2, a standard biodegradation rate was assumed. The step-by-step process for the T-test can be viewed in Appendix C.

Using the Mathcad program to calculate the biodegradation rates for each process, graph E-7 shows the differences found between the two soil types and the influence of reinjection. In section 4.4.2.1, it discusses briefly an apparent inhibition when the concentration of 900 mg PG was present. The figure on page E-7 clearly shows a significant decrease in biodegradation when the reinjection process was not utilized. However, the HC does not appear to have the same results. The increased initial concentration proved to have a significant increase in biodegradation. The results of the T-test support the same results as the graphs. The results of the statistical tests can be viewed below in Table 4.5.

TABLE 4.5

STATISTICAL SUMMARY OF T-test

FOR REAPPLICATION TESTS

TEST CONDUCTED	FINDINGS OF TEST
TEST4: The reapplication process of contaminant significantly affects total oxygen uptake.	HC SOIL: **No significant** difference between reapplication/no reapplication runs SS SOIL: **Significant** difference between reapplication/no reapplication runs

There may be a point in the concentration level where we would find similar

results with the HC soil as we found with the SS. With iterations done to test for the maximum concentration allowable to be present and continue with a significant increase over the reinjection, it is possible to continue to increase the biodegradation rate over the same period of time. The variation between those bottles reinjected and those bottles which received a 1-time injection can be viewed in Table 4.4. This Table proves a summary of the quantitative results for the respiration and biodegradation rates for the various soil loading concentrations.

4.3 RESPIROMETER REPRODUCIBILITY AND REPEATABILITY

To validate the collection method used to complete this research, proving consistent results between experiments and different bottles within each experiment became an issue. By doing a plot of the mean cumulative oxygen uptake all nine bottles reinjected three times with 300 mg from AMRUN1 it is possible to prove that this research is reproducible. Checking for a reasonable variation between bottles can do this. Plot D-3 shows only a slight variation that is acceptable between bottles and leads us to believe the results of the research are reproducible

Comparing the results of AMRUN1 and AMRUN2 for sandy soil can verify the experiments are repeatable. Using the same process as suggested above, the variation between experiments was graphed. However, in this case, the mean of

four random bottles from RUN1 was used to compare the mean of the four bottles in RUN2. Finding a low variation between the two experiments suggests it is possible to repeat the results. Since there were 61 intervals with AMRUN2, only 49 with AMRUN1, only 49 intervals were compared. A graphical representation of the data can be viewed in Appendix D, page D-4.

4.4 PROPYLENE GLYCOL BIODEGRADATION

Although some of the bottles varied in each experiment, the majority of the bottles with the same treatment scheme (see B-1 for treatment design) had similar respiration rates. During the two experiments, there was a visible difference in the soil samples in the bottle after the experiment had been running for about 5 days. In bottle 14 in RUN 2, the sandy soil had a dime-size mold growth on the surface of the soil (see page D-10). From the data results, it is obvious that the respiration in this bottle was increased over that of the other three bottles with the same concentration of PG-water solution. In another case, three bottles were set out for a couple of days to do a pH test on the soil. After two days, a green plant had sprung up in one of the bottles. This seed would have caused a different respiration rate had it happened during one of the experiments. Another source of variation between bottles in the experiments could be the presence of insects. Since the respirometer is so sensitive, even the smallest of bugs can cause a detectable difference.

4.4.1 Analysis of Data for High Clay Soil

The results from the HC soil helped validate the notion that this research can be reproduced. The main difference between RUN1 and RUN2 was the lag time experienced in RUN1.

4.4.1.1 Analysis of Data for RUN1

There appeared to be a slight delay in the ability to stabilize the respiration rate in RUN1. On page D-7, the O_2 uptake by interval for all ten HC bottles is graphed. Initially, most bottles took two intervals (12 hours) to reach approximately 1,000 ul/hr respiration rate. This lag time may have been caused by population growth in the microorganisms. In phase 3, interval 40, all the bottles reach their maximum respiration rate. At this point, the population growth has reached a steady state point and the degradation process is at its optimal point. After the second injection, all bottles appear to have been shocked by the addition of contaminant and experienced that same lag phase as seen in phase one. The final injection of PG appears to have taken less time for the soil to begin rapid biodegradation. With the quick adjustment to the injection, the bottles finally reach their maximum biodegradation rate. Table 4.2 summarizes the final biodegradation rates for each of the soils.

4.4.1.2 Analysis of Data for RUN2

The results for the HC in RUN2 brought about a slightly different view. Only 4

bottles (verses 9 in RUN1) were treated with the three-stage reinjection process of 300 mg. The remainder of the bottles were initially spiked with one injection of the PG/water solution. The figure on page D-8 has been labeled with the amount of PG added to each bottle per kg of soil used.

Unlike the lag period experienced in the HC RUN1, RUN2 only had a slight delay (12 hours) in reaching the stabilized respiration point. The results for phase two are questionable and unexplained. The machine appeared to be running correctly, no leakage or restrictive errors could be found and, according to the software, the machine was continuing normal operations. Phase three figures appear to continue respiration rates at the maximum level for the four bottles with the three reinjections of 300 mg (3X300 mg) and the bottle with the one injection of 900 mg. However, both sets of bottles with 300 mg and 600 mg, reduced in respiration rates and began climbing back towards the control bottle. As expected, the bottles injected with 300 mg began this climb sooner than the 600 mg bottles. As the amount of PG decreased, and the increased microbial population was operating at its maximum oxygen uptake, these bottles were expected to drop back down to background respiration rates. Since the experiment was limited to a predetermined period of time, the termination point was before the 600 mg bottles had time to stabilize. A reasonable expectation would be for these bottles to stabilize at the same respiration rate as the 300 mg bottles. The fact that the 600 mg bottles achieved the same maximum

respiration rate, indicates that the same microbial population was present in both sets of bottles. Therefore the respiration rate in the 600 mg bottles likely could not approach any closer to control values than was observed in the final sampling of the 300 mg bottles.

4.4.2 Analysis of Data for Sandy Soil

The results seen between experimental runs for the SS were much more repeatable then the results seen with the HC. Consistent with the HC soils, the same changes were made to the experimental setup for SS between RUN1 and RUN2. Details concerning the breakdown of bottles, contaminant injections and concentrations can be seen in Appendix B.

4.4.2.1 Analysis of Data for RUN 1

The figure on page D-9 graphically depicts the oxygen consumption of O_2 seen in the sandy soil for RUN1. As seen with the HC experiments, SS also experienced lag times. Forty-two hours after injection of PG/water into the bottles, respiration rates finally increased to attain a significant difference in O_2 uptake over that of the control bottle. Upon reinjection, the new addition of contaminant almost overloaded the soil system. The lag time increase to almost sixty hours. At which point there were only thirty-six hours left for the bottles to biodegrade the contaminant before the next injection period.

Following the final injection, very little respiration took place in phase three. Almost all bottles stabilized at approximately 300 ul/hr (see page D-12 for the mean of the intervals for the nine bottles). This stabilization respiration rate increased over that of the control bottle, however, points in phase one and two well exceeded this stabilized level. Due to the high oxygen uptake levels in the first two phases, the stabilized level reached in phase three indicates a decrease in ability to degrade the contaminant. The bottles injected with lower concentrations appeared to have increased the respiration rates. When an inhibitor is present, one common circumstance, which may affect the length of time prior to rapid biodegradation, is the concentration of the chemical present. Until the population of the microorganisms is adequate and the biomass adjusts to the PG concentration, the maximum respiration rate will not be seen. At 3X300 mg concentrations of PG/water solution, the maximum respiration rate was not found. Indicating that the soil system may have been overwhelmed.

4.4.2.2 Analysis of Data for RUN 2

In an attempt to find the maximum respiration rate for SS, smaller concentrations were used to contaminate the soil. For all nine bottles injected with contaminate, only phase one has the same results as discussed for RUN1 (reference figure on page D-10). All four bottles with 3X300 mg and the one bottle with 900 mg followed the same pattern as described in RUN1. The results from the sets of bottles with 300 mg and 600 mg concentrations are more interesting.

After phase one, both bottles injected with 300 mg immediately made a dramatic increase in respiration rate. They both stabilized by interval fifty (hour 300 of experiment) and were joined at a similar stabilization point in phase two by bottle 14. As described earlier, the mold spot in bottle 14 is probably responsible for altering the respiration rate in that bottle compared to the other bottles with the same design treatments. Since the same maximum respiration rate was achieved by bottle 14 and the two bottles of 300 mg, it is believed that this is the maximum respiration rate of SS (1200 ul/hr). At the very end RUN2, both 300 mg bottles appear to be climbing back towards the control bottle. Assuming that the increase in population will have the same affects on the SS as it did in the HC soil, it is suspected that these two bottles would have stabilized slightly under the control bottle. The results of the bottles injected with 600 mg contaminant had an increased respiration rate over the bottles with 900 mg bottles in phase three, but less of an increase than that seen with the 300 mg bottles. For the last twenty-one intervals, the 600 mg bottles were constantly increasing their respiration rates. Given enough time, these bottles would have been expected to continue their climb down to the maximum stabilization rate and followed the 300 mg bottles.

5. CONCLUSIONS AND RECOMMENDATIONS

5.1 Conclusions

The overall objective to this research was to determine if it might be possible to land treat ADF. This research indicates the soil type has a large effect on biodegradation rate. Respirometer studies indicate indigenous microorganisms are readily able to degrade PG. The results of the high clay soil show significantly higher capabilities to biodegrade the PG/water solution than the sandy soil. With a reapplication approach, the ability of the sandy soil to degrade the solution could be increased. Table 5.1 summarizes the findings of the two soil types.

TABLE 5.1

HIGH-CLAY AND SANDY SOIL SUMMARY RESULTS

SOIL	REINJECTION		NO REINJECTION	
	BIODEGRADATION RATE (mg/day/ kg)	PERCENT DEGRADED	BIODEGRADATION RATE (mg/day/ kg)	PERCENT DEGRADED
HIGH-CLAY	157.5	20.0	188	27.78
SANDY SOIL	49.5	6.22	11	1.49

Loading the two soils with several different concentrations and calculating the respiration rates and biodegradation rates, it is possible to suggest contaminant-

loading rates can also affect a soil's ability to begin rapid biodegradation. Biodegradation in a sandy soil could be enhanced by applying lower concentrations of PG and make use of the reapplication theory. However, the high clay soil could be loaded with a much higher concentration and achieve the stabilization point much quicker. Reapplication of the contaminant to the soil proved to be a valuable method for the sandy soil. However, the results of the high clay soil and reapplication did not appear to be useful for increasing the biodegradation rate.

With the soil used in these experiments, it was not possible to show that a lime additive was a helpful method to increase biodegradation rates. There was no significant difference between the bottles with lime and the bottles without lime. Therefore, the lime addition did not help nor hinder the biodegradation.

5.2 Improvements/Limitations

5.2.1 Keep Machine Running

The results of these experiments could have been improved and easier to analyze if the machine had not been shut down every four days. During the two reapplications of PG into the bottles, the machine had to be shut down. It is possible to use a syringe to inject the bottles with contaminant, however if this method is used, an appropriate method would have to be developed to ensure a

complete mix of the contaminant to the soil. This would eliminate the spikes/phases in the graphed data points.

5.2.2 Scheduling Experiments

Another possible improvement to the research would be to schedule all experiments for more time than predicted. This would make it possible to keep the machine running a couple of extra days to watch any new developments in the batch system.

5.2.3 Respirometer Location

The final suggestion for running the respirometer in research would be to either move the machine to a cooler room in the building or conduct the experiments during the winter months. Since the soil bottles need to be held at a constant temperature in the incubator, keeping the room constant would help keep the bottles at a steady temperature. We also found that conducting the error checks on the machine were easier to complete when the room was less humid and cooler.

5.2.4 Use of Gas Chromatograph

The major limitation of this research was the inability to conduct quantitative chemical analysis for PG. The PG could not be detected using the UV absorption divided array detector on the high performance liquid chromatograph

(HPLC), and gas chromatographic (GC) analyses were not available. PG concentrations remaining in each bottle were calculated based on O_2 consumption using the Mathcad template (Appendix E). For continued research in this area, the use of the GC could validate the biodegradation rates determined with the spreadsheet.

5.3 Follow-On Research

5.3.1 Gas Chromatograph

The use of a gas chromatograph would help determine exact concentrations of contaminant in the soil after the experiments were completed. This could assist in determining a more accurate biodegradation rate and also give indications of whether or not complete mineralization took place (end products of just CO_2 and H_2O).

5.3.2 Focus on High-Clay Soil

Having found data that supports that the high clay soil has the capability to biodegrade PG much more rapidly than sandy soil, a more in-depth study could be done with just high clay soil. Determining the maximum soil loading rate to provide the best situation for the most rapid biodegradation would be a valuable piece of data for developing a land treatment system.

5.3.3 Evaluate Other Chemicals Present in ADF

Although PG is the primary constituent of ADF, there are many other chemicals added to the fluid. If it is possible to determining another chemical present in the ADF used by the Air Force which might be toxic to the soil microorganisms may be interesting to run through the respirometer. Corrosion inhibitors and wetting agents are among the types of chemicals that may be worthy of further study.

5.4 SUMMARY

Propylene glycol is readily biodegradable under aerobic conditions by indigenous soil microorganisms. The rate of biodegradation increases with soil organic content increased and decreased as the sand fraction increased. The ability to prove that it is possible to land treat ADF in the Air Force appears to be viable depending on other additives present in ADF.

Appendix A: Soil Characterization and Analysis

A private contractor completed the analysis on the two soil samples. The soils were sent out on 5 Aug 97 and the results were sent back on 14 Aug 97. Both physical and chemical analyses were completed on the soils. The following pages are the complete laboratory results received from the contracting company.

Appendix B: Experimental Design

The following pages document the experimental design used to complete this research. The bottle designation and contamination process for the two experiments is also given. Experiment one, AMRUN1 was active from 3 July to 15 July and experiment two, and AMRUN2 was active from 11 August to 23 August. To see the schedule for the bottles being reinjected with contaminant please see Table 3.4 and Table 3.5.

DESIGN OF TREATMENTS TO MICROCOSMS: AMRUN1 & AMRUN2

SOIL TYPE	AMRUN1		AMRUN2				
	PG Concentration		PG Concentration				
	HC 11400 mg/kg SS 10600 mg/kg	0.0 mg/kg	11400 mg/kg 10600 mg/kg	0.0 mg/kg	NO REINJECT 3800 mg/kg 3530 mg/kg	NO REINJECT 7600 mg/kg 7060 mg/kg	NO REINJECT 11400 mg/kg 10600 mg/kg
HC	**1, 2, 3, 4, 5, 6, 7, 8,9**	**10**	**1, 2, 3, 4**	**10**	**8,9**	**6, 7**	**5**
SS	**11, 12, 13,14, 15,16,17,18, 19**	**20**	**11, 12, 13, 14**	**20**	**18, 19**	**16, 17**	**15**

*Note: PG concentration is mg PG/Kg soil. The numbers in the boxes represent the number of the bottle used for each treatment during the experiment.

Report Number: F97220-056
Account Number: 96600

A & L GREAT LAKES LABORATORIES, INC.

3505 Conestoga Drive • Fort Wayne, Indiana 46808-4413 • Phone 219-483-4759 • Fax 219-483-5274

To: AFIT/ENV
2950 P STREET
WRIGHT-PATTERSON AFB, OH 4543

For: PR # F61TNV71690100

Attn: CAPT LAURA JOHNSON

P.O. Number: F33600-97-M-0460

Date Received: 8/8/97 Date Reported: 8/12/97

SOIL TEST REPORT

SAMPLE NUMBER	LAB NUMBER	ORGANIC MATTER %	PHOSPHORUS BRAY P1 ppm-P	PHOSPHORUS BRAY P2 ppm-P	POTASSIUM K ppm	MAGNESIUM Mg ppm	CALCIUM Ca ppm	SODIUM Na ppm	pH SOIL pH	pH BUFFER pH	Cation Exchange Capacity meq/100g	PERCENT BASE SATURATION % K	% Mg	% Ca	% H	% Na
1A	7188	5.1	10 *L*		106 *M*	300 *H*	2100 *H*		7.3		13.3	2.0	18.8	79.1		
1B	7189	0.8	1 *VL*		24 *VL*	130 *VL*	5050 *VH*		8.1		26.4	0.2	4.1	95.7		
1C	7190	0.6	2 *VL*		26 *VL*	135 *VL*	5300 *VH*		8.0		27.7	0.2	4.1	95.7		
1D	7191	5.4	9 *VL*		104 *M*	295 *H*	2200 *H*		7.4		13.7	1.9	17.9	80.1		

VL = VERY LOW *L* = LOW *M* = MEDIUM *H* = HIGH *VH* = VERY HIGH

SAMPLE NUMBER	SULFUR S ppm	ZINC Zn ppm	MANGANESE Mn ppm	IRON Fe ppm	COPPER Cu ppm	BORON B ppm	SOLUBLE SALTS mmhos/cm	NITRATE NO_3-N ppm	AMMONIUM NH_4-N ppm	BICARB-P P ppm	COMMENTS

REPORT NUMBER: F97220-056
ACCOUNT NUMBER: 96600

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REPORT OF ANALYSIS

TO: AFIT/ENV
2950 P STREET
WRIGHT-PATTERSON AFB, OH 4543

DATE RECEIVED: 8/8/97
DATE REPORTED: 8/12/97
PAGE: 1
P.O. NUMBER: F33600-97-M-0460

ATTN: CAPT LAURA JOHNSON RE: PR # F61TNV71690100

LAB NO.	SAMPLE ID	ANALYSIS	RESULT	UNIT	METHOD
7188	1A	Sand	43	%	MSA Part 1 (1986) pp 404-408
		Silt	34	%	MSA Part 1 (1986) pp 404-408
		Clay	23	%	MSA Part 1 (1986) pp 404-408
		Soil Textural Class	Loam		MSA Part 1 (1986) pp 383-385
7189	1B	Sand	85	%	MSA Part 1 (1986) pp 404-408
		Silt	8	%	MSA Part 1 (1986) pp 404-408
		Clay	7	%	MSA Part 1 (1986) pp 404-408
		Soil Textural Class	Loamy Sand		MSA Part 1 (1986) pp 383-385
7190	1C	Sand	87	%	MSA Part 1 (1986) pp 404-408
		Silt	6	%	MSA Part 1 (1986) pp 404-408
		Clay	7	%	MSA Part 1 (1986) pp 404-408
		Soil Textural Class	Loamy Sand		MSA Part 1 (1986) pp 383-385
7191	1D	Sand	41	%	MSA Part 1 (1986) pp 404-408
		Silt	34	%	MSA Part 1 (1986) pp 404-408
		Clay	25	%	MSA Part 1 (1986) pp 404-408
		Soil Textural Class	Loam		MSA Part 1 (1986) pp 383-385

Appendix C: Statistical Method

Statistical verification must be completed to validate the results found in this research. With the use of the statistics package Statistix, it is possible to test the hypotheses. Using the cumulative oxygen data of four random bottles of each soil type, and coordinating them by PG concentration, then by replication number and soil type, the data is set up for the Statistix program. The program partitioned the sums of squares (SS) and then generated the degrees of freedom (DF), mean of squares (MS), F statistic, p values and other data needed to test the various hypotheses. The raw data from the general AOV function for each run follows each data set.

This section provides the statistical verification for the following hypotheses tests:

TEST 1:
H_o: PG and SOIL do not interact to affect CUMO2
H_a: PG and SOIL do interact

TEST 2:
H_o: There is NO difference between HC and SS soils
H_a: There IS a difference between HC and SS soils

TEST 3:
H_o: There is NO difference between soil with lime and soil without lime
H_a: There IS a difference between soil with lime and soil without lime

TEST 4:
H_o: There is NO difference between soil reinjection and no reinjection
H_a: There IS a difference between soil reinjection and no reinjection

Test two and three utilize the Tukey pairwise comparison of means. The design for test four is a single sided T-test. A 95% confidence interval was used during tests two, three, and four.

TEST 1: RUN 1 Do PG AND Soil Interaction Affect Cum O_2

H_o: PG and SOIL do not interact to affect CUMO2

H_a: PG and SOIL do interact

CUM O2: from RUN1 with 49 intervals
PG: 1=0 mg/kg 2=900 mg/kg
SOIL: 1=HC 2=SS
Rep: 1, 2, 3, 4

CASE	CUM 02	PG	Rep	Soil
1	140486	1	1	1
2	66522	1	2	1
3	116032	1	3	1
4	120409	1	4	1
5	513492	2	1	1
6	436005	2	2	1
7	368705	2	3	1
8	407266	2	4	1
9	36477	1	1	2
10	30326	1	2	2
11	29941	1	3	2
12	24560	1	4	2
13	93507	2	1	2
14	78715	2	2	2
15	108123	2	3	2
16	111415	2	4	2

ANALYSIS OF VARIANCE TABLE FOR CUMO2 for RUN1

SOURCE	DF	SS	MS	F	P
PG (A)	1	1.506E+11	1.506E+11	120.66	0.0000
SOIL (B)	1	1.714E+11	1.714E+11	137.27	0.0000
A*B	1	6.395E+10	6.395E+10	51.23	0.0000
RESIDUAL	12	1.498E+10	1.248E+09		
TOTAL	15	4.009E+11			

TEST @ α=0.05

Ho: PG and SOIL do not interact to affect CUMO2
Ha: PG and SOIL do interact

Rejection region, $F > F_{\alpha, v1, v2}$

Mean Square for Interaction, MS (AB) = 6.395*10^10
Mean Square for Error, MSE = 1.248*10^9
F Statistic for Interaction = MS (AB)/MSE = 51.25
$F_{\alpha, v1, v2} = F_{0.05, 1, 12} = 4.75$

51.25 > 4.75 $\therefore$ reject Ho FACTORS DO INTERACT

*NOTE: F value was found on page 709, table A.7 Devore. This was a double check to ensure Statistix was being used correctly.

TEST 1: RUN 2 Do PG AND Soil Interaction Affect Cum O_2

CUM O2: from RUN2 with 61 intervals
PG: 1=0 mg/kg 2=900 mg/kg
SOIL: 1=HC 2=SS
Rep: 1, 2, 3, 4

CASE	CUM 02	PG	Rep	Soil	
1	174891	1		1	1
2	82813	1		2	1
3	144448	1		3	1
4	149897	1		4	1
5	505839	2		1	1
6	487571	2		2	1
7	493155	2		3	1
8	473603	2		4	1
9	45410	1		1	2
10	37752	1		2	2
11	37273	1		3	2
12	30575	1		4	2
13	93415	2		1	2
14	44622	2		2	2
15	71925	2		3	2
16	209289	2		4	2

ANALYSIS OF VARIANCE TABLE FOR CUMO2 for RUN2

SOURCE	DF	SS	MS	F	P
PG (A)	1	1.756E+11	1.756E+11	100.43	0.0000
SOIL (B)	1	2.357E+11	2.357E+11	134.78	0.0000
A*B	1	8.121E+10	8.121E+10	46.44	0.0000
RESIDUAL	12	2.099E+10	1.749E+09		
TOTAL	15	5.135E+11			

TEST @ α=0.05

Ho: PG and SOIL do not interact to affect CUMO2
Ha: PG and SOIL do interact

Rejection region, $F > F_{\alpha, v1, v2}$

Mean Square for Interaction, MS (AB) = 8.12*10^10
Mean Square for Error, MSE = 1.749 *10^9
F Statistic for Interaction = MS (AB)/MSE = 46.43
$F_{\alpha, v1, v2} = F_{0.05, 1, 12} = 4.75$

46.43 > 4.75 $\therefore$ reject Ho FACTORS DO INTERACT

*NOTE: F value was found on page 709, table A.7 Devore. This was a double check to ensure Statistix was being used correctly.

TEST 2: RUN 1 Significant Difference Between Soil Types

H_o: There is NO difference between HC and SS soils

H_a: There IS a difference between HC and SS soils

TUKEY PAIRWISE COMPARISON OF MEANS

PG & SOIL Factors on O_2 Uptake Response

Level of Significance	α=0.05
Levels of factor a	a=2
Levels of factor b	b=2
Number of replications	n=4
MSE from 2-way ANOVA	MSE=1.248E9
Variance of D_{hat} (2MSE/n)	$s2\{D_{hat}\}$=6.24E8
Std Deviation of D_{hat}	$s\{D_{hat}\}$=24,980

Tukey multiple

Where q = [0.95, 4, 12] = 4.20

$$T := \frac{1}{\sqrt{2}} \cdot q \cdot (1 - \alpha, ab, (n-1) \cdot ab)$$

$\therefore$ T=2.970

Confidence interval $95\%CI=\pm T^*s\{D_{hat}\}$

95%CI=± 74,191

THE DATA		
FACTORS SOIL/PG	LEVEL OF PG	
	0	900 mg/kg
HC (μ_{ij})	110863	431367
SS ($\mu_{i'j'}$)	30326	97940

Difference between means: $D=(\mu_{ij})-(\mu_{i'j'})$

If the difference between each pair (D) is greater than half the confidence interval, then there is a significant difference the pairs.

PAIR	D	HALF CI	Significant Diff?
0,HC*SS	80537	74191	YES
900,HC*SS	333427	74191	YES

TEST 2: RUN 2 Significant Difference Between Soil Types
TUKEY PAIRWISE COMPARISON OF MEANS

PG & SOIL Factors on O_2 Uptake Response

Level of Significance	α=0.05
Levels of factor a	a=2
Levels of factor b	b=2
Number of replications	n=4
MSE from 2-way ANOVA	MSE=1.749E9
Variance of D_{hat} (2MSE/n)	$s2\{D_{hat}\}$=8.745E8
Std Deviation of D_{hat}	$s\{D_{hat}\}$=29,572

Tukey multiple

Where q = [0.95, 4, 12] = 4.20

$$T := \frac{1}{\sqrt{2}} \cdot q \cdot (1 - \alpha, ab, (n-1) \cdot ab)$$

∴ T=2.970

Confidence interval $95\%CI=\pm T^*s\{D_{hat}\}$

95%CI=± 87,829

THE DATA		
FACTORS SOIL/PG	LEVEL OF PG	
	0	900 mg/kg
HC (μ_{ij})	138012	490042
SS ($\mu_{i'j'}$)	37753	104813

Difference between means: $D=(\mu_{ij})-(\mu_{i'j'})$

If the difference between each pair (D) is greater than half the confidence interval, then there is a significant difference the pairs.

PAIR	D	HALF CI	Significant Diff?
0,HC*SS	100259	87829	YES
900,HC*SS	385229	87829	YES

TEST 3: Lime Additive Comparision

H_o: There is NO difference between soil with lime and soil without lime

H_a: There IS a difference between soil with lime and soil without lime

RUN 2 data was adjusted to 49 readings so the comparison between cum O_2 intervals were equal (49).

ANALYSIS OF VARIANCE TABLE FOR CUMO2

SOURCE	DF	SS	MS	F	P
PG (A)	1	2.649E+09	2.649E+09	1.42	0.2571
SOIL (B)	1	4.133E+11	4.133E+11	220.85	0.0000
A*B	1	5.751E+08	5.751E+08	0.31	0.5895
RESIDUAL	12	2.246E+10	1.871E+09		
TOTAL	15	4.390E+11			

TUKEY PAIRWISE COMPARISON OF MEANS
PG & SOIL Factors on O_2 Uptake Response

Level of Significance	α=0.05
Levels of factor a	a=2
Levels of factor b	b=2
Number of replications	n=4
MSE from 2-way ANOVA	MSE=1.871E9
Variance of D_{hat} (2MSE/n)	$s2\{D_{hat}\}$=9.355E8
Std Deviation of D_{hat}	$s\{D_{hat}\}$=30,586

Tukey multiple

Where q = [0.95, 4, 12] = 4.20

$\therefore$ T=2.970 $T := \frac{1}{\sqrt{2}} \cdot q \cdot (1 - \alpha, ab, (n-1) \cdot ab)$

Confidence interval 95%CI=$\pm$T*$s\{D_{hat}\}$

95%CI=$\pm$ 90,840

THE DATA		
FACTORS SOIL/PG	SOIL	
	HC (μ_{ij})	SS ($\mu_{i'j'}$)
No lime	431367	97940
Lime	393640	65453

Difference between means: $D=(\mu_{ij})-(\mu_{i'j'})$

If the difference between each pair (D) is greater than half the confidence interval, then there is a significant difference the pairs.

PAIR	D	HALF CI	Significant Diff?
HC,Lime*Nolime	37727	90840	NO
SS,Lime*Nolime	32487	90840	NO

TEST 4: Does Reinjection of Contaminant Increase the Biodegradation Rate

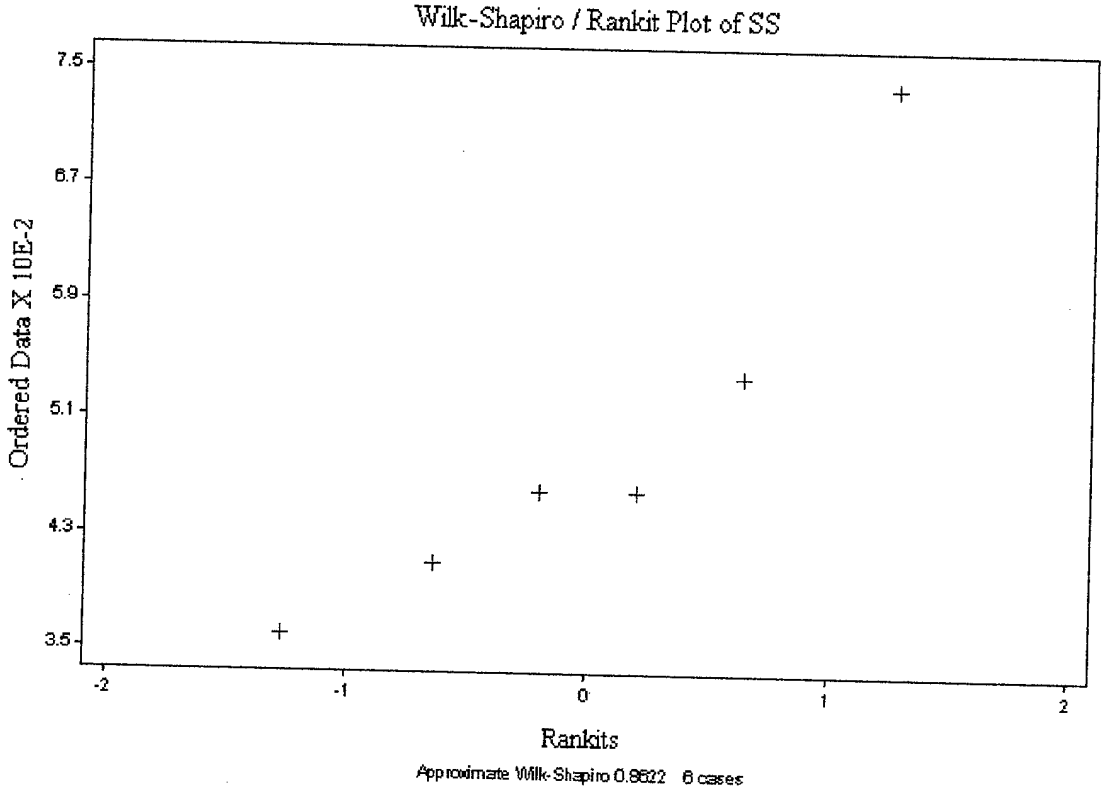
H_o: There is NO difference between soil reinjection and no reinjection
H_a: There IS a difference between soil reinjection and no reinjection

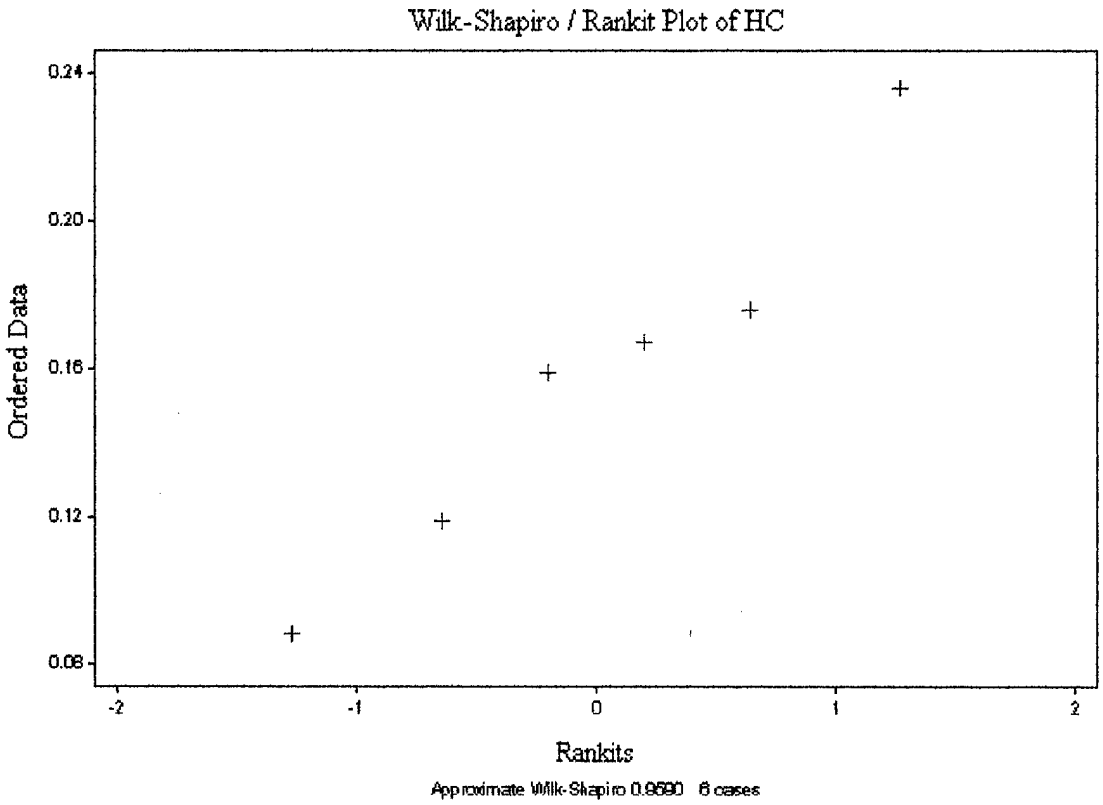
The Statistix's Wilk-Shapiro test was done to determine if the distribution for each population was approximately normal. The results of the tests can be seen below.

CHART C-1

Wilk-Shapiro Normality Tests of Biodegradation Rates

of Sandy and High Clay Soils





Knowing that the distribution of both soil biodegradation rates is approximately normal, the T-test can be performed. Using the single sided T-test, the standard mean was set to equal the biodegradation rate found in RUN2 for the one bottle initially injected with 900 mg PG. It was assumed that this value is the true representation of the biodegradation of soil injected with 900 mg PG. Then the mean of the biodegradation rates from each phase for each soil was compared to that standard. Both RUN1 and RUN2 experiments had three separate phases, so a total of six rates were averaged for each soil.

H_o: $\mu = \mu_o$ No difference between reinjection and no reinjection results
H_a: $\mu \neq \mu_0$ There is a difference between reinjection and no reinjection results

$t_{\alpha, n-1}$ Test Statistic value = $t^* = \frac{x_{bar}-\mu}{(s/n^{0.5})}$

Rejection region for a level 0.05 test

Either $t^* \geq t_{\alpha/2, n-1}$ $t^* \leq -t_{\alpha/2, n-1}$

Soil	μ	μ_o	s	n	t*	t	REJECT H_o
HC	0.188	0.1575	0.0508	6	7.038	2.571	NO
SS	0.011	0.0495	0.0134	6	-1.47	2.571	YES

NO difference between reinjection/injection for HC.

Definite difference between reinjection/injection for SS.

*Note: t value was found in table A.5 in Devore (1995).

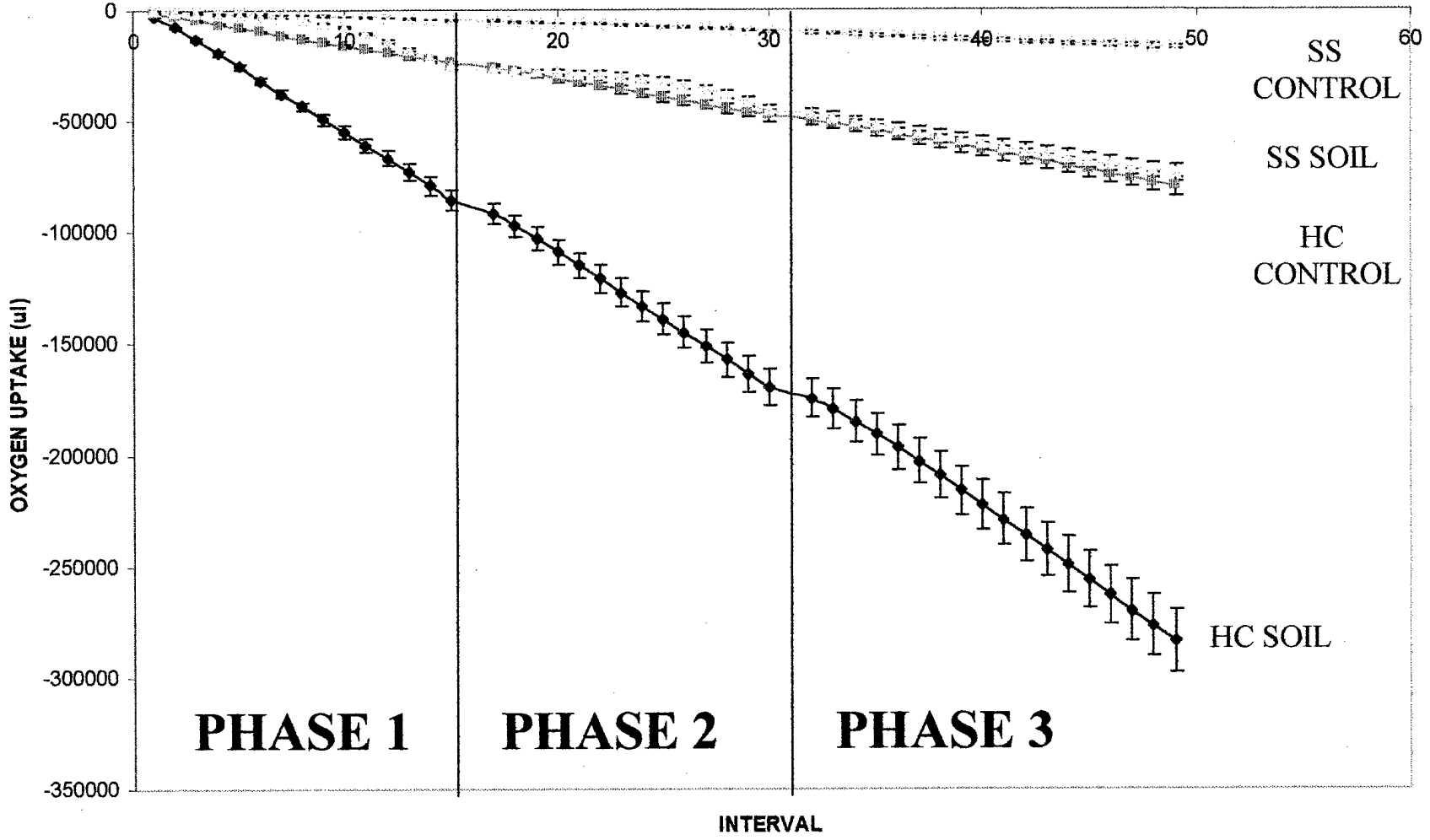
Appendix D: Data Analysis Graphs

The following pages represent the graphical work referenced in chapter four. Monitoring the oxygen uptake (respiration) rates of the sample bottles made it possible to determine the affect of various concentration levels of solution on each batch system. The following graphs plot oxygen uptake for each interval (reading) of the experiment. Both experiments were set up with 6 hour intervals and ran for 49 intervals (AMRUN1) and 61 intervals (AMRUN2).

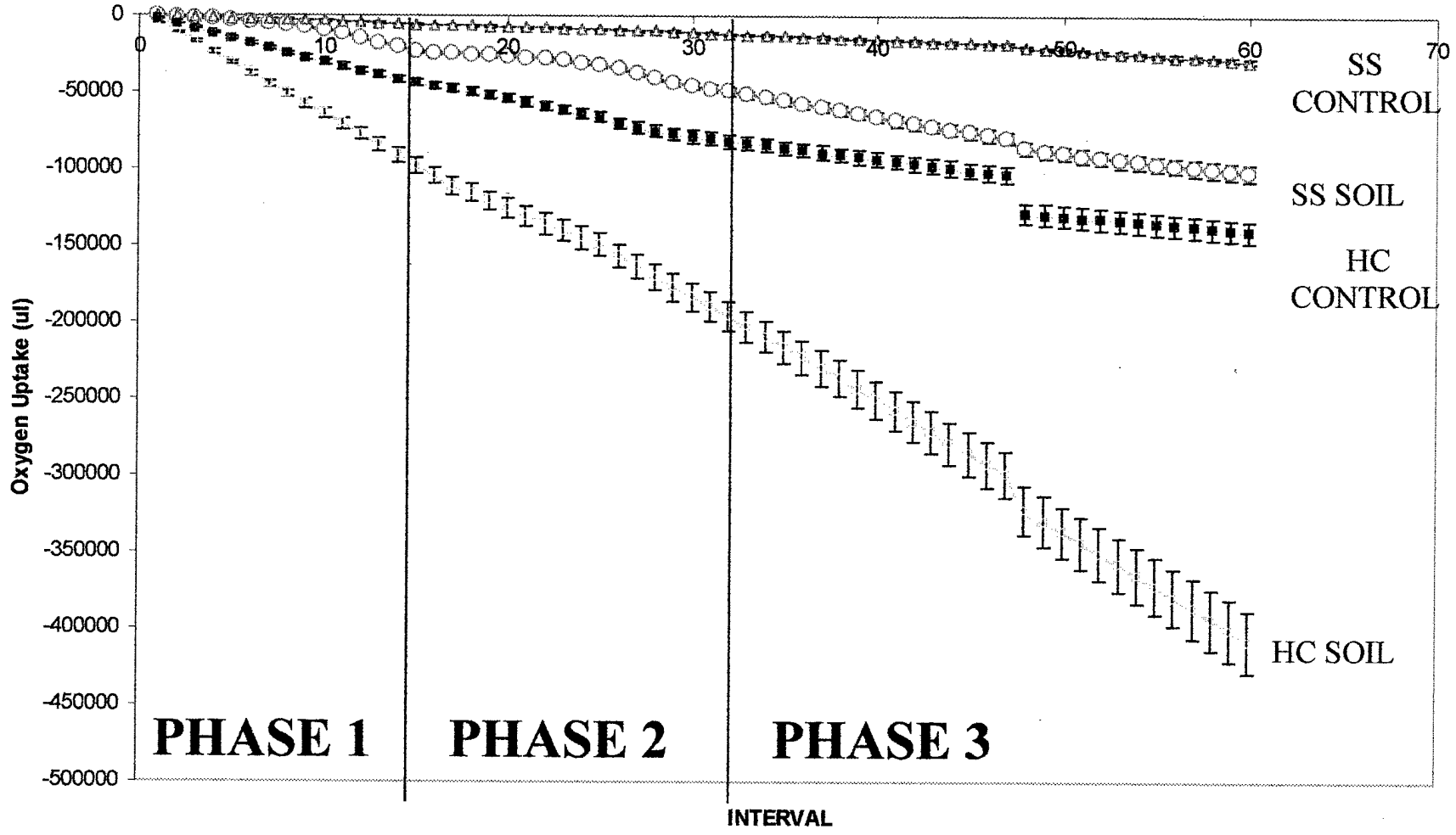
The following list summarizes the order of graphs found in this appendix:

PAGE	GRAPH TITLE
D-1	RUN1: HC VS SS WITH THREE INJECTIONS OF 300 mg/kg
D-2	RUN2: HC VS SS WITH THREE INJECTIONS OF 300 mg/kg
D-3	HC REPRODUCTION GRAPH
D-4	SS REPEATABILITY GRAPH
D-5	HC RUN1 VS RUN2 RESULTS
D-6	SS RUN1 VS RUN2 RESULTS
D-7	RUN1 HC: O2 UPTAKE FOR EACH INTERVAL
D-8	RUN2 HC: O2 UPTAKE FOR EACH INTERVAL
D-9	RUN1 SS: O2 UPTAKE FOR EACH INTERVAL
D-10	RUN2 SS: O2 UPTAKE FOR EACH INTERVAL
D-11	RUN1 HC: EXTRAPOLATION OF MEAN VS CONTROL
D-12	RUN1 SS: EXTRAPOLATION OF MEAN VS CONTROL
D-13	RUN2 HC: EXTRAPOLATION OF MEANS AND CONTROL
D-14	RUN2 SS: EXTRAPOLATION OF MEANS AND CONTROL

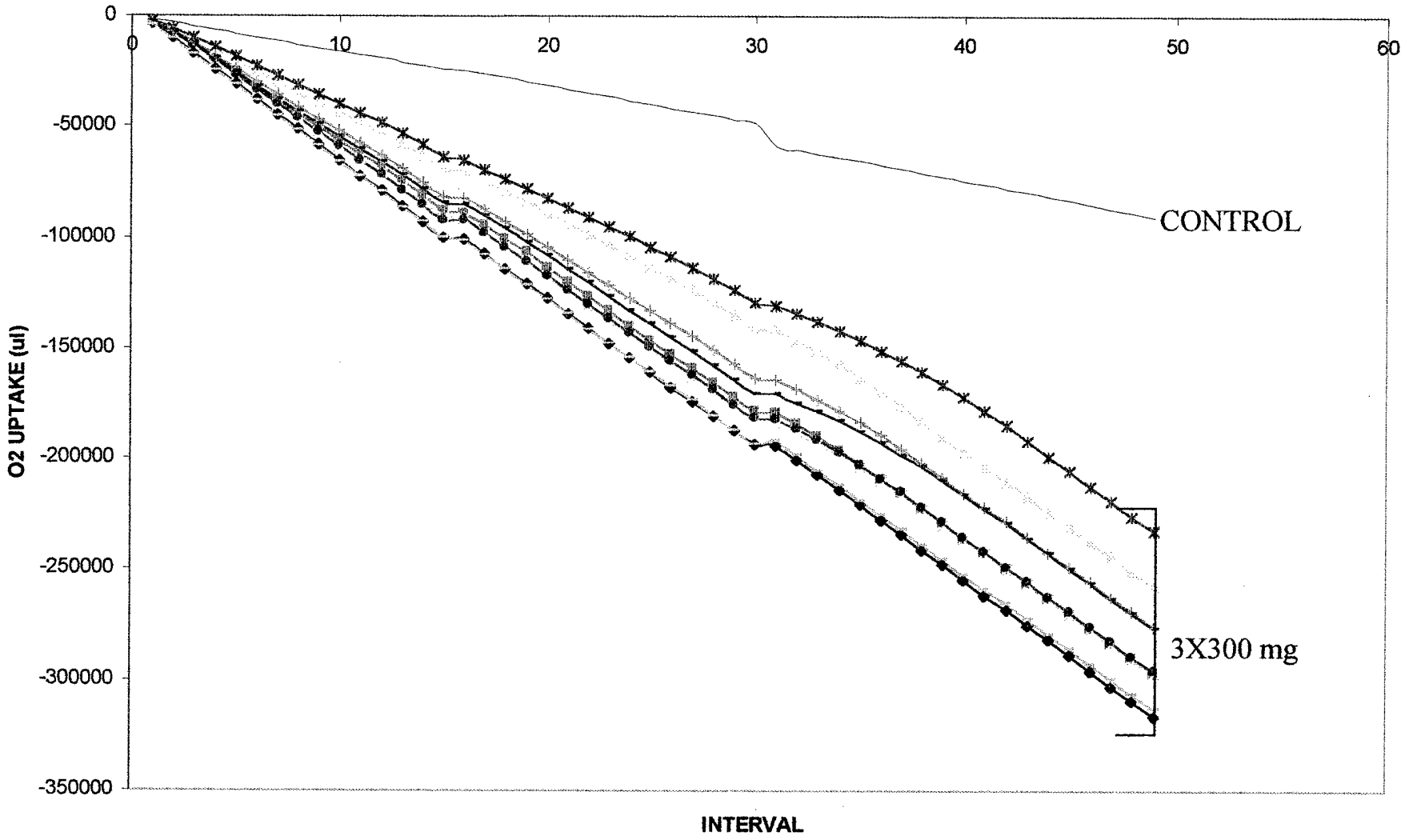
RUN 1: HIGH CLAY VS SANDY SOIL BOTH WITH THREE INJECTIONS OF 300 mg PG INTO SOIL (900 mg PG total)



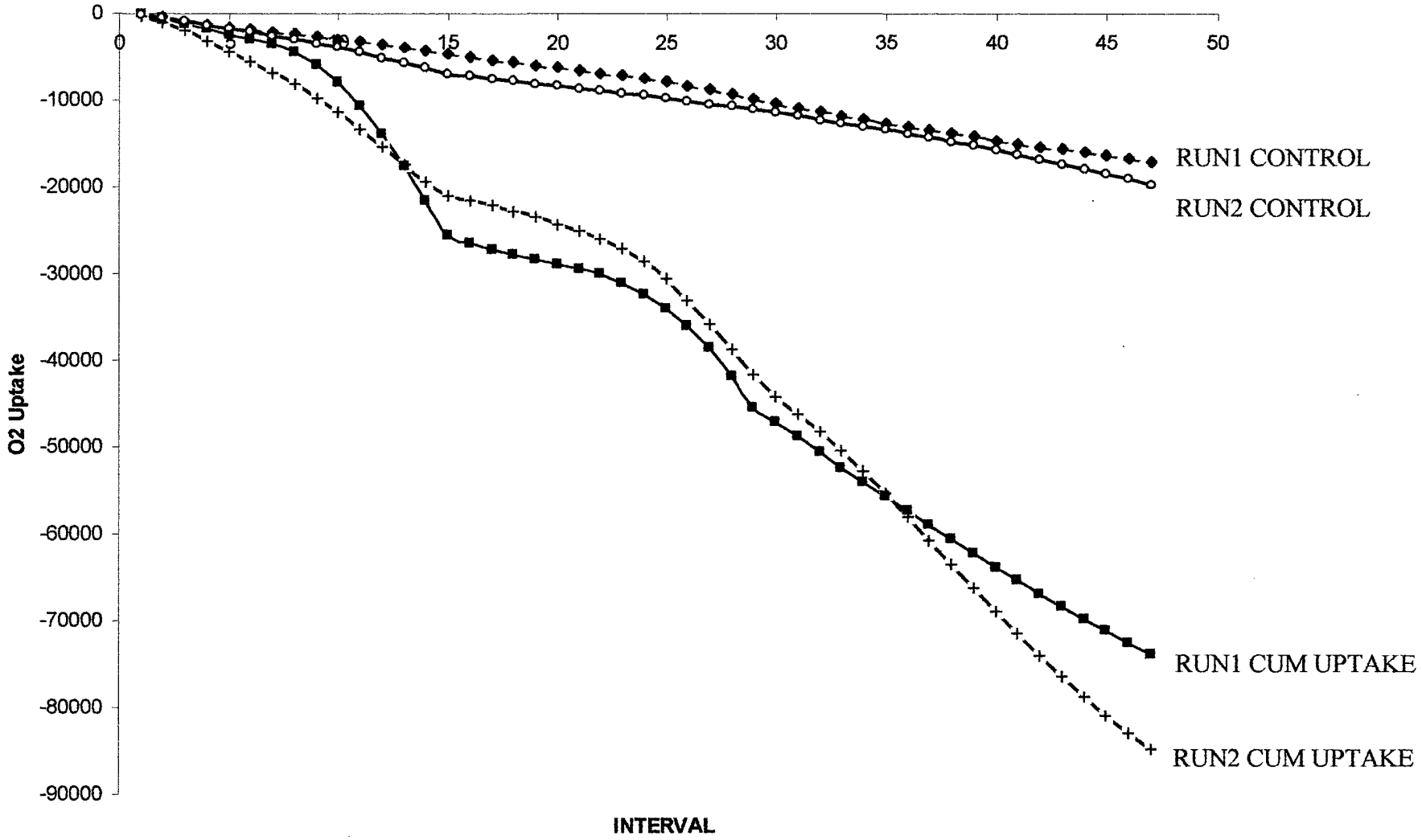
RUN2: HIGH CLAY VS SANDY SOIL BOTH WITH THREE INJECTIONS OF 300 mg PG INTO SOIL (900 mg PG total)

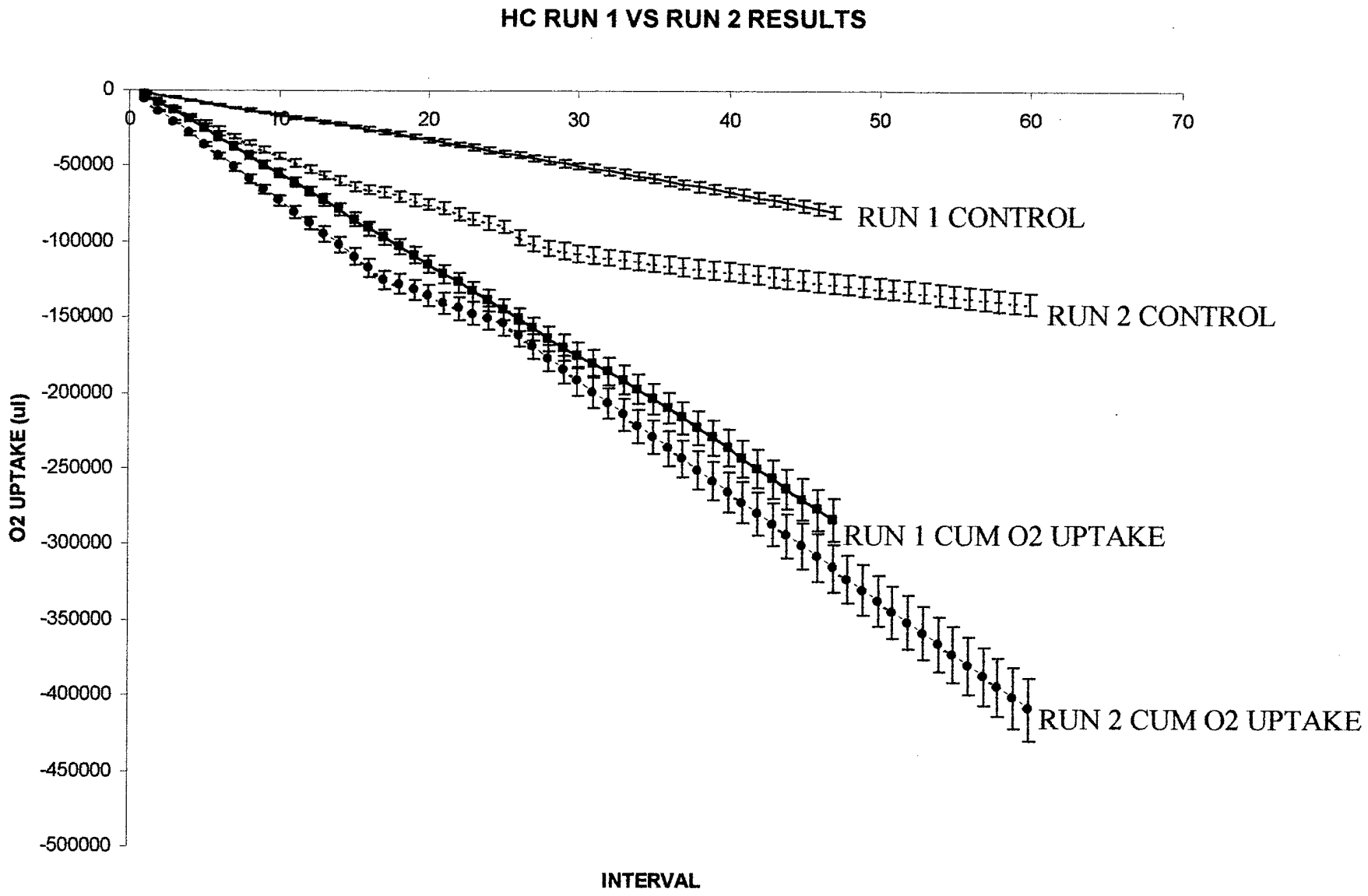


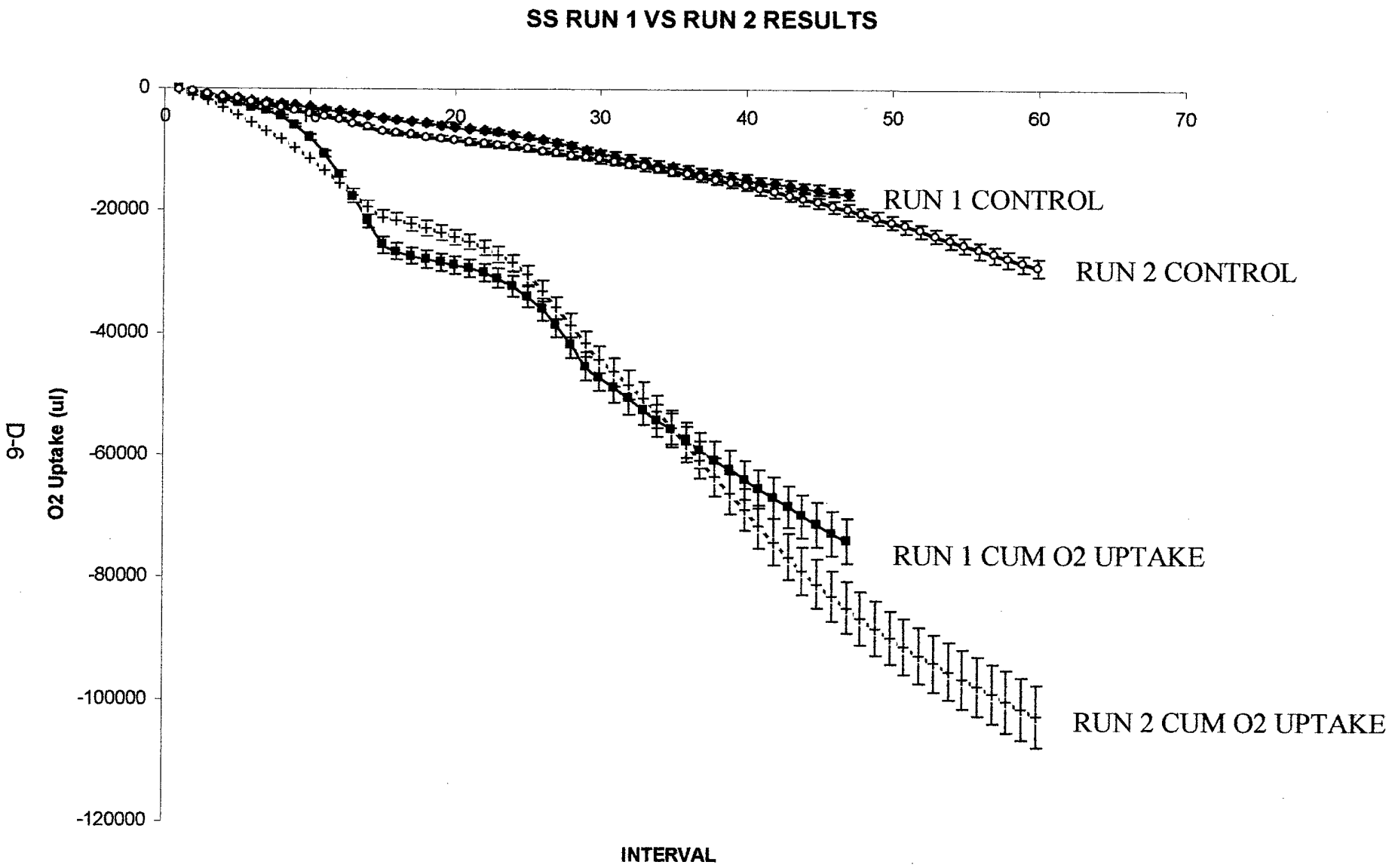
HC REPRODUCTION GRAPH

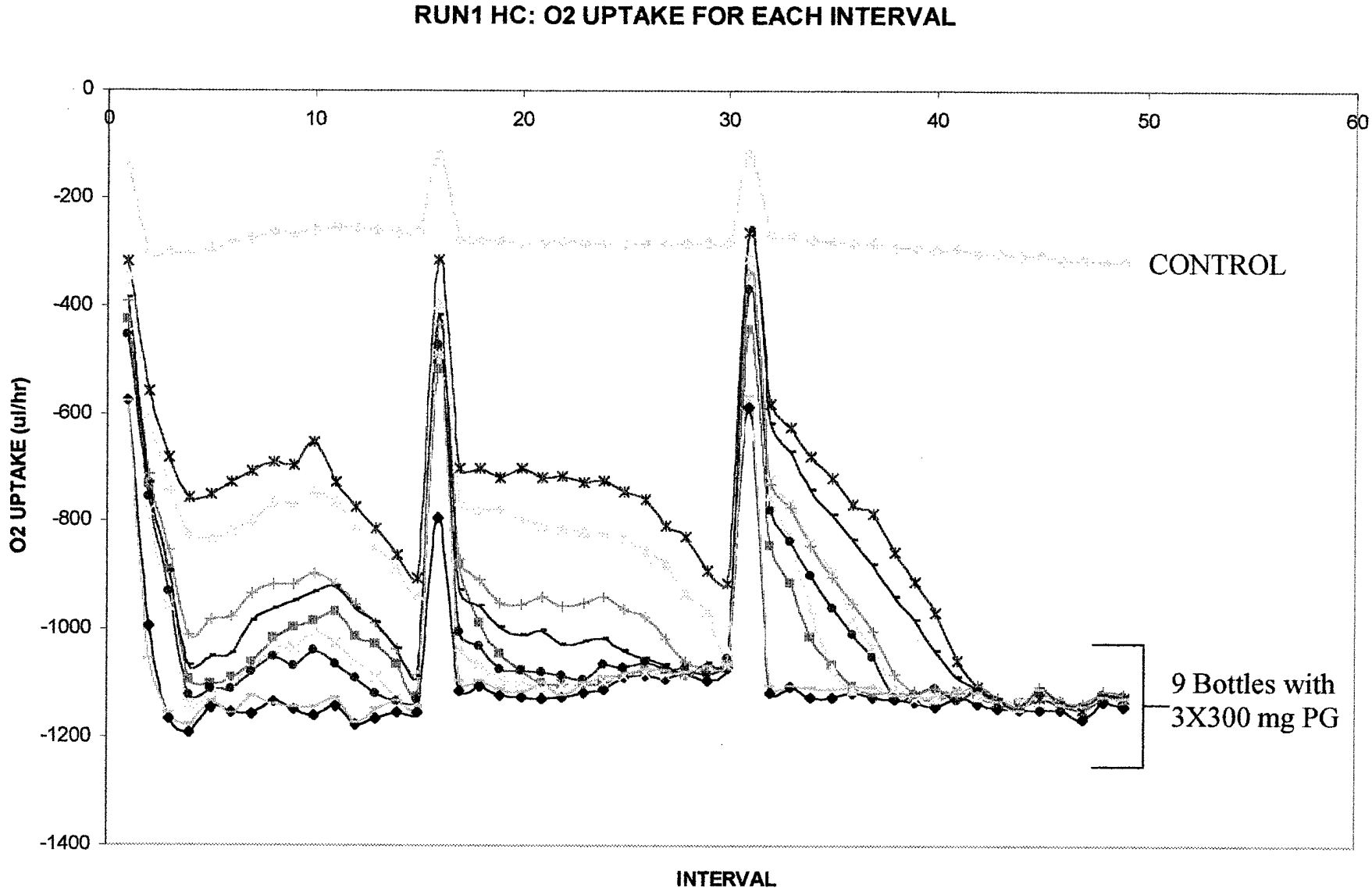


SS REPEATABILITY GRAPH

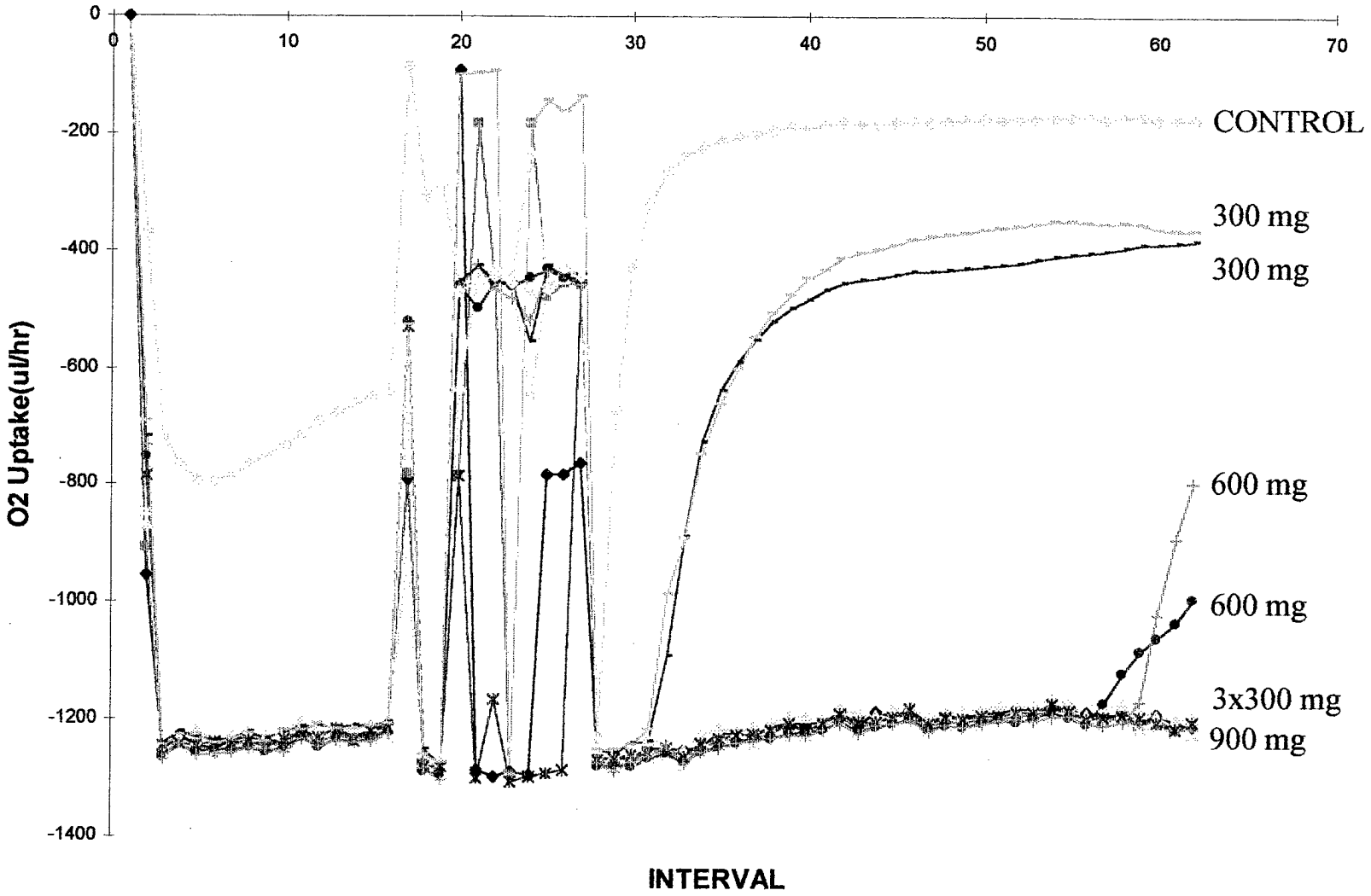


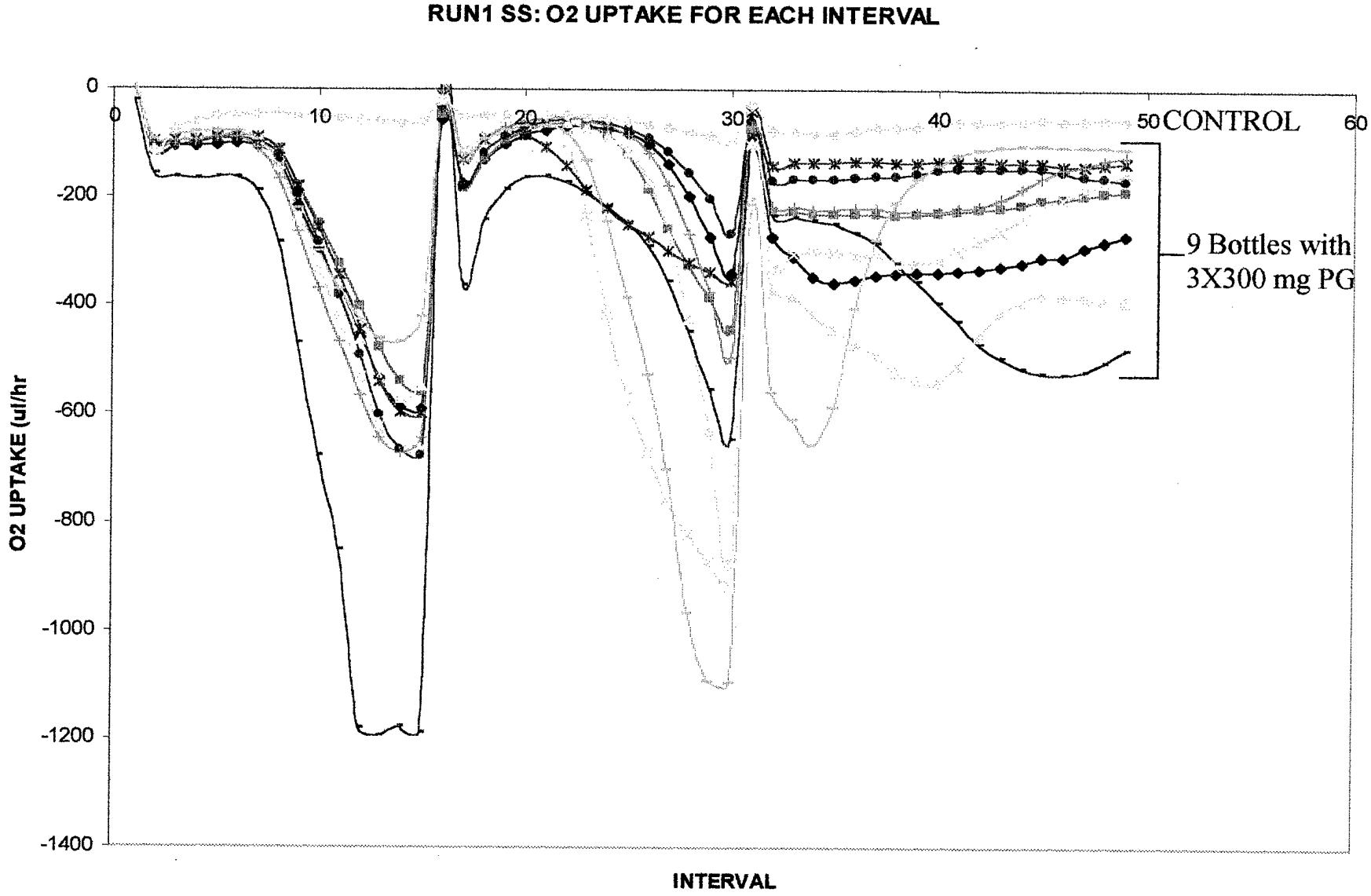

HC RUN 1 VS RUN 2 RESULTS
0
-50000
-100000
-150000
-200000
-250000
-300000
-350000
-400000
-450000
-500000
O2 UPTAKE (ul)
0
10
20
30
40
50
60
70
INTERVAL
RUN 1 CONTROL
RUN 2 CONTROL
RUN 1 CUM O2 UPTAKE
RUN 2 CUM O2 UPTAKE

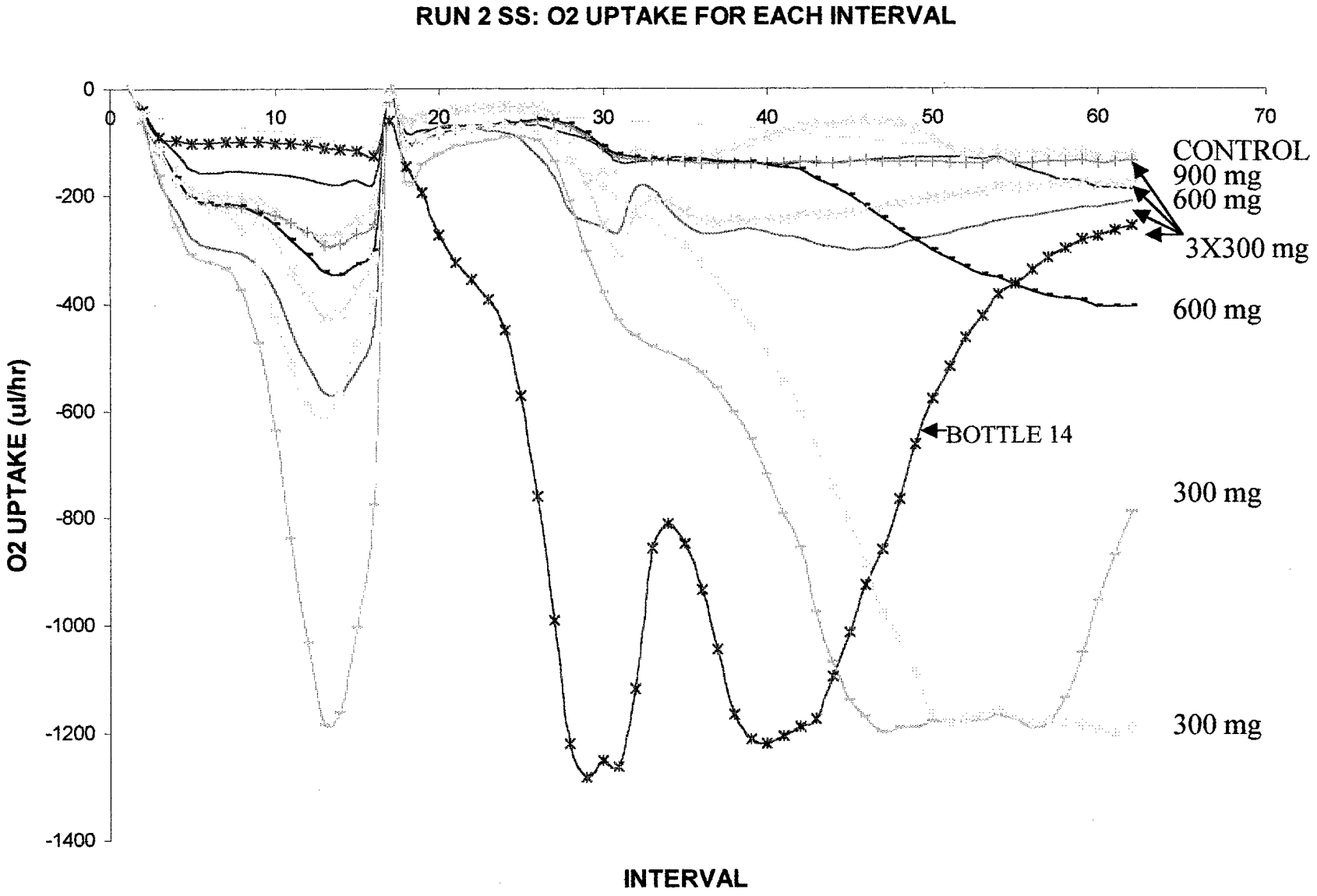

SS RUN 1 VS RUN 2 RESULTS
0
-20000
-40000
-60000
-80000
-100000
-120000
0
10
20
30
40
50
60
70
O2 Uptake (ul)
INTERVAL
RUN 1 CONTROL
RUN 2 CONTROL
RUN 1 CUM O2 UPTAKE
RUN 2 CUM O2 UPTAKE

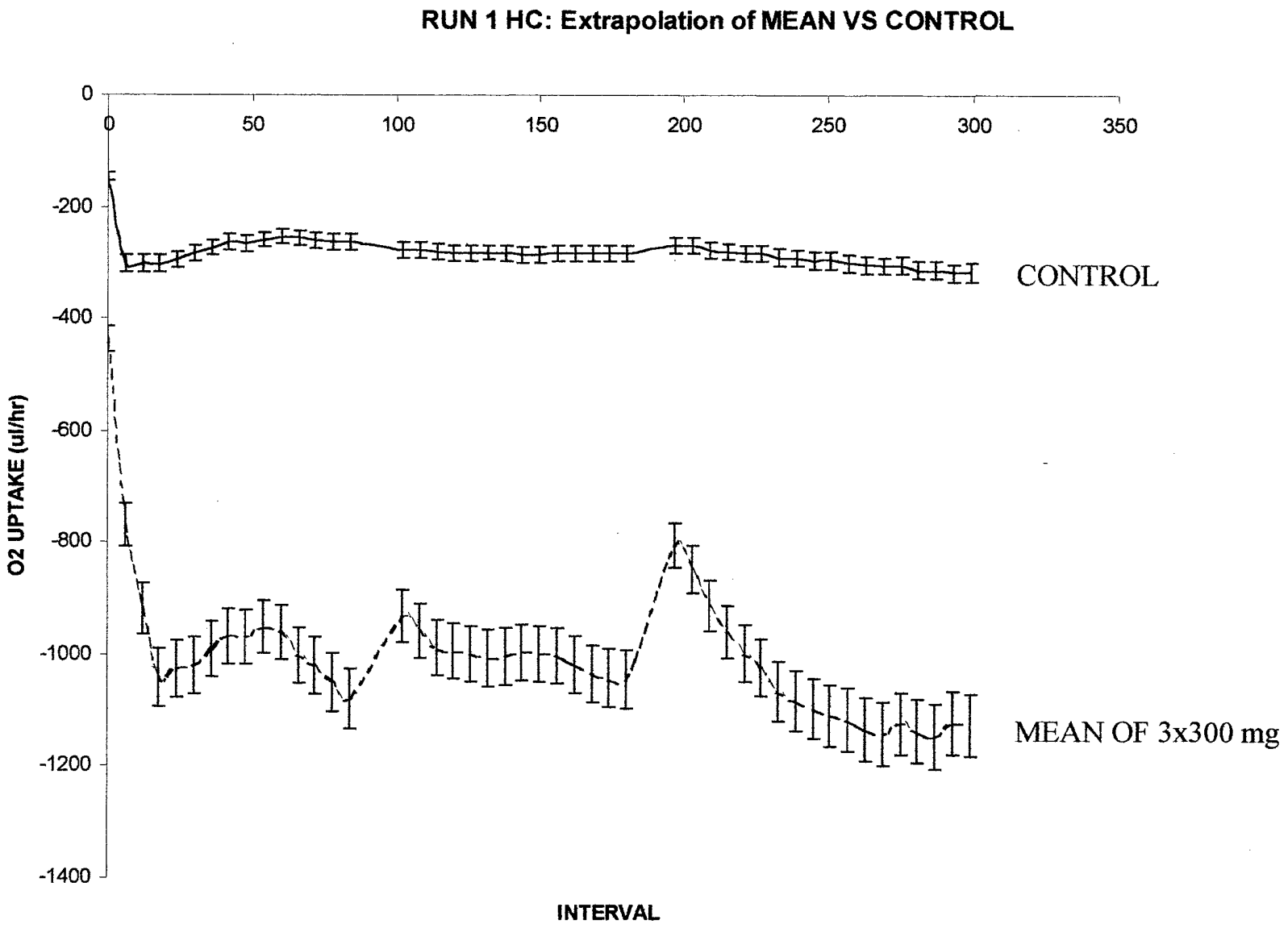

RUN1 HC: O2 UPTAKE FOR EACH INTERVAL
O2 UPTAKE (ul/hr)
0
-200
-400
-600
-800
-1000
-1200
-1400
0
10
20
30
40
50
60
INTERVAL
CONTROL
9 Bottles with
3X300 mg PG

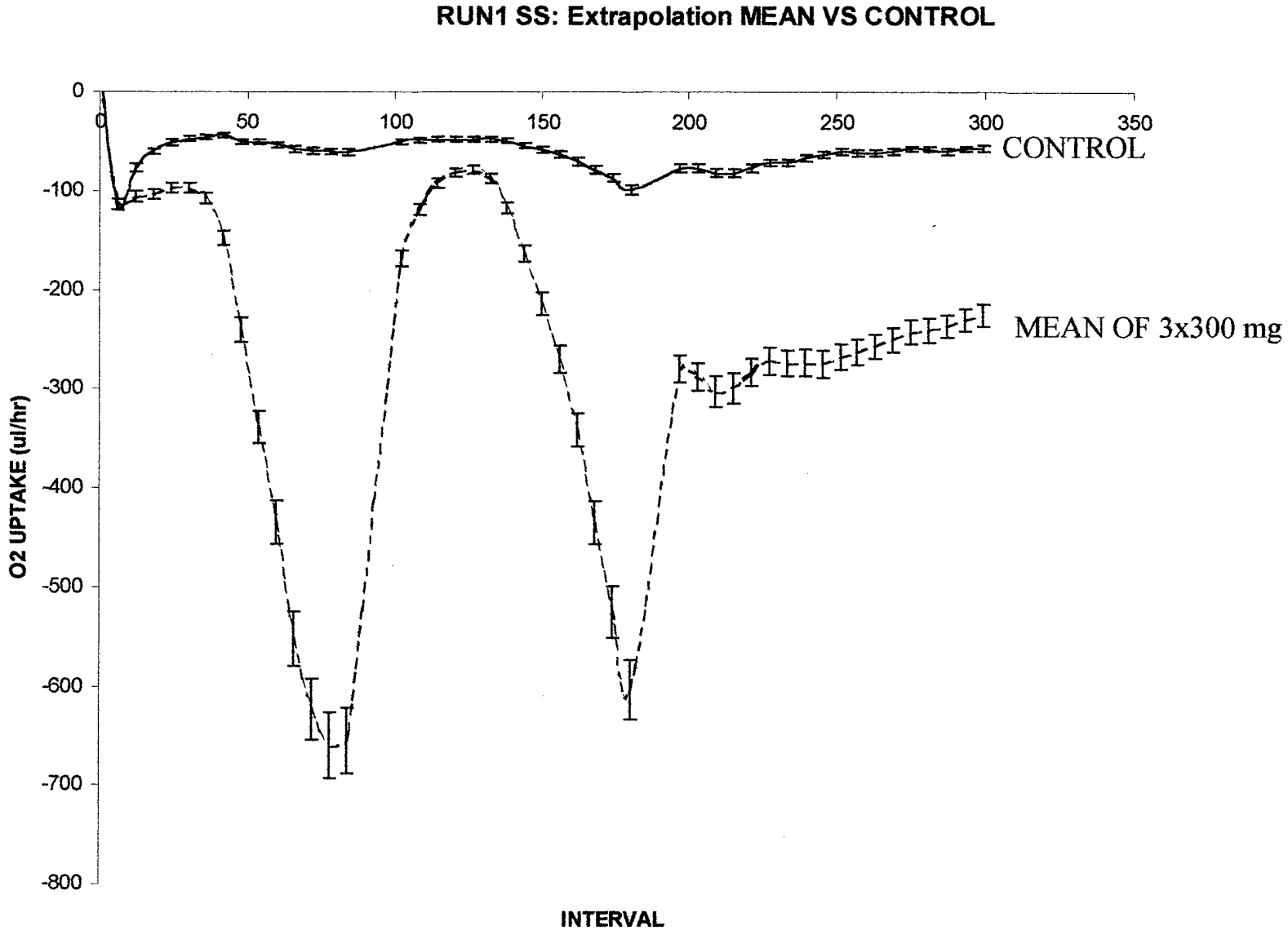
RUN 2 HC: O2 UPTAKE FOR EACH INTERVAL

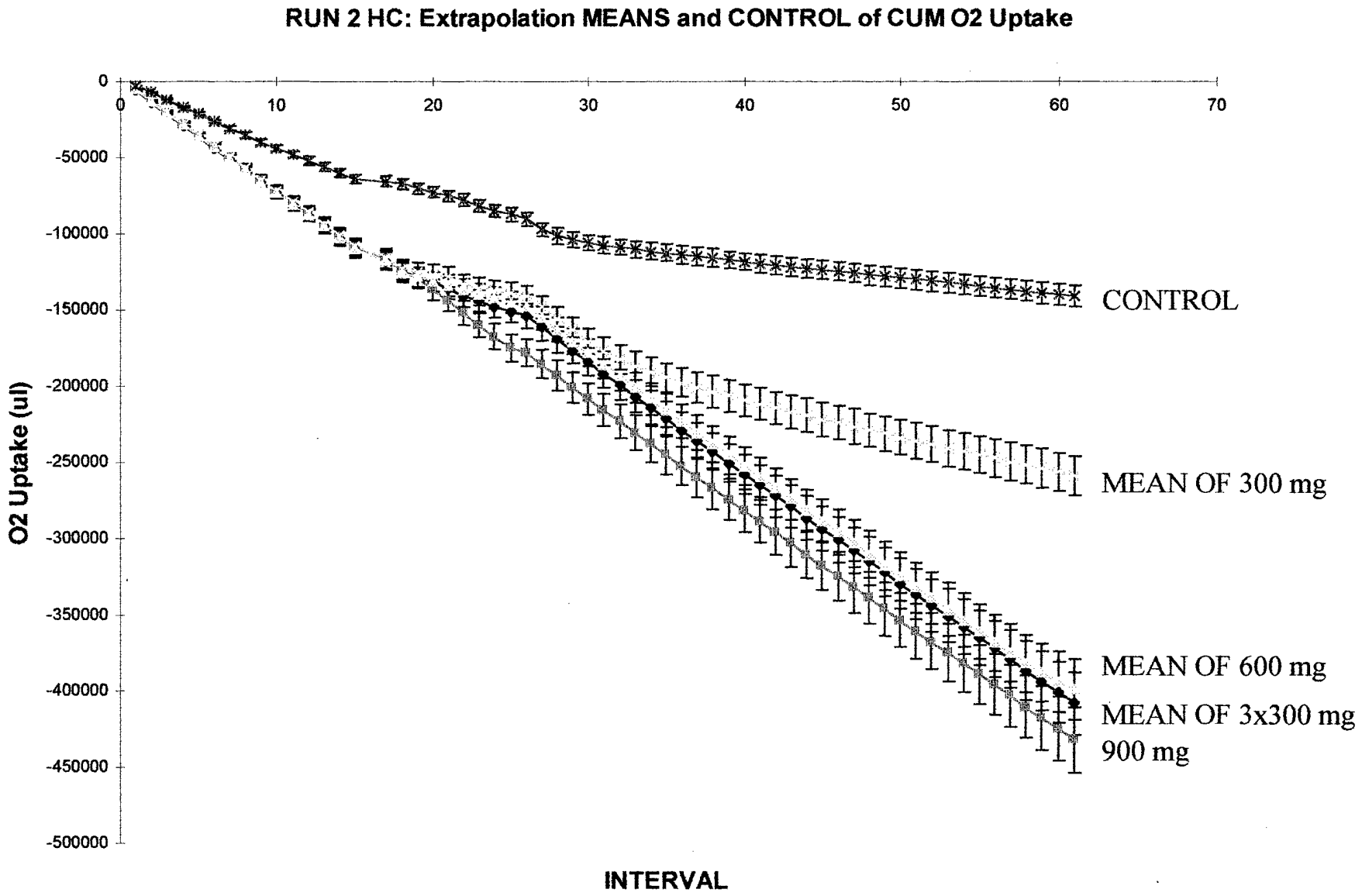


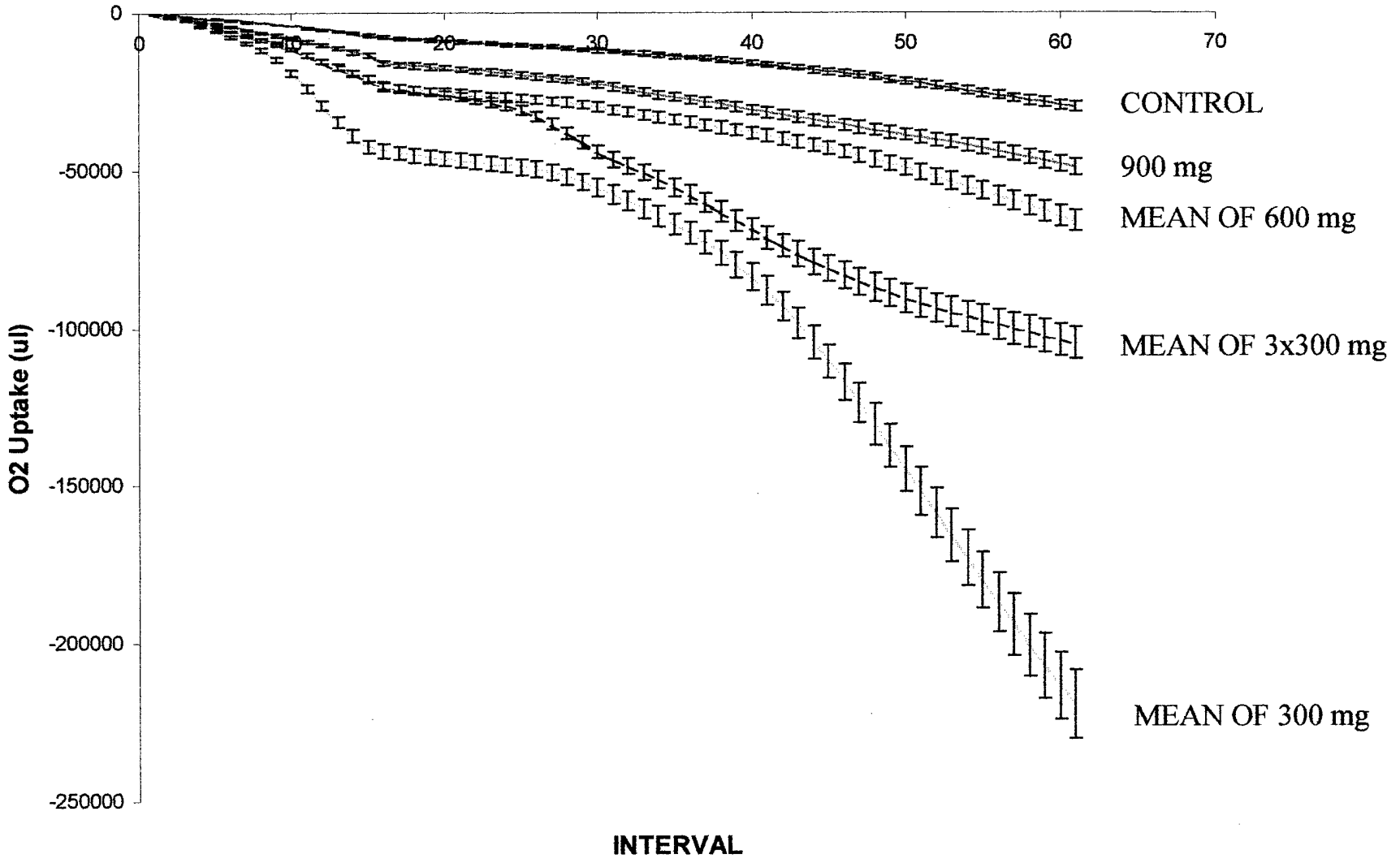

RUN1 SS: O2 UPTAKE FOR EACH INTERVAL
O2 UPTAKE (ul/hr
0
-200
-400
-600
-800
-1000
-1200
-1400
0
10
20
30
40
50
60
CONTROL
9 Bottles with
3X300 mg PG
INTERVAL


RUN 2 SS: O2 UPTAKE FOR EACH INTERVAL
O2 UPTAKE (ul/hr)
0
-200
-400
-600
-800
-1000
-1200
-1400
0
10
20
30
40
50
60
70
INTERVAL
CONTROL
900 mg
600 mg
3X300 mg
600 mg
BOTTLE 14
300 mg
300 mg


RUN 1 HC: Extrapolation of MEAN VS CONTROL
0
0
50
100
150
200
250
300
350
-200
-400
-600
-800
-1000
-1200
-1400
O2 UPTAKE (ul/hr)
INTERVAL
CONTROL
MEAN OF 3x300 mg


RUN1 SS: Extrapolation MEAN VS CONTROL
0
-100
-200
-300
-400
-500
-600
-700
-800
0
50
100
150
200
250
300
350
CONTROL
MEAN OF 3x300 mg
O2 UPTAKE (ul/hr)
INTERVAL


RUN 2 HC: Extrapolation MEANS and CONTROL of CUM O2 Uptake
0
-50000
-100000
-150000
-200000
-250000
-300000
-350000
-400000
-450000
-500000
O2 Uptake (ul)
0
10
20
30
40
50
60
70
INTERVAL
CONTROL
MEAN OF 300 mg
MEAN OF 600 mg
MEAN OF 3x300 mg
900 mg


RUN 2 SS: Extrapolation MEANS and CONTROL of CUM O2 Uptake
0
-50000
-100000
-150000
-200000
-250000
O2 Uptake (ul)
0
10
20
30
40
50
60
70
INTERVAL
CONTROL
900 mg
MEAN OF 600 mg
MEAN OF 3x300 mg
MEAN OF 300 mg

Appendix E: Respiration Ratio and Biodegradation Accounting

To determine the amount of PG degraded due to microbial activity, the following Mathcad program was developed by Jim Baker (1995). As discussed in chapter two, by using the equation for theoretical oxygen demand, it is possible to calculate the amount of oxygen required to convert an organic material to carbon dioxide, water and ammonia. This appendix contains the following pages:

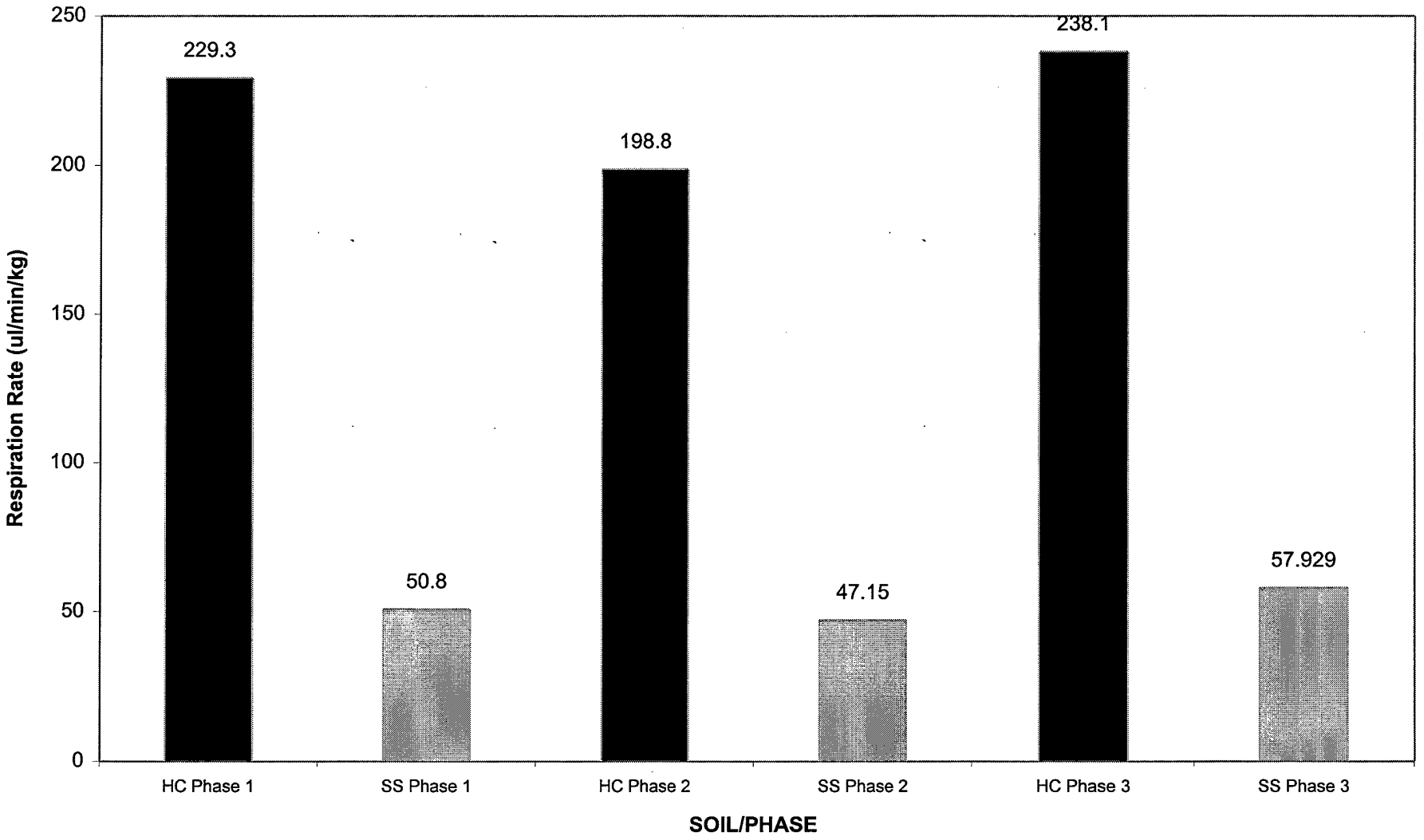
PAGE	SUBJECT
E-1	OXYGEN CONSUMPTION PER PHASE FOR EACH TEST
E-2	QUANTIFICATION OF BIODEGRADATION
E-3	HC VS SS RESPIRATION RATE
E-4	HC VS SS BIODEGRADATION RATE
E-5	HC RUN1 VS RUN2
E-6	SS RUN1 VS RUN2
E-7	NO REINJECTION VS REINJECTION
E-8	Mathcad TEMPLATE (page 1)
E-9	Mathcad TEMPLATE (page 2)

OXYGEN CONSUMPTION PER PHASE FOR EACH OF THE TESTS

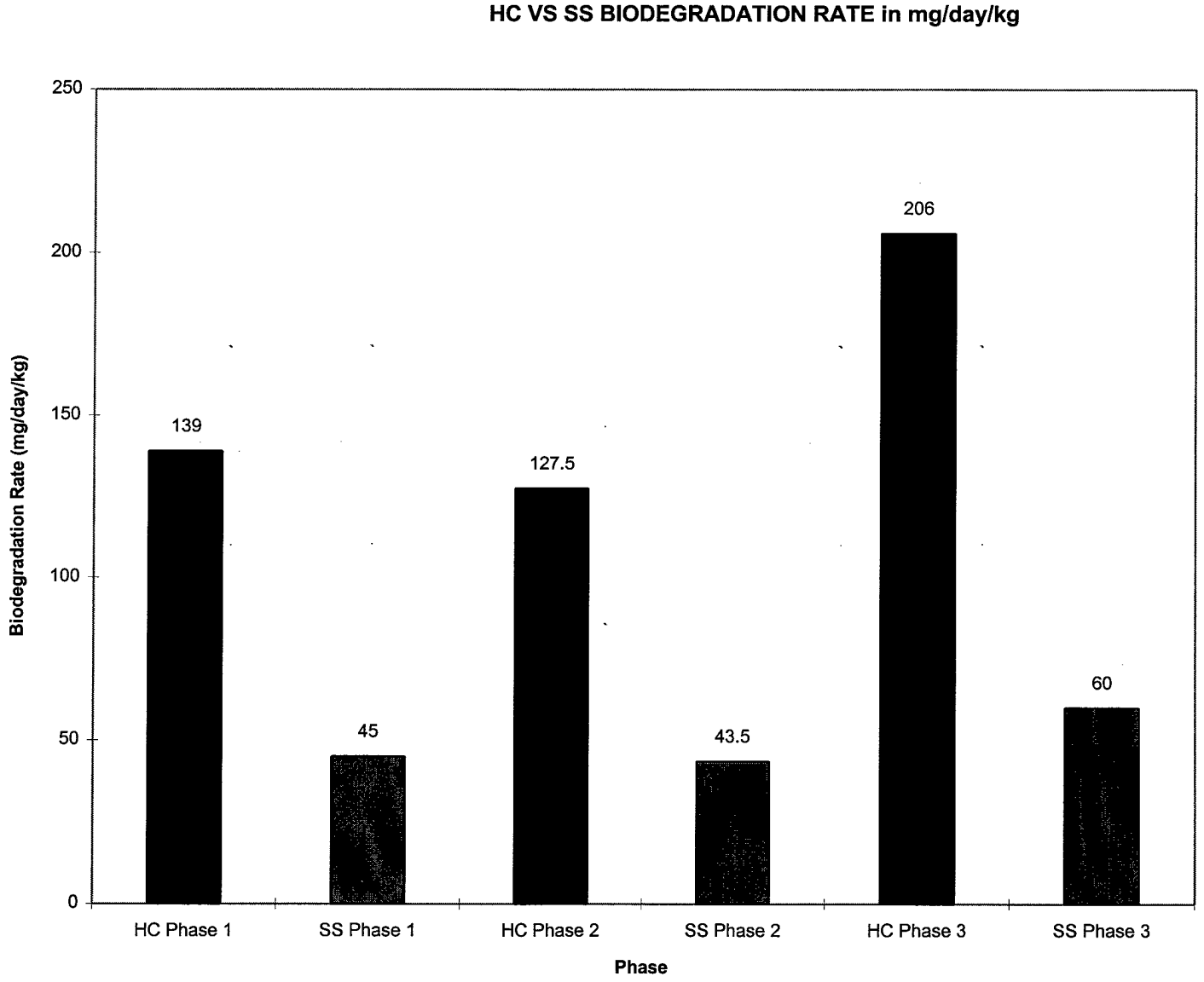
PG added	HC 3x3 RN1			HC 3x3 RN2			SS 3x3 RN1			SS 3x3 RN2		
each phase		CUM O2	Readings		CUM O2	Readings		CUM O2	Readings		CUM O2	Readings
0.3 ml	Phase 1	85735	15		109932	15		25605	15		21028	15
0.3 ml	Phase 2	84095	14		74221	14		19776	14		20622	14
0.3 ml	Phase 3	113664	18		224079	31		28516	18		60765	31
PG added	HC CONTROL RN1			HC CONTROL RN2			SS CONTROL RN1			SS CONTROL RN2		
each phase		CUM O2	Readings		CUM O2	Readings		CUM O2	Readings		CUM O2	Readings
0.0 ml	Phase 1	24198	15		63685	15		4788	15		7041	15
0.0 ml	Phase 2	23730	14		42253	14		5111	14		4120	14
0.0 ml	Phase 3	31926	18		34896	31		7206	18		18103	31
		HC						SS				
	Tot PG	CUM O2	Readings				Tot PG	CUM O2	Readings			
	0.3 ml	258666	60				0.3 ml	218089	60			
	0.6 ml	399180	60				0.6 ml	63799	60			
	0.9 ml	432261	60				0.9 ml	46838	60			
CONTRO	0.0 ml	140835	60			CONTRO	0.0 ml	29264	60			

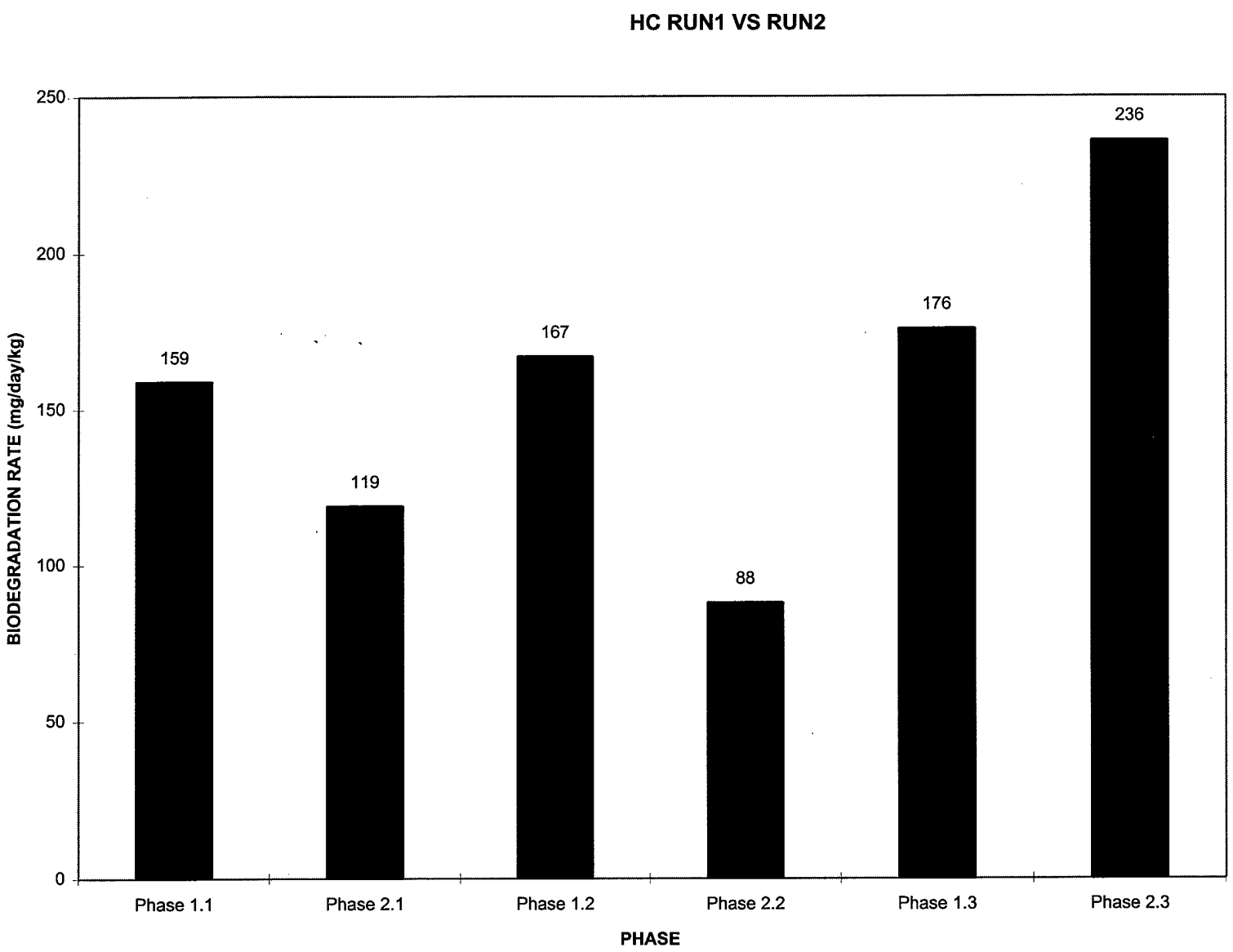
			QUANTIFICATION OF BIODEGRADATION								
SOIL		Mean Total Oxygen Cons, ul	Dry Soil kg	Respiration Rate ml/day/kg	Total PG Consumed ml	Original Qty PG Added, ml	% Lost to Biodeg	Biodegradation Rate mg/day/kg	AVE	Biodegradation Rate mg/day/kg	% Lost to Biodeg
HC RUN 1	Phase 1	85735	0.079	12.05	0.047	0.3	15.9	159	HC	157.5	20
	Phase 2	84095	0.079	12.67	0.046	0.3	16.7	167			
	Phase 3	113664	0.079	13.32	0.063	0.3	17.6	176	SS	49.50	6.22
	TOTALS			38.04	**0.156**	**0.9**	**17.33333**				
HC RUN 2	Phase 1	109932	0.079	15.46	0.035	0.3	11.9	119			
	Phase 2	74221	0.079	11.18	0.024	0.3	8.8	88			
	Phase 3	224079	0.079	15.25	0.145	0.3	23.6	236			
	TOTALS			41.89	**0.204**	**0.9**	**22.66667**				
SS RUN 1	Phase 1	25605	0.085	3.35	0.016	0.3	5.3	54			
	Phase 2	19776	0.085	2.77	0.011	0.3	3.74	41			
	Phase 3	28516	0.085	3.11	0.016	0.3	5.435	46			
	TOTALS			9.23	**0.043**	**0.9**	**4.777778**				
SS RUN 2	Phase 1	21028	0.085	2.7	0.011	0.3	3.567	36			
	Phase 2	20622	0.085	2.89	0.013	0.3	4.21	46			
	Phase 3	60765	0.085	3.84	0.045	0.3	5.04	74			
	TOTALS			9.43	**0.069**	**0.9**	**7.666667**				
HC RUN 2	00 mg P	258666	0.079	9.1	0.09	0.3	30.06	76			
o re-injec	00 mg P	399180	0.079	14.03	0.198	0.6	32.95	167			
	900 mg P	432261	0.079	15.2	0.223	0.9	24.78	188			
SS RUN 2	00 mg P	218089	0.085	7.13	0.144	0.3	48.16	113			
o re-injec	00 mg P	63799	0.085	2.09	0.026	0.6	4.4	21			
	900 mg P	46838	0.085	1.53	0.013	0.9	1.49	11			

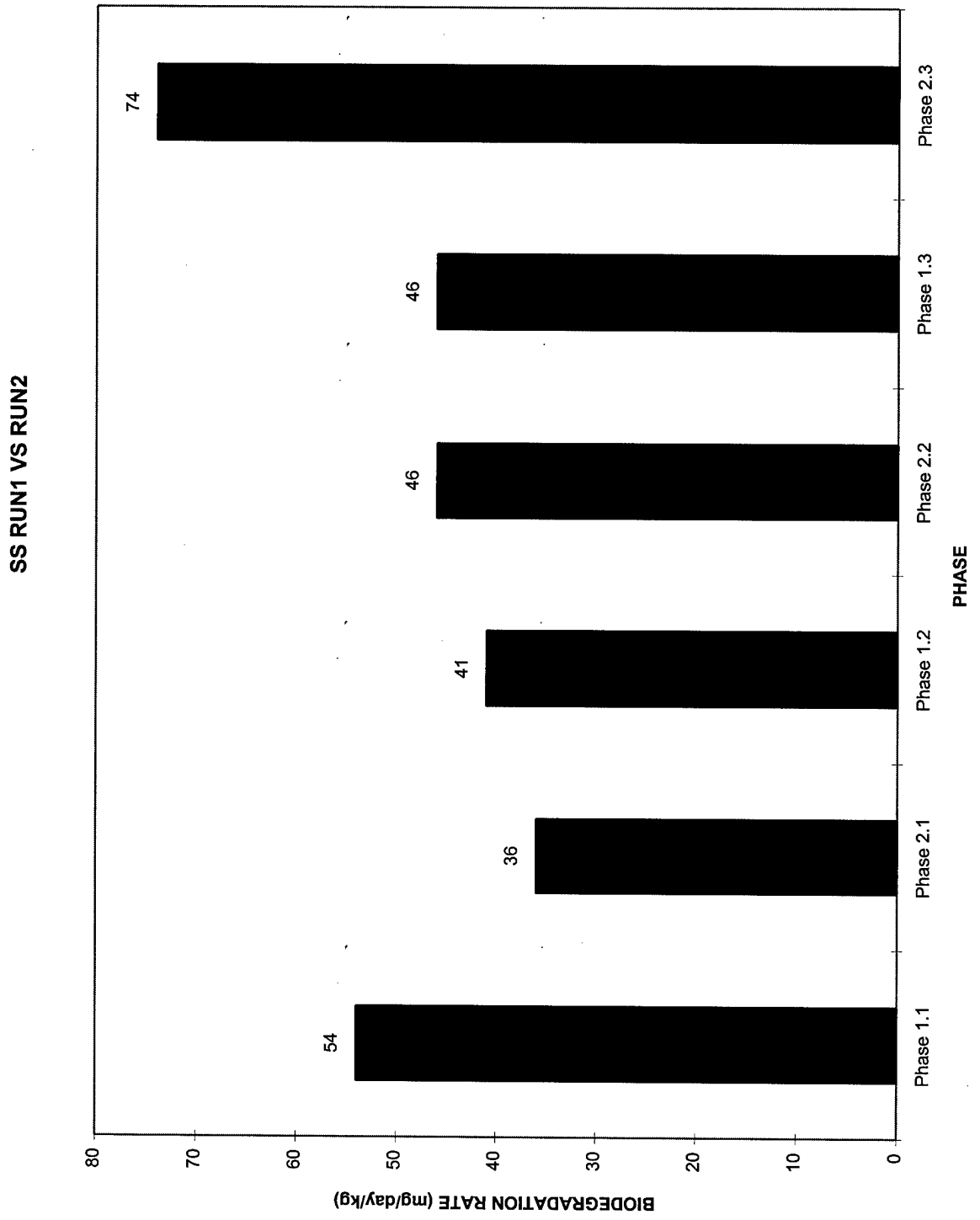
HC VS SS RESPIRATION RATE



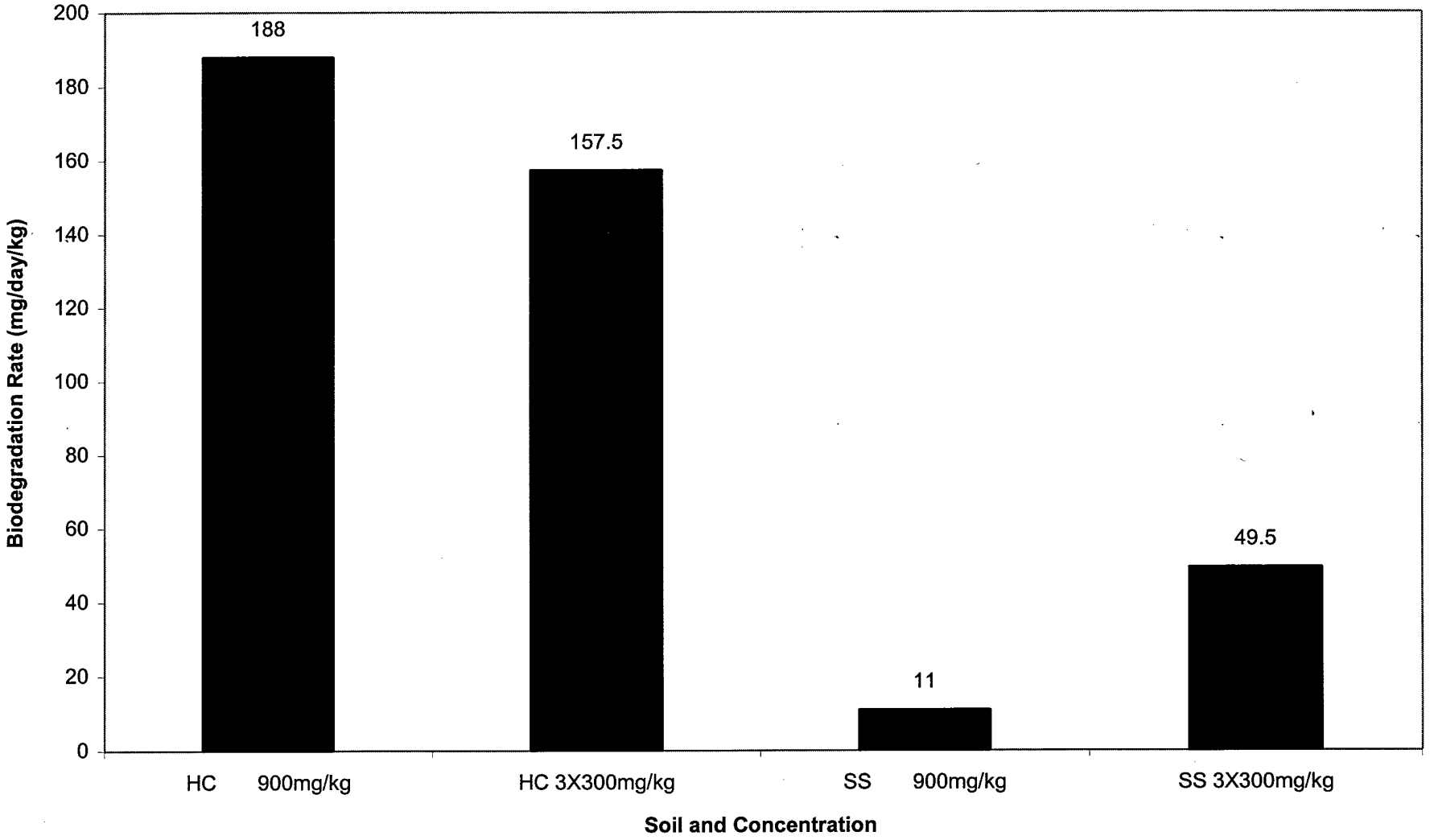
HC VS SS BIODEGRADATION RATE in mg/day/kg




HC RUN1 VS RUN2
BIODEGRADATION RATE (mg/day/kg)
250
200
150
100
50
0
159
119
167
88
176
236
Phase 1.1
Phase 2.1
Phase 1.2
Phase 2.2
Phase 1.3
Phase 2.3
PHASE


SS RUN1 VS RUN2
BIODEGRADATION RATE (mg/day/kg)
0
10
20
30
40
50
60
70
80
54
36
41
46
46
74
Phase 1.1
Phase 2.1
Phase 1.2
Phase 2.2
Phase 1.3
Phase 2.3
PHASE

NO REINJECTION VS REINJECTION



SAMPLE CALCULATIONS FOR QUANTIFICATION OF HYDROCARBON BIODEGRADATION FROM RESPRIOMETER DATA (OXYGEN CONSUMPTION)

Conversion from ul of oxygen consumed to ml of hydrocarbon consumed and intermediate steps using information on page E-1.

$v := 46838$ microliters of oxygen consumed in treatment

$v_{act} := v - 1912$ Adjusting for background O_2 readins in empty microcosms

$V := \frac{v_{act}}{1000000}$ Converting from microliters to liters

$P := 1$ Standard Atompspheric pressure, atm

$t := 30$

$T := 273 + t$ Converting to degrees Kelvin

$R := 0.082058$ Gas constant, L-atm/deg K-mol

$n := \frac{P \cdot V}{R \cdot T}$ Perfect gas law

$n = 0.002$ The number of moles of oxygen consumed

$intv := 6$ Sampling interval, one sample per 6 hours

$time := 60 \cdot intv \cdot 60$ Total time of experiment (min) for 47 intervals

$soil := 0.085$ Weight of soil, Kg

$resp_{rate} := \frac{\frac{v}{time}}{soil}$

$resp_{rate} = 25.511$ RESPIRATION RATE, ul/min/kg dry soil

$V_{0\%} := 0.029264 - \frac{1912}{1000000}$ Background respiration of soil without PG

$V_{hc} := V - V_{0\%}$ Respriation attributable to hydrocarbon respiration

$n_{hc} := \frac{P \cdot V_{hc}}{R \cdot T}$ Moles of O_2 attribuatable to hyrdocarbon respiration

$ratio := 4$ Number of moles of O_2 to mineralize 1 mole $C_3H_8O_2$

$MW := 76.094$ Molecualr weight of $C_3H_8O_2$, gm/mol

$spgr_{hc} := 1.0$ Number of milliliters per gram of PG

$hc := \frac{n_{hc}}{ratio} \cdot MW \cdot spgr_{hc}$

$hc = 0.013$ VOLUME of PG CONSUMED, ml

$hc_{orig} := 0.9$ Orignal volume of fuel added, ml

$percent_{lost} := \frac{hc \cdot 100}{hc_{orig}}$

$percent_{lost} = 1.494$ % PG LOST TO BIODEGRADATION

$days := \frac{time}{60 \cdot 24}$ Number of days experiment ran

$degrade_{rate} := \frac{\frac{hc}{days}}{soil}$

$degrade_{rate} = 0.011$ PG BIODEGRADATION RATE, ml/day/kg soil

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Vita

Second Lieutenant AnnMarie Halterman-O'Malley was born on August 7th, 1972 in Stanley, Wisconsin. She spent her childhood in the small town of Stanley and graduated from Stanley-Boyd High School in the Spring of 1991. After graduating from High School, 2Lt Halterman-O'Malley attended the United States Air Force Academy Prepatory School for a year and then continued her education at the Air Force Academy. On May 29th, 1996, she graduated with a Bachelor of Science in Civil Engineering. Commissioned a Second Lieutenant on the same day, Wright-Patterson Air Force Base and the Air Force Institute of Technology are her first assignment. Upon completion of the AFIT program, Second Lieutenant Halterman-O'Malley will be assigned to Civil Engineering at Aviano Air Base, Italy.

Second Lieutenant Halterman-O'Malley's husband David is also in the Air Force and currently flies F-16s Luke Air Force Base, Arizona.

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

Public reporting burden for this collection of information is estimated to average 1 hour per response, including the time for reviewing instructions, searching existing data sources, gathering and maintaining the data needed, and completing and reviewing the collection of information. Send comments regarding this burden estimate or any other aspect of this collection of information, including suggestions for reducing this burden, to Washington Headquarters Services, Directorate for Information Operations and Reports, 1215 Jefferson Davis Highway, Suite 1204, Arlington, VA 22202-4302, and to the Office of Management and Budget, Paperwork Reduction Project (0704-0188), Washington, DC 20503.

1. AGENCY USE ONLY *(Leave blank)*	2. REPORT DATE	3. REPORT TYPE AND DATES COVERED
	December 1997	Masters Thesis

4. TITLE AND SUBTITLE
Biodegradation of Aircraft Deicing Agents in various Soil Types

5. FUNDING NUMBERS

6. AUTHOR(S)
ANNMARIE HALTERMAN-O'MALLEY, 2Lt, USAF

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)
Air Force Institute of Technology
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Wright Patterson AFB, OH 45433-7765

8. PERFORMING ORGANIZATION REPORT NUMBER
AFIT/GEE/ENV/97D-09

9. SPONSORING/MONITORING AGENCY NAME(S) AND ADDRESS(ES)
WL/MLSE
Wright Patterson AFB, OH 45433

10. SPONSORING/MONITORING AGENCY REPORT NUMBER

11. SUPPLEMENTARY NOTES

12a. DISTRIBUTION AVAILABILITY STATEMENT
Approved for Public Release; Distribution Unlimited

12b. DISTRIBUTION CODE

13. ABSTRACT *(Maximum 200 words)*

The primary concern of this research effort was to monitor the behavior of a simulated aircraft deicing fluid in various soil systems. An automated respirometer monitored the behavior of the microbial activity in soil that was contaminated with aircraft deicing fluid (ADF). Reapplication of the contaminant to the soil, one time soil loading variations and a lime additive analyzed in an attempt to maximize the biodegradation of the contaminant.

Analysis of the oxygen consumption rates of the deicing agents, provide biodegradation rates and the amount of time required for treatment of propylene glycol-based ADF. Since deicing agents do not sorb strongly to soil, determining the effects of the contaminant in different soil types helps optimize biodegradation. During the 660 hours of experimental time, the effectiveness of the high clay system was significantly higher than that of the sandy soil. The sandy soil results were increased greatly when a reapplication process was implemented. The high-clays most effective system was a one time high concentration injection of PG.

Reapplication of the contaminant, lime additions and varying concentrations of contaminant were the methods used to attempt to optimize the batch system. These methods were typically effective for one of the two soil types, but rarely worked on both soils.

14. SUBJECT TERMS	15. NUMBER OF PAGES
Biodegradation, Micro-Oxymax Respirometer, Oxygen Uptake, Propylene Glycol, Soil, Land Treatment, Respiration	120
	16. PRICE CODE

17. SECURITY CLASSIFICATION OF REPORT	18. SECURITY CLASSIFICATION OF THIS PAGE	19. SECURITY CLASSIFICATION OF ABSTRACT	20. LIMITATION OF ABSTRACT
Unclassified	Unclassified	Unclassified	UL