BIOREMEDIATION OF PETROLEUM HYDROCARBONS IN SOIL

Activated Sludge Treatability Study

By

J. E. LA RUE-VAN ES

A Thesis
Submitted to the Faculty of Graduate Studies
in Partial Fulfilment of the Requirements
for the Degree of

MASTER OF SCIENCE

Department of Civil Engineering
University of Manitoba
Winnipeg, Manitoba

ISBN 0-315-85954-7

Canada

BIOREMEDIATION OF PETROLEUM HYDROCARBONS IN SOIL

ACTIVATED SLUDGE TREATABILITY STUDY

BY

J.E. LA RUE-VAN ES

A Thesis submitted to the Faculty of Graduate Studies of the University of Manitoba in partial fulfillment of the requirements for the degree of

MASTER OF SCIENCE

ABSTRACT

Batch activated sludge treatability studies utilizing petroleum hydrocarbon contaminated soils (diesel oil and leaded gasoline) were conducted to determine: (1) initial indigenous biological activity in hydrocarbon-contaminated soils; (2) limiting factors of microbiological growth by investigating nutrient addition, chemical emulsifiers, and co-substrate; (3) acclimation of an indigenous population of microorganisms to utilize hydrocarbons as sole carbon source; and (4) temperature effects.

Soil samples were taken from three different contaminated sites. Four sequencing batch reactors were run from site one (southern Manitoba), three from site two (northern Manitoba), and two from site three (northern Manitoba). Substrate (diesel fuel) and nutrient were added as determined by laboratory analysis of orthophosphate, ammonia nitrogen, chemical oxygen demand (COD), and total organic carbon (TOC). Substrate was made available to the bacterial mass by experimenting with the use of four different chemical emulsifiers. All reactors were also monitored with respect to other chemical, physical, and biological parameters. Laboratory analysis followed Standard Methods.

Indigenous microorganisms capable of biotransforming hydrocarbons seem to be present in all the contaminated soil samples received from all sites. Microscopic analysis of reactors revealed no visible activity at the beginning if the study and presence of flagellated protozoa, paramecium, rotifers, and nematodes at the end of a year. Nutrient requirements (nitrogen, phosphorous) and the limiting factors in microorganisms growth have been determined for each particular site. A co-substrate was used initially to enhance bacterial mass growth. Use of an emulsifier was deemed necessary initially to make the hydrocarbons available to the microbial population. Temperature effects study (site one, temperature decreased gradually from 22 oC to 12 oC) showed a decrease in removal (TOC) and an emerging presence of filamentous bacteria. A second temperature study (site two, temperature to decrease gradually from 22 oC to 4 oC) also showed a decrease in removal.

Removal efficiencies, in terms of chemical oxygen demand, range from 50% to 90% in reactors from site one (16 months ongoing at room temperature, no waste sludge). Acclimation of indigenous microorganisms to hydrocarbons is possible and could reduce remediation time of contaminated soils.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF GRAPHS

ABBREVIATIONS

LUST	-	Leaking Underground Storage Tank
COD	-	Chemical Oxygen Demand
TOC	-	Total Organic Carbon
MLSS	-	Mixed Liquor Suspended Solids
MLVSS	-	Mixed Liquor Volatile Suspended Solids
TKN	-	Total Kjeldahl Nitrogem
DO	-	Dissolved Oxygen
COD_i	-	Chemical Oxygen Demand, influent
COD_e	-	Chemical Oxygen Demand, effluent
L	-	litre
NH_3-N	-	ammonia nitrogen (mg/l)
Ortho P	-	orthophosphorous
Total P	-	total phosphate
ThOD	-	Theoretical Oxygen Demand
C:N:P	-	carbon: nitrogen: phosphorous
^oC	-	degrees Celcius
BTEX	-	Benzene, Toluene, Ethylbenzene, Xylene
TPH	-	Total Petroleum Hydrocarbon
PAH	-	Polycyclic Aromatic Hydrocarbon
VOC	-	Volatile Organic Carbon
ppb	-	parts per billion

ACKNOWLEDGEMENTS

This thesis is dedicated to my children and their future.

Thanks Nicole, Peter, and Joelle.

I wish to thank W. Kwan of Geokwan Environmental Ltd., Manitoba Hydro, especially P. Janzen and J. Mackay, as well as T. Wingrove of UMA Engineering Ltd. for their research support.

This thesis could never have been attempted without the expert and patient technical laboratory support from J. Tingley and most especially W. Trebacz.

I have been most fortunate that Prof. A. Sparling agreed to advise me, with help from Prof. D. Shields. Thank-you Prof. J. Graham for pointing me their way and to Prof. B. Lence for encouraging me to become a grad student.

To all the graduate students in 406-C for your company.

The encouragement and interest given to me by my parents throughout my life made this thesis possible.

David, what can I say. **We made it!**

1. INTRODUCTION

An environmental problem of recent significance is the contamination of soil and groundwater by hydrocarbon spills. Petroleum contaminated soils and groundwater can result from: leaking underground storage tanks (LUST), petroleum pipeline breaks, spills of petroleum products, leaking above ground tanks, tanker spills, leaks from petroleum refineries and bulk storage facilities (Newton 1990), as well as refinery residues, coal tar sites, chemical processing sites, and wood treating sites. (Sherman and Stroo 1989) Risks of spills are also created during the production, transportation and refining of crude oil, as well as the distribution and marketing of refined products.

Bioremediation is a developing soil treatment technology that biodegrades petroleum hydrocarbons aerobically and completely to nontoxic end-products of carbon dioxide and water. It is interesting to note that in 1986, Mackay and Hoag mused that perhaps soil treatment would be the next growth industry.

1.1 Scope of the Problem

It has been estimated that 20 - 25% of all storage tanks at petroleum retail outlets in Canada are leaking or suspected to be leaking. (Environ. Sci. & Eng. 1991) In many instances, LUSTs pose a major threat to drinking water supplies as only

1 gallon of gasoline can render 1 million gallons of water unsuitable for consumption. Redevelopment of urban areas that were formerly used for industry is also an issue of great concern because much of the soil on these lands has been contaminated with petroleum hydrocarbons. (Environ. Sci. & Techn. 1991) As well as contamination of ground water, LUST can create explosion hazards from the accumulation of hydrocarbon vapours under buildings and can degrade utility lines that may come into contact with the leaking petroleum hydrocarbons. (Lingineni 1992)

The scope of the problem is large. Toronto's industrial port contains an estimated 2 million tonnes of contaminated soil which is expected to cost $160/tonne and take approximately 10 years to clean up. (Piper 1991) In Canada at present, there are approximately 200,000 UST. Of these, 70,000 are located at retail gasoline stations. The remaining 130,000 UST are used in manufacturing, transportation, commercial and agricultural industries. "If 20% of the 200,000 USTs in Canada are leaking, the total remediation cost could be many tens of billions of dollars - the same order of magnitude as the annual Canadian Federal Government Deficit." (Environ. Sci. & Eng. 1991) Bioremediation usually has lower costs associated with it than other remediation technologies due to lower mechanical and energy requirements.

1.2 Petroleum Hydrocarbons

The most common petroleum hydrocarbons contaminating soil and groundwater include: gasoline, diesel, and fuel oils. Each is a complex mixture composed of many organic chemical compounds. "Crude oil is a complex mixture made up of approximately 11 to 13% hydrogen and 84 to 87% carbon by weight. Of the 18 series of hydrocarbons identified in crude oils, paraffins, olefins, polymethylenes, acetylenes, turpenes, and benzenes are those found most often. Crude oil contains, on the average, approximately 1 % polynuclear aromatic hydrocarbons." (Custance 1992) Crude oil then is very rich in hydrocarbons which results in a very high C:N (carbon:nitrogen) ratio.

Figure 1 shows a gas chromatograph of crude oil, identifying the constituents which range from light hydrocarbons to heavy hydrocarbons. Some chemical and physical properties of diesel fuel are shown in **Table 1.**

TABLE 1: CHEMICAL/PHYSICAL PROPERTIES OF DIESEL FUEL Adapted from Custance 1992

Parameter	Value
Density (g/cm^3)	0.84 g/cm^3
Aqueous Solubility (mg/l)	0.2 g/m^3 (0.2 mg/l)
Vapour Pressure (mmHg)	0.03
Diffusion Coefficient in Air (cm^2/s)	4.63×10^{-2}
Henry's Law Constant ($atm\text{-}m^3/mol$)	4.2×10^{-2}
Log organic carbon: water partition coefficient	3.04
Biodegradation ($year^{-1}$)	1 year

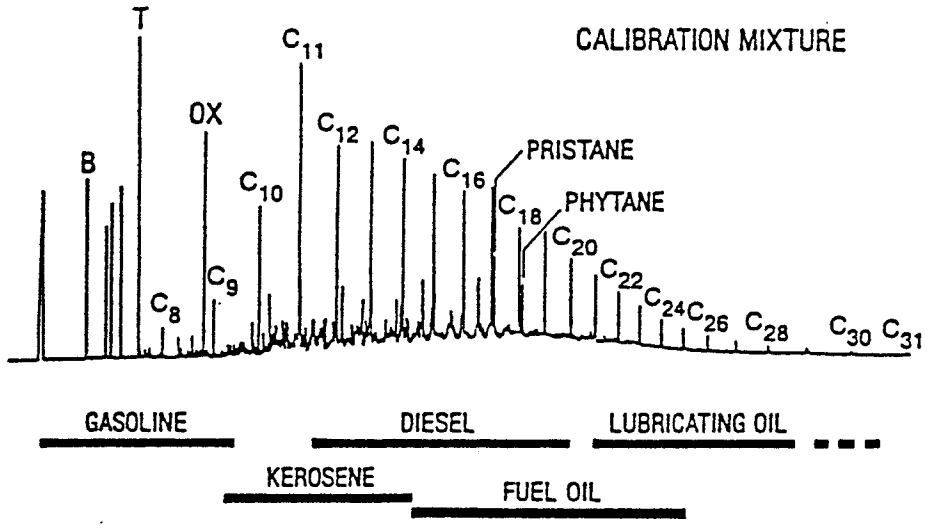


FIGURE 1: PETROLEUM HYDROCARBONS (Galaska 1990) Adapted from Senn and Johnson, 1985

In gasoline, the organic compounds generally have low solubility, low volatility, and strong adsorption characteristics. The primary gasoline constituents have monocyclic aromatic hydrocarbons which include benzene, toluene, ethylbenzene and xylene (BTEX). They are of the greatest concern because of their toxicity and mobility. "All oils and oil products differ in toxicity. In general, the lighter oils such as diesel fuel and gasoline cause the greatest short term damage, whereas heavy oils such as crude may cause acute toxic damage." (Nichols 1989)

Benzene is also a known carcinogen. Benzene has a much greater solubility in water than xylene, and can be stripped

out as it is flushed with water. Xylene has the lowest solubility in water and adsorbs to clays in soil. Some of these compounds are usually contained in the vadose zone (soil area above the water table) because they are readily adsorbed to clay and the organic fractions of the soil. This makes them less mobile and thus they are not likely to make their way downward to the water table. (Newton 1990)

"The physical, chemical, and biological properties of these chemicals in a complex petroleum product has (sic) a major effect on the distribution of the compound in a soil/gas/liquid matrix." (Galaska 1990) High solubility compounds are most likely to be present in the aquifer itself. High volatility compounds are most likely present in the soil gases and the atmosphere. Therefore, on-site or in-situ biological remediation of petroleum hydrocarbon contaminated soils and groundwater must address the particular organic compound which is present.

1.3 "Typical" Spill Mechanisms

Soil consists of four phases: mineral matter (sand, clays, etc), organic matter, water and air. When petroleum hydrocarbons leak into the subsurface environment, they percolate downwards and spread laterally. A small spill may not reach the groundwater. The petroleum hydrocarbons may be held in the pores of the soil particles. A large spill may

reach the ground water table and form a saturated zone above the water table.

Petroleum hydrocarbon contamination can be present in many phases including dissolved, floating and suspended. Because diesel oil and gasoline are complex mixtures, their behaviour is much more complex. Downey and Elliott (1990) found that "fuels trapped within the micropores of the soil were largely inaccessible to the nutrients and oxygen that were being provided".

Figures 2 and **3** show views of a "typical spill". The spread and adsorption rate of a fuel oil spill will depend mostly on the permeability of the soil and its water content. At the same time that the spill is spreading over the soil and absorbing into the soil, mass transfer to the air is taking place. Because of all these mechanisms, the properties of the fuel oil will change.

Figure 4 is a conceptual model showing how petroleum hydrocarbons are distributed among the soil organic matter phase, water phase, and air phase in soil. (Qiu and McFarland 1991)

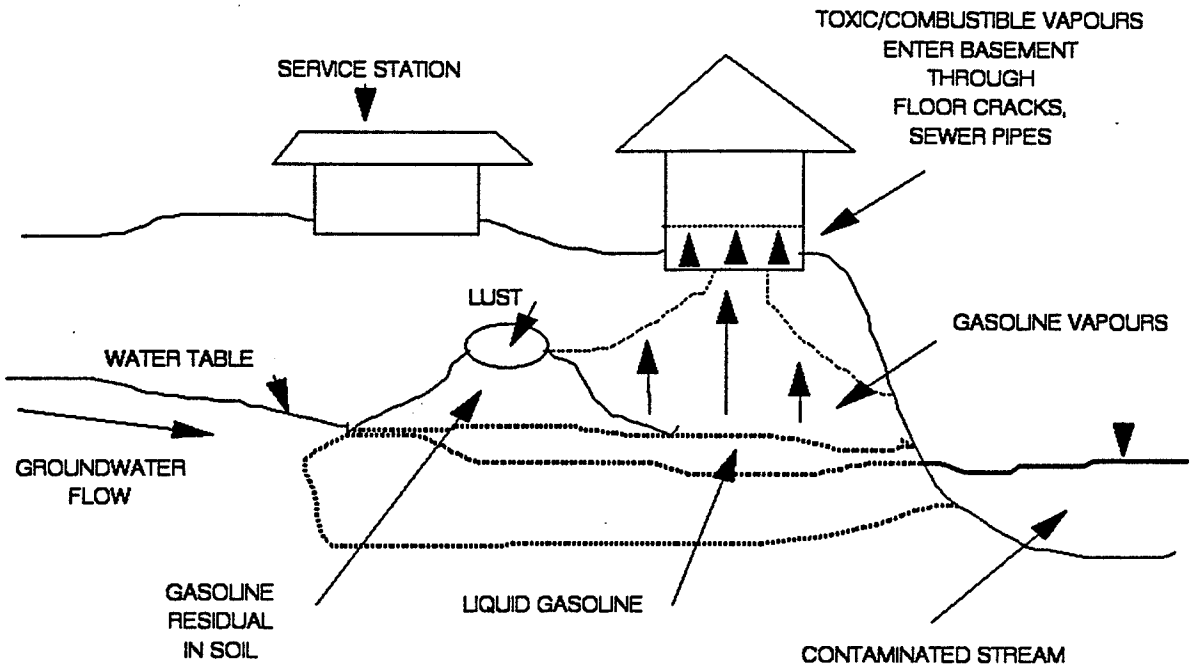


FIGURE 2: GASOLINE CONTAMINATION OF SOILS AND GROUNDWATER

(Wolowich 1991)

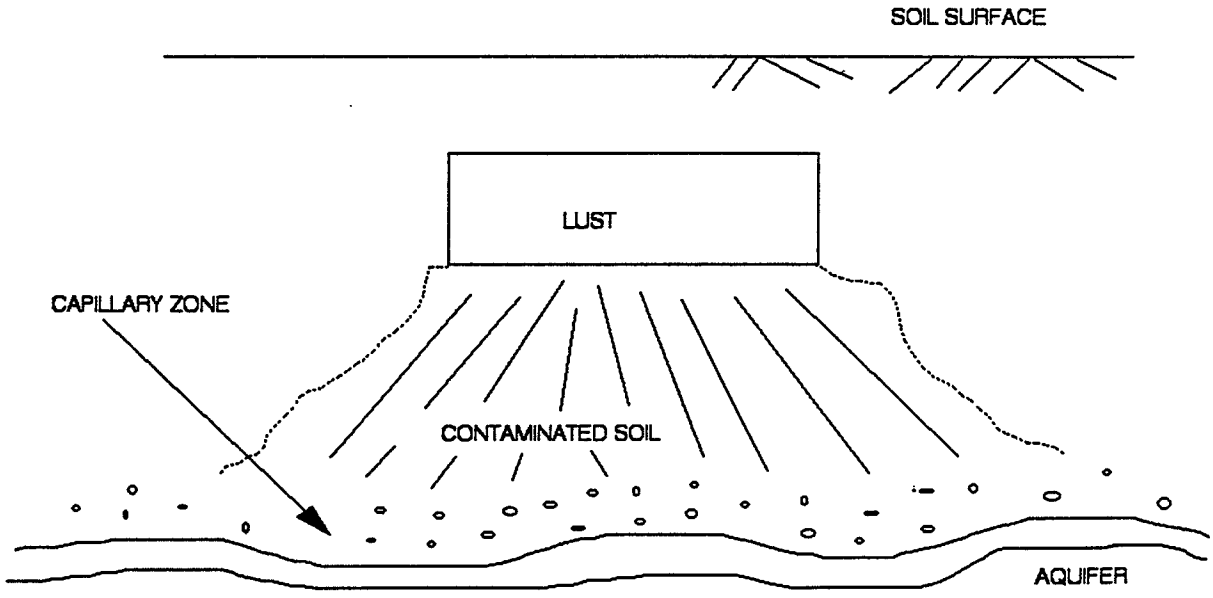
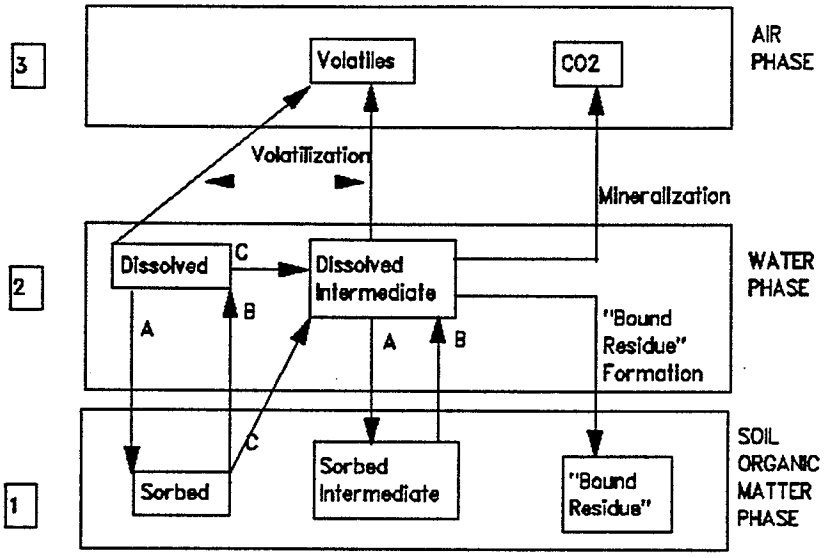


FIGURE 3: "TYPICAL" SPILL SITE



A = Adsorption
B = Desorption
C = Degradation

FIGURE 4: PETROLEUM HYDROCARBON DISTRIBUTION AMONG 3 COMPARTMENTS (Qiu & McFarland 1991)

1.4 Remediation Strategies

Remediation strategies have included: chemical fixation; soil washing; enhanced biodegradation; soil venting (soil vapour extraction); thermal treatment (low and high temperature); surface bioremediation (land farming); solidification/stabilization; asphalt incorporation; soil leaching; solvent extraction; slurry phase bioremediation; and landfill disposal. (Srivastava 1990) Soil flushing (washing) and soil excavation have been found to be very expensive and inefficient in residual contaminant removal. (Lingineni 1992)

Soil venting becomes expensive if air emissions are to be remediated as well. Thermal treatment has demonstrated excellent removal efficiencies of organics but is extremely expensive. If off-gas treatment is not provided, contaminants may only have been transferred to another phase - the atmosphere. In-situ bioremediation is a developing technology that does not transfer contaminants but renders them harmless.

1.5 Bioremediation

Biotechnology is a very old science. For millions of years organic materials have been naturally decomposing. Without this process, the earth would be buried under a blanket of leaves. For thousands of years waste has been biologically managed and the majority of the microorganisms used in this biological waste management have been extracted from soil and water.

A technology that is a viable treatment alternative and which can safely, effectively and permanently remediate petroleum contaminated soils is bioremediation. Bioremediation is a more or less viable remediation technology for contaminated soil and groundwater depending on the site characteristics which could include the presence of indigenous microorganisms and suitable site geology. Soil, contaminated with petroleum hydrocarbons, may have the capacity to detoxify, degrade, and inactivate the toxic organic chemicals.

Because of the increasing costs of current technologies, the enthusiasm of regulatory agencies to innovative technology, and the more stringent regulatory requirements, bioremediation is beginning to play an important role in soil and groundwater contamination clean-up.

1.5.1 Definition of Bioremediation

"Bioremediation is a process that uses the soil's naturally occurring microorgansms to decompose toxic or hazardous substances." (Hopper 1989) Bioremediation follows the thesis that biodegradation is a naturally occurring process in all soils. "The underlying premise of in situ bioremediation is the Ubiquity Principal which states that all types of bacteria are available at all times everywhere." (Major 1991)

"All living systems require sources of energy to develop and sustain their populations." (Torpy 1989) Microorganisms in the soil use organic compounds that contain carbon. Bioremediation works because the organic compounds in the hazardous substances can be utilized as food and energy for the microorganisms. These microorganisms, which use enzymes in the process of organic decomposition, feed on the organics (carbon) found in the soil and require oxygen and water to survive. By metabolizing the organic compounds in the soil, microorganisms derive energy and carbon which will be

incorporated into new cell mass. (Mahaffey 1991, Torpy 1989) "Biotransformation refers to the conversion of a compound or its intermediates to the next product in the biochemical pathway." (McFarland et al 1991) Incorporation of certain amounts of nutrients such as nitrogen and phosphorous is necessary for microbial growth.

Microorganisms used in the degradation process may be indigenous to the site (biostimulation) or they may be especially selected (bioaugmentation). In biostimulation, the enrichment conditions are identified, quantified and then applied. In bioaugmentation, the organisms are supplied as a mass inoculum from a proprietary inoculum or by way of enrichment for the active microbes indigenous in the waste. Thus bioremediation is a microbiological process that depends on the growth and activities of a population of bacteria and other microorganisms. With the right selection of enrichment of a microbial population, it may be possible to stimulate and increase biological activity thereby degrading a contaminant.

Bioremediation then, is a biological method that can use indigenous microorganisms environmentally enriched to aerobically metabolize contaminants. The end products of this mineralization include carbon dioxide and water (which in themselves do not pose any environmental concerns) and biomass (Bouwer 1989), which provides for a "final, ecologically sound

solution to toxic waste problems". (Zitrides 1990) Thus, bioremediation is a remediation technology that is considered to be a true destruction process. In this process, contaminants are permanently remediated and then require no further treatment.

In-situ bioremediation, using indigenous species, stimulates the activity of the microorganisms by the addition of nutrients and oxygen. The growth of the microorganisms will depend in part on the temperature, pH, moisture, oxygen, and nutrient levels in the soil. The optimal environmental conditions for aerobic metabolism are:

1. temperature 15°C to 45°C, mesophilic;
2. pH 5.5 to 8.5, near neutral;
3. optimum nutrient ratio;
4. DO greater than 0.2 mg/l in soil pores; and
5. redox potential greater than 50 mV. (Andreottola 1991)

Bioremediation is limited only by the understanding of the microbial ecology and physiology of polluted sites and involves "a strong interaction between the microbial community and the physical and geochemical environment, creating a dynamic environment in which contaminants are degraded". (Major 1991)

1.5.2 In-situ, Ex-situ Bioremediation

Bioremediation as a remediation strategy, can be used in-situ or ex-situ. In-situ implies that the contaminated soil and groundwater are disturbed the least amount possible. Contamination in the soil is treated without removal from the area which had been contaminated. An advantage of in-situ biological treatment is the production of a biologically active soil. Ex-situ technologies are those where the contaminated soil is excavated. When excavated soil is removed from the site for treatment, such as in land farming, it is called off-site treatment. The "clean" soil may or may not be returned after treatment. On-site treatment can be in-situ but most commonly implies that the contaminated soil is extracted, treated at the site, and then put back. In-situ bioremediation is an appropriate method when it is impossible or too expensive to excavate contaminated soil. (Andreottola and Acaia 1991)

The in-situ bioremediation process requires a subsurface matrix that will be permeable enough to allow oxygen, nutrients and contaminant-degrading microbes to enter and travel. However, most contaminated sites have irregular geology, have been previously disturbed by construction and/or have multiple or unknown sources of contamination. (Torpy 1989)

In-situ technologies can have many advantages, such as:

- minimum intrusion to site (therefore less disruptive),
- more cost-effective (excavation and hauling are expensive),
- work well in high permeability soils (sand and gravel),
- require small above-ground surface area, and
- contaminant particulates and vapours are minimized.

In-situ technologies also have some disadvantages which may include: longer treatment times may be required; removal efficiencies and monitoring of remediation effectiveness may be difficult to obtain; and they often do not work well in low permeability material such as clay.

1.5.3 Enrichment Conditions

Since in-situ bioremediation stimulates microbiological activity in the soil which in turn causes degradation of the contaminant, it follows that microbiological activity can be stimulated by modifying any one or combination of geochemical conditions, physical conditions, nutrients, and microorganisms. Thus, environmental conditions can be optimized by supplying oxygen, nutrients, circulating water and/or increasing temperature.

One of the most important aspects in bioremediation is the carbon:nitrogen:phosphorous (C:N:P) ratio. The importance of

mineral nutrient addition (N,P) for decomposition of oil has been widely recognized. (Cook and Westlake 1974) Microorganisms require carbon for growth and energy, and nitrogen for protein synthesis. In order to prosper, "bacteria require about 10 parts carbon to 1 part nitrogen". (Westlake and Cook 1973) A range of C:N:P of 100:10:1 to 100:10:5 was recomended by Torpy (1989). Thus when oil spills on soil, the carbon to nitrogen ratio becomes unbalanced and there is a nitrogen deficiency. A deficiency of phosphorous may also be aggravated by an oil spill. The rate of decomposition will be dependent on this ratio of C:N:P. What is ultimately of the most importance to biodegradation is the availability of nutrient rather than the ratio.

The limiting nutrient in bioremediation is most likely nitrogen. Rasiah (1991) reported that nitrogen amendment enhanced the carbon mineralization of an oily waste significantly and that the greatest enhancement in waste carbon mineralization occurred when the waste-C:fertilizer-N (WC:FN) ratio was in the range of 18 to 22:1. Carbon need not be supplied as it is one of the key elements of the petroleum hydrocarbon molecule. However, this carbon may not always be easily available to the active microorganisms and in this circumstance "easy" carbon is added only to the extent that the active population remains large. Typically, nutrients added to soil for enrichment include inorganic salts such as

ammonium chloride, ammonium nitrate, sodium nitrate, sodium phosphate, and potassium phosphate. Trace nutrients are rarely required because they are rarely limiting in the open environment.

The rate and extent of biological degradation will therefore be a function of: (1) limitations of mass transfer; (2) lack of capable microflora; (3) complexity and variability of waste, including the nature and concentration of the waste as well as the presence of other organic substrates; (4) soil and site characteristics (including soil pH, salinity, dissolved oxygen levels, soil moisture content, soil permeability, oxidation-reduction potential, temperature); (5) nutrient availability; and (6) toxic or inhibitory compounds. (Hickman 1989, Mahaffey 1991)

The major rate-limiting steps in biodegradation have been discussed by Srivastava (et al 1990). In order to accelerate bioremediation, several strategies were explored: (1) mass transfer of waste material to microorganisms could be increased; (2) the contaminant could be made more soluble (although low solubility limits migration to groundwater, it also limits microbial degradation); (3) oxidation of PAH could occur, making them biologically more available; and (4) enrichment cultures of pollutant degrading microorganisms for aerobic environments could be developed.

In order for bioremediation to be successful, oxygen must be supplied to the microorganisms. There are many alternatives available for the supply of oxygen:

(1) air sparging (porous stone at bottom of well);

(2) injection of aerated/oxygenated water;

(3) venting (vacuum withdrawal or injection);

(4) hydrogen peroxide addition; and

(5) encapsulation of air in surfactant bubbles. (Major 1991)

Aerobic conditions could also be maintained in contaminated soil through the use of extraction and injection wells (flushing) containing oxygen saturated water or by the use of irrigation pipes. The wells could also be the vehicle for enrichment techniques such as nutrient addition.

Successful bioremediation then, will be the combined action of basic microbiological processes and sound bioprocess engineering and will require:

1. favourable environmental conditions including pH, oxygen concentration, influent organics concentration, concentration of inorganic nutrients (N in the form of ammonia and P in the form of orthophosphate) and temperature;
2. suitable microbial populations; and
3. absence of high concentrations of toxic/inhibitory chemicals.

1.5.4 Laboratory Treatability Studies

Skladany (1988) pointed out that laboratory evaluations are required for assessing the biodegradation potential of a site and could be done by placing soil in a reactor, modifying conditions and monitoring. Soil/water slurry experiments with petroleum hydrocarbon contaminated soil have been conducted by Srivastava (1990).

McFarland (et al 1991) reported the necessity of laboratory treatability studies to develop remediation strategies for contaminated soils. To evaluate soil bioremediation requires: (1) laboratory screening; (2) bench-scale testing; (3) pilot-scale testing; and (4) addressing unique design concerns related to mass transfer and the partitioning of nutrients and/or contaminants in the soil matrix.

The necessity of nutrient addition should be determined using laboratory bench-scale treatability studies. Enrichment conditions for enhancing bioremediation were discussed by Golueke and Diaz (1990) and included nutritional aspects as well as environmental factors. Nutritional aspects included: (1) setting up an enrichment culture; (2) ensuring that the concentration of nontoxic substances with easy carbon be at a minimum so that organisms capable of using the carbon in the toxic contaminant would survive and thrive; (3) identifying growth factors; and (4) nutrient availability (such as

phosphate and nitrogen). Environmental factors included pH level, temperature, and oxygen concentration (aeration). The optimum temperature for biodegradation will be that of the active microbes. The authors concluded that enrichment must be continued throughout the treatment.

Laboratory assessment of biodegradation potential using a standardized laboratory protocol provides a basis for comparison of many different sites. However, the conditions used in the lab will not be the conditions encountered in the open environment.

1.5.5 Proof of Biodegradation

"All microorganisms need a source of sulfur (usually sulfate), nitrogen (usually nitrate, ammonium), and phosphorous (phosphate), as well as the trace elements...Energy may be supplied solely as organic molecules for heterotrophs." (O'Leary 1989) Confirmation of biodegradation could be shown by a mass balance between contaminants, nutrients, and end-products. Adaptation such as shown by enhanced numbers of protozoan predators provides "essential auxiliary evidence for in-situ biodegradation". (Madsen 1991)

Proof of in-situ biodegradation will require demonstration of a decrease in the mass of contaminant due to microbiological activity. Madsen (1991) also states that "in-situ

biodegradation of organic contaminants is very difficult to prove" and has shown examples of investigations that have successfully proven in-situ biodegradation. Evidence of in-situ biodegradation could include: increase in microbial numbers or biomass and activity of the requisite organisms (enhanced numbers of protozoa or other metabolically stimulated members of the microbial community), adaptation/acclimation response, mass balances, determinations of loss of contaminant, production of expected end products, production of metabolic intermediate compounds, loss of co-reactants (i.e. oxygen, nitrate), biodegradation kinetics, and manipulation of the field site (such as adding nutrient) to determine a relative response (field controls). (Major 1991)

"Site data often include total petroleum hydrocarbon (TPH) as diesel and BTEX concentrations. BTEX concentrations are relatively low in diesel. Therefore, BTEX concentrations in both soils and groundwater from diesel spills are generally below the detection limit or regulatory criteria and are not reliable indicators of diesel contamination." (Custance 1992)

"General categories like...total organic carbon (TOC) or chemical oxygen demand (COD) are useful and inexpensive parameters for monitoring the progress of biodegradation" both in the lab and in the actual field implementation. (Woodward 1988)

The target waste should be characterized physically, chemically, and biologically. Chemical characterization using COD and TOC monitors the progress of biodegradation. (Canter 1990) TOC is a direct parameter for the carbon concentration, thus decreasing concentrations of TOC values will indicate mineralization of the organic contaminant. (Staps 1989) Biological characterization monitors the initial microbial consortium and changes in toxicity during biodegradation.

1.5.6 Bioremediation Costs

A survey on how bioremediation is being applied was reported by the Groundwater and Soil Remediation Program (GASReP 1990). Contaminated site sizes ranged from 90 to 25,000 square meters, the water table was generally 1 to 8 meters and hydraulic conductivity of subsurface sediments was from 10^1 to 10^{10} m/s. The kinds of hydrocarbon most frequently treated included aromatics, creosote/coal tar/PAH, and chlorinated aliphatics. Contaminants that were least likely to be treated using bioremediation included multi-ring cyclic hydrocarbons (greater than 4 rings). The bioremediation phase of the treatments lasted anywhere from 2 months to 3 years. Lighter hydrocarbons typically showed faster clean-up times and were reduced by 2 to 3 orders of magnitude.

The Canadian Petroleum Products Institute claims that in-situ

bioremediation can have removal efficiencies between 46% to 99% after a few months of treatment. Costs are said to be site-specific with initial equipment and installation costs of $30,000 to $150,000 (Cdn 1991 $) for a site of 500 to 2500 tonnes and operation/maintenance/monitoring costs of $10,000 to $50,000/year. "Overall costs are on the order of $15-$50/tonne." (Intera 1991)

Bioremediation is a particularly attractive remediation technology because of the potential it has to ultimately destroy organic contaminants at much lower capital and operating costs than many other technologies. Lower costs could be attributed to:

1. use of the natural soil environment as the treatment medium;
2. work performed by the indigenous soil microbial population; and
3. lower energy requirements.

2. SIGNIFICANT PARAMETERS OF BIOREMEDIATION

2.1 Enrichment

Many pilot projects and studies have been used to demonstrate that petroleum hydrocarbons in the form of diesel fuel are amenable to biological treatment with environmental enrichment. Laboratory studies to determine whether on-site soil microbes were present and capable of degrading contaminants under conditions conducive to biodegradation were performed by Mahaffey (et al 1991) and field evidence for biodegradation of organic compounds was compiled by McCarty (et al 1984). Previous work has also shown that petroleum hydrocarbons can be biologically degraded. (Sherman and Stroo 1989)

Studies were performed on accidental and experimental oil spills in Alberta, Alaska, and the N.W.T. under northern climatic conditions. Microbial populations (mixed cultures) capable of degrading crude oil were found at all the sites. (Westlake 1973) The need for oxygen as an electron acceptor and fertilizer (N, P) as a growth stimulant was reported by Hutchinson (1974) and Cook and Westlake (1974). "These investigations have shown that the normal indigenous flora in the soils that we have investigated have the capability of degrading crude oil if supplemented with fertilizer containing nitrogen and phosphorous. Inoculations with "bacterial cocktails" were not beneficial under these conditions." (Cook

and Westlake 1974) Other field and laboratory studies determined that the factors which influence the microbial utilization of crude oil include nutrient availability and the form that nutrient takes (i.e., fertilizer), temperature, and seeding with oil-utilizing bacteria. "The laboratory studies relating chemical composition of oil to biodegradability have shown that bacterial populations developed on high quality, crude oils have very little ability to digest low quality oils. However, populations developed on low quality oils can utilize high quality oils." (Cook and Westlake 1973)

Biodegradability studies carried out by Tabak (et al 1981) reported that monocyclic aromatics such as benzene, toluene, and nitrobenzene, exhibited significant biodegradation with rapid acclimation. Laboratory studies performed by Mahaffey (et al 1990) determined that the greatest degree of microbial contaminant degradation in heavily petroleum hydrocarbon contaminated soils occurred with enrichment by elevating oxygen levels and adding inorganic nutrients. Oxygen consumption was used as the indicator of aerobic microbial activity due to the fact that an oxygen demand is exerted during aerobic biodegradation of the contaminant (i.e., COD). No effect on biodegradation was observed with the addition of growth factor and surfactant.

Many successful applications of aerobic bioremediation systems were observed by Pheiffe (et al 1990) during their assessment of European contaminated soil treatment techniques. At one contaminated gasoline site where hydrogen peroxide was used as the oxygen source, the observed biodegradation rate was 10 mg C/kg/day. At another bioremediation site, the diffusion of organics from the soil particles was seen to be the rate limiting step. The conclusion of Pheiffe was that in-situ bioremediation could be useful when polishing an effluent to lower concentrations of contaminants. Biological techniques, including in-situ bioremediation of contaminated soil, in The Netherlands showed that in soil, a C:N:P ratio of 250:10:3 is considered optimal for biodegradation. They also reported that "there is much uncertainty about the efficacy of the addition of microorganisms to the subsoil and the possibilities of transporting bacteria through the soil" as "generally 95% of the soil population tends to adsorb on soil particles whereas only 5% can be transported". (Staps 1989) They also questioned the effect of adding detergents to soils to aid in solubilization of contaminant.

At an aviation fuel spill site in the U.S.A., Wilson (et al 1989) reported that almost all hydrocarbons had been removed at a field demonstration of infiltration wells injecting chemically amended water (380 mg/l ammonium chloride, 190 mg/l disodium phosphate and 190 mg/l potassium phosphate as well as

oxygen in the form of hydrogen peroxide).

A pilot bioremediation of petroleum contaminated soil resulted in a 73% decrease of BTEX compounds and an 86% reduction in PAH compounds with enrichment using nitrogen and a bacterial suspension. (Barnhart and Myers, 1990) In-situ bioreclamation of a service station in Montreal using enrichment with nutrients and oxygen caused total VOC to drop from 15,000 ppb to < 100 ppb after 6 months. (Tribe and Brown 1990) A pilot land-farm bioremediation site in Ontario (petroleum hydrocarbon contaminated soil), showed a 73% reduction in BTEX components, 86% reduction in total PAH with a 92% reduction of 2 and 3-ring compounds, 80% reduction in 4-ring compounds, and 65% reduction in 5-ring compounds with addition of nutrient and bacteria.

Site assessment at a site contaminated with nonvolatile petroleum hydrocarbons for 30 years, indicated that a significant number of hydrocarbon degrading bacteria existed and that their growth seemed to be limited by the oxygen and nutrient conditions in the soil. Bioremediation using enrichment techniques removed 94% of the contamination, the vapour extraction system volatilized 2%, and the phase-separated hydrocarbon recovery removed 4%. BTEX was 420 mg/kg initially and by the end of the project was below detection limits. (Nelson 1993) Thus if an indigenous population is

small, it can be made larger by a treatment regime based on the enrichment approach of maintaining nutritional and environmental conditions that favour the active microbes.

In the remediation techniques used to cleanup the 1989 Exxon Valdez oil spill, bioremediation was the most successful, accelerating recovery fivefold where fertilizer was added to encourage indigenous hydrocarbon degraders. Indigenous and hydrocarbon degrading bacteria were also found to be present at another petroleum hydrocarbon contaminated site in the U.S.A.. When enrichment using oxygen, nitrogen, and phosphorous was supplied, a 75% removal of hydrocarbon was noted. (Galaska et al 1990)

A laboratory treatability study conducted by McFarland (1991) on an acclimated soil (previously contaminated with petroleum hydrocarbons) and on an unacclimated soil, showed that with enrichment by addition of manure and pH adjustment, PAH degradation was stimulated and occurred at a faster rate in acclimated soils. Biological activity in a treatability study conducted by Sherman and Stroo (1989) was monitored with respect to oxygen uptake rates, COD reduction and bacterial cell counts. Contaminated soil receiving nutrient, a growth inducer and inoculum (acclimated petroleum hydrocarbon degraders) showed the greatest rate of degradation.

microbes, (3) no enhancement with addition of enriched organisms, and (4) no inhibitory effects of crude oil at concentration of 10,000 mg/l. This study also found that "Inoculation "seeding" experiments have repetitively demonstrated that specific cultures of oil-degrading bacteria fail to enhance the hydrocarbon degradation capability of natural environments because they typically disappear from dominant microflora". (Tabak et al 1990)

Lindstrom (et al 1991) applied fertilizer (N:P ratio of 10.6:1) to determine if natural microbial degradation of stranded oil could be enhanced. Results showed increases in in-situ numbers of hydrocarbon degraders and increased mineralization potential along with decreases in dissolved oxygen levels.

2.2 Microbial Ecology

The microorganism population in surface soils includes bacteria, actinomycetes, fungi, algae and protozoa which are biochemically complex and capable of producing unique enzymes with the ability to degrade many organic wastes. (Ross and Phung 1982)

"Most bacteria are quite small, being rods, cocci, or filaments that range from 0.5 to 1 micrometer in diameter." (O'Leary 1989) More than 30 genera of bacteria, filamentous

fungi and yeast are known to degrade oil. Microbial degradation of petroleum hydrocarbons involves more than 200 species of bacteria, yeast, and fungi including Acinetobacter, Arthrobacter, Mycobacteria, Actinomycetes, Pseudomonas (bacteria) and Cladosporium and Scolecobasidium (yeasts). (Cheremisinoff 1987) Removal of organics is also influenced by the amount of viable biomass. (Hamoda 1987)

In an active microbial community, biomass is constantly transformed into carbon dioxide, humic material and microbial biomass as shown in **Figure 5.**

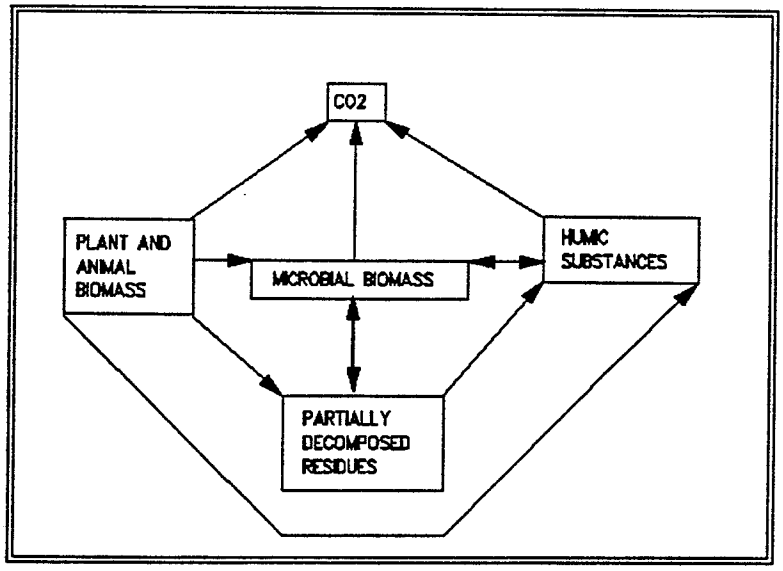


FIGURE 5: SOIL RECLAMATION PROCESSES (Tate and Klein 1985)

A question that arises when oil is spilled onto soil is whether or not oil-utilizing microorganisms are present. Case histories supporting stimulation of indigenous microorganisms to degrade organic contaminants by adjusting certain physical and chemical conditions are reported by Piotrowski (1991). Addition of acclimated culture to PAH contaminated soil increased biodegradation rates in the lab. (Srivastava 1990) Viable and hydrocarbon-degrading bacteria were found in all soil samples from a fuel-oil contaminated site. (Kampfer et al 1991)

Parkinson (1973) states that the overall effect of crude oil on the indigenous soil microorganisms is that "certain microorganisms will be killed or inhibited by the crude oil, and certain chemo-organotrophic microorganisms will increase in numbers and activity due to their capability to metabolize hydrocarbons". "The absence of adequate environmental conditions and not of suitable microorganisms is probably more important in determining the rapidity and extent of petroleum degradation in soil." (Hornick 1983) However, Yong (1987) feels that "Fresh hydrocarbon spills in non-preconditioned anaerobic soils may not undergo self-decontamination even if large amounts of nitrogen and phosphorous nutrients are added because of the absence of non-adapted microbial species".

Many researchers have found that oil-inundated soil was the

best source of hydrocarbon-oxidizing microorganisms. Hodge (et al 1991) used soil previously exposed to petroleum hydrocarbons as a microbial seed. This soil was found to have a very high rate of hydrocarbon degradation. "Some species of soil bacteria and petroleum-degrading microorgansms have been reported to be capable of utilizing benzene, ethylbenzene, and toluene as a sole carbon source and the metabolic pathways involved in the microbial oxidative degradation of these compounds have been established." (Tabak 1981) Pseudomonas putida F1 was shown to initiate oxidation of toluene through the enzyme system of toluene dioxygenase. The aromatic substrates (benzene, toluene, and ethylbenzene) were also shown to have been oxidized to hydroxylated products cis-1,2,dihydrodiol and cis-2,3-dihydrodiol. (Zylstra 1988,1989)

Kampfer (et al 1991) conducted a microbiological characterization of a fuel-oil contaminated site and showed that "all groundwater and soil samples contained methylotrophic, denitrifying, sulphate reducing, anaerobic, and hydrocarbon degrading bacteria". Both gram-positive and gram-negative bacteria were common to all soil samples. A total of 39 bacteria isolated from one soil sample were able to grow on fuel oil as their sole carbon source.

Microbial assessment by plate count techniques could introduce a culturing bias. By enumerating hydrocarbon-degrading

bacteria, information can only be provided on the presence or lack of bacteria, not on their activity or non-activity. The use of media to culture the biomass might also underestimate the active biomass by repressing the growth of oligotrophic microorganisms which survive and grow at low organic substrate concentrations. Plate counts of all naturally occurring bacteria are considered meaningless as it is the active bacteria we are interested in. (Kampfer 1991)

"Bioremediation using indigenous bacteria is desirable." (EPA 1991) "The efficacy of microbial inoculants is also in question. Little or no data is provided on how effective the added microorganisms were compared to the stimulated indigenous species." (Major 1991)

Adaptation of a microbial community requires a continuous culture with a low specific growth rate. This condition has the advantage of "selecting for organisms with good scavenging capacity for organic carbon". (Grady 1985) The use of glucose/glutamic acid as an auxiliary carbon source allows the growth of organisms that are incapable of degrading the compound of interest but "in the early stages of culture development, before an organism may be present that can attack the target substrate, this provides continuous exposure of many organisms to it". (Grady 1985) Thus the evolution of pathways and then enzymes can be produced in a community of

microorganisms. "It is as if individual bacteria in a community have access to a large library of outside information which endows them with extraordinary adaptive capability." (Grady 1985)

Parkinson (1973) reported that a northern Canadian soil contaminated with crude oil showed increases in soil respiration and bacterial numbers. Soil temperature, moisture, and fertilizer treatment (N,P) were seen to influence microbial degradation of crude oil. Fertilizer treatment of the contaminated soil showed the largest amount of microbial activity. The role of common soil fungus in the biodegradation of crude oil was also explored.

Factors affecting microbial fate in the subsurface include: 1. the nature of the organism; 2. climate (for example, rainfall); and 3. the nature of the soil. (Bitton and Gerba 1984) van Elsas and Trevors (1991) reported that the possible risk of genetically engineered microorganisms is essentially zero as they are rapidly degraded in the environment and gene transfer is negligible. "The only impact on the environment is probably some stimulation of microbial turnover processes at the expense of the extra source of C, N, P, S and some trace elements added in the form of cellular biomass."

The survival of the specific enriched microbial population in

contaminated soil would be dependent on a continuous amount of the original enriching substrate. If the contaminant was adsorbed, the enriched microbial population might continue to survive and thrive on the slowly desorbed materials. (Kaufman 1983)

2.3 Volatilization

Two basic mechanisms contribute to the observed loss of contaminant: biodegradation and volatilization. It is difficult to determine the rate of mass loss due to volatilization. Rifai (1988) reports research by Chiang (et al 1987) that calculates the mass loss due to volatilization using Henry's law for benzene to be 5% of the total mass at one site.

The relative importance of volatilization and biotransformation was assessed by Bouwer (1989) and Blackburn (1985). Biotransformation with enrichment was found to be the major removal mechanism in both cases. In a strip-pit pond contaminated with alkylbenzenes and chlorobenzene (Bouwer 1989), it was discovered that volatilization rates exceeded the natural biotransformation rates, but enhanced biotransformation rates (with addition of nutrient) were more than an order of magnitude faster than volatilization rates. This study also demonstrated that indigenous microorganisms were capable of effecting bioremediation of the aromatic

contaminants as long as adequate oxygen was supplied. Kincannon (et al 1983) reported that benzene and methylene chloride were principally removed by biotransformation in laboratory activated sludge reactors. Similarly Blackburn (et al 1985) showed that 82% of toluene was biotransformed and 12% was volatilized. Sorption was found to be of minor importance.

Park (et al 1990) investigated the influence of abiotic losses and volatilization on 14 PAH (polycyclic aromatic hydrocarbon) compounds and found that significant volatilization occurred in the 2-ring compounds (between 14 and 30%) whereas volatilization of all the other higher-ringed compounds was less than 0.1%. Results of this study showed that PAH compounds were destroyed and detoxified by biological transformation (as the major removal mechanism).

Removal mechanisms for toxic priority pollutants were investigated in an activated sludge study by Kincannon (et al 1983) who found that the major removal mechanism for benzene was biodegradation (89%) followed by stripping (16%). They also noted that when biodegradation was the major removal mechanism, the effluent TOC achieved was much lower than from stripping alone. Research on evaporation of gasoline on beach sand showed that about 60% of the gasoline fraction evaporated after 1 hour. (Bergueiro et al 1989)

2.4 Emulsifiers

An aquifer or soil contaminated by a limited soluble organic involves complex processes including vertical and lateral migration (unsaturated zone), trapping by capillary forces (unsaturated and saturated zones) and adsorption. When trapped by capillary forces as discrete drops (sometimes called ganglia), hydrocarbons may extend over several pores. "Once trapping occurs, mobilization of individual drops....is usually not possible for the water velocities achievable in practicable pump and treat operations." (Bury and Miller 1993) Large molecular weight petroleum hydrocarbons have a tendency to absorb in the pores of soil aggregates and adsorb to soil particles. Because these molecules aggregate, they become too large to be available to the microorganisms for growth. Thus the degree to which petroleum hydrocarbons are degraded is a function of their degree of solubilization.

The key components of emulsifiers are one or more surface-active agents or surfactants which contain molecules with hydrophillic and lipophilic (oil compatible) or hydrophobic parts. (Hall 1989) Surfactants (emulsifiers) can lower the capillary forces and thus mobilize these trapped drops as well as solubilize the trapped organics into micelles. When a surfactant solubilizes the petroleum hydrocarbons, the molecules may then become more available for microbial utilization. Thus, applying an emulsifier reduces the

oil/water interfacial tension and allows the oil to break into droplets.

TABLE 2: SOLUBILITY OF PETROLEUM HYDROCARBONS Merck Index

Benzene C_6H_6	Soluble in 1430 parts water
Toluene C_7H_8	Very slightly soluble in water
Xylene C_8H_{10}	Practically insoluble in water
Ethylbenzene $C_6H_5C_2H_5$	Practically insoluble in water

The solubility of petroleum hydrocarbons is low in water and decreases as the molecular weight or number of rings increases. Solubility of a contaminant is very important in determining the rate of dispersal and exposure to microorganisms. Low solubility compounds limit microbial degradation because these compounds are in effect unavailable to the microorganism as food.

There are three mechanisms discussed in the literature, by which bacteria take up sparingly soluble substrates:

1. bacterial cells interact with aqueous dissolved hydrocarbon;
2. cells have direct contact with hydrocarbon drops which are

larger than the cells; and

3. cell interact with solubilized hydrocarbon which is much smaller than the cells.

There is confirmation that solubilization of hydrocarbons in small micelles of surfactants greatly increased the rate of degradation. "Adding surfactants not only facilitates emulsification of the oil, with a resulting increase in interfacial area, but also provides micelles for solubilization." (Bury and Miller 1993) They also stated that the surfactant itself should not be a threat to the environment (that is, it should be readily biodegradable).

In order to improve the mass-transfer limitation of petroleum hydrocarbons, two techniques were developed by Srivastava (et al 1990), which included the use of bioemulsifiers and chemical emulsifiers to solubilize PAH's. Their research showed that bioemulsifiers were the most effective in freeing PAH's from soil because they enhanced solubility and increased the degradation rate. They also determined that chemical emulsifiers increased the solubility of PAH's by several orders of magnitude but were not as effective as the bioemulsifiers and that greater microbial growth was evident when enrichment with emulsifier was used. Green (1989) found that emulsifiers increased the rate but not the extent of biodegradation of petroleum hydrocarbons.

Surfactant addition was shown to significantly increase the aqueous phase concentration of biphenyl and anthracene demonstrating the feasibility of surfactant-aided restoration of contaminated aquifers. (Vigon and Rubin 1989)

A treatability study conducted by Seech (1992) on a soil contaminated with heavy oils, asphalt and coal tars, using organic soil amendments, alone or combined with a surfactant showed that biodegradation was enhanced using fully-contaminated soil, an organic amendment (10%) and a surfactant.

A study conducted by Aronstein (et al 1991) determined that low concentrations of surfactants may promote mineralization of sorbed aromatic compounds in contaminated soils by increasing desorption and biodegradation. Surfactants at high concentrations may inhibit the microorganisms that have the capacity to metabolize the polluting compounds.

Wunderlich (et al 1992) experimented with surfactant use in the remediation of groundwater contaminated with dense nonaqueous-phase liquids and found that organic contaminant solubility increased by 3 orders of magnitude.

2.5 Temperature

The biodegradation of petroleum hydrocarbons is slowed under

low temperature conditions. Thorpe and Hellenbrand (1987) calculated that the theoretical biodegradation rate for crude oil in beach sand at 0 °C was 8.4 mg/m^2/day and at -5°C was 4.2 mg/m^2/day from data which gave rates of 40 mg/m^2/day at 15°C, 18 mg/m^2/day at 10°C, and 13 mg/m^2/day at 5°C.

The data strongly suggest that the environmental parameter of temperature may have a major influence on petroleum hydrocarbon degradation. "Temperature is another factor which determines a population's oil-utilizing capabilities. Enrichments obtained at 4°C were able to metabolize that same oil at 30°C but those obtained at 30°C had little effect on the same oil at 4°C." (Cook and Westlake 1974)

2.6 Oxygen

The importance of oxygen to biotransformation is described by Grady, Jr. (1985, 1989) in reviews of the microbiological basis of biodegradation and oxygen uptake curves. In a modelling study conducted by Wu (et al 1990), the rate of biodegradation of contaminants in soil was affected by oxygen supply as well as resistance of the contaminant to migration within the pore network.

In an enhanced in-situ biodegradation study, Taylor and Jaffe (1991) determined that "increasing the oxygen concentration in the injection water, increasing the well-pumping rate, and

introducing oxygen through multiple injection wells all result in improved levels of bioremediation without causing excessive biofouling".

A major conclusion in a case study by Mahaffey (et al 1991) was the necessity of an oxygen delivery system as well as more studies to determine the correlation between oxygen and contaminant removal. A feasibility testing program to develop a site-specific bioremediation strategy was initiated.

In the first phase of the study, a microbiological evaluation was performed to determine if indigenous microorganisms were present which could degrade the waste, which was composed of oil, phenol, PCP, PAH, and other petroleum hydrocarbon fractions. Mahaffey (et al 1991) concluded that: (1) contaminant concentration could vary within a site; (2) indigenous microorganisms capable of degrading the toxics were present; (3) removal efficiency was related to location; (4) total contaminant degradation appears to be related to total contaminant concentration; (5) biodegradation was not increased with addition of growth factors or surfactants; and (6) total bioremediation of a site may involve injection of microorganisms to redistribute biodegradation potential at a site.

The second phase of the study involved the development of

several in-situ bioreclamation processes which included: (1) surface bioreclamation (land farming); (2) surface bioreclamation after free product recovery; and (3) subsurface bioreclamation following soil washing. Options (2) and (3) were seen to have the potential for viable, cost-effective bioremediation.

2.7 Summary

"One of the most significant mechanisms acting to destroy organic chemicals in the environment is biodegradation." (Dang et al 1989) During biodegradation, organic compounds are ultimately destroyed when they are used as substrate for biomass growth.

Soil is a heterogeneous and dynamic environment in which the growth and activity of microorganisms is affected by soil factors and, in the case of petroleum hydrocarbon spills, waste factors.

Soil factors would include:

1. water;
2. temperature;
3. soil pH;
4. aeration or oxygen supply;
5. available nutrients; and
6. soil texture and structure.

Waste factors would include:

1. chemical composition of the waste;
2. physical state;
3. its C:N ratio;
4. water content and solubility;
5. chemical reactivity and dissolution effects on soil organic matter;
6. volatility;
7. pH;
8. biochemical oxygen demand;
9. chemical oxygen demand; and
10. nature of the indigenous microflora. (Parr et al 1983)

Microbial degradation is a significant process in the dissipation of many organic chemicals in soil but volatilization, adsorption, and leaching (abiotic factors) may limit the availability of the contaminant to biodegradation.

"Biodegradation of organic chemicals by organisms includes enzymatic attack by dehalogenation, dealkylation, hydrolysis, oxidation, reduction, ring cleavage, and condensation reactions." (Ambrose et al 1988) This chemical degradation can occur metabolically or through co-metabolism (non-utilization of the organic chemical for growth substrate).

Limitations of bioremediation could include:

1. clogging of screens with microbial growth;
2. maintenance of stable oxygen;
3. fluctuations in water table which may cause a release in bound hydrocarbons;
4. production of bioemulsifying agents;
5. clay units which act as hydrocarbon sinks;
6. precipitation of phosphorous;
7. fuel residuals in vadose zone inaccessible to the passing water which might include nutrient and oxygen; (Major 1991) as well as,
8. sensitivity to toxins; and
9. difficulty in containing volatile organic compounds.

Difficulties with addition of inorganic nutrients for in-situ bioremediation could include:

1. interaction of ions with the subsurface components;
2. ammonium ion binding to mineral surfaces by cation exchange reactions; and
3. phosphates precipitating out as metal salts. (Morgan and Watkinson 1992)

When phosphorous precipitates out in groundwater becoming an insoluble salt, clogging could occur as well as reduction in biological activity due to the reduction of available phosphorous.

Benefits of using bioremediation could include:

1. no excavation;
2. complete mineralization (to carbon dioxide and water);
3. minimal mechanical requirements; and
4. lower energy requirements and thus lower overall costs.

3. OBJECTIVE

Batch activated sludge treatability studies utilizing petroleum hydrocarbon contaminated soils (from three different sites in Manitoba) were conducted to determine:

(1) initial indigenous biological activity in hydrocarbon-contaminated soils;

(2) limiting factors of microbiological growth by investigating nutrient addition, chemical emulsifiers, and co-substrate;

(3) removal efficiencies; and

(4) temperature effects.

4. MATERIALS AND METHODS

4.1 Experimental System

The experimental system consisted of calibrated batch reactors as shown in **Figure 6.**

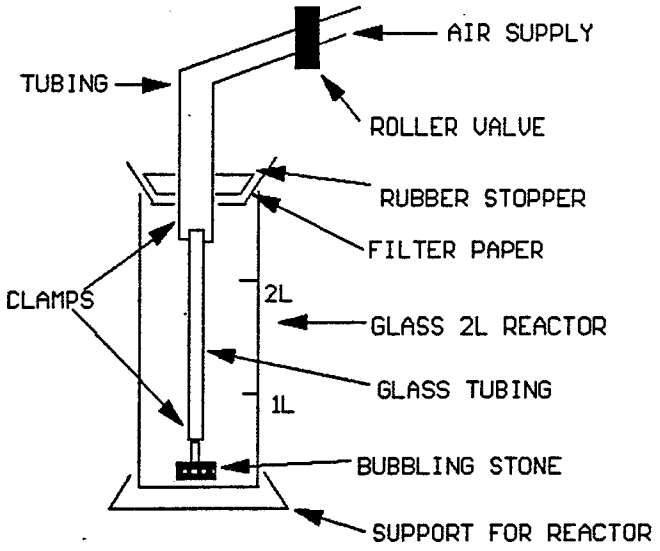


FIGURE 6: BATCH REACTORS SYSTEM

A cover was used to minimize evaporation. Air flowed through a distribution system that included controls to a diffuser which kept solids in suspension during aeration.

4.1.1 Brandon - INITIAL PHASE

The experimental system consisted of seven 2L glass batch reactors at room temperature, set up as shown in **Figures 6** and **7.** Each of the batch reactors consisted of a 2 litre capacity glass tube. The two litre volume reactors were calibrated to measure 1L and 2L volumes. The top consisted of filter paper and a rubber stopper with an inlet port on top. This cover was used to minimize evaporation.

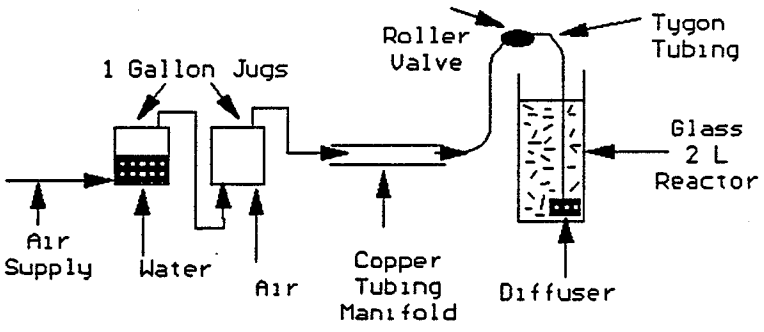


FIGURE 7: EXPERIMENTAL SYSTEM

Air flowed through two one-gallon jugs, (the first jug contained water, the second jug contained air, in order to eliminate surge) and then to a distribution system which consisted of a copper tube with several outlet ports. Outlet

ports had tygon tubing attached with a roller valve to control flow to each reactor. A glass tube with bubbling stone completed the air distribution to each reactor. This diffuser kept solids in suspension during aeration.

Two handfuls of contaminated soil were placed in each reactor, and tap water was added to the 2-litre mark. Reactor and source of soil is shown in **Table 3**. Each reactor had 0.1 ml diesel oil added and was stirred well. This soil-water slurry was aerated gently for 24 hours.

After 24 hours, the air was turned off and each batch reactor was allowed to settle for one hour. Most of the supernatant was pumped out using a peristolic pump and the soil/water slurry at the bottom was thrown out. Each reactor was rinsed with tap water and then one litre of the supernatant was returned to be used as seed for the batch activated sludge reactors. A sample size of approximately 200 ml was saved from the excess supernatant for initial laboratory analyses.

The reactors were then fed C:N:P as detailed in the schedule of **Table A-1, Appendix A**, and tap water was added to the 2L mark. On a daily basis, the following procedure was utilized:

(1) settling one hour,

(2) removing effluent 200 ml sample,

(3) decanting supernatant to the one litre mark,

(4) feeding, and

(5) filling reactor to the 2 litre mark with tap water.

One hour after feeding, a sample of approximately 7 ml was removed from the settled reactor at the 1L mark using a 10 ml volumetric pipette.

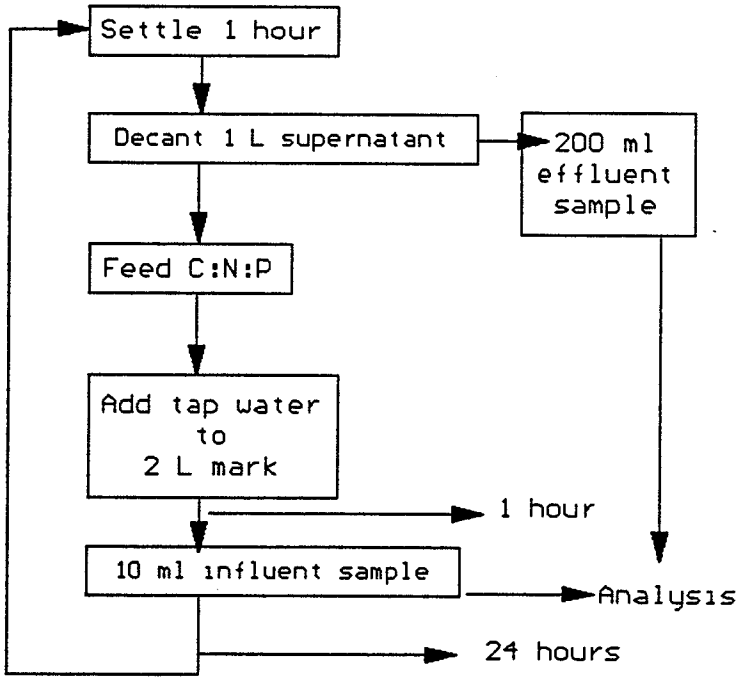


FIGURE 8: FEEDING FLOWCHART

4.1.2 Brandon - PHASE I

From this intial phase, the reactors most likely to succeed at bioremediation were selected (1-0-A, 2-6-C, 1-6-F). From these three reactors, eight 2L batch reactors were run at room

temperature as detailed in the INITIAL PHASE. The feed schedule during PHASE I is detailed in **Table A-2, Appendix A.**

4.1.3 Brandon - PHASE II

On Day 49, two of the reactors from PHASE I were moved to a cold chamber and two were left at room temperature. The activated sludge from all the other reactors in PHASE I was amalgamated for PHASE III.

The experimental system consisted of 2 batch reactors set up in a cold chamber and was consistent with PHASE I of the experiment except for the air distribution system. Air flowed through a flowmeter and then an erlenmeyer flask before being split into 2 lines, one line to serve each reactor. A thermometer measuring degrees Celsius was installed in the cold chamber.

On day 49 of PHASE I of the experiment, Reactors 1-0-B and 2-6-C were moved to the cold chamber. The initial temperature was 24 ^{o}C. This was decreased gradually to 12 ^{o}C by day 69. Sampling remained the same as in PHASE I.

4.1.4 Brandon - PHASE III

PHASE III of the experiment consisted of three 20L reactors and one 25L reactor at room temperature. The reactors were plastic pails with covers to minimize evaporization. The

activated sludge from the 2 litre reactors in PHASE I was utilized for the large reactors and no supernatant was wasted until the reactors were filled to the 20 and 25L mark, respectively.

The procedure of feeding included:

(1) one hour settling time,
(2) decanting 5 litres supernatant from which an effluent 200 ml sample was taken,
(3) feeding, and
(4) filling to the mark with tap water.

One hour after feeding, a 50 ml influent sample was taken which was allowed to settle 30 minutes. The supernatant from this sample was used for all influent COD samples. The feed schedule is outlined in **Appendix A, Table A-3.**

An overview of all phases of Brandon is detailed in **Figure 9.**

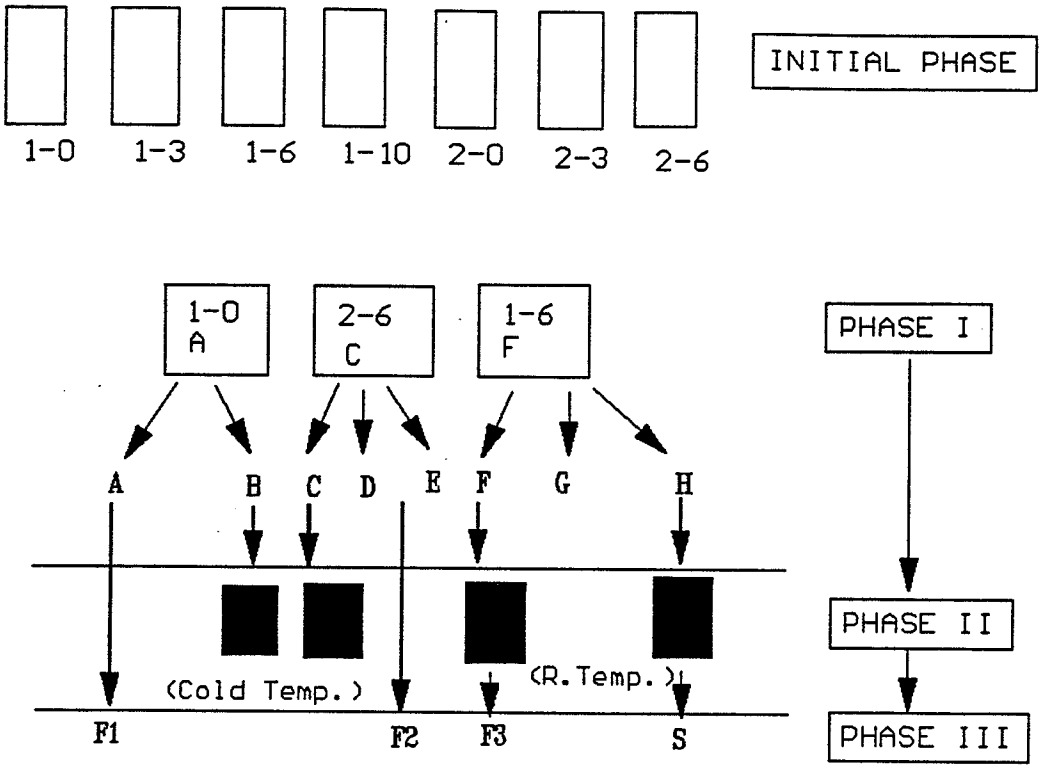


FIGURE 9: OVERVIEW OF BRANDON

4.1.5 Bakers Narrows

The experimental system was essentially the same as used in PHASE I (Brandon) for one reactor. Soil from a sample area about 6 feet away from Bore Hole #10 and 0.5 meter deep was used as the seed.

4.1.6 Pukatawagan

The experimental system was essentially the same as used in PHASE I (Brandon) for three reactors. Three bagfuls of contaminated soil (A,B,C) were received the beginning of July,

1992. Three 2-L reactors were set up, one reactor for each sample of contaminated soil (A,B,C). Two handfuls of contaminated soil were placed in each reactor, and tap water was added to the 2-litre mark. Each reactor had a few drops of diesel fuel added and was stirred well. All other methodology was essentially the same as in PHASE I (Brandon).

4.2 Enrichment

Each 2L batch reactor was given: (1) nutrient, substrate, and co-substrate in a ratio of 100 C: 7 N: 1 P; and (2) chemical emulsifier in order to lower surface tension and allow oil to be distributed in the liquor; then (3) filled to the 2 litre mark with tap water and aerated gently. See flow chart, **Figure 8.**

Each 20L and 25L reactor was finally given only nutrient and substrate in a ratio of 100 C: 7 N: 1 P. Initially, a co-substrate and chemical emulsifier were added as required. See **Table A-3** in **Appendix A.**

Nutrient ratio was determined from "Groundwater and Soil Contamination Remediation" (McCarty 1990), p. 49, "...with an example of the requirements for remediating a ground water contaminated with 1000 gal (6000 lb) of hydrocarbon, say, gasoline.....Along with this, about 875 lb of nitrogen (ammonia or nitrates) would need to be included."

Confirmation was found in "Biological Treatment of Hazardous Waste" (Torpy 1989). "Generally, the range of carbon:nitrogen:phosphorous is between about 100:10:1 to 100:10:5 depending on the type of treatment used, and the phase (liquid or solid) in which the contaminant is found."

A co-substrate consisting of glucose and glutamic acid was considered necessary to acclimatize the indigenous seed. From Standard Methods (1989), p. 5-6, "In general, for BOD determinations not requiring an adapted seed, use a mixture of 150 mg glucose/L and 150 mg glutamic acid/L as a "standard" check solution. Glucose has a high and variable oxidation rate but when it is used with glutamic acid, the oxidation rate is stabilized and is similar to that obtained with many municipal wastes." From this information, a 2L stock solution of dextrose and glutamic acid was made containing 15 mg dextrose and 15 mg glutamic acid.

A 1000 mg/L nitrogen nutrient solution was made from 3.82 grams ammonium chloride dissolved in one litre tap water. A 100 mg/L phosphorous nutrient solution was made up with 50% of phosphorous from potassium phosphate monobasic (0.2195 g) and 50% of phosphorous from potassium phosphate dibasic (0.281 g) dissolved in one litre of tap water. The substrate was diesel fuel.

Chemical emulsifiers used included: a phosphate dishwasher detergent (Cascade); Stearic Acid; Sodium Lauryl Sulphate; and a phosphate-free dishwasher detergent (Biodish). A chemical emulsifier was necessary to reduce surface tension and thus the possibility of air stripping. Secondary reagents were also prepared for ammonia analysis, COD, TOC, nitrate, nitrite, total phosphorous and orthophosphorous. See Standard Methods (1989).

Nutrient addition to the reactors was accomplished every few days as detailed in **Tables A-1, A-2, A-3** in **Appendix A**, **Table B-1** in **Appendix B**, and **Table C-1** in **Appendix C.**

4.3 Analytical Monitoring/Methods

An effluent supernatant sample was taken from the settled activated sludge reactors (1 hour settling time) before each feed addition. All analyses were performed on this settled unfiltered supernatant, with the exception of MLSS (mixed liquor suspended solids) and microscopic monitoring which were performed on the mixed liquor before the reactors were settled.

The treatment performance of the biological systems was monitored with respect to: COD (Chemical Oxygen Demand), ammonia, orthophosphate, and TOC (Total Organic Carbon). Each batch reactor was also monitored with respect to: pH, and

occasionally dissolved oxygen, temperature, total phosphate, nitrite, nitrate, and TKN (Total Kjeldahl Nitrogen). The organic removal efficiency was assessed by chemical oxygen demand (COD) measured daily and total organic carbon measured two to three times per week. Well-mixed liquor was analyzed under the microscope regularly.

All analyses were performed according to Standard Methods (1989) as follows:

1. **Chemical Oxygen Demand**; Closed Reflux Colorimetric Method,
2. **Total Organic Carbon**; Persulfate-Ultraviolet Oxidation method,
3. **Ammonia Nitrogen**; Semi-Micro Kjeldahl Method with titration to the end point using 0.01 N HCl titrant and Boric Acid indicator,
4. **Total Kjeldahl Nitrogen**; Semi-Micro Kjeldahl Method,
5. **Nitrite/Nitrate**; Automated Cadmium Reduction Method,
6. **MLSS**; Total Suspended Solids Dried at 103°C - 105°C,
7. **pH**; Electrometric Method,
8. **Orthophosphate**; Automated Stannous Chloride Method,
9. **Total Phosphate**; Automated Stannous Chloride Method.

In PHASE III and for BAKERS NARROWS, samples were stored before analysis with the exception of COD. Phosphate samples were frozen for up to one month. Nitrogen samples were acidified to pH 2 and stored in the refrigerator for up to one

month. TOC samples were acidified with one drop H_2SO_4 (sulfuric acid) and analyzed within 3 days. pH and microbiological analyses were conducted immediately.

All samples taken for the PUKATAWAGAN study were not refrigerated or acidified; all analyses took place immediately.

4.4 Other Materials

Other material required for this experimental study included: siphon tubing; 1L and 2L volumetric flasks; 10 ml volumetric pipettes; 50 ml beakers; analytical balance; microscope; clamps; standard laboratory equipment for COD, nitrite, nitrate, ammonia, TKN, pH, dissolved oxygen, temperature, TOC, orthophosphorous and total phosphorous; as well as reagents.

5. RESULTS

5.1 Brandon

Appendix A presents results of all the laboratory analyses conducted during 95 days on 8 - 2L batch reactors and for 500 days on 3 - 20L batch reactors at room temperature, as well as 2 - 2L batch reactors in the cold chamber.

Table 3 shows reactors and the source of the contaminated soil for the initial trial. **Table 4** displays the results of

laboratory analyses on all the initial samples. **Table 5** presents reactors and source of contaminated soil for PHASE I.

TABLE 3: REACTOR AND SOURCE OF SOIL - INITIAL TRIAL

Source	Reactor	Depth (feet)
Site 1	TP1-0	0
	TP1-3	3
	TP1-6	6
	TP1-10	10
Site 2	TP2-0	0
	TP2-3	3
	TP2-6	6

TABLE 4: LABORATORY ANALYSES - INITIAL SAMPLES - DAY 0
(expressed as mg/l except for pH)

	1-0	1-3	1-6	1-10	2-0	2-3	2-6
NH_3-N	0	0	0	0	0	0	0
NO_2	0	0	0	0.04	0.03	0.05	0.05
NO_3	0	0	0	0.31	0.07	0.4	0.2
COD	70	130	120	175	75	195	180
pH	8.2	8.1	8.0	7.9	7.8	7.4	7.7
Ortho P	0.2	0.1	0.15	0.2	0	0.25	0.35
Total P	0.6	0.2	0.15	0.2	0.1	1.7	0.6
TOC	24.58	46.60	42.80	77.27	22.89	94.74	84.85

TABLE 5: REACTOR AND SOURCE OF SOIL - PHASE I

Reactor #	Site	Depth (feet)
1-0-A	TP1	0
1-0-B	from 1-0-A, Day 8	-
2-6-C	TP2	6
2-6-D	from 2-6-C, Day 8	-
1-6-F	TP1	6
1-6-E	from 1-6-F, Day 8	-
1-6-G	from 1-6-F, Day 17	-
1-6-H	from 1-6-F, Day 17	-

5.2 Bakers Narrows

Appendix B presents initial results of laboratory analyses conducted during 250 days for 2 - 2L reactors, one at room temperature (22 °C) and one cooled down from 22 °C to 5 °C. **Table 6** presents temperature data for Reactor BN10 - Cold.

TABLE 6: TEMPERATURE DATA - REACTOR BN10 - COLD

Date	Temperature (°C)
October 23	17
26	16
November 3	14
23	9
31	7
December 5	6
21	5

Difficulties were encountered with keeping the refrigerator at a constant temperature (the unit froze in November and warmed up to room temperature in December); on December 5, Reactor BN10 - Cold was placed in a HAAKE K Thermal Unit at a constant temperature of 5 °C.

5.3 Pukatawagan

Analytical results for all laboratory analyses are shown in **Appendix C** for Reactors A, B, and C.

6. DISCUSSION

6.1 Brandon

In the INITIAL PHASE, previous to PHASE I and lasting 30 days, seven 2L reactors (TP1-0, TP1-6, TP2-0, TP2-3, TP2-6, TP1-3, TP1-10) were given 77 mg ThOD (theoretical oxygen demand) daily from all sources of carbon (See **Table A-1**). Nitrogen and phosphate were also given in a ratio of 100:7:1. The ThOD from the dextrose/glutamic acid solution was decreased by 3 mg/l and the ThOD from the diesel fuel was increased daily until all the ThOD came from the diesel fuel. At this time, it was observed that a black, oily scum was floating on top of most of the seven reactors. Thereafter, until the end of this trial run, half of the ThOD was from the dextrose/glutamic acid and half of the ThOD was from the diesel oil.

For the first eight days during this INITIAL PHASE, all

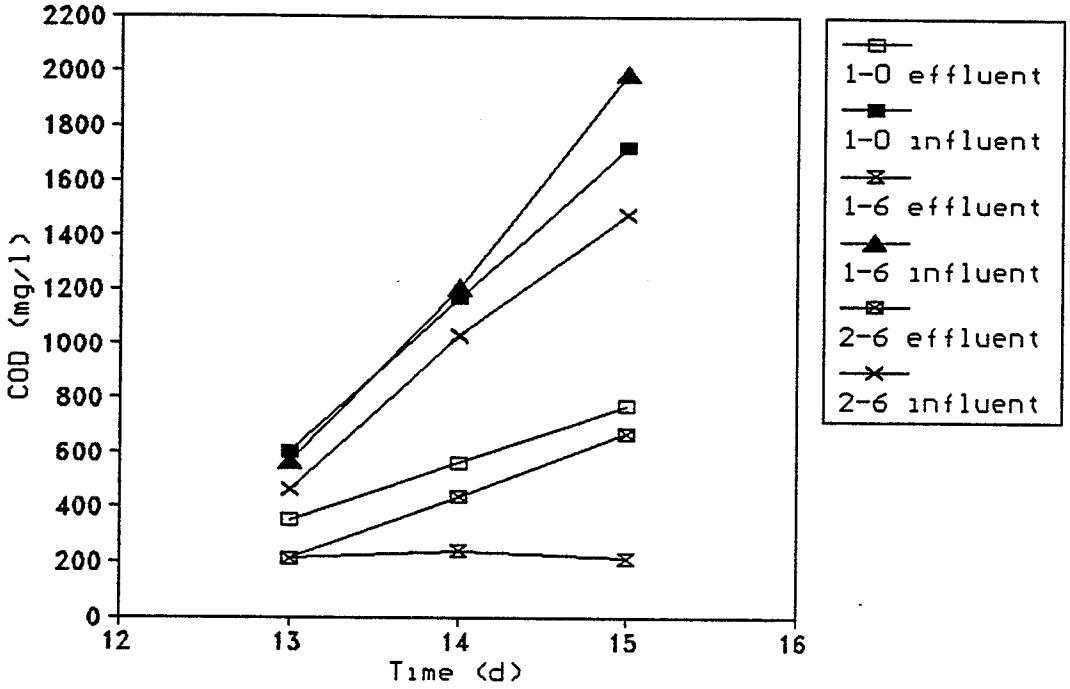
reactors were monitored with respect to: ammonia-nitrogen, nitrate, nitrite, chemical oxygen demand, pH, total phosphate, orthophosphate, and total organic carbon.

As shown in **Tables A-4** through **A-10**, the pH varied from 6.3 to 7.5. Nitrite was initially determined to be about 0.03 mg/l in all the samples but was not detected after the fifth day. Ammonia-nitrogen was 3 to 5 mg/l initially and decreased gradually after 5 days, measuring 1 to 2.5 mg/l in all the reactors. Total phosphate increased gradually, however the orthophosphate decreased slightly during this initial time period.

By day 13, COD sampling was performed on the influent (30 minutes after nutrient addition to reactor) and the effluent (24 hours after nutrient addition). **Graph A-1**, COD (mg/l) vs. Time (d) for Reactor 1-0, shows that COD removal was approximately 50% at this time. Effluent COD started increasing on Day 14 until the reactors showed no removal (i.e., COD influent = COD effluent). It is clear during this initial phase that COD removal was occurring before the reactors crashed.

Graph 1 shows total COD versus time. From this graph and the slopes of the lines, the three reactors with the highest COD removal rate were selected as the most likely to succeed in

bioremediation for PHASE I. Three reactors were initially started: 1-0-A; 2-6-C; and 1-6-F.



GRAPH 1: TOTAL COD vs TIME - INITIAL PHASE

6.1.1 PHASE I

Three batch reactors were begun in PHASE I. After day 5, the number of reactors was doubled by decanting the supernatant into another reactor instead of wasting it. Finally, eight reactors were operating. See **Figure 9**, page 53, and **Table 5**, page 60.

All reactors were given nutrient, co-substrate, substrate, and chemical emulsifier. The process of settling one hour,

removing sample for analysis, and aerating 24 hours was repeated until percent removal of COD was greater than 50%. When COD removal was 50% or more, the reactors were fed again. See **Table A-2** for feed schedule. At least 50% of the carbon source came from substrate and chemical emulsifier. On subsequent feed days, one litre of the supernatant was wasted from each reactor, with a 200 ml sample saved for analysis. See **Appendix D** for detailed calculation of nutrient and substrate addition.

Feed ratio was 100 C: 7 N: 1 P. Carbon and nitrogen in the dextrose/glutamic acid solution can be calculated (see **Appendix D**). Carbon, nitrogen, and phosphorous from substrate/chemical emulsifier were determined by: COD, TOC, total phosphorous, orthophosphorous, ammonia, nitrate, and nitrite analysis. The organic content of the substrate is reflected by the COD and TOC. See **Tables 7** and **8**.

Three different amounts of substrate (0.1 ml, 0.5 ml, 1.0 ml) were added to 3 capfuls of chemical emulsifier (Cascade), in one litre of tap water, well mixed and analyzed. The decision to use 3 capfuls of chemical emulsifier was made by adding lesser and greater amounts to 0.5 ml of diesel oil to one litre of tap water, mixing well, and visually observing whether the oil emulsified or floated to the top after a waiting period of at least two hours.

TABLE 7: LABORATORY ANALYSES OF SUBSTRATE

Substrate/ Chemical Emulsifier	COD mg/l	TOC mg/l	Total P mg/l	Ortho P mg/l	NO_2 mg/l	NO_3 mg/l	pH
0.1 ml	21	120	4.1	0.4	0.08	0	9.6
0.5 ml	550	215	4.0	0.4	0.09	0	9.5
1.0 ml	930	300	3.8	0.5	0.1	0	9.55

The three different concentrations of substrate were also analyzed for ammonia and were determined to have 0 mg NH_3-N/l. The dextrose/glutamic acid solution was also analyzed for: TOC, COD, total phosphorous, orthophosphorous and ammonia. From these results, it was decided that the nutrient/substrate broth for PHASE I would be 0.5 ml substrate, 3 capfuls chemical emulsifier, 20 ml of 7.5 g/L dextrose/glutamic acid and 14 ml of 1000 mg/L N from Ammonium Chloride to one liter tap water.

TABLE 8: LABORATORY ANALYSES OF DEXTROSE/GLUTAMIC ACID

Analyses	**7.5 g/l dextrose/glutamic acid**
TOC	7764 mg/l
COD 1:10 dilution	9300 mg/l
COD 1:100 dilution	14,000 mg/l *
Total phosphate	0.2 mg/l
Orthophosphate	0 mg/l
Ammonia	0 mg/l

* probably this is the more correct determination due to the dilutions

The first choice of chemical emulsifier was a dishwasher detergent that had phosphate (Cascade was used in this study). Three capfuls of this chemical emulsifier readily dissolved 0.5 ml oil in one litre tap water, did not foam when mixed and had one of the necessary nutrients for growth of the microorganisms (i.e. phosphate). Three capfuls is approximately equal to 0.5 grams.

Graph A-37 in **Appendix A** shows COD versus Time for Reactor 1-0-A in PHASE I. The % removal of COD by day 4 was 89%. From COD and TOC analyses, it was determined that about 54% of available carbon was from the substrate/chemical emulsifier and 39% of available carbon was from the dextrose/glutamic acid. Reactor 1-0-A also had a residual of about 11% of the total available carbon. Assuming 100% of carbon from the dextrose/glutamic acid solution was consumed, calculations show that 73% of carbon from the substrate/chemical emulsifier was removed. These results confirmed that biotransformation was a possibility. The next phase of the experimental study was to determine rate of nutrient addition that would achieve a steady-state condition.

COD analyses determined rate of nutrient addition. After 75% removal, as determined by COD analyses, nutrients were added. A steady-state appeared to have been achieved by day 25 and every other day additions of the same concentration nutrient/substrate began. Percent removal of COD slowed down

and through observation it appeared that an oily film was building up on the surface of the supernatant. Microscopic analyses after day 23 confirmed that when the rate of C:N:P addition increased (same concentration), % COD removal was lower (fewer ciliated protozoa were present).

Graph A-13 in **Appendix A** shows that a buildup of nitrogen began occurring at this time. **Graph A-29** also shows a buildup of orthophosphorous. Nutrient amounts were changed accordingly. The phosphate in the chemical emulsifier was seen to be the culprit and efforts were made to search for other more suitable chemical emulsifiers.

The following emulsifiers were analyzed: Stearic Acid; Oleic Acid; and Sodium Lauryl Sulphate. Stearic Acid did not dissolve well. COD results of one hour feed after day 12 indicate reactors with stearic acid had much less carbon available than was calculated. Observation was made that stearic acid was not dissolving well in reactor supernatant and therefore was not emulsifying the diesel oil. Stearic acid was used in only half of the reactors, the other half had no chemical emulsifier added at that feed. Oleic Acid did seem to emulsify the diesel oil but did not dissolve in tap water and both substrate and oleic acid floated on top even after overnight mixing. Sodium Lauryl Sulphate was used with some success at first but foaming became an unmanageable problem. Finally a phosphate-free dishwasher

detergent was used which did not foam, was easily dissolved and appeared to emulsify 0.5 ml oil readily. From the feed schedule in **Appendix A** can be found addition of nutrient and substrate as found necessary by laboratory analyses.

TOC was monitored 2-3 times weekly from a filtered sample. When percent COD removal was high, TOC was low. This confirmed microbiological activity and removal of carbon from the diesel oil. TOC remained low on the third day after each feed until day 31 when every other day feedings of the same concentration (0.5 ml substrate) were begun and Biodish was the chemical emulsifier. It soon became apparent that all reactors were stressed and nutrient/substrate addition (0.5 ml substrate) every third day was instituted again. The percent COD removal was lower, and TOC was increasing just before nutrient addition. A different tactic might be to feed smaller amounts of substrate daily but what is most desired is a steady-state condition in the reactors.

Graph A-7 shows an increase in effluent TOC at about day 31 when the chemical emulsifier was changed to Biodish (phosphate-free biodegradable dishwasher detergent). The TOC of Biodish is shown in **Table 12** and **Graph 2**. Biodish contributed to the TOC and was more efficient in emulsifying the diesel fuel than the other chemical emulsifiers used. Biodish also increased the influent and effluent TOC. However, both Ammonia-N shown in

Graph A-13 and orthophosphorous in **Graph A-29** decreased from day 35, suggesting microbiological activity was increasing.

pH of the supernatant was monitored 2-3 times weekly and varied from 7.4 to 8.6. A pH of 7.5 to 8.5 is necessary to sustain microbiological life. As time progressed the colour of the supernatant in the reactors changed colour from almost clear to a medium yellowish-brown, which could account for the increase in pH.

Nitrite and nitrate were monitored occasionally in filtered samples. On day 17, very low concentrations of nitrite in the order of 0.05 to 0.07 mg/L were determined in all reactors, however, only one reactor showed presence of nitrate.

Nitrogen, as measured from analysis of ammonia, increased from very low concentrations on day 0 to almost 10 mg/L by day 12 when Cascade was used as chemical emulsifier as shown in **Graph A-13**. At this point, ammonia was monitored daily and was seen to decrease when use of the high phosphate dishwasher detergent as the chemical emulsifier was discontinued. Sodium Lauryl Sulphate was used alternatively with the phosphate dishwasher detergent to reduce the build-up of ammonia. However, the sodium lauryl sulphate began to foam uncontrollably. On day 31, Biodish was used as chemical emulsifier and ammonia nitrogen in the effluent measured 0 mg/l. Influent ammonia nitrogen for the

entire PHASE I was 7 mg/l or 14 mg N per reactor. Clearly nutrient removal was occurring. By day 39 nitrogen from ammonia was reduced to zero, this analysis was performed on a sample taken 3 days after nutrient/substrate addition. Since 14 mg/l of N (from ammonium chloride and dextrose/glutamic acid) was added to the reactors on feed days, this showed removal and confirmed microbiological activity.

Phosphate was measured both as total phosphate and as orthophosphate. Samples for total phosphate analysis were autoclaved as detailed in the Standard Methods (1989). Total phosphate in all the reactors was 0.1 mg/L on day 0 and increased to 18-34 mg/L by day 12. Cascade added from day 0 to day 12 contributed a large amount of phosphate. The other chemical emulsifiers also contained some phosphate. Orthophosphate behaved in the same manner with an increase apparent when phosphate laden chemical emulsifier was used. On day 31, Biodish was added which contained no phosphate. Orthophosphorous shown in **Graph A-29** shows a clear decline in effluent orthophosphorous concentration. This nutrient removal was occurring due to microbiological activity. No additional phosphate was added until day 43 when 2 mg P per reactor supplemented the original nutrient feed. See **Table A-2**. It was calculated that 2 mg/L phosphorous was necessary each nutrient addition. Again, these results confirmed the choice to use a phosphate-free chemical emulsifier and control addition of

phosphorous by use of a reagent. The graph of phosphorous over time shows definite removal of phosphorous and also confirms microbiological activity.

Dissolved oxygen and temperature were determined on day 24 to confirm that the batch reactors were operating at room temperature and contained sufficient dissolved oxygen to sustain growth. The average temperature of the reactors was room temperature (approximately 24 degrees C). The amount of dissolved oxygen varied from 7.70 mg/l to 8.05 mg/l. This amount of oxygen is more than sufficient to sustain growth. The variation in dissolved oxygen can be explained by the use of the roller valve to regulate the air flow. The valve is not an exact regulator.

TABLE 9: DISSOLVED OXYGEN AND TEMPERATURE

Reactor	Dissolved Oxygen (mg/l)	Temperature (degrees C)
A	7.90	23.5
B	8.05	23.0
C	7.85	24.0
D	7.70	24.0
E	8.05	23.5
F	7.75	24.0
G	7.95	23.5
H	8.10	23.5

Microscopic examination of all reactors was conducted weekly. Microscopic examination before day 19 showed no activity. A microscope analysis of reactors 1-0-B and 2-6-D was conducted on day 19. Reactor 1-0-B showed many ciliated protozoa (8 on one floc). Reactor 2-6-D showed fewer ciliated protozoa (1 to 2 per view) and a few algae which were thought to be *Melosira*. The sample came from sludge after the reactors were settled for one hour. On day 24, a microscopic analysis on reactors 1-0-B and 2-6-C showed many ciliated protozoa. At this time, the sample was taken from the 1 litre mark of thoroughly mixed reactors. A microscopic analysis of reactors 1-6-G, 2-6-D, and the seed batch was conducted on day 34. Reactor 1-6-G showed a few ciliated protozoa. Reactor 2-6-D showed 8 to 10 ciliated protozoa per view in a very active state. The seed showed even more than 10 ciliated protozoa per view that were extremely active. The sample was taken from well-mixed liquor at the 1 litre mark. On day 42, reactor 1-6-F showed some small movement but nothing could be identified. No ciliated protozoa were found to be present. On this day, the seed batch was viewed as well and was found to have more than 6 ciliated protozoa per view. The amount of nutrient/substrate fed to the large reactors was manipulated in order to achieve a steady-state. By day 98, all four PHASE III reactors had rotifers and protozoa present.

By the end of the summer, tap water used in the

nutrient/substrate broth was allowed to sit for a few hours in order to allow most of the chlorine to dissipate.

Visual observation of the reactors include noticing a change of colour of the supernatant from almost clear to a yellowish-brown over the 45-day period. Biomass did not begin to accumulate visually until about day 20 when flocs of sludge were observed in the supernatant during the hour of settling. After day 20, biomass also began to accumulate on the outside walls of the reactor at the edge of the supernatant and was regularly scraped down after that. On day 21, an oily film was noticed on top of most of the reactors. Since sodium lauryl sulphate was used as the chemical emulsifier on day 20 and foamed quite a lot, it was thought that the oil was not emulsified but was lifted out on the bubbles and the residual diesel oil was left floating on the surface. On day 23, the reactors were given "Cascade" in order to prevent this occurrence from happening again. Problems were also encountered with oil working its way through tubing connections and causing dismantling of connections.

6.1.2 Brandon - PHASE II

PHASE II of this experiment began on day 49 when two of the reactors which had showed excellent carbon removal were moved to the cold chamber. One reactor was chosen from each contaminated area, namely 1-0-B and 2-6-C. The temperature was decreased gradually from 24 °C to 12 °C. Two reactors were left at room

temperature (F and S). The only variable changed during this phase was the temperature of the reactors in the cold chamber.

TABLE 10: TEMPERATURE OVER TIME FOR REACTORS 1-0-B AND 2-6-C

Day	Temperature (degrees Celsius)
49	24
52	24
54	21
55	20
58	17
59	17
62	16
67	14
69	13

pH of the supernatant was monitored 2 to 3 times weekly. The pH of the room temperature reactors was consistently higher than the pH of the cold reactors as shown in **Graph A-5**. However, the pH was still in the acceptable range for microbiological growth.

TOC was monitored regularly until day 69. The TOC analyzer was giving many time-out errors and some samples had sludge apparent in the bottom of the test tube after analysis causing the analytical results to be questionable. TOC of the room temperature reactors was almost three times the TOC of the reactors in the cold chamber. A decline in TOC was evident in

the room temperature reactors but not the cold temperature reactors. See **Graph A-8.**

Effluent ammonia-nitrogen was analyzed and determined to be zero from day 49 to day 95 for the reactors in the cold chamber as well as the reactors at room temperature.

Orthophosphate analysis was also conducted. Orthophosphate in the influent was on the average higher than the orthophosphorous in the effluent as shown in **Graphs A-30** and **A-31** for both room temperature and cold reactors. Nutrient removal (orthophosphorous decline) also suggests microbiological activity.

Influent and effluent COD for reactor 1-0-B and F are shown in **Graphs A-40** and **A-41.** COD removal is shown clearly for the room temperature reactors. Effluent COD and influent COD for the room temperature reactors shows a general decline but results are inconclusive. There were problems encountered with obtaining a representative influent sample due to the time for emulsification of the oil, dissolution of the emulsifier, and possibility of oil droplets clinging to the glassware (especially the pipettes).

Microscopic analysis of the reactors kept in the cold chamber during days 49 to 98, showed the emergence of many filamentous

bacteria along with a decline in the number of ciliated protozoa. This may explain the settleability of both reactors which was excellent in the beginning, but gradually became very poor. The supernatant also became cloudy and very light coloured as opposed to the reactors at room temperature which were becoming more darkly coloured over time.

6.1.3 Brandon - PHASE III

PHASE III consisted of three 20 L reactors and one 25 L reactor at room temperature, run from day 98 to day 600. Laboratory analysis began at day 200. Initially Biodish as chemical emulsifier was added. No emulsifier was added after day 400.

From day 200 to day 325 the reactors were not decanted. After day 325, 5 L of supernatant was withdrawn on each feed day. Feed addition after day 400 consisted of nutrient (N,P) and carbon only from diesel fuel. Effluent TOC remained low in all the reactors after day 400. Even though no chemical emulsifier was added at this time, it appeared that the oil was undergoing a natural emulsification.

When chemical emulsifier (Biodish) was not added to the reactors anymore, the pH declined as shown in **Graph A-6**. When biodish was added the pH increased to betweem 8.2 to 8.7. pH varied from 7.00 to 7.60 when no biodish was added.

TOC (**Graphs A-9, A-10, A-11,** and **A-12**) showed a decline when chemical emulsifier and co-substrate were not added to the reactors. Initial TOC at day 0 was 55.03 mg/l, 98.77 mg/l, and 69.68 mg/l for Reactors 1-0-A, 2-6-C, and 1-6-F. After 500 days, effluent TOC for reactors F1, F2, and F3 was 73.9 mg/l, 56.9 mg/l, and 81.3 mg/l. Clearly TOC removal of the diesel fuel was occurring.

Graphs A-17, A-18, A-19, and **A-20** show a decline in ammonia N over time, with a residual being apparent in the reactors around day 350 and day 410-470. High levels of nitrite/nitrate were evident in all reactors around day 350. At day 0 and during the initial part of PHASE I nitrite/nitrate was always less than 0.5 mg/l. No nitrogen was added in the feed after day 350 until a decline in NO_2/NO_3 was evident. This is shown in **Graphs A-21** and **A-22**. Influent ammonia nitrogen from day 200 to 350 was 200 mg for each reactor and effluent ammonia nitrogen was always less than 1 mg/l. Nutrient removal is evident. Despite losses due to un-ionized ammonia, microbiological activity flourished under microscopic examination.

From day 200 to 325, no settling and decanting of supernatant was conducted. **Graphs A-25, A-26, A-27,** and **A-28** show an increase in total phosphate at this time. **Graphs A-32, A-33, A-34,** and **A-35** also show an increase in orthophosphorous. From day 325, the regular procedure of settling and decanting 5

litres before feed was reinstated. TOC, COD, total phosphate, and orthophosphorous show an obvious decrease at this point.

Microbiological examination during this time showed great activity in all the reactors. A healthy population of active protozoa, flagellated protozoa, rotifers, and vorticella was observed on a regular basis. See **Table A-33** in **Appendix A** for detailed report. The presence of flagellated protozoa emerged when the substrate given the reactors was only the diesel fuel.

A scum on top of all the reactors was observed at feeding time. Upon examination under the microscope, it was observed that biomass was attaching itself to the oil that was dispersed on the top of the mixed liquor. This was at the time that no chemical emulsifier was added to the reactors. At initial feed time, the oil was on top of the mixed liquor. After 1 hour, there was a considerable increase in "scum". By 12 hours, this scum was noticeably smaller in volume. It has been suggested in the literature that "bacteria" possess their own natural emulsifiers. It appeared to me that the biomass present in the reactor was "emulsifying" the diesel fuel.

6.2 Bakers Narrows

pH was acceptable for microbiological activity the whole of the experiment. pH levelled out to around 8.5 for Reactor BN10-RT (Room Temperature, 22 ^{o}C) and can be seen to be decreasing to

about 8.0 for Reactor BN10-C (Cold, 5 °C). See **Graph B-1.**

Ammonia-N was much higher in the cold reactor than the room temperature reactor. Nitrite/nitrate was present at a fairly high concentration in BN10-RT around day 82 but decreased quickly because nutrient addition was adjusted accordingly. Orthophosphate decreased steadily over the 120 days from around 4.0 to less than 0.5 mg/l. MLSS (mixed liquor suspended solids) also increased, confirming the increase in microbiological activity, until day 100 when the contents of the reactor were split in two to make reactor BN10-C. MLSS initially declined in the cold reactor while an increase in MLSS is evident in the room temperature reactor.

COD and TOC, shown in **Graphs B-6** and **B-7**, were decreasing until day 150. At this point chemical emulsifier and co-substrate were discontinued in the feed. Reactors were only given carbon from substrate (diesel fuel) from that day on. Effluent TOC shown in **Graph B-8** is less for the warm reactor than the cold reactor. The cold reactor also had a higher effluent COD. **See Graph B-9.**

Microbiological activity increased steadily from day 0. Microbiological activity declined intially in the cold reactor but was seen to be increasing although this reactor was never as active as the room temperature reactor.

6.3 Pukatawagan

The feed consisted of substrate, co-substrate, chemical emulsifier and nutrient. The COD of the synthetic feed was approximated as follows:

(1) chemical emulsifier, 200 mg/L as determined by COD analyses;
(2) fuel oil (COD 450,000 mg/l determined by the U.S. Navy), thus 0.5 ml in the feed will exert 225 mg/l COD;
(3) glucose/glutamic acid has 60 mg C as per calculations in **Appendix D**; and
(4) nitrogen and phosphorous in the feed exert no COD.

Thus, influent COD (synthetic feed) was approximately 450 mg/l. Nutrient ratio of 100:7:1 implies that, for every 450 mg/l COD (or about 180 mg/l TOC), 14 mg/l N and 2 mg/l P are required. Glucose/Glutamic acid provides 7 mg/l N. An addition of 7 mg/l is required from the 1000 mg/l N stock to make a total of 14 mg/l N. Initial analyses of orthophosphate showed values of 0.8 to 2.2 mg/l in the reactors. Thus initially, only 1 mg/l of the 1000 mg/l P stock solution was added. The nutrient additions of N and P were manipulated depending on the laboratory results of ammonia N and orthophosphate of the supernatant effluent. See **Appendix D** for detailed calculations.

Table 11 records some of the data from laboratory analyses of the feed. There is great difficulty in obtaining consistent results due to the difficulty in getting diesel oil to go into

solution (even with the chemical emulsifier). Factors affecting the dissolution of the oil included the temperature of the water, rate at which stirring occurred, amount of oil clinging to the magnetic stirrer or sides of the volumetric flask, and amount of chemical emulsifier used. Thus the COD of fuel oil as determined by the U.S. Navy was used (1 ml fuel oil = 450 mg COD).

TABLE 11: COD/TOC OF THE FEED

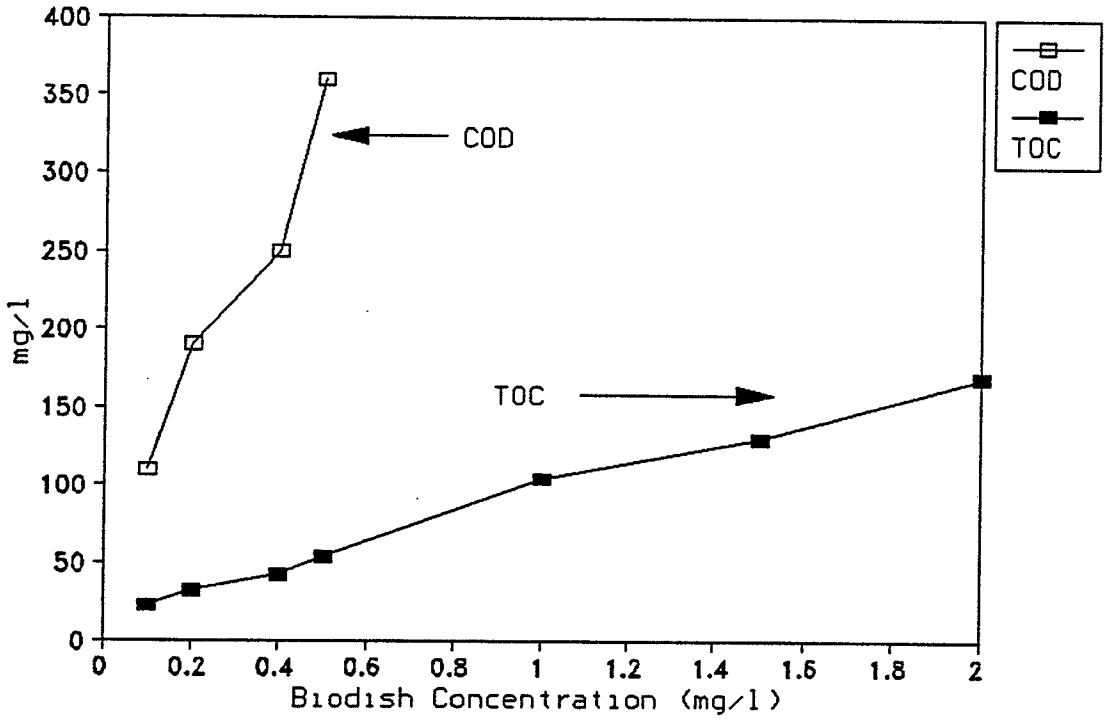
	COD (mg/l)			TOC (mg/l)
	July 13	July 16	July 28	
# 1	-	-	200	103.9/78.1/81.76
# 2	1000	400	680	215.2/255.5/172.1/177.5
# 3	-	-	510	142.1 *

 # 1 - 1 g Biodish/l
 # 2 - 0.5 ml oil plus 1 g Biodish/l
 # 3 - 0.5 ml oil plus 1 g Biodish plus 10 ml dextrose-glutamic acid/l
***** - filtered sample

Table 12 records COD and TOC data of the chemical emulsifier (Biodish) alone. Biodish contains no nitrogen and phosphorous. **Graph 2** shows this relationship.

TABLE 12: BIODISH COD/TOC

Biodish Concentration (g/l)	COD (mg/l)	TOC (mg/l)
0.1	-	22.12
0.2	-	32.26
0.4	-	42.26
0.5	110	53.14
1.0	190	103.9
1.5	250	129.9
2.0	360	169.4



GRAPH 2: TOC/COD OF BIODISH

The microbiological monitoring detailed in **Table C-6, Appendix C,** verifies that nutrient depletion was due mainly to microbiological activity. There was no visible microbiological activity on day 1 of the treatability study. By day 9, microbiological activity had increased, biomass was beginning to accumulate on the sides of the reactors, and orthophosphate in the effluent was declining. Mixed liquor suspended solids also continued to increase in reactors B and C as shown in **Graph C-4.**

pH of the reactors (**Graph C-1**) varied from 7.15 to 8.10. This pH is not harmful to microbiological activity but could possibly affect the amount of ammonia in solution available as nutrient. The percent un-ionized ammonia in aqueous solutions can be determined from part of the following table found in "Manitoba Surface Water Quality Objectives". (Williamson 1986)

TABLE 13: UN-IONIZED NH_3 AMMONIA IN AQUEOUS AEROBIC SOLUTION

pH values	Temperature		
	15 °C	20 °C	25 °C
7.00	0.0184	0.0184	0.0184
7.25	0.0184	0.0184	0.0184
7.50	0.0186	0.026	0.026
7.75	0.031	0.043	0.043
8.00	0.035	0.050	0.050
8.25	0.035	0.050	0.050

Thus, at pH of 8.00 and a temperature of 20 °C, the percent of

un-ionized ammonia is about 5%. From Figure 4-1, "Advanced Wastewater Treatment" (Culp and Culp 1971), at pH of about 8.00 and temperature of 20 °C, there is a distribution of about 5% NH_3 ammonia and 95% NH_4^+ ammonium ion. Thus, only 5% of the synthetic feed of ammonium chloride would have been liberated into the air (i.e., 5% of 14 mg/l = 0.7 mg/l). 13.3 mg/l N from the ammonium chloride would have been readily available to the microorganisms as nutrient. On July 9, the reactors were fed 14 mg/l N. By July 13, Reactor A had 1.72 mg/l residual N as ammonia; Reactor B had 2.69 mg/l residual N as ammonia; and Reactor C had 0.49 mg/l residual N as ammonia. **Graph C-2** charts Ammonia-N vs Time for Reactors A, B, C. Despite losses due to the un-ionized ammonia, microbiological growth and activity was evident. This nutrient depletion by microbial activity is also confirmed in the declining orthophosphate in the effluent supernatant.

Graph C-5 records the chemical oxygen demand over time for the duration of the treatability study. Acclimation of the bacterial seed was evident by day 65. Reactor A began with a Day 1 effluent COD of about 210 mg/l and ended on Day 65 with an effluent COD of about 170 mg/l.

Graph C-6, TOC over Time, shows a gradual increase of Total Organic Carbon of the effluent supernatant until about Day 40 when a decline in TOC is evident.

7. SUMMARY

This project has assessed under laboratory conditions the ability of indigenous bacteria to degrade petroleum hydrocarbons (diesel fuel) when proper conditions are provided to enhance growth. The enrichment conditions provided for this batch activated sludge treatability study included oxygen and nutrient addition. Chemical emulsifier and co-substrate were initially considered for enrichment and were eventually eliminated from the enrichment regime.

Indigenous microorganisms capable of biodegrading petroleum hydrocarbons seem to be present in the batch activated sludge reactors which were seeded with contaminated soil from three different sites in Manitoba (two of these sites in northern Manitoba). The acclimation period for all reactors appeared to be about three weeks. A chemical emulsifier was considered necessary for the dissolution of the diesel fuel and to prevent air stripping of the diesel fuel initially. Several chemical emulsifiers were experimented with and eliminated. Biodish, a biodegradable non-phosphate non-foaming dishwasher detergent, was chosen as the chemical emulsifier of choice. The batch reactors were dosed with nutrient and substrate 100: 7:1. The presence of an active microbial population and the depletion of nutrient suggested bioremediation of the diesel fuel.

8. CONCLUSIONS

1. Acclimation of indigenous microorganisms (from contaminated soil) to petroleum hydrocarbons is possible.

2. Microbiological growth was observed to increase significantly from no visible activity at day 0 to a very active biomass including flagellated protozoa, rotifers, paramecium, worms, stalked ciliated protozoa, and ciliated protozoa (sole carbon source: diesel fuel).

3. The acclimation period of microorganisms to diesel fuel appears to be about three weeks.

4. Nutrient requirements (nitrogen, phosphorous) to enhance microorganism growth have been determined for all three sites.

5. Nutrient/substrate ratio was determined to be 100:7:1.

6. Organic removal efficiencies in terms of chemical oxygen demand (COD) were between 50% and 90% for PHASE I.

7. Microbiological activity and removal (COD, TOC) decreased in the reactors run at cold temperatures.

9. RECOMMENDATIONS

- predominant species in the acclimated sludge could be identified.

- removal rates, in the field, of a microbiologically stimulated versus an augmented contaminated site could be explored.

- geotechnical limitations to bioremediation, such as pore size, should be identified.

REFERENCES

Ambrose, R.B., et al. (1988) "Waste Allocation Simulation Models." *Journal WPCF*, Vol. 60, No. 9, 1646-1655.

Andreottola, G. and Acaia, C. (1991) "Contaminated Soil Reclamation - A State of the Art." *Environmental Impact of Hazardous Wastes*, Rydzyna, Poland, 109-128.

Aronstein, B.N., et al. (1991) "Effect of Surfactants at Low Concentrations on the Desorption and Biodegradation of Sorbed Aromatic Compounds in Soil." *Environmental Science and Technology*, Vol. 25, No. 10, 1728-1731.

Barnhart, M.J. and Myers, J.M. (1989). "Pilot Bioremediation Tells All About Petroleum Contaminated Soil." *Pollution Engineering*, October, 110-112.

Bergueiro, J.R., et al. (1989) "Research on the Evaporation of Gasoline on Beach Sand." *Spill Technology Newsletter*, Environment Canada, Vol. 14, No. 2, 10-15.

Bitton, G. and Gerba, C.P. (1984) *Groundwater Pollution Microbiology*, Wiley, New York.

Bouwer, E.J. (1989). "Biotransformation of Aromatics in Strip-Pit Pond." *Journal of Environmental Engineering*, Vol.115, No.4, August, 741-755.

Bowers, A.R., et al. (1988) "Toxicity Reduction in Industrial Wastewater Discharges." *Pollution Engineering*, February, 68-72.

Bury, S.J. and Miller, C.A. (1993) "Effect of Micellar Solubilization on Biodegradation Rates of Hydrocarbons." *Environmental Science and Technology*, Vol. 27, No. 1, 104-110.

Canter, L.W. (1990) "Current Practices and Applications of Ground Water and Soil Contamination/Remediation: Successes and Failures." *Ground Water and Soil Contamination Remediation*, National Academy Press, Washington, DC, 104-132.

Cheremisinoff, P.N. (1987) "Biotechnology: Treating Industrial/Municipal Wastes and Wastewater." *Pollution Engineering*, September, 74-87.

Clesceri, et al. (1989) *Standard Methods For the Examination of Water and Wastewater*, American Public Health Association.

Cook, F.D. (1973) "Biodegradability of Northern Crude Oils." Task Force on Northern Oil Development, *Environment Canada*, October, 1-115.

Cook, F.D. and Westlake, D.W.S. (1974) "Microbiological Degradation of Northern Crude Oils." *Task Force on Northern Oil Development*, Environment Canada, 1-127.

Custance, S.R., et al. (1992) "Environmental Fate of the Chemical Mixtures: Crude Oil, JP-5, Mineral Spirits, and Diesel Fuel." *Journal of Soil Contamination*, Vol. 1, No. 4, 379-386.

Culp, R.L. and Culp, G.L. (1971) *Advanced Wastewater Treatment*, Van Nostrand Reinhold Environmental Engineering Series.

Dang, J.S., et al. (1989) "Evaluation of Biodegradation Kinetics With Respirometric Data." *Research Journal WPCF*, Vol. 61, No. 11/12, 1711-1721.

Downey, D. and Elliot, M.G. (1990) "Performance of Selected In Situ Soil Decontamination Technologies: An Air Force Perspective." *Environmental Progress*, Vol. 9, No. 3, 169-173.

Environmental Science and Engineering (1991) "Leaking Storage Tank Costs Could Rival Our Federal Deficit." *Environmental Science and Engineering*, July, 38-41.

Environmental Science and Technology (1991) "Treating Contaminated Soil." *Environmental Science and Technology*, October, 70.

EPA (1991) *Stabilization Technologies for RCRA Corrective Actions*, U.S. Environmental Protection Agency, Office of Research and Development, Washington, DC, 42-54

Fox, J.C., et al. (1981) *Sewage Organisms: A Color Atlas*, The Metropolitan Sanitary District of Greater Chicago, 19-22.

Galaska, E.G., et al. (1990) "Biological Treatment of Groundwater, Soils and Soil Vapors Contaminated With Petroleum Hydrocarbons." *44th Purdue Industrial Waste Conference Proceedings*, Lewis Publishers, Inc., Chelsea, Michigan, 11-21

GASReP (1990) *Petroleum Contaminated Soils: Regional and National Action Plans*, Canadian Petroleum Products Institute.

Gaudy Jr., A.F.and Gaudy, E.T. (1980) *Microbiology for Environmental Scientists and Engineers*, McGraw-Hill Book Company, 210-215.

Gouleke, C.G. and Diaz, L.F. (1990). "Bioremediation for Hazardous Wastes." *Biocycle*, February, 54-55.

Grady Jr., C.P.L. (1990) "Biodegradation of Toxic Organics: Status and Potential." *Journal of Environmental Engineering*, September/October, Vol. 116, No. 5, 805-828.

Grady Jr., C.P.L. (1989) "Determination of Biodegradation Kinetics Through the Use of Electrolytic Respirometry." *Water Science Technology*, Vol. 21, 957-968.

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

Green, G. (1989) *The Use of Surfactants in the Bioremediation of Petroleum-Contaminated Soils*, U.S. Department of Commerce, 1-26.

Hall, S.K. (1989) "Oil Spills At Sea." *Pollution Engineering*, December, 59-63.

Hamoda, ,M.F. and Al-Haddad, A.A. (1987) "Investigation of Petroleum Refinery Effluent in an Aerobic Fixed-Film Biological System." *Journal of the Institution of Water and Environmental Management*, Vol. 1, August, 239-246.

Hickman, G.T., et al. (1989) "Effects of Site Variations on Subsurface Biodegradation Potential." *Journal WPCF*, Vol. 61, No. 9, 1564-1575.

Hodge, D.S., et al. (1991) "Treatment of Hydrocarbon Fuel Vapors in Biofilters." *Environmental Technology*, Vol. 12, 655-662.

Hopper, D.R. (1989) "Cleaning Up Contaminated Waste Sites." *Chemical Engineering*, Vol.96, No. 8, 94-110.

Hornick, S.B. (1983) "The Interaction of Soils With Waste Constituents." *Land Treatment of Hazardous Wastes*, Park Ridge, New Jersey, 4-19.

Hosler, K.R. (1992) "Ex-situ Bioremediation of Contaminated Soil." *Wastewater Technology Centre Newsletter*, No. 22, March, 7-8.

Hutchinson, T.C., et al. (1974) "Oil Spill Effects on Vegetation and Soil Microfauna at Norman Wells and Tuktoyaktuk, N.W.T." *Task Force on Northern Oil Development*, 1-111.

Intera (1991) *Manual of Petroleum Contaminated Soil Treatment Technologies*, The Canadian Petroleum Products Institute, November.

Kampfer, P. (1991) "Microbiological Characterization of a Fuel-Oil Contaminated Site Including Numerical Identification of Heterotrophic Water and Soil Bacteria." *Microbial Ecology*, Vol. 21, 227-251.

Kaufman, E. (1983) "Fate of Toxic Organic Compounds in Land-Applied Wastes." *Land Treatment Of Hazardous Wastes*, Park Ridge, New Jersey, 77-151.

Kincannon, D.F. et al. (1983) "Removal Mechanisms for Toxic Priority Pollutants." *Journal WPCF*, February, 157-163.

Lindstrom, J.E., et al. (1991) "Microbial Populations and Hydrocarbon Biodegradation Potentials in Fertilized Shoreline Sediments Affected by the T/V Exxon Valdez Oil Spill." *Applied and Environmental Microbiology*, Vol. 57, No. 9, 2514-2522.

Lingineni, S. and Dhir, V.K. (1992) "Modelling of Soils Venting Processes to Remediate Unsaturated Soils." *Journal of Environmental Engineering*, Vol. 118, No. 1, Jan/Feb, 135-152.

Mackay, D. and Hoag, G.E. (1986) "A Perspective on the Behaviour of Chemicals Spilled in Soil." *Proceedings of the Third Annual Technical Seminar on Chemical Spills*, Montreal, Quebec, 123-142.

Madsen, E.L. (1991). "Determining In Situ Biodegradation: Facts and Challenges." *Environmental Science and Technology*, Vol. 25, No. 10, October, 1663-1773.

Mahaffey, W.R., et al. (1991) "Developing Strategies for PAH and TCE Bioremediation." *Water Environment and Technology*, October, 83-88.

Major, D. (1991) "Reviewing the Options in Bioremediation of Ground Water." *Environmental Science and Engineering*, September, 33-38.

Major, D. (1991) "The Current State-of-the-Art of In Situ Bioremediation: Considerations, Limitations, Potential and Future Directions." *Proceedings of the First Annual GASReP Symposium*, Ottawa, Ontario.

Martin Jr., J.H., et al. (1991) "Estimating Oil and Grease Content of Petroleum-Contaminated Soil." *Journal of Environmental Engineering*, Vol. 117, No. 3, 291-299

McCarty, P.L. (1990) "Scientific Limits to Remediation of Contaminated Soils and Ground Water." *Groundwater and Soil Contamination Remediation*, National Academy Press, Washington, DC, 38-52.

McFarland, M.J., et al. (1991) "Use of Treatability Studies in Developing Remediation Strategies for Contaminated Soils." *Environmental Biotechnology for Waste Treatment*, Plenum Press, New York, 163-174.

Merck Index, The (1983) Tenth Edition, Merck and Co. Inc., USA.

Morgan, P. and Watkinson, R. (1992) "Factors Limiting the Supply and Efficiency of Nutrient and Oxygen Supplements for the In Situ Biotreatment of Contaminated Soil and Groundwater." *Water Resources*, Vol. 26, No. 1, 73-78.

Nelson, C.H. (1993) "A Natural Cleanup." *Civil Engineering*, March, 57-59.

Newton, J. (1990). "Remediation of Petroleum Contaminated Soils." *Pollution Engineering*, December, 46-52.

Nichols, A.B. (1989) "Alaskan Oil Spill Shocks the Nation." *Journal WPCF*, Vol. 61, No. 7, 1175-1185.

O'Leary, W. M. (1989) *Practical Handbook of Microbiology*, CRC Press, Inc., 1, 55-66, 337-347

Opatken, E., et al. (1989) "Biological Treatment of Leachate From a Superfund Site." *Environmental Progress*, Vol. 8, No. 1, 12-18.

Park, K.S., et al. (1990) "Transformation of PAHs in Soil Systems." *Journal of Environmental Engineering*, Vol. 116, No. 3, 632-640.

Parkinson, D. (1973) "Effects of Oil Spills on Microorganisms." *Task Force on Northern Oil Development*, October, 1-49.

Parr, J.F., et al. (1983) "Factors Affecting the Degradation and Inactivation of Waste Constituents in Soils." *Land Treatment of Hazardous Wastes*, Park Ridge, New Jersey, 20-49.

Pheiffer, T.H., et al. (1990) "EPA's Assessment of European Contaminated Soil Treatment Techniques." *Environmental Progress*, Vol. 9, No. 2, 79-86.

Piotrowski, D.A. and Yost, K.W. (1991) "Intercept Trench Technology for Remediating Waste Oil Contaminated Soil and Groundwater: A Case Study." *44th Purdue Industrial Waste Conference Proceedings*, Lewis Publishers, Inc., Chelsea, Michigan, 65-74.

Piper, A. (1991) "Managing Soil Contamination: Cleaning Up the Dirt." *Engineering Dimensions*, Association of Professional Engineers of Ontario, July/August, 20-21.

Qiu, X. and McFarland M. (1991) "Bound Residue Formation in PAH Contaminated Soil Composting Using Phanerochaete chrysosporium." *Hazardous Waste and Hazardous Materials*, Vol. 8, No. 2, 115-126.

Rasiah, V., et al. (1991) "Effect of N Amendment on C Mineralization of an Oily Waste." *Water, Air, and Soil Pollution*, No. 59, 249-259.

Rifai, H.S., et al. (1988) "Biodegradation Modelling at Aviation Fuel Spill Site." *Journal of Environmental Engineering*, October, Vol. 114, No. 5, 1007-1029.

Robinson, K.G., et al. (1990) "Bioremediation Removes Gasoline Residues." *Pollution Engineering*, August, 76-79.

Ross and Phung (1982) "Soil Incorporation: Land Farming of Industrial Waste." *Toxic and Hazardous Waste Disposal*

Sergy, G. (1986) *The Baffin Island Oil Spill Project, B.I.O.S.*, Environment Canada and Canadian Petroleum Association, 19-23.

Sherman, D. and Stroo, H. (1989) "Biological Treatment of PAH's in Soil Residues in Simulated Land Treatment Systems." *Specialty Conference on Environmental Engineering*, ASCE, July, 726-733.

Skladany, G.J. (1988) "Onsite Biological Treatment of an Industrial Landfill Leachate: Microbiological and Engineering Considerations." *First Annual Conference on Genetically Engineered or Adapted Microorganisms in Hazardous Waste Treatment*, Washington, DC.

Srivastava, V.J., et al. (1990). "Bioremediation of Former Manufactured Gas Plant Sites." *44th Purdue Industrial Waste Conference Proceedings*, Lewis Publishers, Inc., Chelsea, Michigan, 49-60.

Staps, S.J. (1990) "International Evaluation of In-Situ Biorestoration of Contaminated Soil and Groundwater." *Proceedings of NATO/CCMS Third International Conference, Demonstration of Remedial Action Technologies for Contaminated Land and Groundwater*, EPA.

Tabak, H., et al. (1981) "Biodegradability Studies With Organic Priority Pollutant Compounds." *Journal WPCF*, Vol. 53, No. 10, 1503-1518.

Tabak, H., et al. (1990) *Laboratory Studies Evaluating the Enhanced Biodegradation of Weathered Crude Oil Components Through the Application of Nutrients*, U.S. EPA, 1-32.

Tate, R.L. and Klein, R. (1985) *Soil Reclamation Processes: Microbiological Analyses and Applications*, Marcel Dekker, Inc.

Tate, R.L., (1985) "Microorganisms, Ecosystem Disturbance and Soil Formation Processes." *Soil Reclamation Processes: Microbiological Analyses and Applications*, Marcel Dekker, Inc., 1-33, 208-229.

Taylor, S.W.and Jaffe, P.R. (1991) "Enhanced In-Situ Biodegradation and Aquifer Permeability Reduction." *Journal of Environmental Engineering*, Vol. 117, No. 1, 25-46.

Thorpe, J.W. and Hellenbrand, K.E. (1987) "Microbial Degradation of Hydrocarbon Mixtures in a Marine Sediment Under Different Temperature Regimes." *Environmental Studies Research Funds*, 1-54.

Torpy, M.F., et al. (1989) "Biological Treatment of Hazardous Waste." *Pollution Engineering*, May, 80-86.

Tribe, R.B. and Brown, R.A. (1990) "In Situ Bioreclamation of a Gasoline Spill in a Low Permeable Heterogeneous Formation." *PACE Workshop*, Winnipeg, MB.

van Elsas, J.D. and Trevors, J.T. (1991) "EnvironmentaL Risks and Fate of Genetically Engineered Microorganisms in Soil." *Journal of Environmental Science and Health*, Vol. A26, No. 6, 981-1001.

Vignon, B.W. and Rubin, A.J. (1989) "Practical Considerations in the Surfactant-Aided Mobilization of Contaminants in Aquifers." *Journal WPCF*, Vol. 61, No. 7, 1233-1240.

Williamson, D.A. (1986) *Manitoba Surface Water Quality Objectives, Manitoba Department of Environment and Workplace Safety and Health*, Water Standards and Studies Report.

Wilson, J.T. et al. (1989) *In Situ Bioremediation of Spills From Underground Storage Tanks: New Approaches for Site Characterization, Project Design, and Evaluation of Performance*, Robert S. Kerr Environmental Research Laboratory, U.S. EPA, 1-56.

Wolowich, E. and Horning, B. (1991) "Remediation of Gasoline Contaminated Ground Water." *Western Canada Water and Sewage Conference*, Winnipeg, 221-232.

Woodward, R.E. (1988) "Bioremediation Feasibility Studies for Haz Waste." *Pollution Engineering*, July, 102-103.

Wu, J.C., et al. (1990) "Modelling and Simulation of Bioremediation of Contaminated Soil." *Environmental Progress*, Vol. 9, No. 1, 47-56.

Wunderlich, R.W. (1992) "In Situ Remediation of Aquifers Contaminated With Dense Nonaqueous Phase Liquids by Chemically Enhanced Solubilization." *Journal of Soil Contamination*. Vol. 1, No. 4, 362-378.

Yong, R.N. and Mourato, D. (1987) "Stimulation of Microbial Biodegradation in a Jet Fuel Contaminated Soils." *Second International Conference on New Frontiers for Hazardous Waste Management*, McGill University, Montreal.

Zitrides, T.G. (1990) "Bioremediation Comes of Age." *Pollution Engineering*, May, 57-62.

Zylstra, G. and Gibson, D. (1991) "Aromatic Hydrocarbon Degradation: A Molecular Approach." *Genetic Engineering*, Vol. 13, 183-203.

APPENDIX A: BRANDON

TABLE A-1: FEED SCHEDULE - INITIAL TRIAL

Day	C		N	P
	Dextrose/ Glutamic Acid (mls)	Diesel Fuel (mls)	(mg)	(mg)
June 19	10.0	1.0	5.36	0.76
June 20	9.0	1.35	5.36	0.76
June 21	8.0	1.7	5.36	0.76
June 22	7.0	2.0	5.36	0.76
June 23	6.0	2.4	5.36	0.76
June 24	5.0	2.8	5.36	0.76
June 25	4.0	3.1	5.36	0.76
June 26	3.0	3.4	5.36	0.76
June 27	2.0	3.9	5.36	0.76
June 28	1.0	4.2	5.36	0.76
June 29	0	4.5	5.36	0.76
June 30	0	4.5	5.36	0.76
July 1	0	4.5	5.36	0.76
July 2	0	4.5	5.36	0.76
July 3	7.0	2.0	5.36	0.76
July 4	7.0	2.0	5.36	0.76
July 5	10.0	1.0	5.36	0.76
July 6	10.0	1.0	5.36	0.76
July 7	10.0	1.0	5.36	0.76
July 8	10.0	1.0	5.36	0.76
July 9	10.0	1.0	5.36	0.76

10 ml Dextrose/Glutamic Acid = 60 mg C

1 ml Diesel Fuel = 17 mg C (my initial laboratory analyses)

TOTAL C = 77 mg as COD

TABLE A-2: FEED SCHEDULE FOR 2 L REACTORS - PHASE I

Day	Diesel Fuel (mls)	Dextrose/ Glutamic Acid (mls)	Emulsifier	N (mg)	P (mg)
5	0.5	20.0	3 capfuls Cascade	14.0	-
8	0.5	20.0	3 capfuls Cascade	14.0	-
12 *	0.5	10.0	1 capful Stearic Acid	-	-
12**	0.5	20.0	-	-	-
17	0.5	10.0	3 capfuls S.L.S.***	-	-
20	0.5	10.0	3 capfuls S.L.S.	5.0	-
23	0.5	20.0	3 capfuls Cascade	14.0	-
26	0.5	10.0	3 capfuls S.L.S.	14.0	-
28	0.5	10.0	3 capfuls Cascade	-	-
31	0.5	10.0	5 capfuls Biodish	-	-
33	0.5	10.0	5 capfuls Biodish	5.0	-
36	0.5	10.0	5 capfuls Biodish	7.0	-
39	0.5	10.0	5 capfuls Biodish	7.0	-
43	0.5	10.0	5 capfuls Biodish	7.0	20.0
46	0.5	10.0	5 capfuls Biodish	7.0	20.0
49	0.5	10.0	5 capfuls Biodish	7.0	20.0

* Reactors A, C, E, G, H
** Reactors B, D, F
*** Sodium Lauryl Sulphate

TABLE A-3: FEED SCHEDULE FOR 20 L REACTORS - PHASE III

Day	Diesel Fuel (mls)	Dextrose/ Glutamic Acid (mls)	Emulsifier	N (mg)	P (mg)
26	4.0	80	24 capfuls S.L.S.	112	-
28	4.0	80	48 capfuls Cascade	-	-
31	10.0	240	18 g Biodish	-	-
33 S	10.0	240	18 g ***	60	-
33 F	5.0	120	9 g	60	-
37	10.0	200	18 g	70	-
40	10.0	200	18 g	70	-
43	10.0	200	18 g	70	-
49	5.0	100	9 g	70	20
52 -	5.0	100	9 g	70	20
August 18	5.0	0	5 g	120	12
Sept. 4	5.0	0	5	60	12
Sept. 11 -	5.0	0	5	60	12
Oct. 1	5.0	0	0	60	12
Oct. 9	5.0	0	0	60	12
Oct. 15	5.0	0	0	60	12
Oct.22	5.0	0	5	60	12
Oct. 29	5.0	0	5	60	12
Nov. 5 -	5.0	0	0	60	12
Jan. 6 -	5.0	0	0	120	12
Feb. 4 -	5.0	0	0	150	14
Feb. 19	5.0	0	0	200	20

S = 25 litre reactor SEED
F = 12 litre reactor FIELD

*** = Biodish as emulsifier of choice from this day on
S.L.S. = Sodium Lauryl Sulphate

LIST OF UNITS FOR INITIAL TRIAL ANALYSES

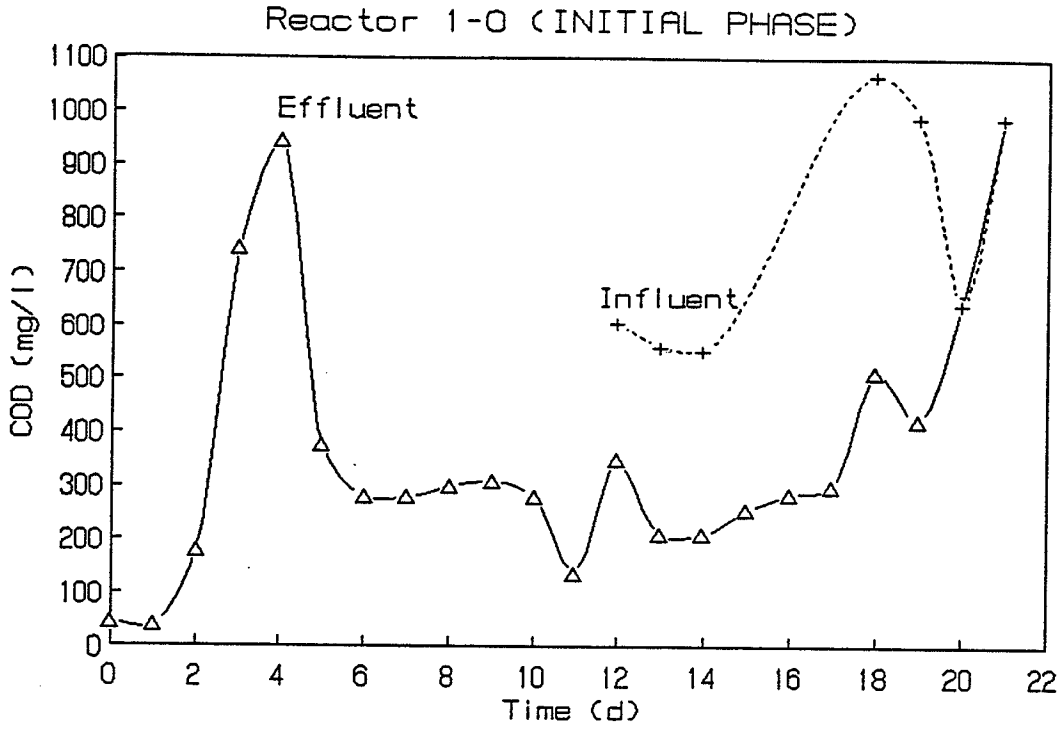
TABLES A-4 TO A-10

NH_3-N	- mg/l ammonia nitrogen
NO_2	- mg/l nitrite
NO_3	- mg/l nitrate
COD_e	- mg/l chemical oxygen demand:effluent
COD_i	- mg/l chemical oxygen demand:influent
T.P.	- mg/l total phosphate
O.P.	- mg/l orthophosphate
TOC	- mg/l total organic carbon

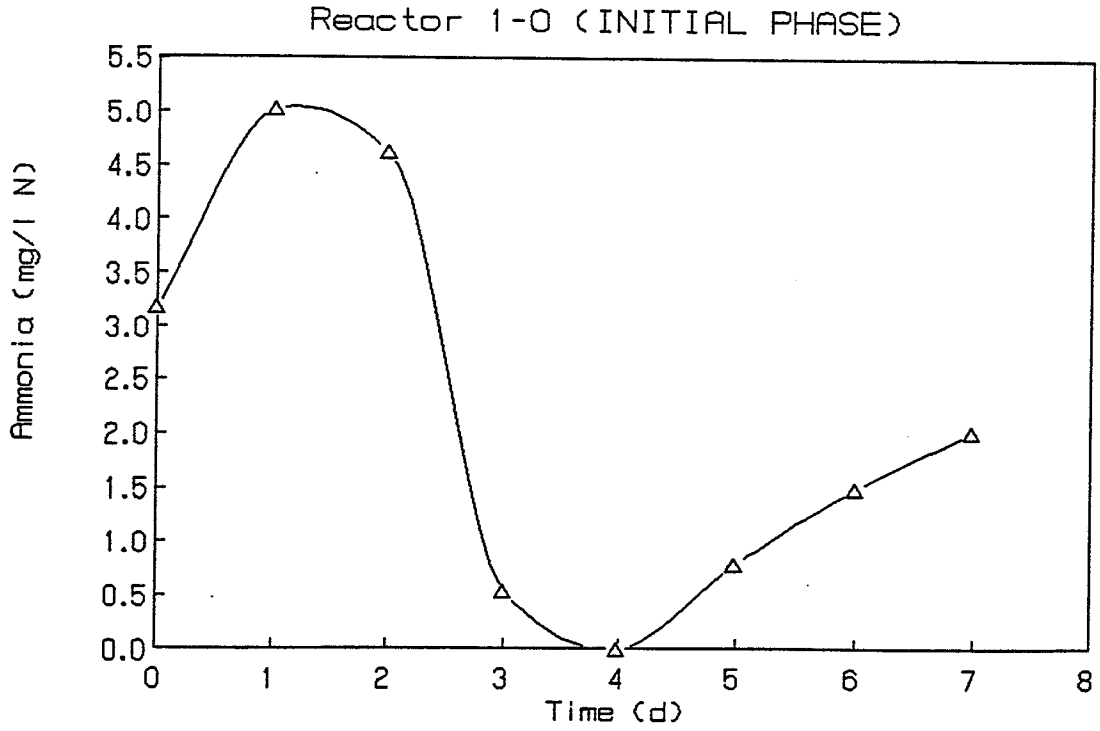
TABLE A-4: INITIAL TRIAL ANALYSES - REACTOR 1-0

	NH_3	NO_2	NO_3	COD_e	pH	T.P. **	O.P. ***	TOC
20	3.175	0	0	45	6.5	>14	13.9	55.03
21	5.025	0.03	0	40	7.25	>14	18.2	54.79
22	4.630	0.03	0	180	7.4	>18	16.8	52.12
23	0.530	0	0	745	7.3	>18	13.7	110.1
24	0.0			945	6.85	>20	12.2	172.3
25	0.795	0	0	375	6.65	>20	10.9	110.2
26	1.500			280	6.75	>20	11.2	92.05
27	2.030			280	6.9		11.55	91.79
				COD_e				**COD_i**
28				300			11.8	
29				310			11.5	
30				280			11.6	
1				135			9.7	
2				350				605
3				210				560
4				210				555
5				255				
6				285				
7				300				
8 *				510				1070
9				420				990
10				640				640
11				990				990

* unfiltered samples from this time
** Total Phosphate
*** Orthophosphate
COD_e Chemical Oxygen Demand - effluent
COD_i Chemical Oxygen Demand - influent



GRAPH A-1: COD (mg/l) vs TIME (d) - REACTOR 1-0



GRAPH A-2: AMMONIA (mg/l) vs TIME (d) - REACTOR 1-0

TABLE A-5: INITIAL TRIAL ANALYSES - REACTOR 1-3

	NH_3	NO_2	NO_3	COD_e	pH	T.P. **	O.P. ***	TOC
20	2.515	0.06	0.02	120	6.3	12.8	10.9	90.82
21	2.775	0.03	0	60	7.4	>14	11.5	58.58
22	3.220	0.03	0	165	7.4	>18	10.2	79.51
23	3.880	0	0	300	6.95	>18	9.1	96.88
24	1.235			255	6.7	>20	8.1	94.56
25	0.446	0.05	0	315	6.4	>20	7.8	117.9
26	0.355			375	6.7	>20	7.1	127.1
27	0			210	7.1		6.4	78.92
				COD_e				COD_i
28				335			6.7	
29				245			5.8	
30				390			6.2	
1				755			6.5	
2				625				915
3				210				330
4				175				330
5				195				
6				270				
7				255				
8 *				1250				990
9				910				955
10				940				985
11				850				1035

* unfiltered samples from this time
** Total Phosphate
*** Orthophosphate

TABLE A-6: INITIAL TRIAL ANALYSES - REACTOR 1-6

	NH_3	NO_2	NO_3	COD_e	pH	T.P. **	O.P. ***	TOC
20	5.161	0	0	75	6.25	12.9	4.8	98.77
21	0.705	0.03	0	105	7.05	>14	5.8	65.98
22	1.629	0.05	0	180	7.2	>18	5.9	78.60
23	2.425	0	0	160	7.35	>18	4.9	93.25
24	1.94			240	6.9	>20	4.1	100.0
25	3.486	0	0	255	6.8	>20	3.0	103.5
26	3.311			235	6.85	>20	2.7	86.83
27	2.295			285	6.8		2.75	98.03
				COD_e				COD_i
28				285			2.5	
29				255			2.3	
30				215			2.1	
1				235			1.6	
2				210				565
3				240				640
4				210				775
5				215				
6				280				
7				345				
8 *				495				940
9				480				1060
10				440				1010
11				540				1070

* unfiltered samples from this time
** Total Phosphate
*** Orthophosphate

TABLE A-7: INITIAL TRIAL ANALYSES - REACTOR 1-10

	NH_3	NO_2	NO_3	COD_e	pH	T.P. **	O.P. ***	TOC
20	0.711	0.05	0	45	6.8	12.85	8.1	61.30
21	4.410	0.03	0	35	7.3	>14	9.5	53.11
22	3.570	0.04	0	60	7.2	>18	8.7	51.80
23	1.365	0	0	215	7.4	>18	7.2	79.12
24	1.59			270	6.7	>20	6.9	104.6
25	2.735	0	0	335	6.9	>20	4.9	104.6
26	2.690			285	6.9	>20	4.2	96.32
27	2.295			270	6.7		3.8	94.37
				COD_e				COD_i
28				285			3.0	
29				700			4.7	
30				315			2.6	
1				205			2.2	
2				205				410
3				235				485
4				165				480
5				160				
6				290				
7				345				
8 *				1150				1040
9				940				970
10				925				985
11				835				925

* unfiltered samples from this time
** Total Phosphate
*** Orthophosphate

TABLE A-8: INITIAL TRIAL ANALYSES - REACTOR 2-0

	NH_3	NO_2	NO_3	COD_e	pH	T.P. **	O.P. ***	TOC
20	5.381	0	0	150	6.2	5.7	11.2	105.9
21	2.955	0.04	0	135	7.1	11.4	7.1	117.0
22	3.175	0.04	0	225	6.75	17.8	8.6	114.3
23	1.985	0	0	840	6.9	>18	8.4	149.9
24	0			880	6.55	>20	8.2	222.6
25	1.105	0	0	540	6.8	>20	7.4	123.5
26	3.000			300	6.7	>20	7.7	110.2
27	2.205			280	6.7		7.9	99.43
				COD_e				COD_i
28				225			6.6	
29				205			5.9	
30				225			5.8	
1				210			5.2	
2				320				120
3				390				510
4				255				540
5				270				
6				225				
7				280				
8 *				565				970
9				365				990
10				375				770
11				435				985

* unfiltered samples from this time
** Total Phosphate
*** Orthophosphate

TABLE A-9: INITIAL TRIAL ANALYSES - REACTOR 2-3

	NH_3	NO_2	NO_3	COD_e	pH	T.P. **	O.P. ***	TOC
20	6.576	0	0	75	6.9	9.8	6.8	69.68
21	4.191	0.05	0	90	7.15	>14	7.6	84.02
22	0.795	0.03	0	130	6.5	>18	6.7	105.8
23	0.925	0	0	165	7.1	>18	6.5	101.6
24	0.090			265	6.9	>20	5.9	98.21
25	1.371	0	0	310	7.0	>20	5.1	85.99
26	2.470			315	6.8	>20	5.1	78.23
27	1.940			310	6.8		5.0	85.30
				COD_e				COD_i
28				300			4.3	
29				255			4.2	
30				225			4.0	925
1				215			3.55	
2				210				725
3				540				490
4				240				345
5				240				
6				310				
7				300				
8 *				560				925
9				420				925
10				310				910
11				495				955

* unfiltered samples from this time
** Total Phosphate
*** Orthophosphate

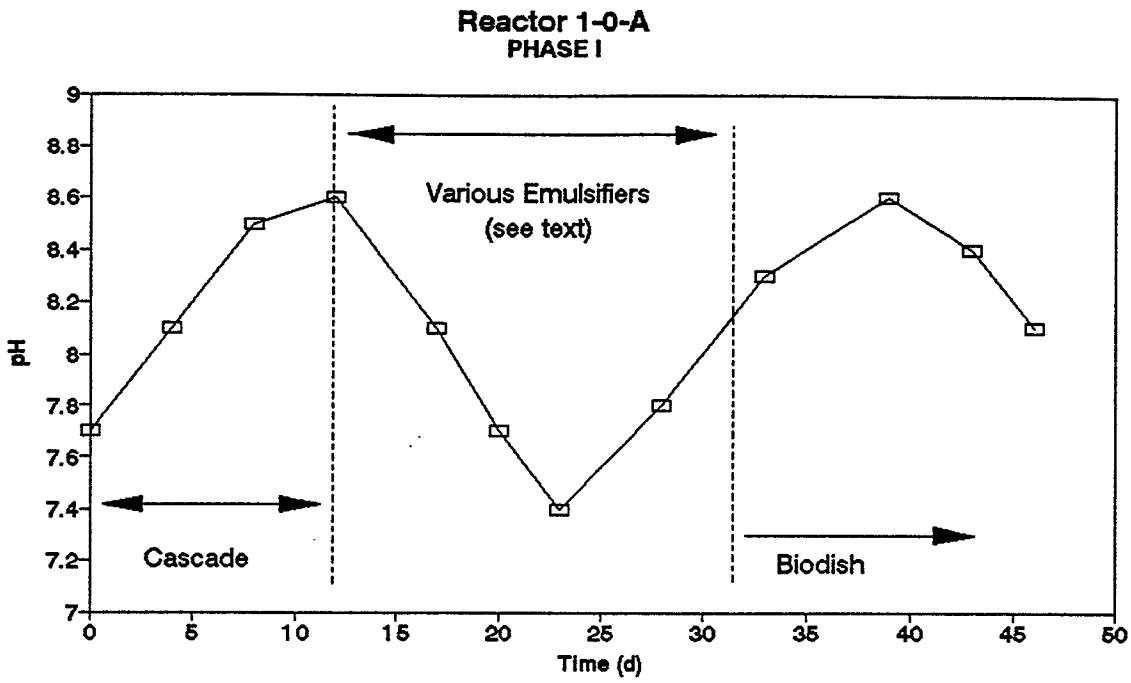
TABLE A-10: INITIAL TRIAL ANALYSES - REACTOR 2-6

	NH_3	NO_2	NO_3	COD_e	pH	T.P. **	O.P. ***	TOC
20	6.091	0.15	0.41	115	7.5	13	13.7	78.22
21	1.629	0.04	0	130	7.4	>14	19	67.69
22	1.060	0.05	0	190	7.35	>18	14.5	77.18
23	3.266	0	0	250	7.1	>18	12.5	72.31
24	1.19			290	7.0	>20	10.0	83.45
25	1.636	0	0	315	7.0	>20	8.4	82.74
26	2.871			290	6.95	>20	8.3	66.72
27	2.470			285	6.7		8.1	74.87
				COD_e				COD_i
28				225			7.5	
29				205			6.9	
30				180			6.8	
1				175			5.4	
2				210				465
3				225				560
4				235				450
5				235				
6				-				
7				315				
8 *				870				1070
9				740				940
10				895				990
11				880				1000

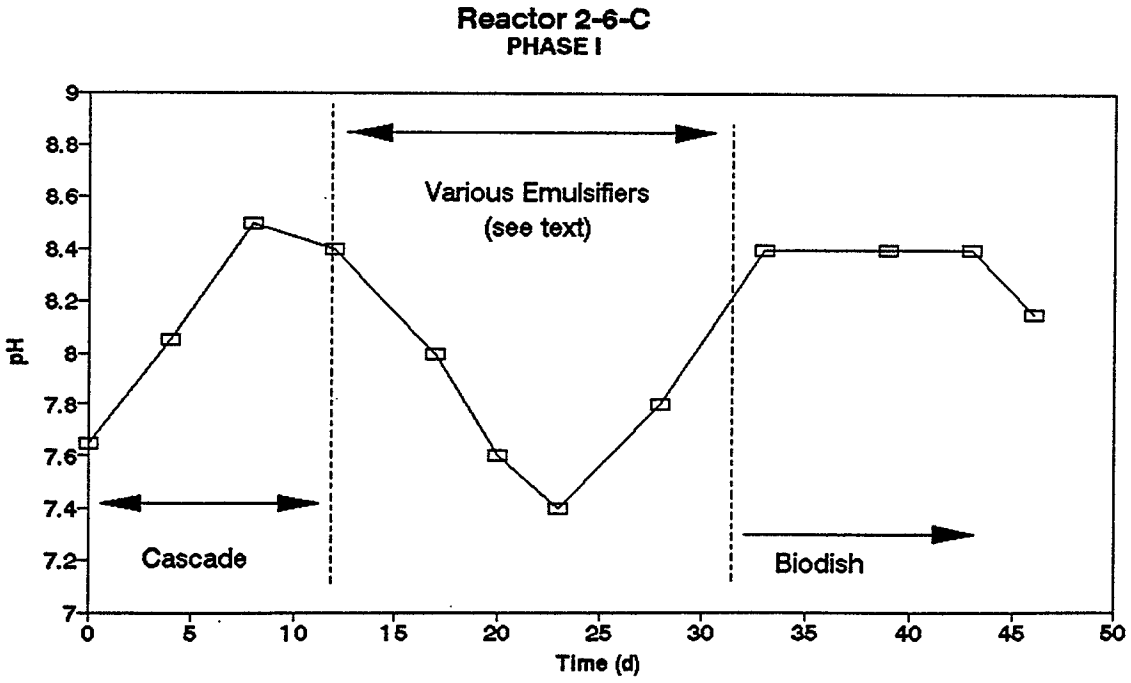
* unfiltered samples from this time
** Total Phosphate
*** Orthophosphate

TABLE A-11: pH - PHASE I - DAY 0 TO DAY 46

Day	1-0 A	1-0 B	2-6 C	2-6 D	1-6 E	1-6 F	1-6 G	1-6 H	Seed
0	7.70		7.65			7.50			
4	8.10		8.05			-			
5	-		-			7.90			
8	8.50	8.50	8.50	8.40	8.40	8.50			
12	8.60	8.40	8.40	8.55	8.25	8.25			
17	8.10	8.10	8.00	8.00	8.10	8.10	7.70	7.95	
20	7.70	7.50	7.60	7.70	7.70	7.60	7.50	7.50	
23	7.40	7.50	7.40	7.40	7.40	7.50	7.40	7.40	
26	-	-	-	-	-	-	-	-	7.80
28	7.80	7.70	7.80	7.90	7.80	7.90	7.80	7.70	7.85
31	-	-	7.80	-	-	8.10	-	-	-
33	8.30	8.30	8.40	8.40	8.40	8.50	8.40	8.50	8.50
35	-	-	-	-	-	-	-	-	8.50
37	-	-	-	-	-	-	-	-	8.10
39	8.60	8.45	8.40	8.35	8.40	8.25	8.30	8.50	-
40	-	-	-	-	-	-	-	-	8.20
43	8.40	8.40	8.40	8.40	8.50	8.30	8.35	8.30	8.70
46	8.10	8.10	8.15	8.15	8.20	8.05	8.10	8.10	-



GRAPH A-3: pH vs TIME (d) - REACTOR 1-0-A - PHASE I



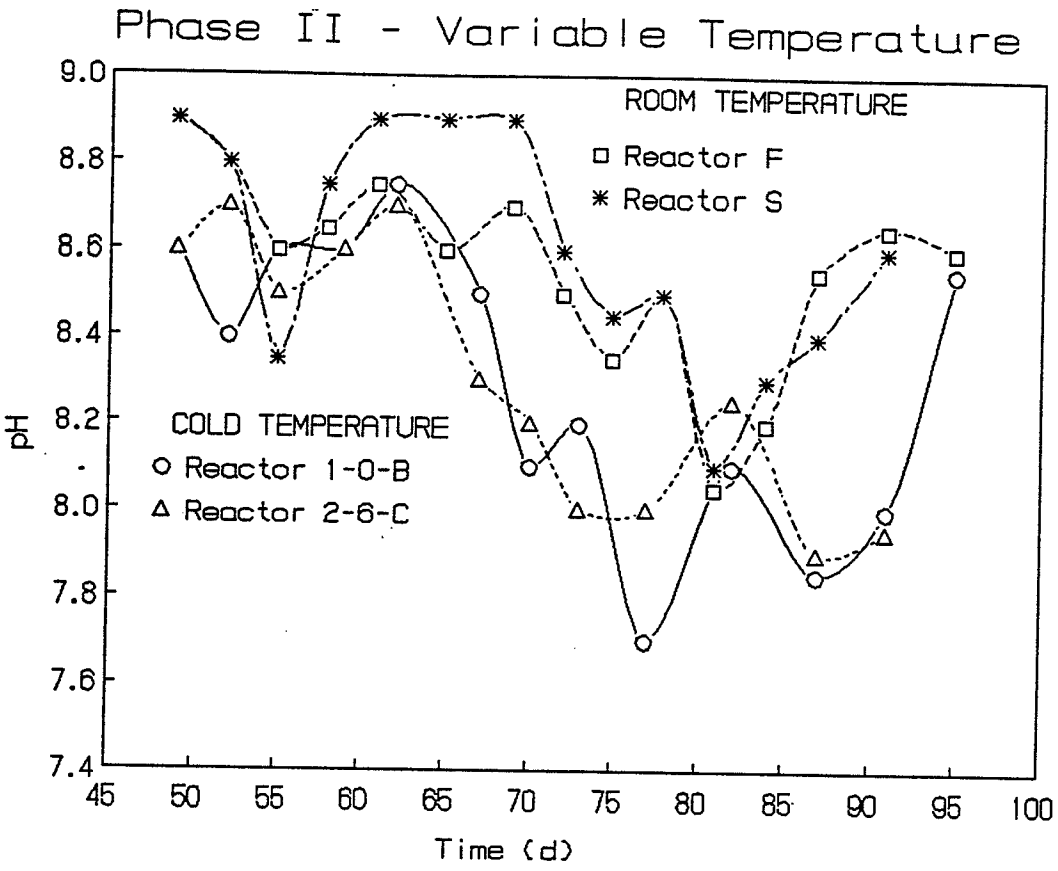
GRAPH A-4: pH vs TIME (d) - REACTOR 2-6-C - PHASE I

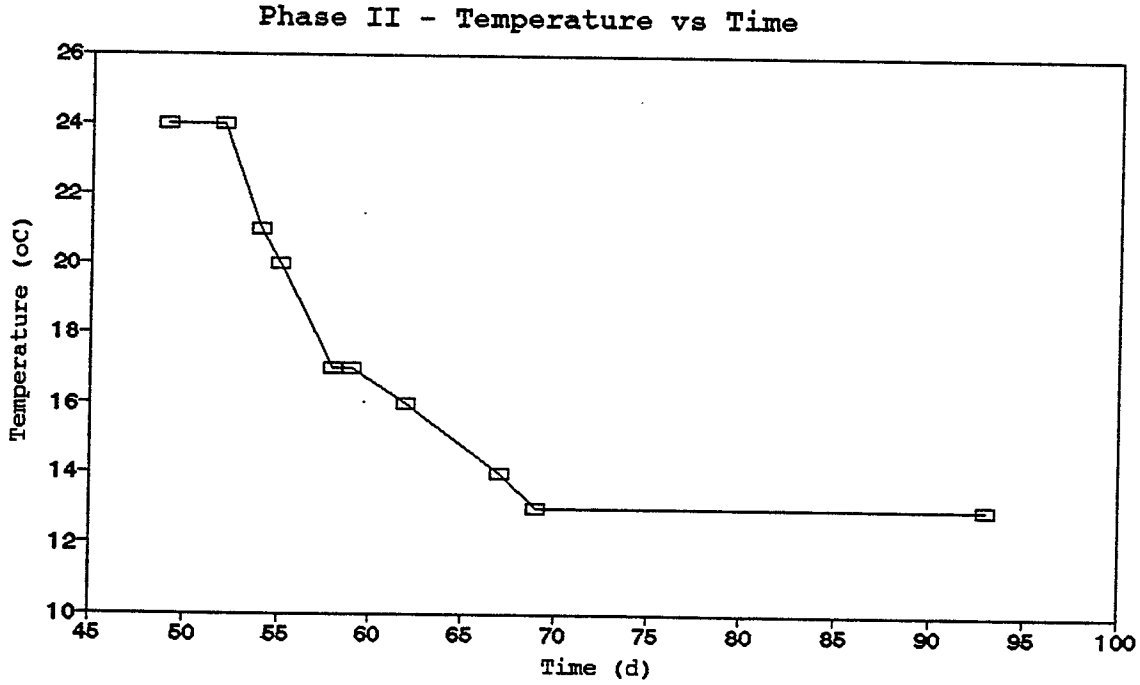
TABLE A-12: pH - PHASE II

Day	Cold Chamber		Room Temperature	
	1-0-B	2-6-C	F	S
49	8.60	8.60	8.90	8.90
49.04	-	-	8.85	8.70
52	8.40	8.70	8.80	8.80
52.04	9.00	8.90	8.80	8.70
55	8.60	8.50	8.60	8.35
58	-	-	8.65	8.75
58.04	-	-	8.70	8.80
59	8.60	8.60	-	-
61	-	-	8.75	8.90
61.04	-	-	8.20	8.70
62	8.75	8.70	-	-
62.04	8.20	7.90	-	-
65	-	-	8.60	8.90
65.04	-	-	8.60	8.60
67	8.50	8.30	-	-
69	-	-	8.70	8.90
69.04	-	-	8.15	8.00
70	8.10	8.20	-	-
70.04	8.30	7.75	-	-
72	-	-	8.50	8.60
72.04	-	-	8.40	8.10

TABLE A-12 CONTINUED:....

Day	Cold Chamber		Room Temperature	
	1-0-B	2-6-C	F	S
73	8.20	8.00	-	-
73.04	8.15	8.05	-	-
75	-	-	8.35	8.45
75.04	-	-	8.70	8.50
77	7.70	8.00	-	-
77.04	7.50	7.50	-	-
78	-	-	8.50	8.50
78.04	-	-	7.50	7.80
81	-	-	8.05	8.10
81.04	-	-	8.20	8.15
82	8.10	8.25	-	-
82.04	7.60	7.65	-	-
84	-	-	8.20	8.30
84.04	-	-	8.30	8.45
87	7.85	7.90	8.55	8.40
87.07	7.65	7.35	8.40	8.30
91	8.00	7.95	8.65	8.60
91.04	7.65	7.45	8.40	8.40
95	8.55	-	8.60	-
95.04	8.55	-	8.40	-

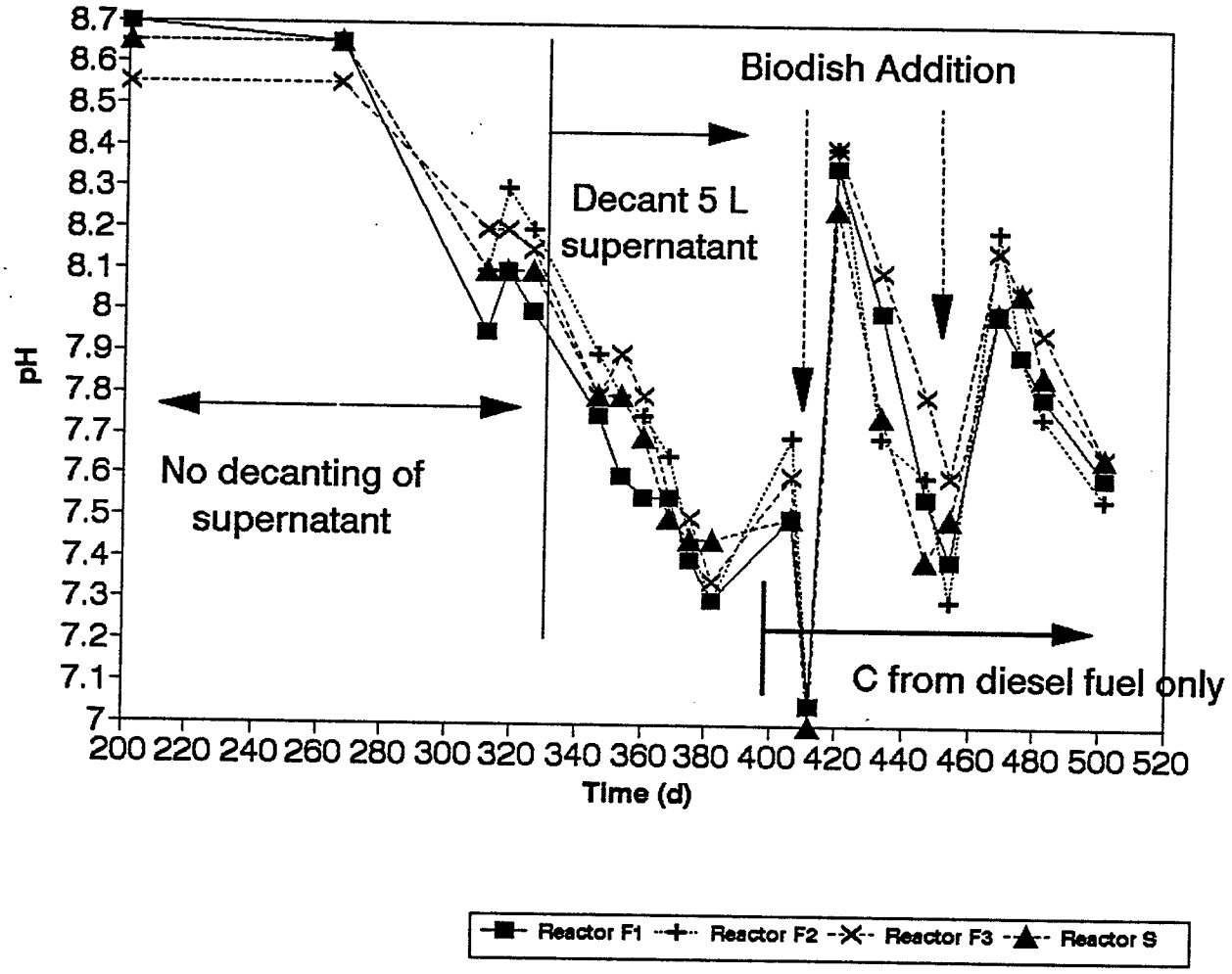




GRAPH A-5: pH vs TIME (d) - (VARIABLE TEMPERATURE) - PHASE II

TABLE A-13: pH - PHASE III

Date	Day	F1	F2	F3	S
Feb. 12	201	8.70	8.65	8.55	8.65
April 17	266	8.65	8.65	8.55	8.65
June 2	312	7.95	8.10	8.20	8.10
8	318	8.10	8.30	8.20	8.10
16	326	8.00	8.20	8.15	8.10
July 6	346	7.75	7.90	7.80	7.80
13	353	7.60	7.80	7.90	7.80
20	360	7.55	7.75	7.80	7.70
28	368	7.55	7.65	7.55	7.50
August 4	375	7.40	7.45	7.50	7.45
11	382	7.30	7.30	7.35	7.45
Sept. 4	406	7.50	7.70	7.60	7.50
10	412	7.05	7.05	7.05	7.00
17	419	8.35	8.40	8.40	8.25
Oct. 1	433	8.00	7.70	8.10	7.75
15	447	7.55	7.60	7.80	7.40
22	454	7.40	7.30	7.60	7.50
Nov. 5	468	8.00	8.20	8.15	8.00
12	475	7.90	7.90	8.05	8.05
19	482	7.80	7.75	7.95	7.85
Dec. 7	501	7.60	7.55	7.65	7.65

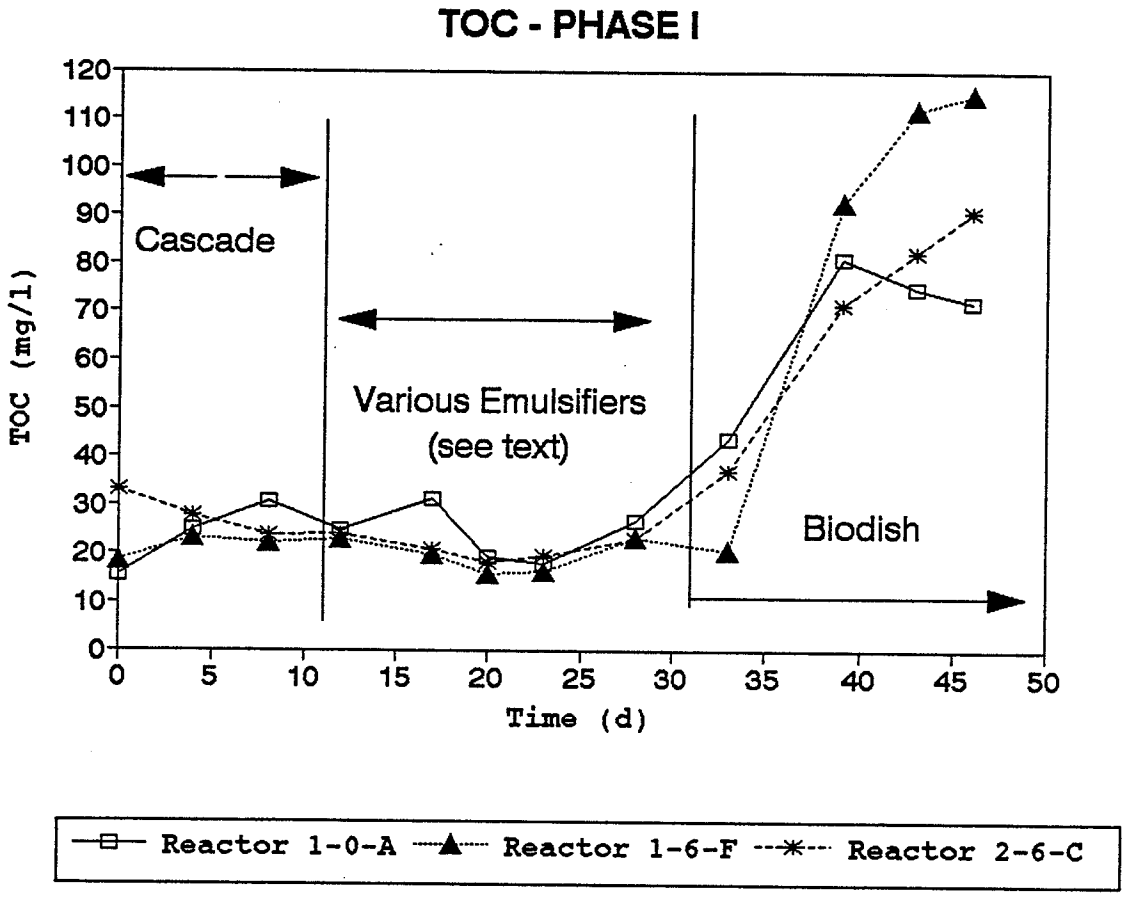


GRAPH A-6: pH vs TIME (d) - PHASE III

TABLE A-14: TOC DATA (mg/l) - PHASE I

	1-0 A	1-0 B	2-6 C	2-6 D	1-6 E	1-6 F	1-6 G	1-6 H	Seed
0	15.5	33.1	33.2	-	-	18.5	-	-	-
4	25.0	-	27.9	-	-	-	-	-	-
5	-	-	-	-	-	23.3	-	-	-
8	30.6	22.5	23.9	25.9	25.	22.2	-	-	-
12	24.8	29.4	24.2	24.5	23.2	23.0	-	-	-
17	31.3	18.1	21.0	22.5	19.1	19.9	16.3	17.5	-
20	19.2	17.9	18.1	18.6	17.1	15.9	13.3	12.9	-
23	18.0	17.8	19.6	17.2	17.3	16.3	15.7	14.2	-
26	-	-	-	-	-	-	-	-	34.2
28	26.7	24.3	23.1	22.6	23.1	23.3	22.9	25.1	30.6
31	-	-	26.5	-	-	22.9	-	-	-
33 *	43.9	37.6	37.0	2.9	30.2	20.7	39.4	48.1	7.2
37	-	-	-	-	-	-	-	-	145
39	81.0	84.2	71.4	56.6	80.5	92.8	67.1	74.1	-
40	-	-	-	-	-	-	-	-	172
43	74.8	74.1	82.3	63.8	86.6	112	73.9	82.9	178
46	71.9	70.0	90.9	70.8	82.9	115	69.7	90.3	-

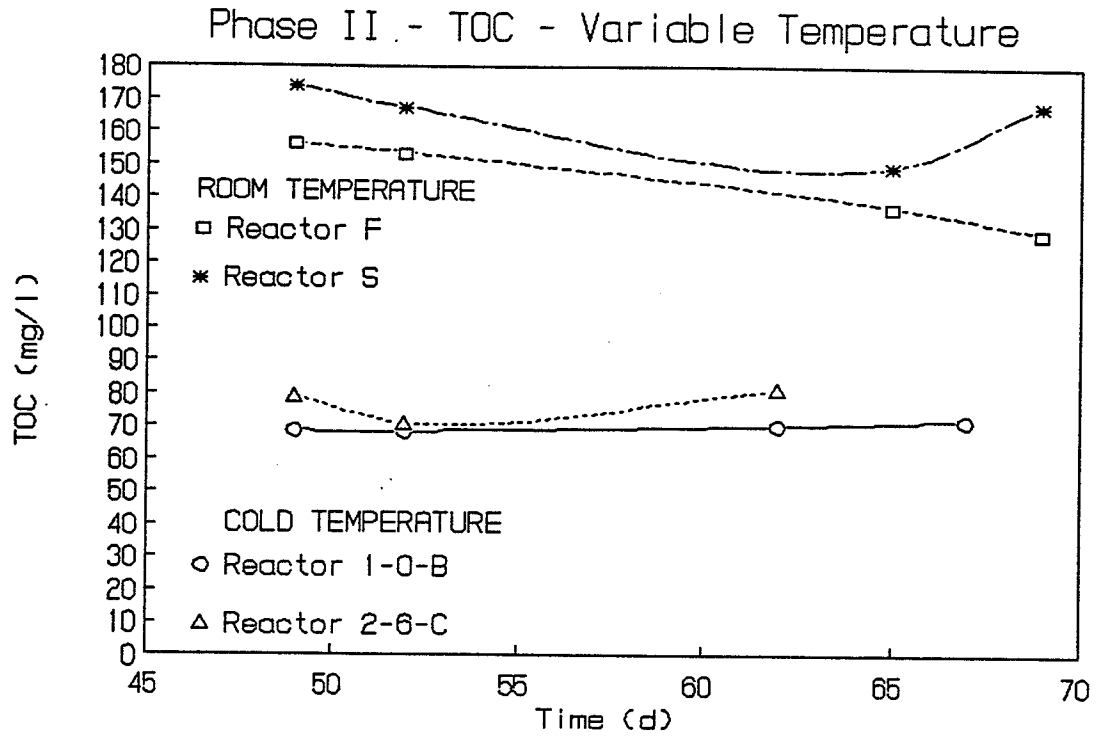
* this data row suspect due to samples being run on the lower range of 10 mg rather than the 400 mg range

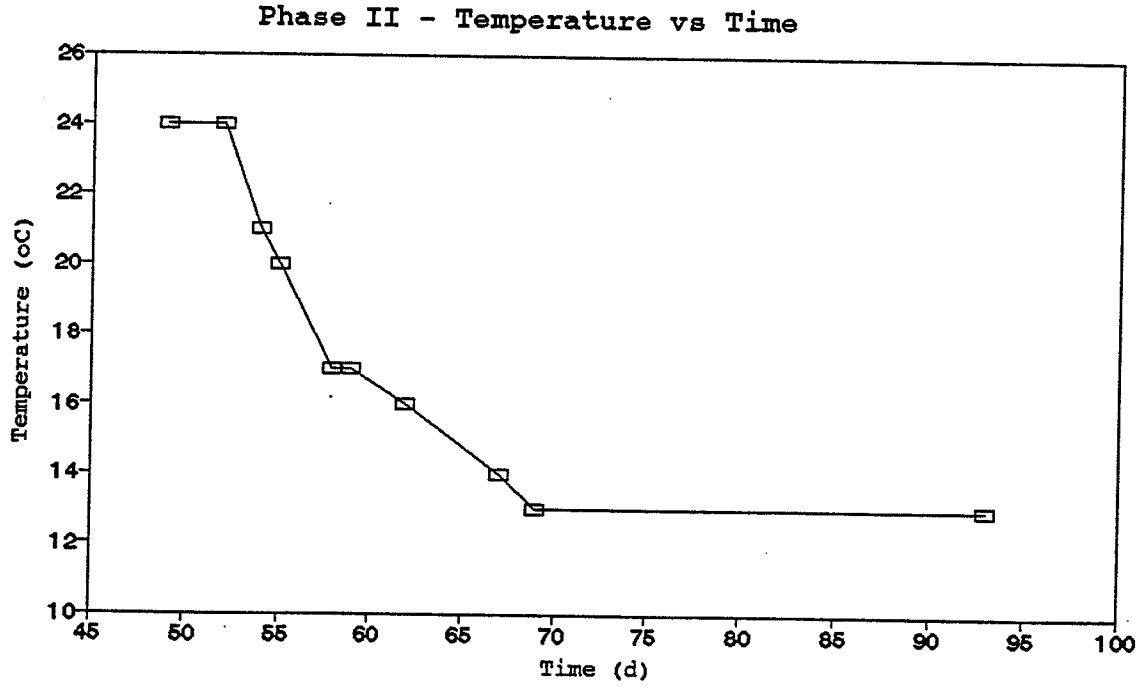


GRAPH A-7: TOC vs TIME (d) - PHASE I

TABLE A-15: TOC DATA (mg/l) - PHASE II

Day	Cold Chamber		Room Temperature	
	1-0-B	2-6-C	F	S
49	68.7	79.1	156.4	174.1
49.04	-	-	171.3	176.0
52	68.6	71.0	153.5	167.3
52.04	113.7	98.3	159.2	178.8
61.04	-	-	142.3	144.2
62	70.5	81.5	-	-
62.04	63.8	87.9	-	-
65	-	-	137.0	149.4
65.04	-	-	155.3	149.8
67	72.2	577.2*	-	-
69	-	-	128.9	168.4

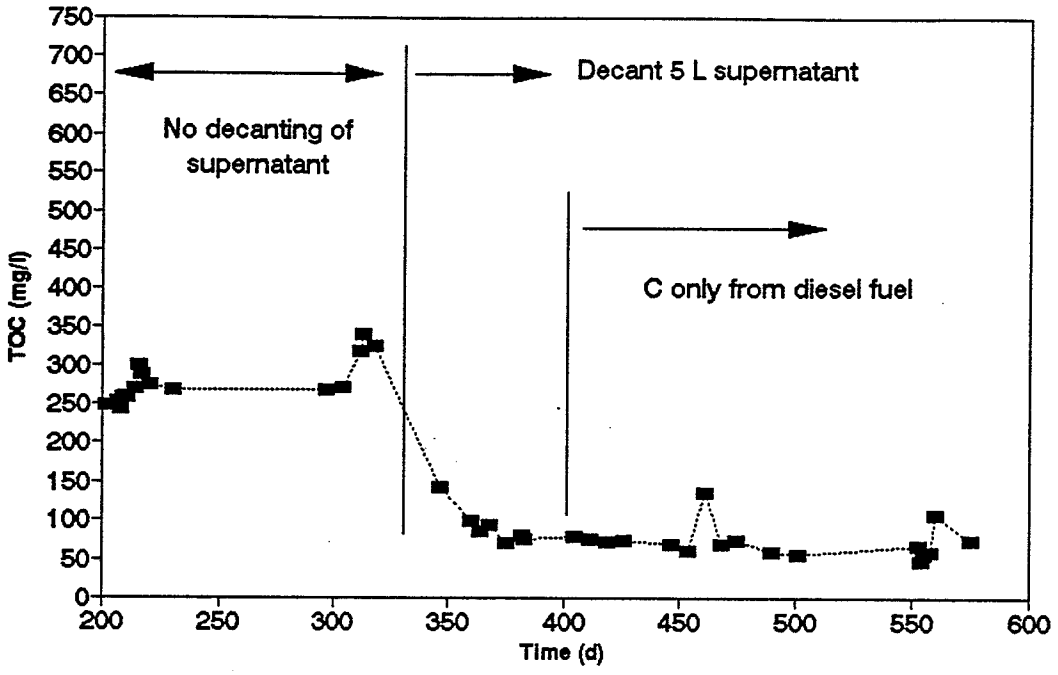




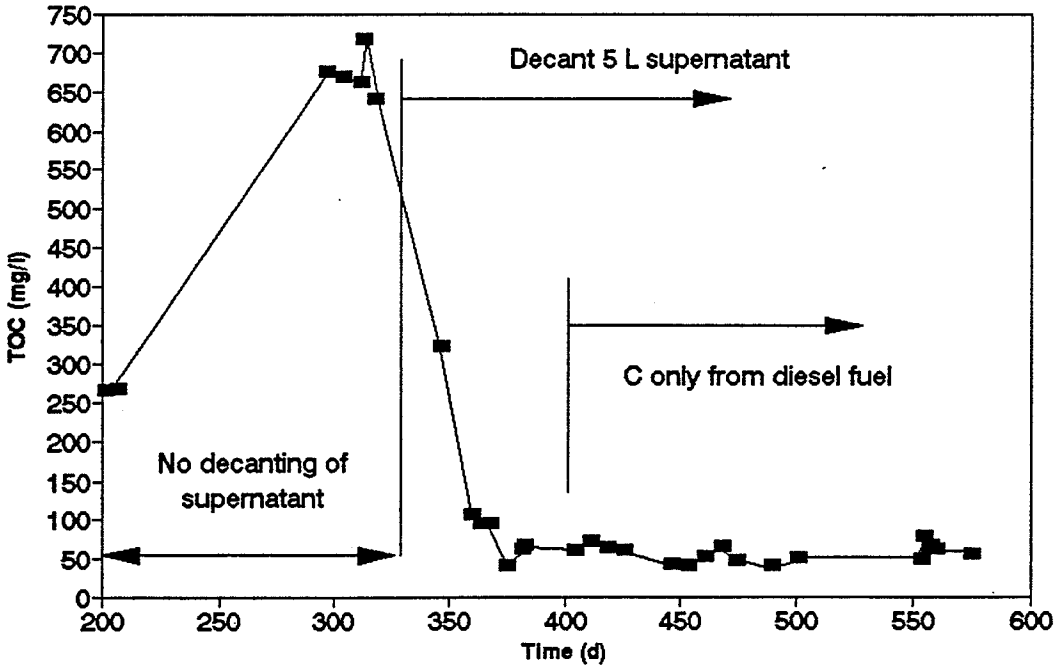
GRAPH A-8: TOC vs TIME (d) - PHASE II

TABLE A-16: EFFLUENT TOTAL ORGANIC CARBON - PHASE III

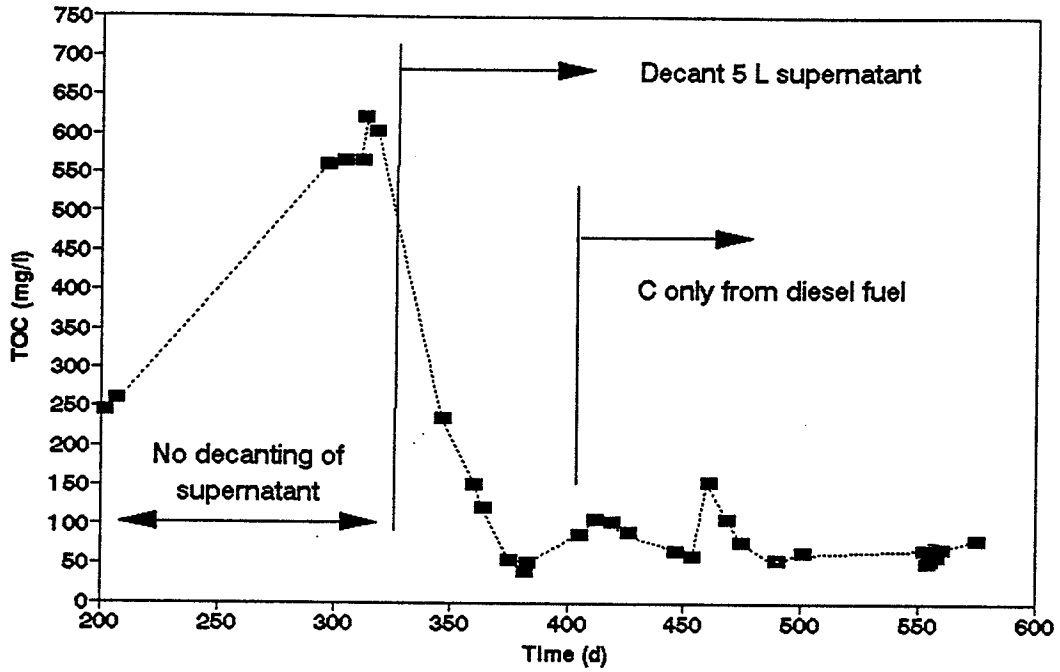
Date	Day	F1 (mg/l)	F2 (mg/l)	F3 (mg/l)	S (mg/l)	Influent (mg/l)
February 13	202	249.3	267.2	246.0	232.9	1085.0
18	207	252.9	269.4	260.2	228.1	
19	208	243.3				
20	209	258.3				
21	210	260.3				
22	211	258.5				
25	214	270.0				
27	216	301.0				
28	217	288.7				
March 3	220	274.9				
13	230	269.1				
May 19	297	269.4	676.5	561.0		
25	304	272.0	671.6	567.3		
June 2	312	317.8	662.6	565.4	461.6	
3	313	340.6	719.4	621.7	503.3	
8	318	325.6	641.2	604.1	477.5	626.3
July 7	347	143.3	323.2	235.4	299.3	
20	360	98.5	108.0	152.0	119.0	
24	364	85.4	95.9	123.0	94.4	
28	368	94.6	96.8		121.8	
August 4	375	71.2	41.3	54.8	61.1	
11	382	81.8	63.1	39.8	70.4	
12	383	75.7	67.3	52.1	44.1	
September 3	405	78.9	59.8	88.8	86.0	
10	412	76.8	72.0	107.3	89.6	
17	419	71.7	65.0	104.1	55.7	
24	426	73.9	59.9	90.8	85.7	
October 15	447	69.2	43.2	66.2	62.7	
22	454	61.6	41.2	60.0	55.5	
29	461	136.6	52.6	155.2	108.8	
November 6	469	69.9	66.0	106.5	83.0	
12	475	75.1	48.5	77.6	84.4	
27	490	59.2	40.0	55.5	51.9	
December 7	501	55.8	50.9	65.0	54.9	
January 28	553	67.1		67.9	61.9	
29	554	48.6	50.3	51.9	56.0	
30	555	56.0	78.8	55.1	63.0	
February 1	557	59.0	65.0	66.5	63.1	
2	558	59.2	68.9	60.3	62.9	
4	560	107.1	62.6	70.1	70.8	
19	575	73.9	56.9	81.3	90.6	



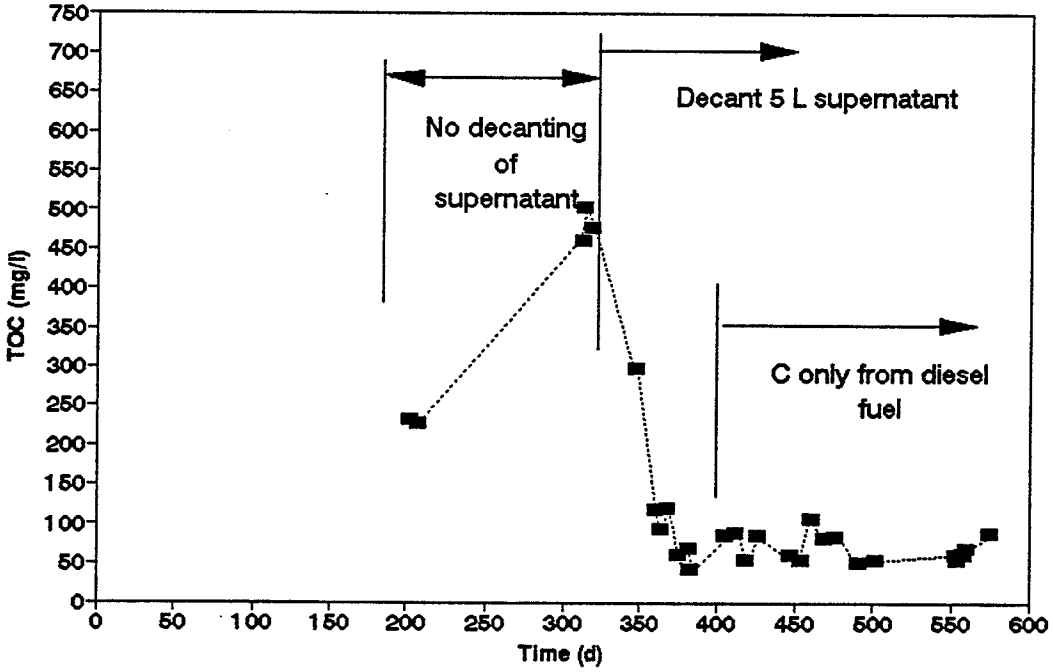
GRAPH A-9: TOC (mg/l) vs TIME (d) - REACTOR F1 - PHASE III



GRAPH A-10: TOC (mg/l) vs TIME (d) - REACTOR F2 - PHASE III



GRAPH A-11: TOC (mg/l) vs TIME (d) - REACTOR F3 - PHASE III



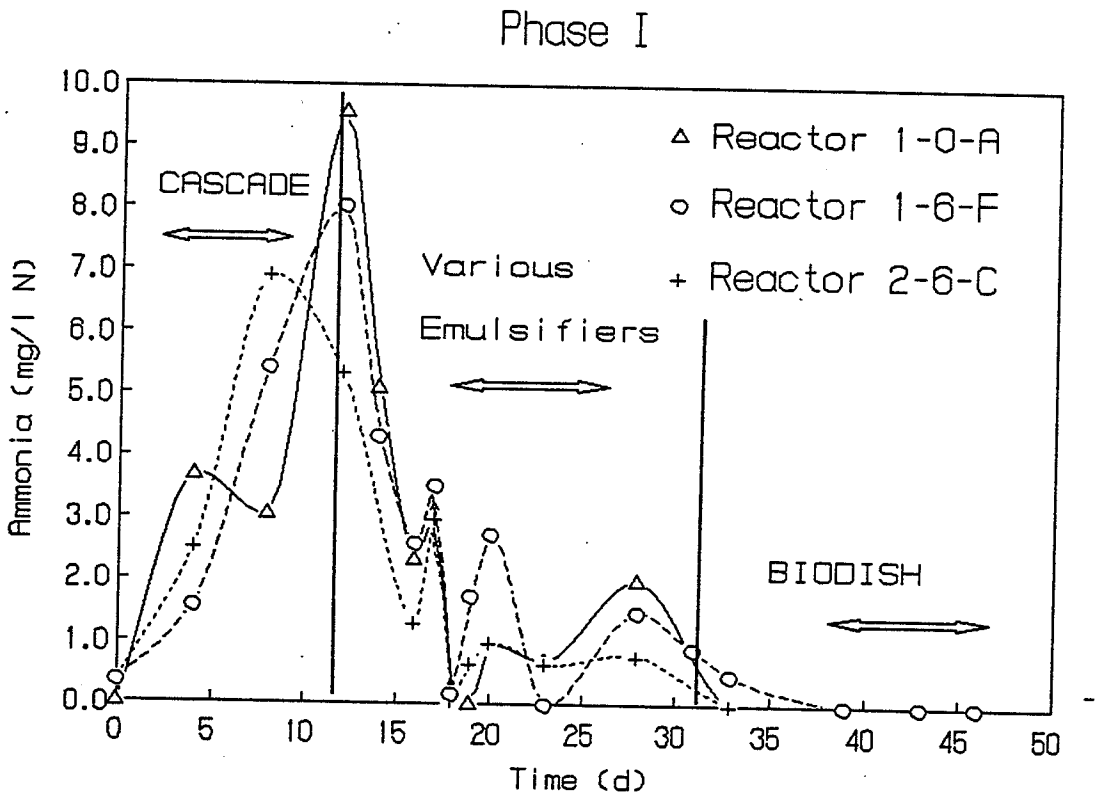
GRAPH A-12: TOC (mg/l) vs TIME (d) - REACTOR S - PHASE III

TABLE A-17-1: AMMONIA DATA (mg/l N) - PHASE I - REACTORS A,B,C,D

Day	1-0-A	1-0-B	2-6-C	2-6-D
0	0	-	0.36	-
4	3.69	-	2.5	-
8	3.05	4.90	6.92	7.28
12	9.58	7.67	5.34	7.90
14	5.11	4.59	-	2.51
16	2.32	2.92	2.41	1.30
17	3.11	3.34	2.88	2.97
18	0.23	0.05	0.65	0.05
19	0	0.19	0.42	0.65
20	0.93	1.30	1.24	0.99
23	0.69	0.42	1.07	0.65
28	1.99	3.07	1.49	0.79
31	-	-	0	-
33	0	0	0	0
39	0	0	0	0
43	0	0	0	0
46	0	0	0	0

TABLE A-17-2: AMMONIA DATA (mg/l N) - PHASE I - REACTORS E,F,G,H,Seed

Day	1-6-E	1-6-F	1-6-G	1-6-H	Seed
0	-	0.36	-	-	-
5	-	1.56	-	-	-
8	3.05	5.43	-	-	-
12	7.85	8.03	-	-	-
14	3.34	4.32	0.95	0.96	-
16	1.99	2.60	0.86	0.86	-
17	2.28	3.53	1.86	2.04	-
18	0	0.18	0	0	-
19	1.44	1.72	0.99	0.93	-
20	2.23	2.74	1.25	2.00	-
23	0	0	0	0.28	-
26	-	-	-	-	5.76
28	3.29	1.49	4.09	4.13	9.52
31	-	0.89	-	-	-
33	0.98	0.47	0	0.51	0
35	-	-	-	-	0
39	0	0	0	0	-
43	0	0	0	0	0
46	0	0	0	0	-



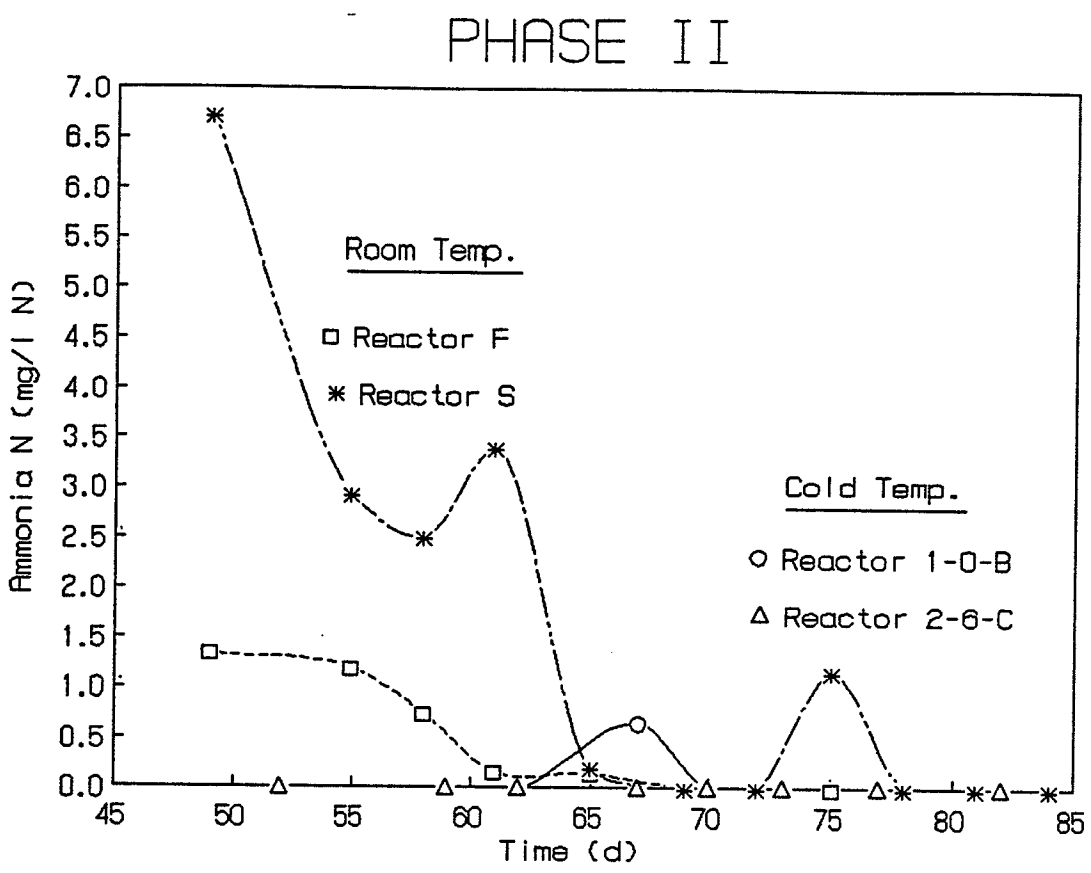
GRAPH A-13: AMMONIA (mg/l N) vs TIME - PHASE I

TABLE A-18: AMMONIA (mg/l N) DATA - PHASE II

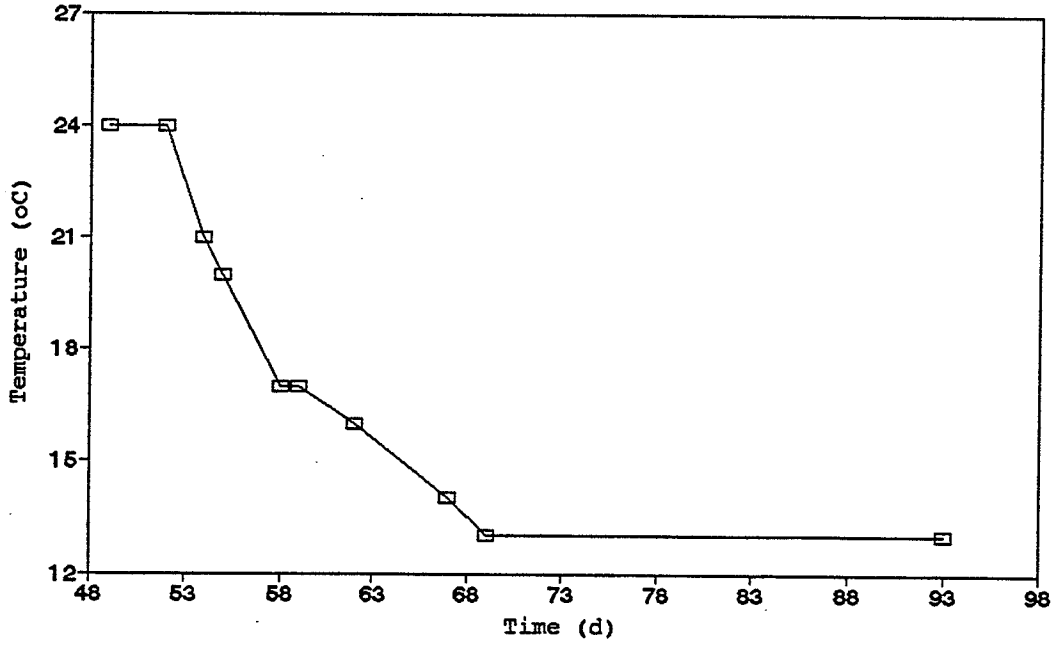
Day	Cold Chamber		Room Temperature	
	1-0-B	2-6-C	F	S
49			1.34	6.72
49.04			3.71	5.11
52	0	0		
52.04	1.49	0.28		
55	0	0	1.19	2.93
58			0.74	2.51
58.04			3.16	4.74
59	0	0		
61			1.26	3.39
61.04			1.09	1.77
62	0	0		
62.04	0.44	0		
65			0.15	0.20
65.04			1.57	1.57
67	0.66	0		
69			0	0
69.04			0	0
70	0	0		
70.04	0	0		
72			0	0
72.04			0	0
73	0	0		
73.04	0	0		
75			0	1.15
75.04			0	0.57

TABLE A-18 CONTINUED:....

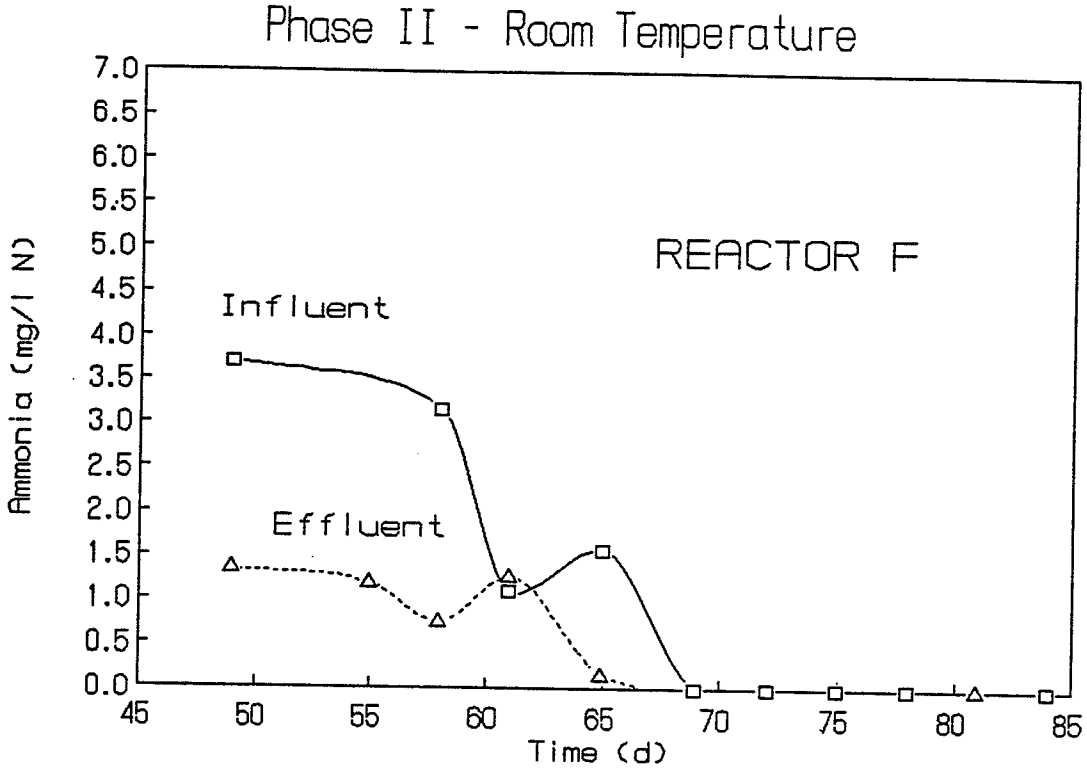
Day	Cold Chamber		Room Temperature	
	1-0-B	2-6-C	F	S
77	0	0		
77.04	0.17	0		
78			0	0
78.04			0	0.62
81			0	0
81.04			0	0
82	0	0		
82.04	0	0		
84			0	0
84.04			0	0.55



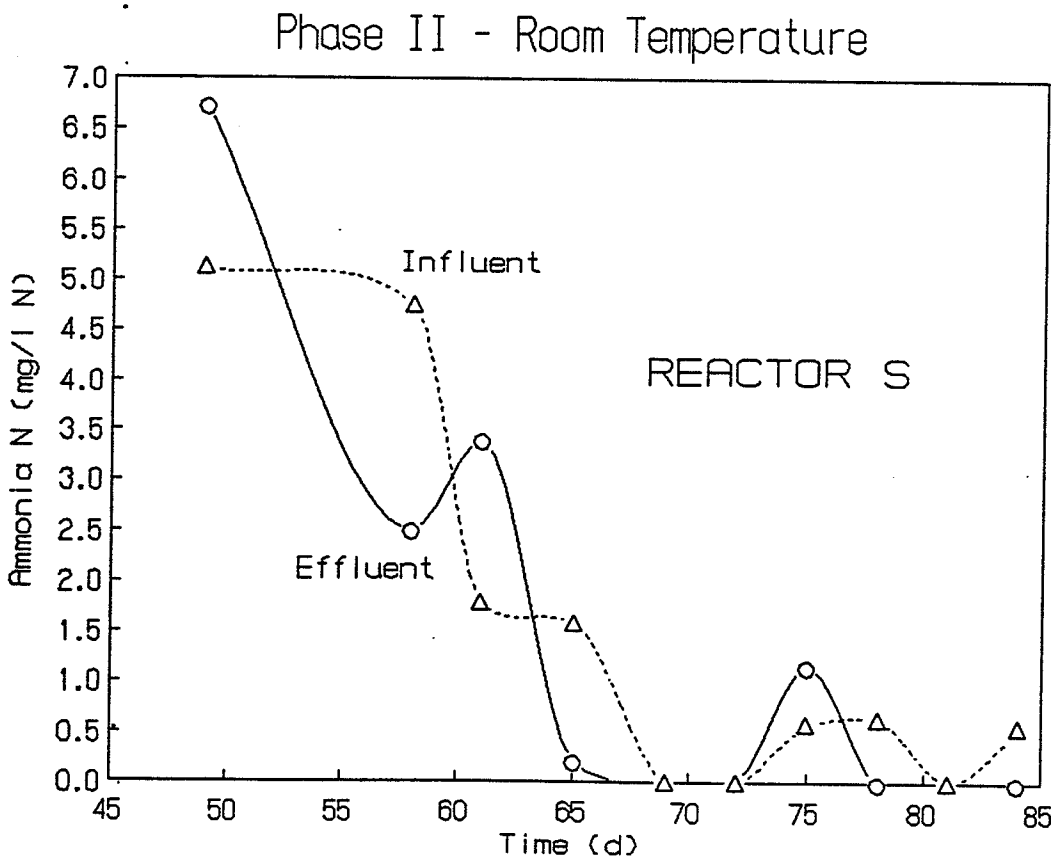
Phase II - Temperature vs Time



GRAPH A-14: EFFLUENT AMMONIA (mg/l N) vs TIME - PHASE II



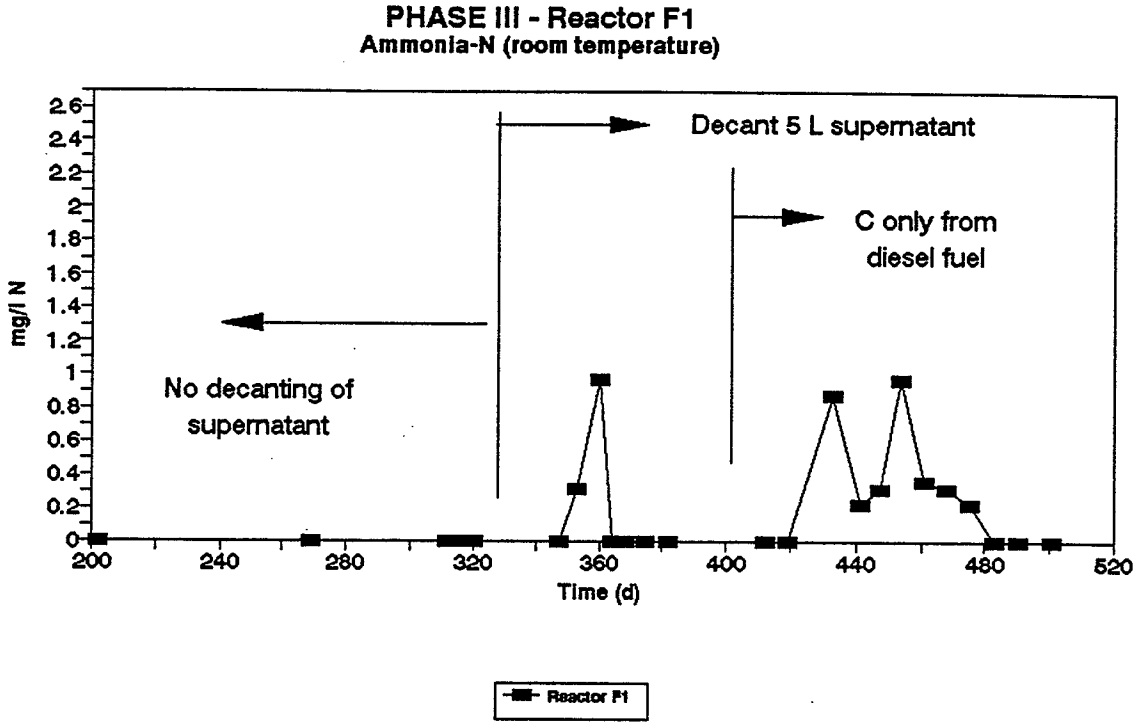
GRAPH A-15: AMMONIA (mg/l N) vs TIME (REACTOR F)



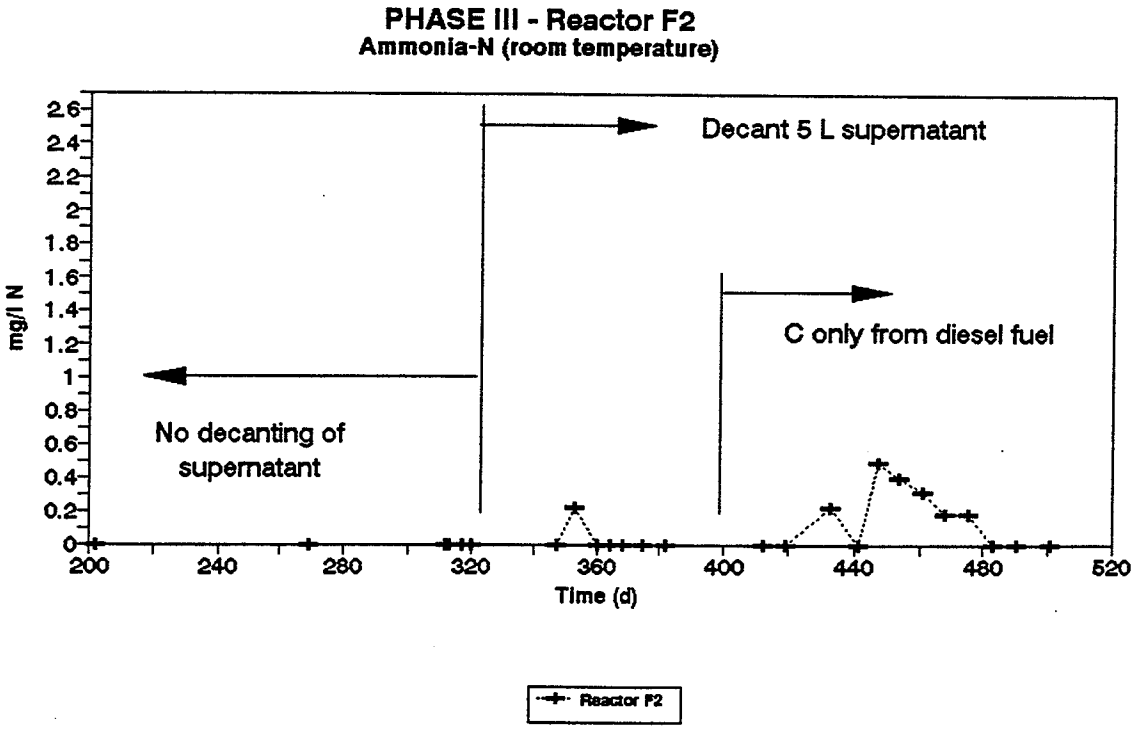
GRAPH A-16: AMMONIA (mg/l N) vs TIME (REACTOR S)

TABLE A-19: AMMONIA (mg/l N) DATA - PHASE III

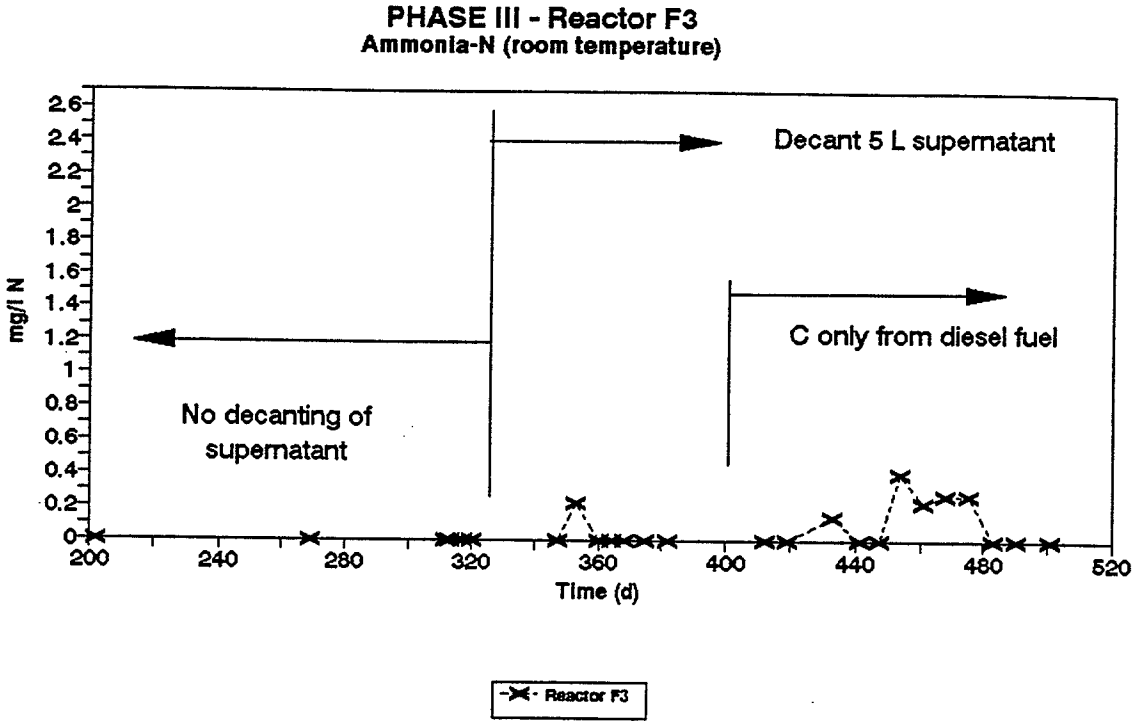
Date	Day	F1	F2	F3	S
Feb.13	202	0	0	0	2.60
April 20	269	0	0	0	0
June 2	312	0	0	0	0
3	313	0	0	0	0
7	317	0	0	0	0
10	320	0	0	0	0
July 7	347	0	0	0	0
13	353	0.31	0.22	0.22	0.14
20	360	0.97	0	0	0
24	364	0	0	0	0
28	368	0	0	0	0
August 4	375	0	0	0	0
11	382	0	0	0	0
Sept. 10	412	0	0	0	0
17	419	0	0	0	0
Oct. 1	433	0.88	0.22	0.14	0.05
9	441	0.22	0	0	0
15	447	0.31	0.49	0	0
22	454	0.97	0.40	0.40	0.89
29	461	0.36	0.31	0.22	0.22
Nov. 5	468	0.31	0.18	0.27	0.22
12	475	0.22	0.18	0.27	0.18
20	483	0	0	0	0
27	490	0	0	0	0
Dec. 7	501	0	0	0	0



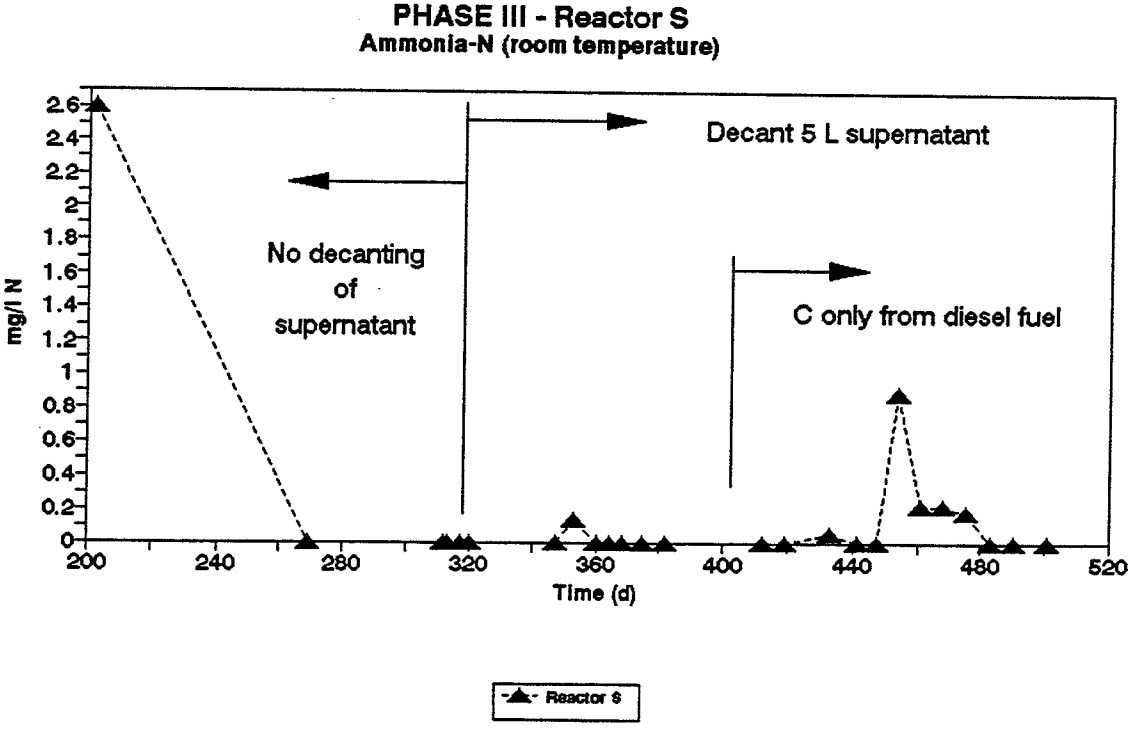
GRAPH A-17: AMMONIA-N (mg/l) vs TIME - PHASE III - F1



GRAPH A-18: AMMONIA-N (mg/l) vs TIME - PHASE III - F2



GRAPH A-19: AMMONIA-N (mg/l) vs TIME - PHASE III - F3



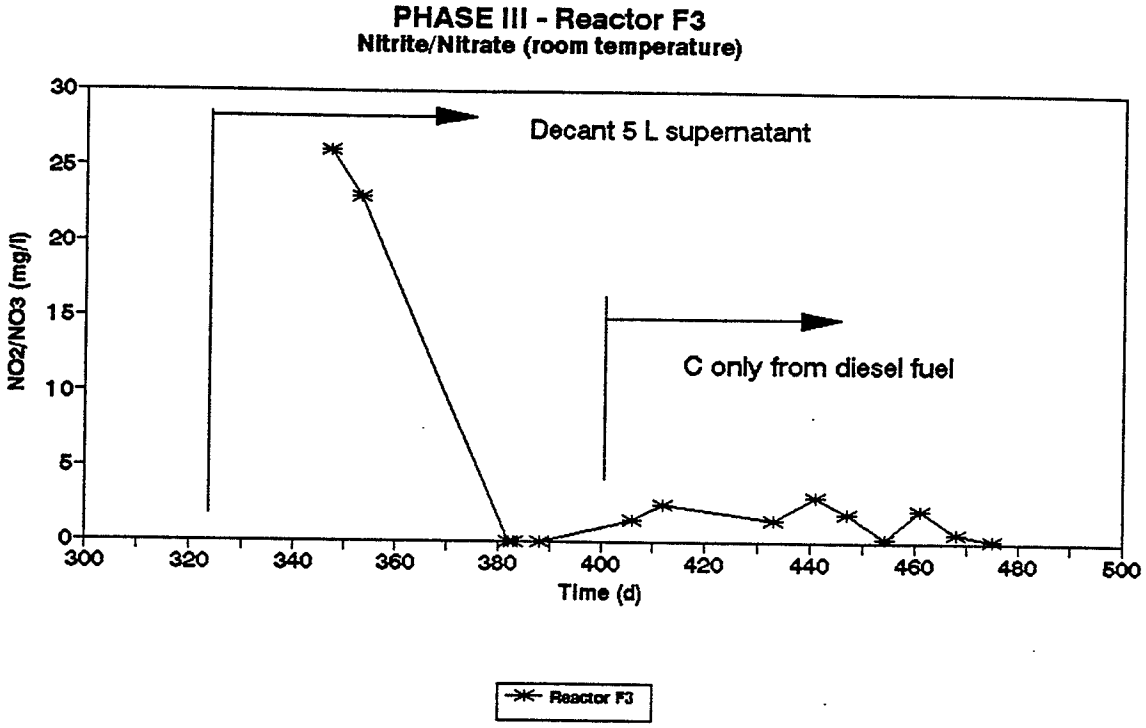
GRAPH A-20: AMMONIA-N (mg/l) vs TIME - PHASE III - S

TABLE A-20: NITRITE/NITRATE (mg/l)- PHASE I

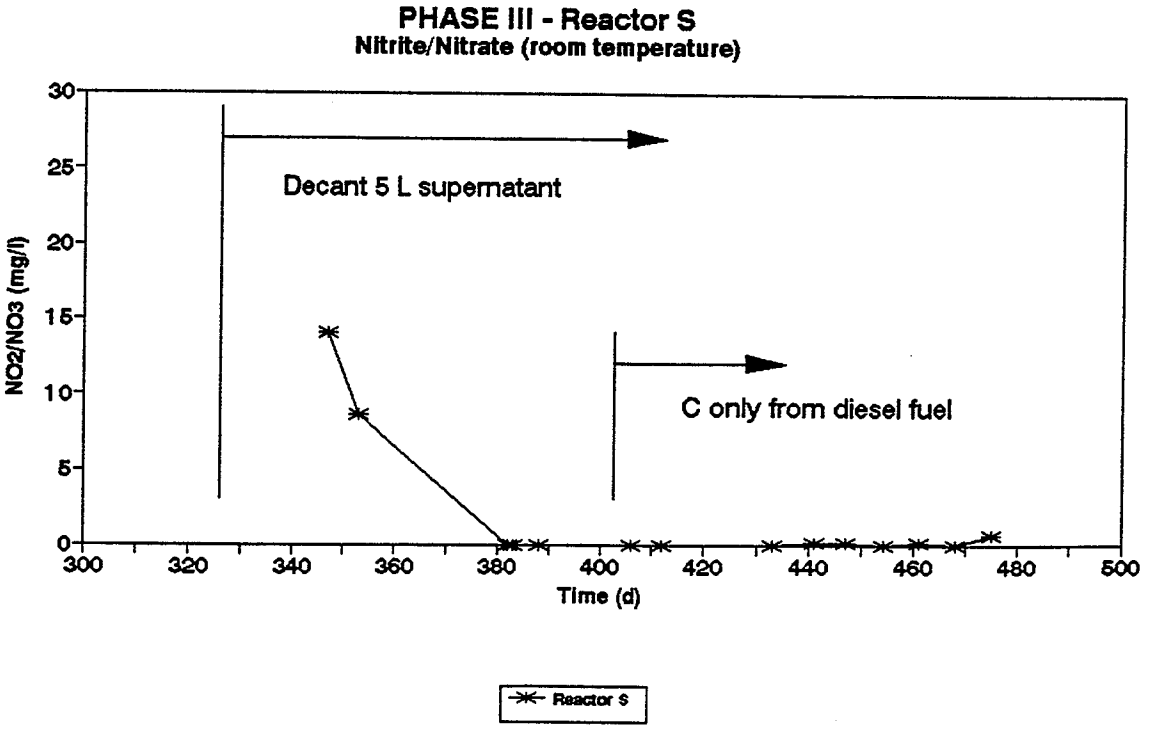
Day	1-0-A		2-6-C		1-6-F	
	NO2	NO3	NO2	NO3	NO2	NO3
0	0	0	0	0	0	0
4	0	0	0	0		
5					0	0
8	0.13	0	0	0	0	0
17	0.05	0.04	0.06	0	0.07	0
20	0	0	0	0	0	0
23	0	0	0	0	0	0
28	0	0	0	0	0	0
33	0	0	0	0	0	0

TABLE A-21: NITRITE/NITRATE (mg/l) - PHASE III

Date	Day	F1	F2	F3	S
July 7	347	24	46	26	14
13	353	23	28	23	8.6
August 11	382	0	0	0	0
12	383	0.1	0	0	0
17	388	0	0	0	0
Sept. 4	406	3.6	0.7	1.4	0
10	412	2.3	0	2.4	0
Oct. 1	433	1.9	0.1	1.5	0
9	441	>2.5	0	3	0.15
15	447	>2.5	0	1.9	0.2
22	454	4	0	0.25	0
29	461	5.5	0.1	2.1	0.2
Nov. 5	468	7	0	0.55	0
12	475	9	0	0.15	0.65



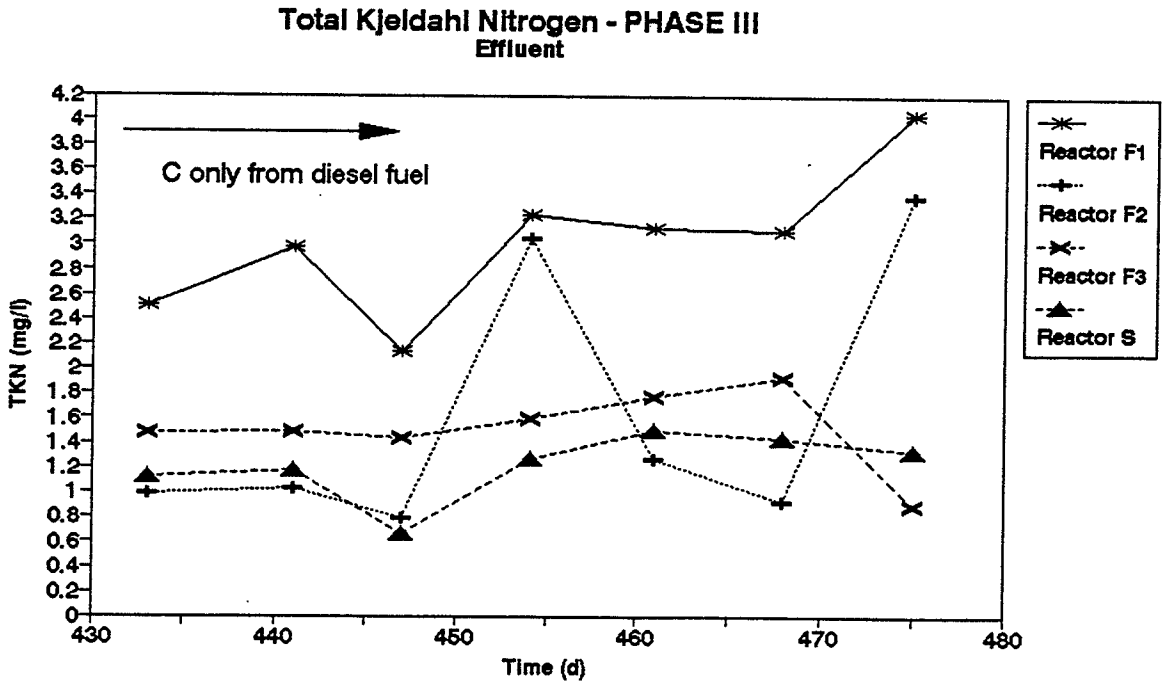
GRAPH A-21: NITRITE/NITRATE (mg/l) vs TIME - PHASE III - F3



GRAPH A-22: NITRITE/NITRATE (mg/l) vs TIME - PHASE III - S

TABLE A-22: TKN (mg/l N) - PHASE III

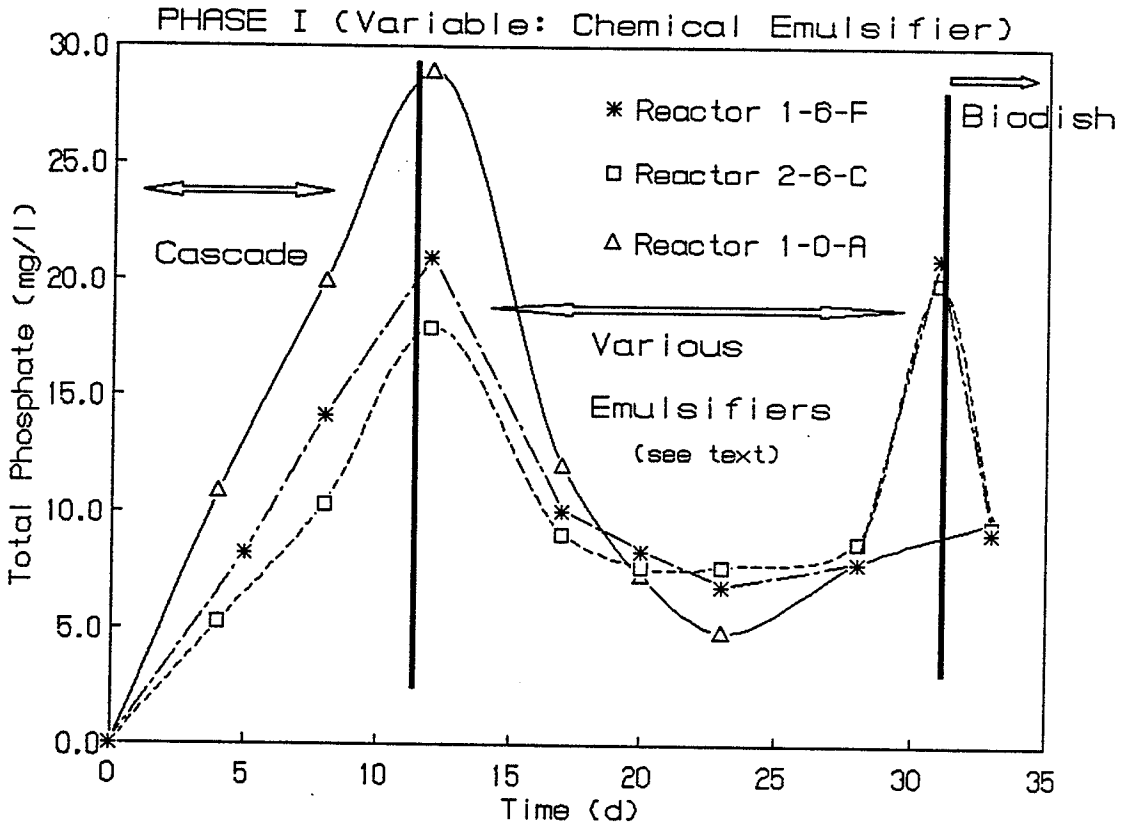
Date	Day	F1	F2	F3	S
October 1	433	2.51	0.99	1.48	1.12
9	441	2.98	1.03	1.49	1.17
15	447	2.14	0.79	1.43	0.66
22	454	3.24	3.05	1.59	1.26
29	461	3.13	1.26	1.77	1.50
Nov. 5	468	3.11	0.92	1.92	1.43
12	475	4.05	3.39	0.90	1.33



GRAPH A-23: TKN (mg/l) vs TIME - PHASE III - ALL REACTORS

TABLE A-23: TOTAL PHOSPHATE DATA (mg/l) - PHASE I

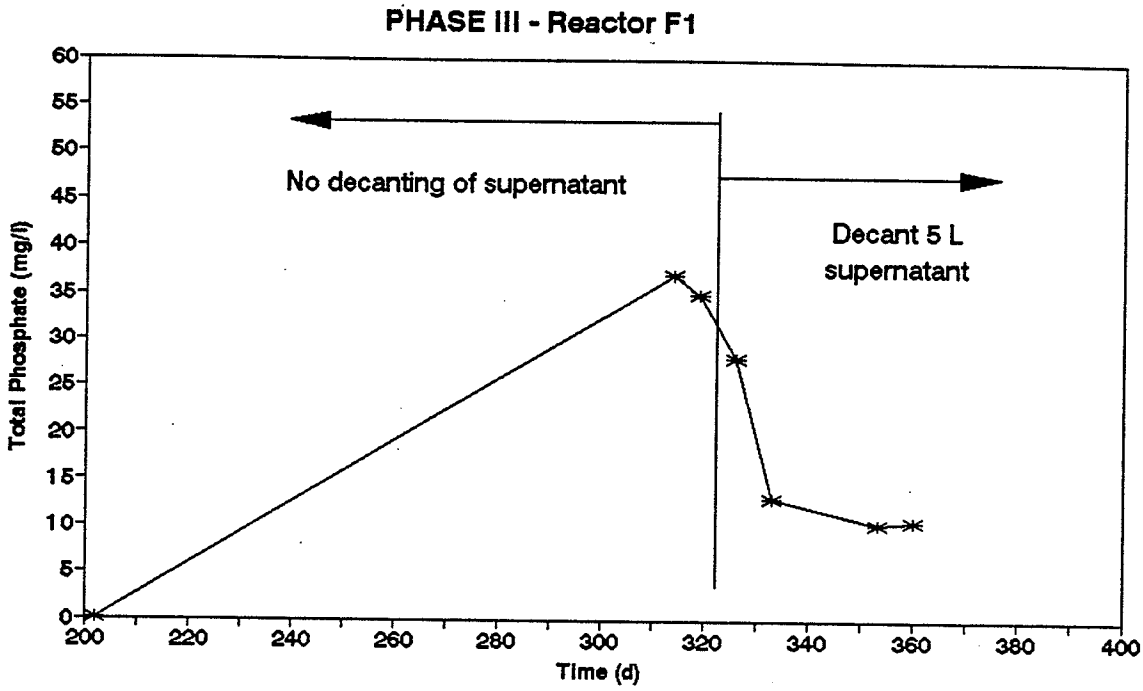
Day	1-0 A	1-0 B	2-6 C	2-6 D	1-6 E	1-6 F	1-6 G	1-6 H	Seed
0	0.1	-	0.1	-	-	0.1	-	-	
4	10.9	-	5.3	-	-	-	-	-	
5	-	-	-	-	-	8.3	-	-	
8	20	20	10.4	13.5	-	14.2	-	-	
12	29	34	18	23	23	21	-	-	
17	12	16	9.1	9.7	10.1	10.1	8.4	8.2	
20	7.3	7.3	7.7	7.0	8.1	8.4	4.7	4.2	
23	4.9	3.8	7.7	7.2	7.3	6.9	2.2	2.0	
26	-	-	-	-	-	-	-	-	15.0
28	7.9	8.2	8.8	8.4	8.7	7.9	7.5	8.7	10.7
31	-	-	20	-	-	21	-	-	-
33	9.7	9.7	9.5	10.0	10.0	9.2	9.8	11.2	32



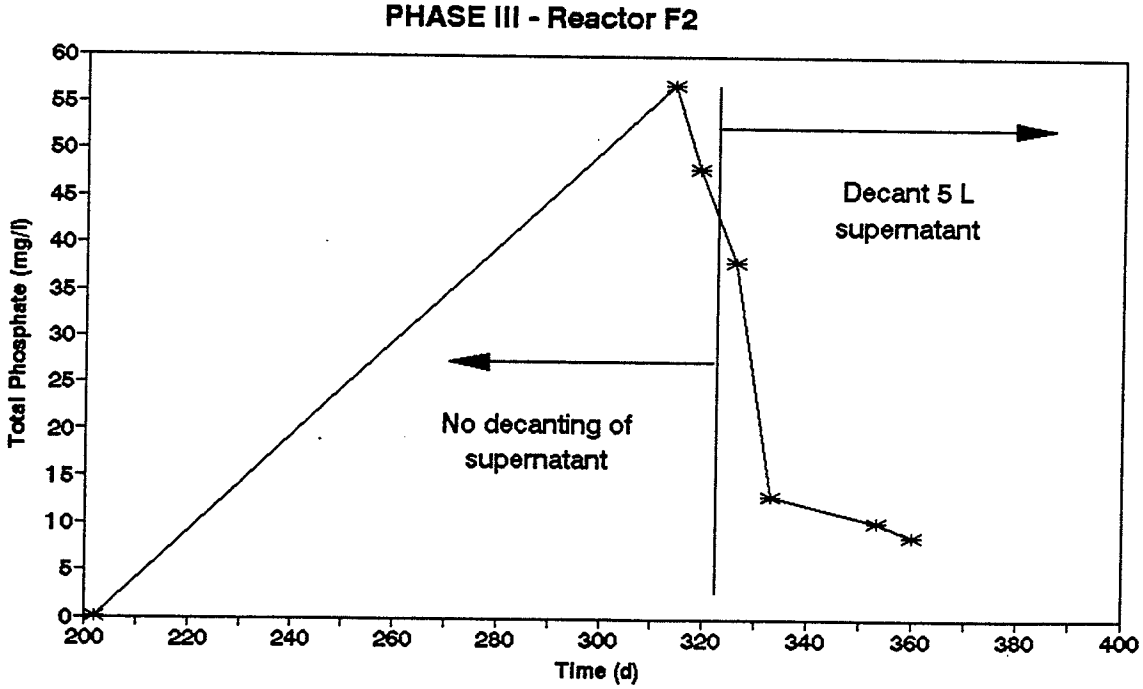
GRAPH A-24: TOTAL PHOSPHATE (mg/l) vs TIME - PHASE I

TABLE A-24: TOTAL PHOSPHATE - PHASE III

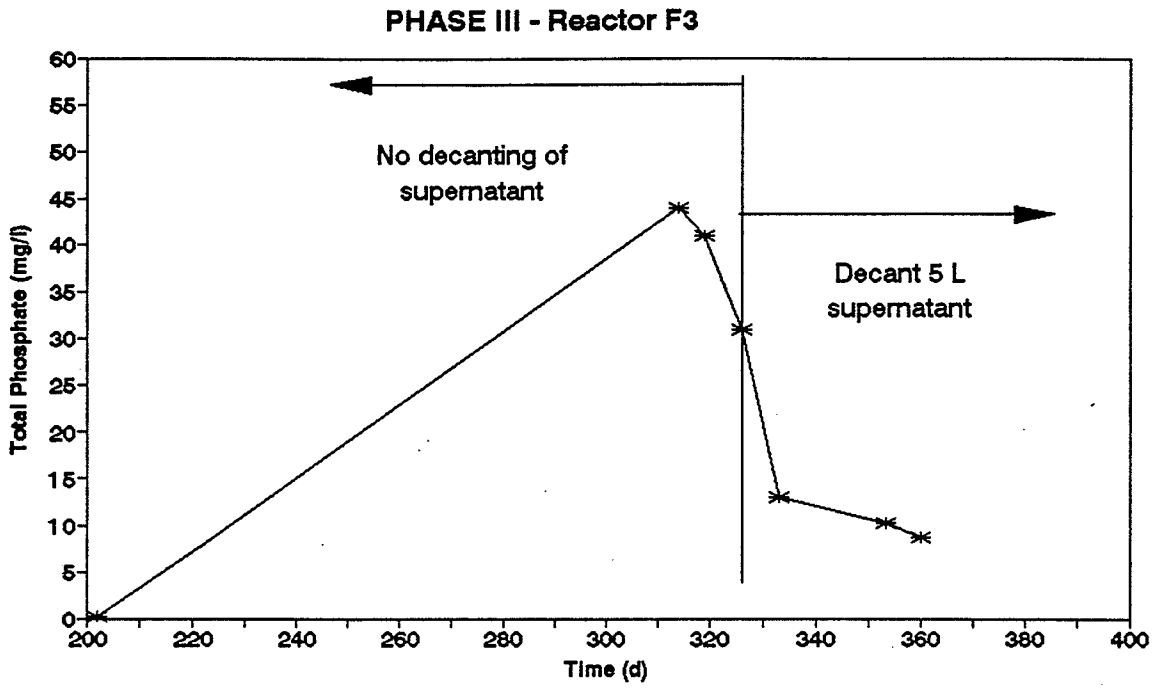
Date	Day	F1	F2	F3	S
February	202	0.1	0.1	0.3	0.1
June 4	314	37	57	44	31
9	319	35	48	41	33
16	326	28	38	31	28
23	333	13	13	13	13
July 13	353	10.3	10.3	10.3	10.6
20	360	10.5	8.8	8.7	27



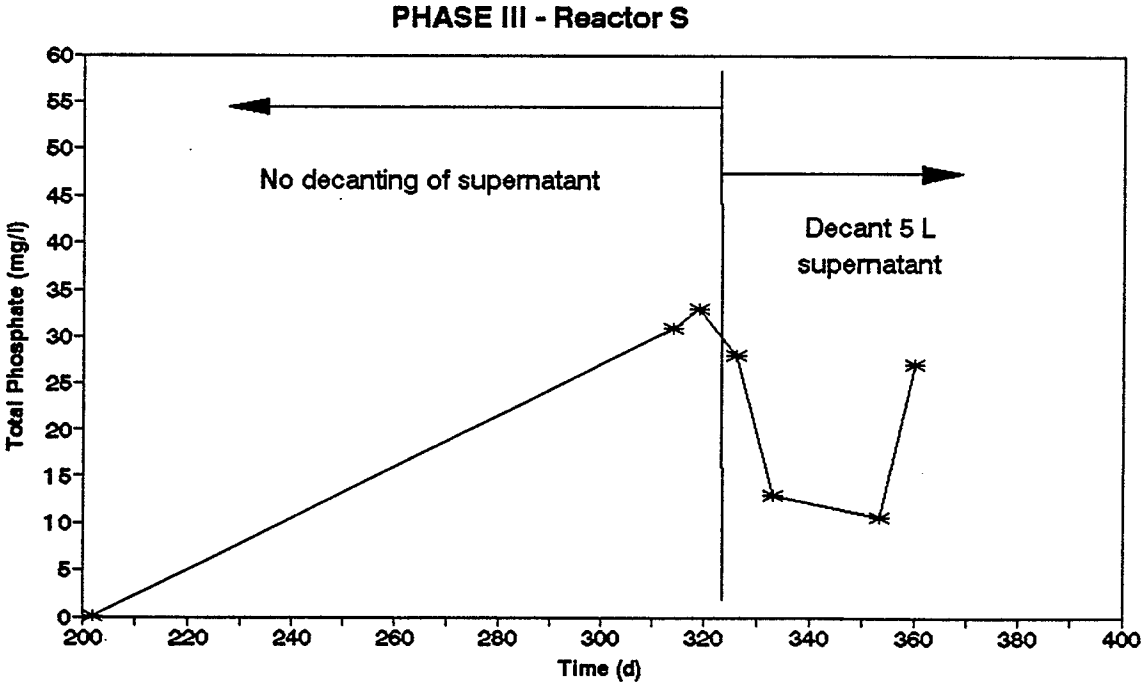
GRAPH A-25: TOTAL PHOSPHATE (mg/l) vs TIME - PHASE III



GRAPH A-26: TOTAL PHOSPHATE (mg/l) vs TIME - PHASE III



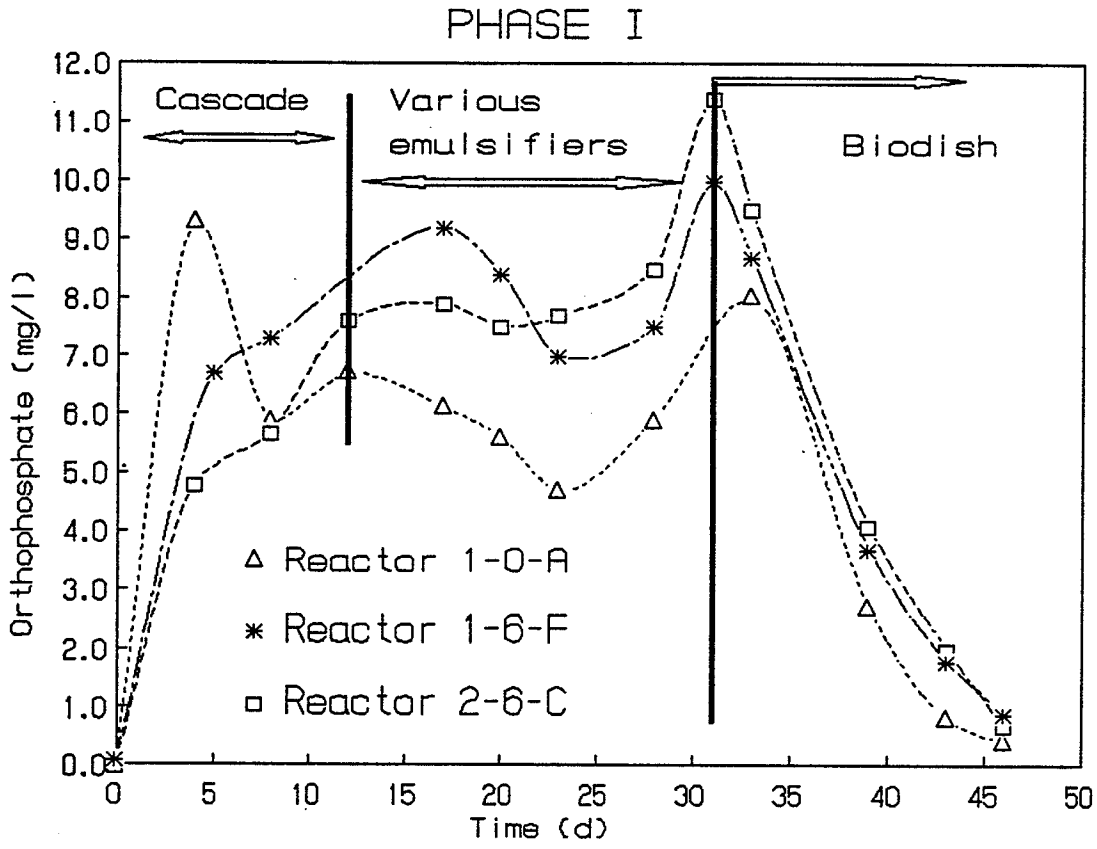
GRAPH A-27: TOTAL PHOSPHATE (mg/l) vs TIME - PHASE III



GRAPH A-28: TOTAL PHOSPHATE (mg/l) vs TIME - PHASE III

TABLE A-25: ORTHOPHOSPHATE DATA (mg/l) - PHASE I

Day	1-0 A	1-0 B	2-6 C	2-6 D	1-6 E	1-6 F	1-6 G	1-6 H	Seed
0	0.1	-	0	-	-	0.1	-	-	
4	9.3	-	4.8	-	-	-	-	-	
5	-	-	-	-	-	6.7	-	-	
8	5.9	4.7	5.7	4.0	7.1	7.3	-	-	
12	6.7	4.6	7.6	5.7	-	-	-	-	
17	6.1	4.5	7.9	6.0	8.7	9.2	4.8	4.8	
20	5.6	5.0	7.5	6.7	8.0	8.4	4.5	3.3	
23	4.7	3.7	7.7	7.1	7.2	7.0	2.0	1.7	
26	-	-	-	-	-	-	-	-	10.2
28	5.9	5.4	8.5	7.7	7.4	7.5	5.4	8.6	10.3
31	-	-	11.4	-	-	10	-	-	-
33	8.0	5.6	9.5	9.5	6.6	8.7	5.7	8.3	24
37	-	-	-	-	-	-	-	-	26
39	2.7	2.5	4.1	3.8	4.4	3.7	2.2	3.1	-
40	-	-	-	-	-	-	-	-	13.7
43	0.8	0.7	2.0	2.1	1.9	1.8	0.3	1.1	10.1
46	0.4	0.2	0.7	1.7	0.8	0.9	0.1	0.5	-



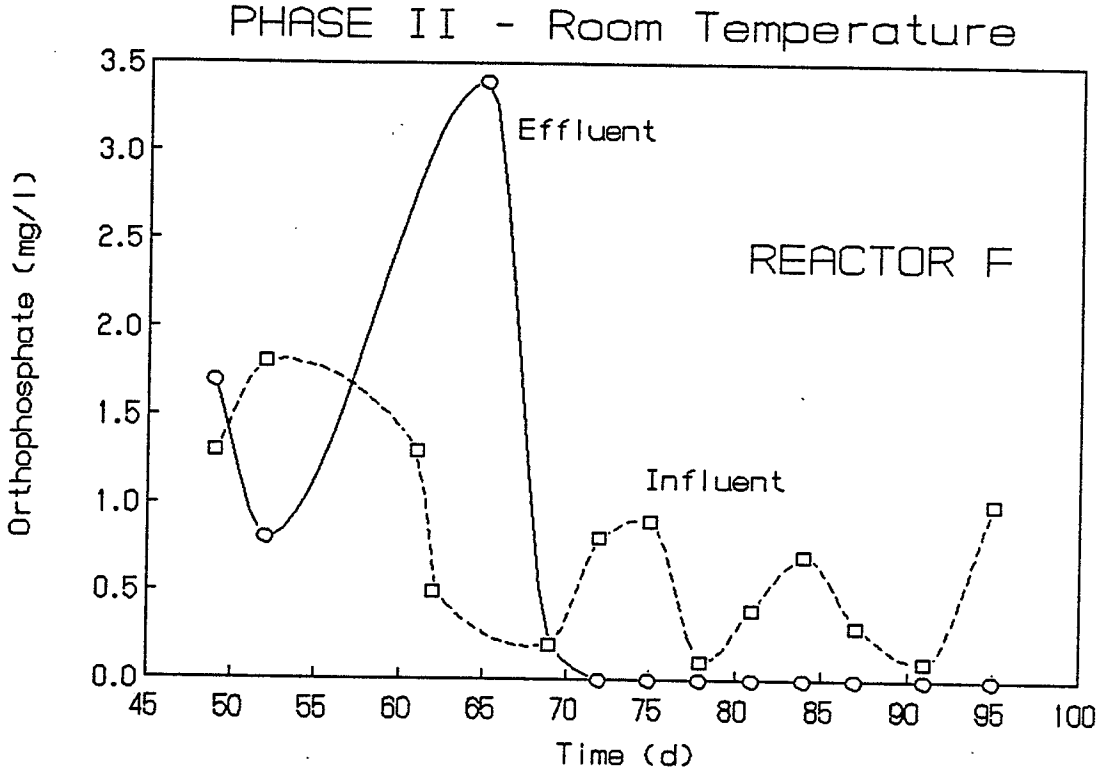
GRAPH A-29: ORTHOPHOSPHATE (mg/l) vs TIME - PHASE I

TABLE A-26: ORTHOPHOSPHATE DATA - PHASE II

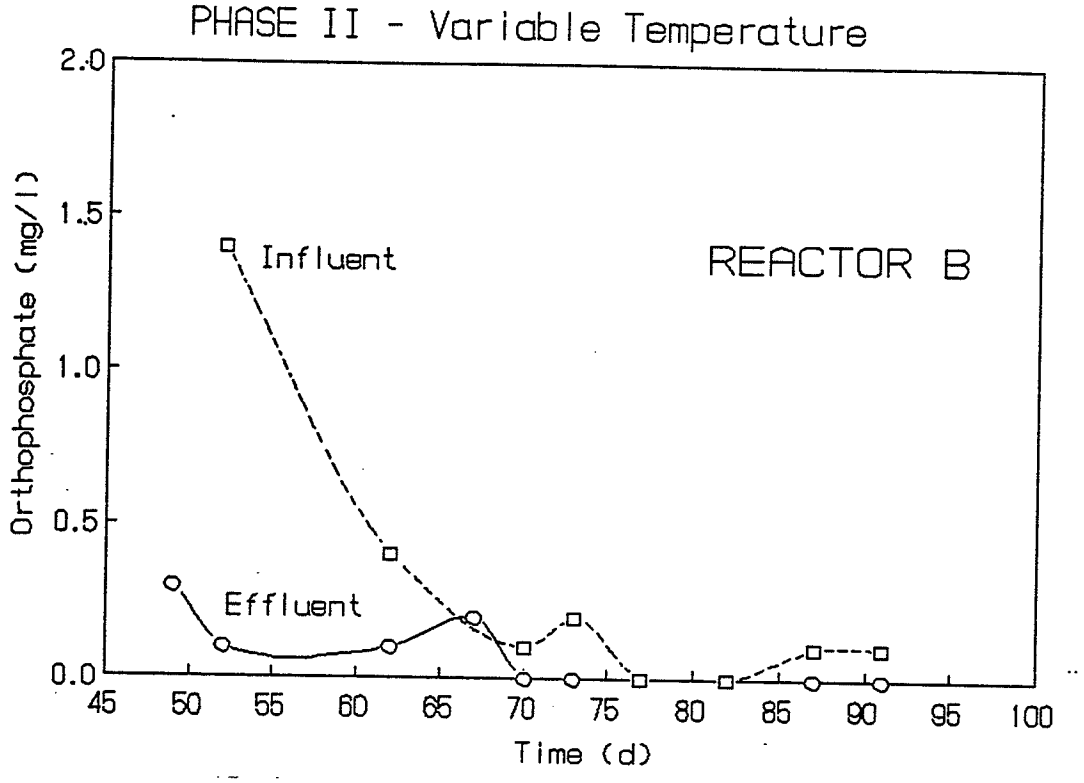
Day	Cold Chamber		Room Temperature	
	B	C	F	S
49	0.3	0.2	1.7	10.0
49.04	-	-	1.3	8.1
52	0.1	0.1	0.8	8.0
52.04	1.4	1.5	1.8	7.1
61.04	-	-	1.3	3.4
62	0.1	0	-	-
62.04	0.4	0.5	0.5	1.3
65	-	-	3.4	3.3
65.04	-	-	-	-
67	0	0	-	-
69	-	-	0.2	1.1
69.04	-	-	0.2	0.7
70	0	0	-	-
70.04	0.1	0	-	-
72	-	-	0	0.5
72.04	-	-	0.8	0.7
73	0	0	-	-
73.04	0	0	-	-
75	-	-	0	0.1
75.04	-	-	0.9	1.2
77	0	0	-	-
77.04	0	0	-	-
78	-	-	0	0.2
78.04	-	-	0.1	0.8

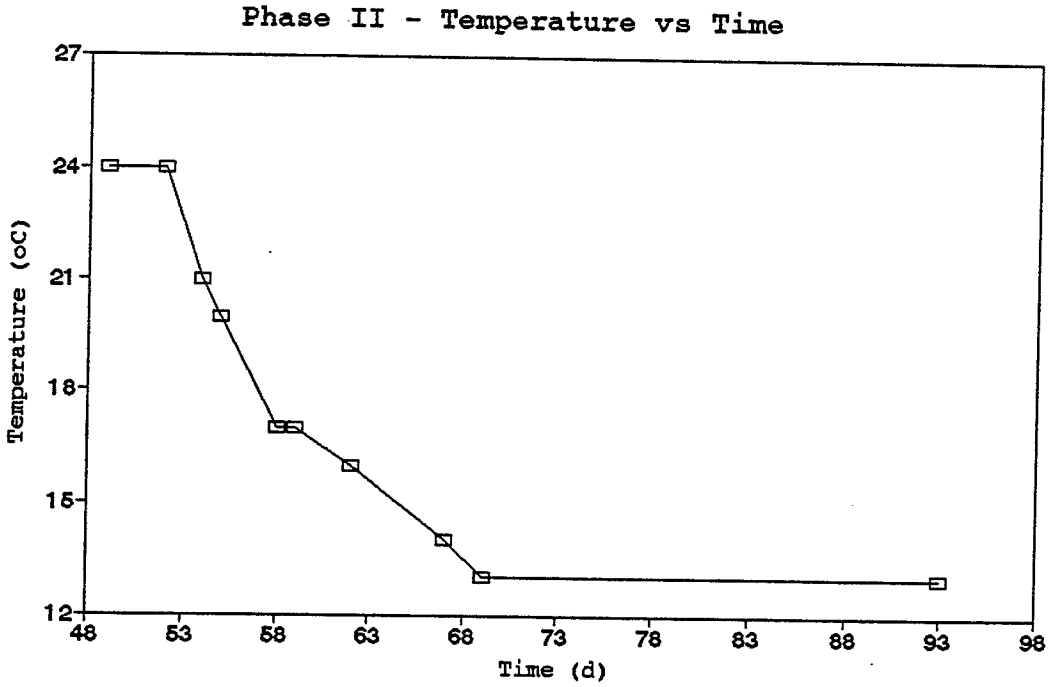
TABLE A-26 CONTINUED:....

Day	B	C	F	S
81	-	-	0	0.2
81.04	-	-	0.4	0.5
82	0	0	-	-
82.04	0	0.1	-	-
84	-	-	0	0.2
84.04	-	-	0.7	0.6
87	0	0.1	0	0.4
87.04	0.1	0.2	0.3	0.7
91	0	0	0	0.7
91.04	0.1	0.3	0.1	1.1
95	-	-	0	1.1
95.04	-	-	1.0	1.8



GRAPH A-30: ORTHOPHOSPHATE (mg/l) vs TIME - PHASE II

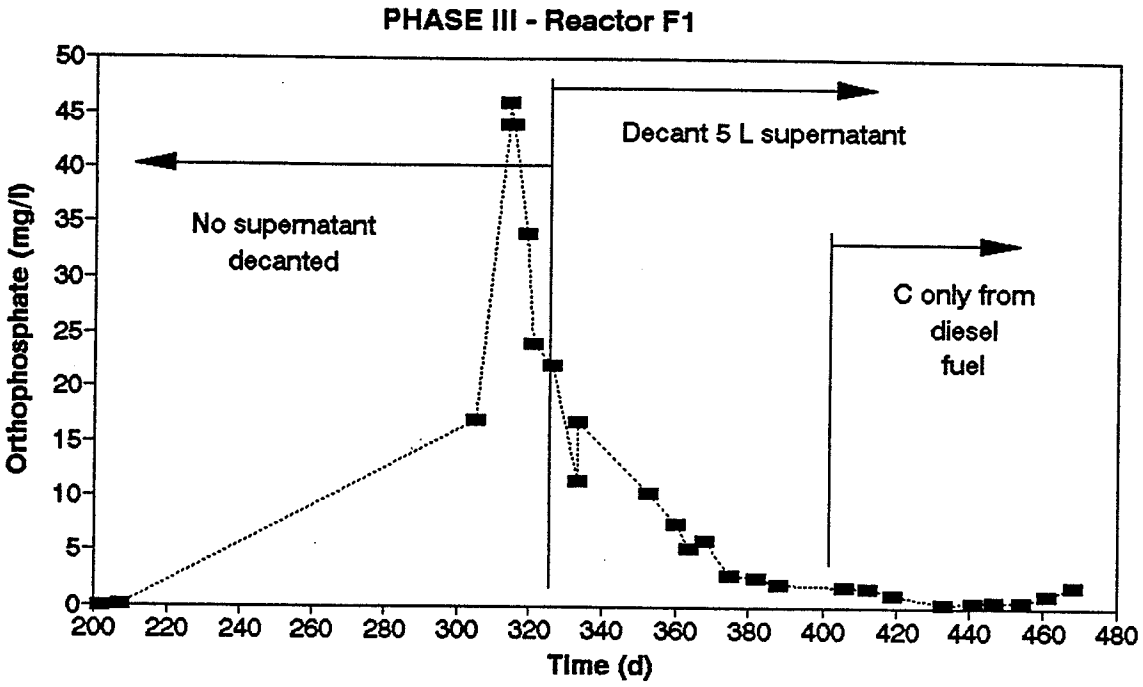




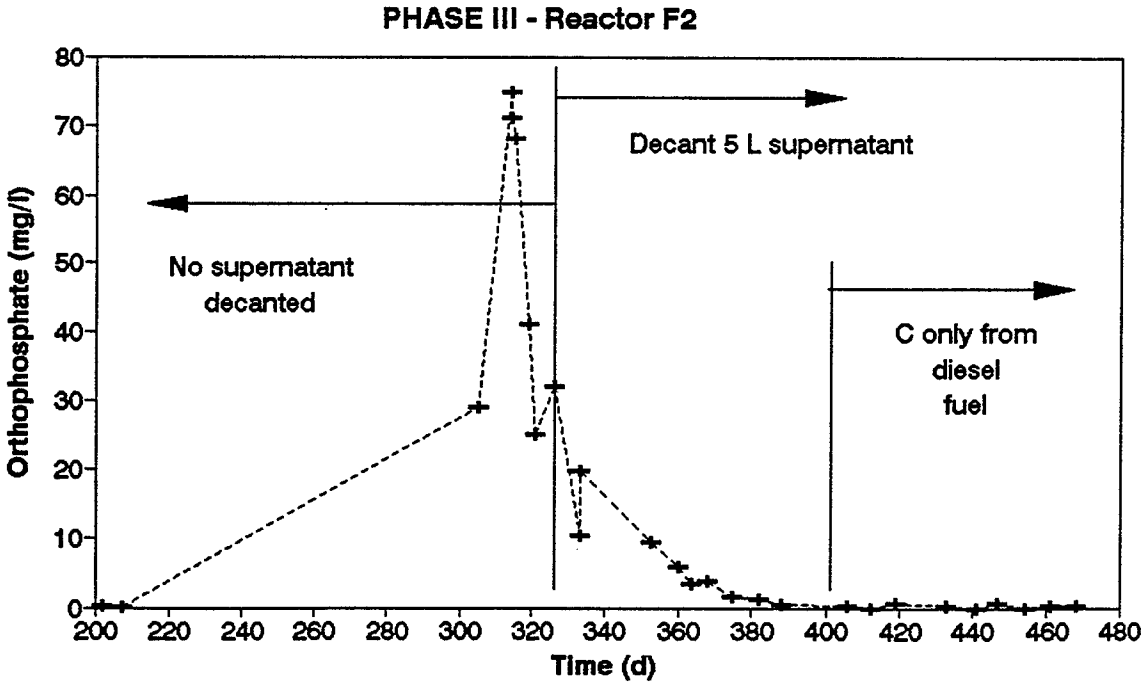
GRAPH A-31: ORTHOPHOSPHATE (mg/l) vs TIME - PHASE II

TABLE A-27: ORTHOPHOSPHATE (mg/l) - PHASE III

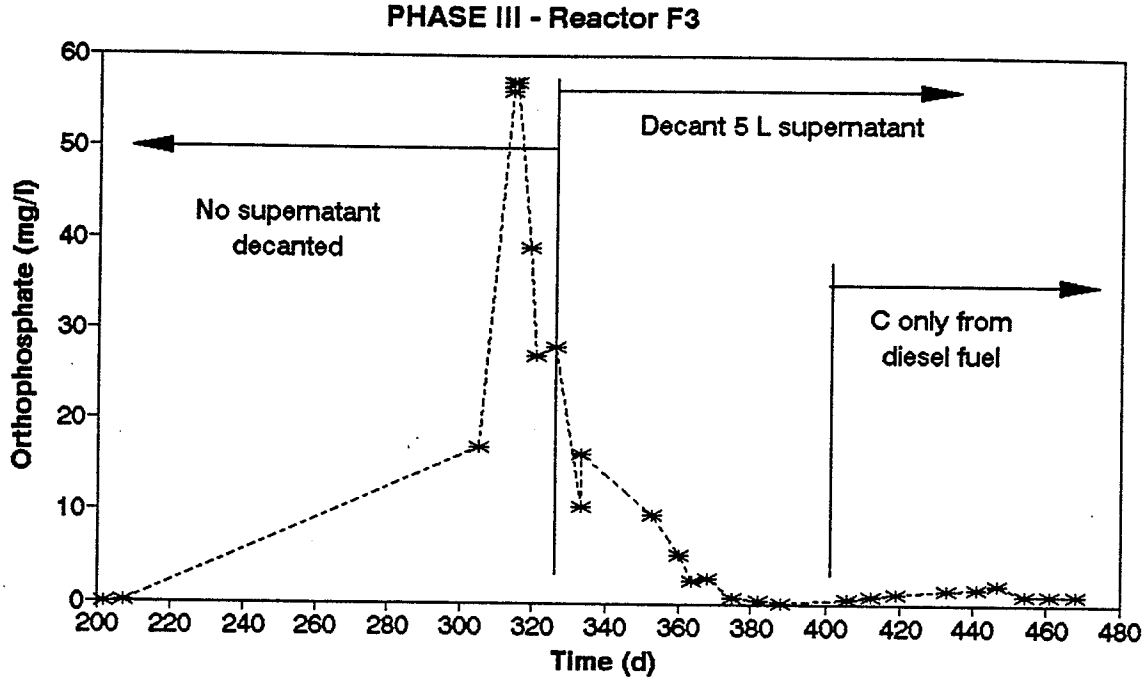
Date	Day	F1 (mg/l)	F2 (mg/l)	F3 (mg/l)	S (mg/l)	Feed (mg/l)
February 13	202	0.0	0.3	0.0	0.0	24.0
18	207	0.1	0.1	0.1	0.1	
May 26	305	17.0	29.0	17.0		
June 4	314	44.0	75.0	56.0	43.0	
Influent (1 hr)	314.04	46.0	71.0	57.0	44.0	
5	315	44.0	68.0	57.0	44.0	
9	319	34.0	41.0	39.0	34.0	21.0
11	321	24.0	25.0	27.0	23.0	
16	326	22.0	32.0	28.0	26.0	
23	333	11.4	10.4	10.4	12.5	
Influent (1 hr)	333.04	16.8	19.8	16.2	17.6	
13	353	10.4	9.4	9.6	10.6	
20	360	7.6	5.8	5.3	7.2	
24	364	5.3	3.4	2.4	5.8	
28	368	6.0	3.9	2.7	5.9	
August 4	375	2.9	1.6	0.6	3.1	
11	382	2.6	1.1	0.3	1.9	
17	388	2.0	0.4	0.1	1.0	
September 4	406	1.7	0.2	0.5	0.1	
10	412	1.6	0.0	0.7	0.0	
17	419	1.1	0.6	1.1	1.0	
October 1	433	0.2	0.2	1.4	0.1	
9	441	0.3	0.0	1.6	0.1	
15	447	0.5	0.5	2.0	0.2	
22	454	0.5	0.0	0.8	0.1	
29	461	1.1	0.2	0.8	0.2	
November 5	468	1.9	0.2	0.8	0.0	



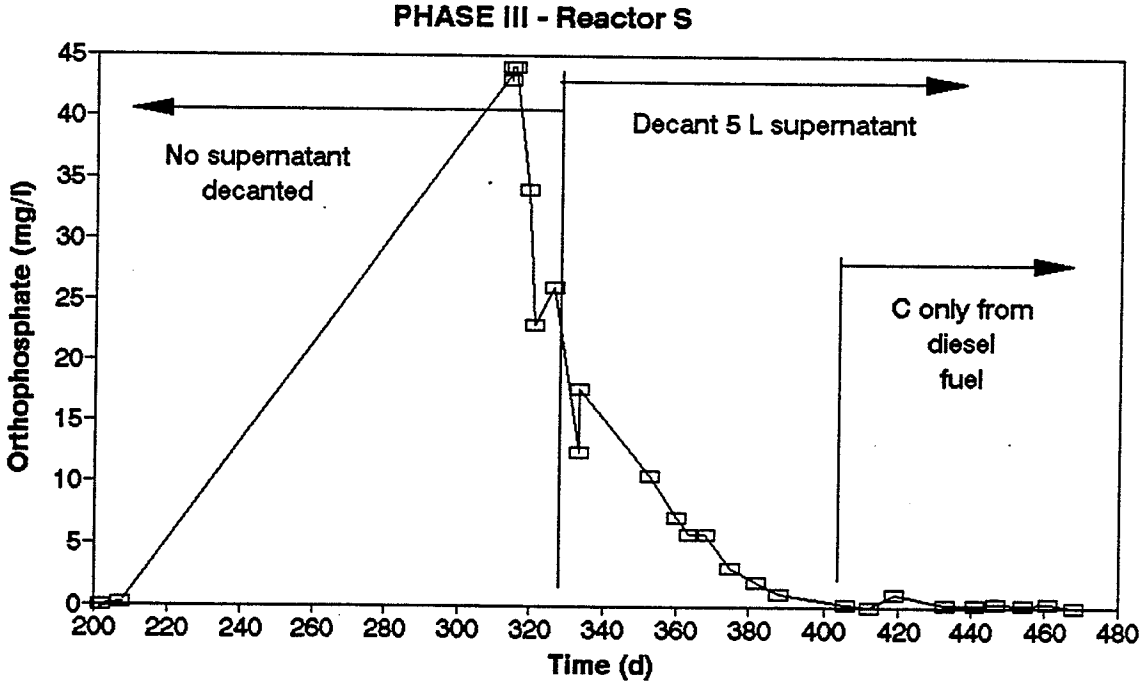
GRAPH A-32: ORTHOPHOSPHATE (mg/l) vs TIME - PHASE III



GRAPH A-33: ORTHOPHOSPHATE (mg/l) vs TIME - PHASE III



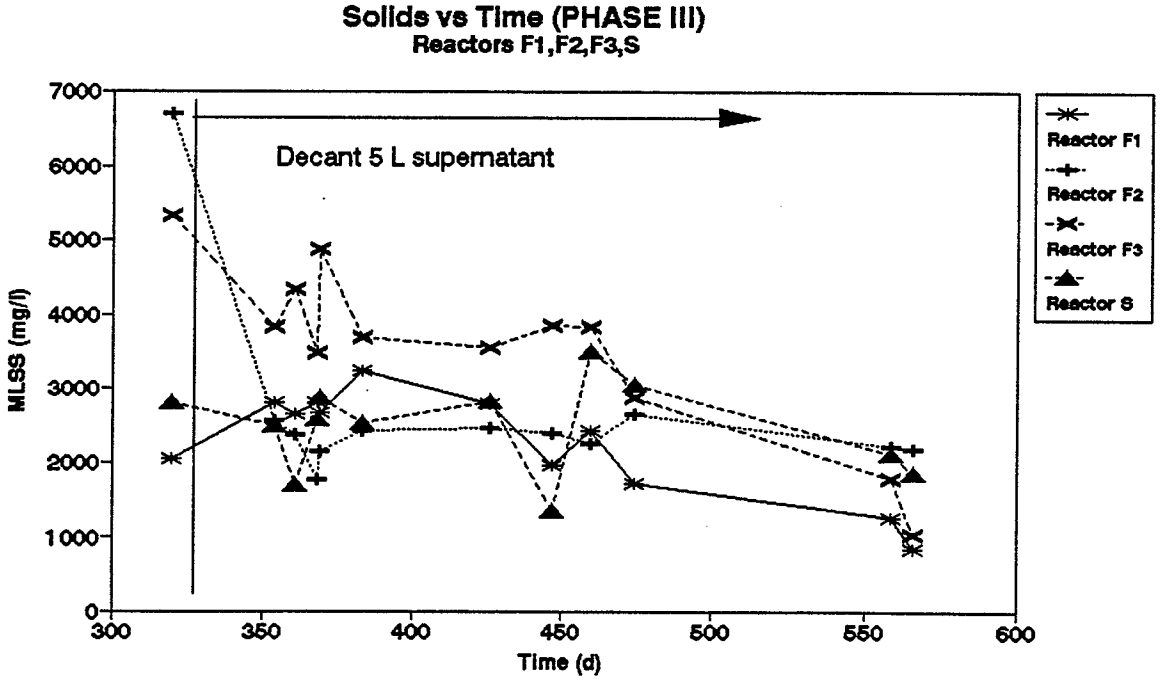
GRAPH A-34: ORTHOPHOSPHATE (mg/l) vs TIME - PHASE III



GRAPH A-35: ORTHOPHOSPHATE (mg/l) vs TIME - PHASE III

TABLE A-28: SOLIDS (mg/l) - PHASE III

Date	Day	MLSS (mg/l)				MLVSS (mg/l)			
		F1	F2	F3	S	F1	F2	F3	S
June10	320	2030	6700	5320	2810	1200	2510	2210	1340
July 14	354	2800	2560	3840	2500				
21	361	2640	2370	4340	1700				
28	368	2800	1760	3480	2570				
29	369	2680	2140	4880	2880				
August 12	383	3250	2430	3700	2520				
September 24	426	2780	2450	3560	2830				
October 15	447	1950	2390	3850	1340				
28	460	2430	2260	3840	3510				
November 12	475	1720	2640	2870	3050				
February 4	559	1260	2220	1780	2100	740	1340	1070	1550
11	566	840	2180	1040	1850	1240	1600	780	1520



GRAPH A-36: MLSS (mg/l) vs TIME - PHASE III - F1, F2, F3, S

TABLE A-29: COD (mg/l) - PHASE I

Day	1-0 A	1-0 B	2-6 C	2-6 D	1-6 E	1-6 F	1-6 G	1-6 H
0	100		210			90		
0.4	400		510			370		
1	460		190			210		
2	320		130			140		
3	130		140			130		
4	50		80			120		
5	-		-			70		
5.04	360	335	330	340	420	460		
6	160	240	80	80	120	70		
7	170	200	70	130	90	80		
8	170	160	80	70	140	110		
8.04	760	450	590	570	560	550		
9	-	-	-	-	-	-		
10	120	150	180	120	160	100		
11	110	140	120	70	120	110		
12	100	120	120	80	120	100		
12.04	220	370	170	330	240	340	130	160
13	90	60	130	60	50	50	50	50
14	150	110	460	470	85	80	50	80
15	-	-	-	-	-	-	-	-
16	110	70	70	70	70	130	50	60
17	70	50	40	50	40	60	30	20
17.04	460	230	250	240	260	200	240	210
18	150	85	90	120	70	40	140	110
19	60	110	50	85	100	85	70	110
20	120	100	130	160	100	140	70	130

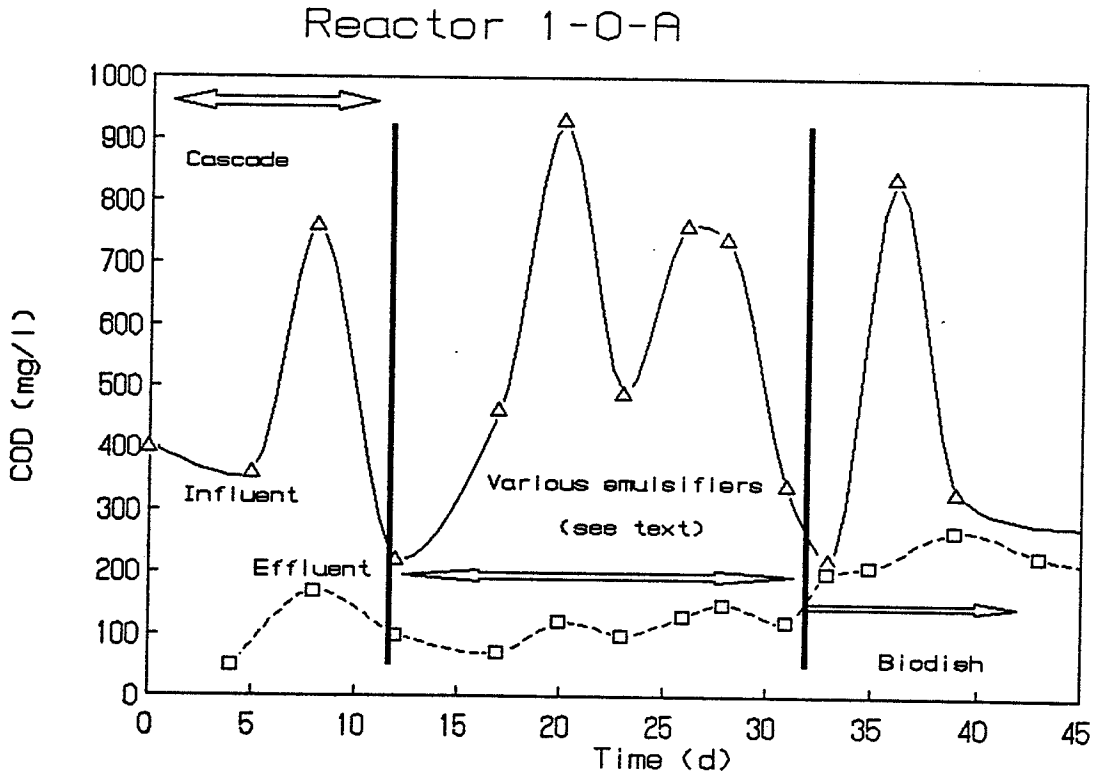
TABLE A-29 CONTINUED:....

Day	A	B	C	D	E	F	G	H
20.04	930	570	920	700	470	620	590	440
21	100	80	40	70	50	30	50	40
22	-	-	-	-	-	-	-	-
23	100	90	85	60	85	30	70	70
23.04	490	700	370	550	450	290	610	520
24	100	110	130	130	140	70	210	270
25	70	130	70	130	130	60	120	160
26	130	130	110	90	120	100	120	130
26.04	760	810	760	910	620	450	680	820
27	130	190	70	50	150	60	130	150
28	150	280	130	130	150	100	220	260
28.04	740	620	860	790	640	330	590	610
31	120	180	170	130	130	70	150	230
31.04	340	820	590	330	820	230	670	910
32	140	320	190	180	130	90	280	430
33	200	330	170	160	130	60	250	270
33.04	220*	600*	220*	200*	250*	140*	590	610
34	330	440	190	180	150	110	450	360
35	210	420	270	170	240	110	420	240
36.04	840	710	930	790	910	820	840	800
37	230	550	330	190	220	270	350	230
39	270	490	320	230	220	420	290	290
39.04	330	790	480	320	400	560	470	810
40	230	280	300	140	180	430	210	270
42	240	280	240	140	240	360	200	260
43	230	310	330	190	290	450	200	330
43.04	-	490	330	-	-	-	270	-

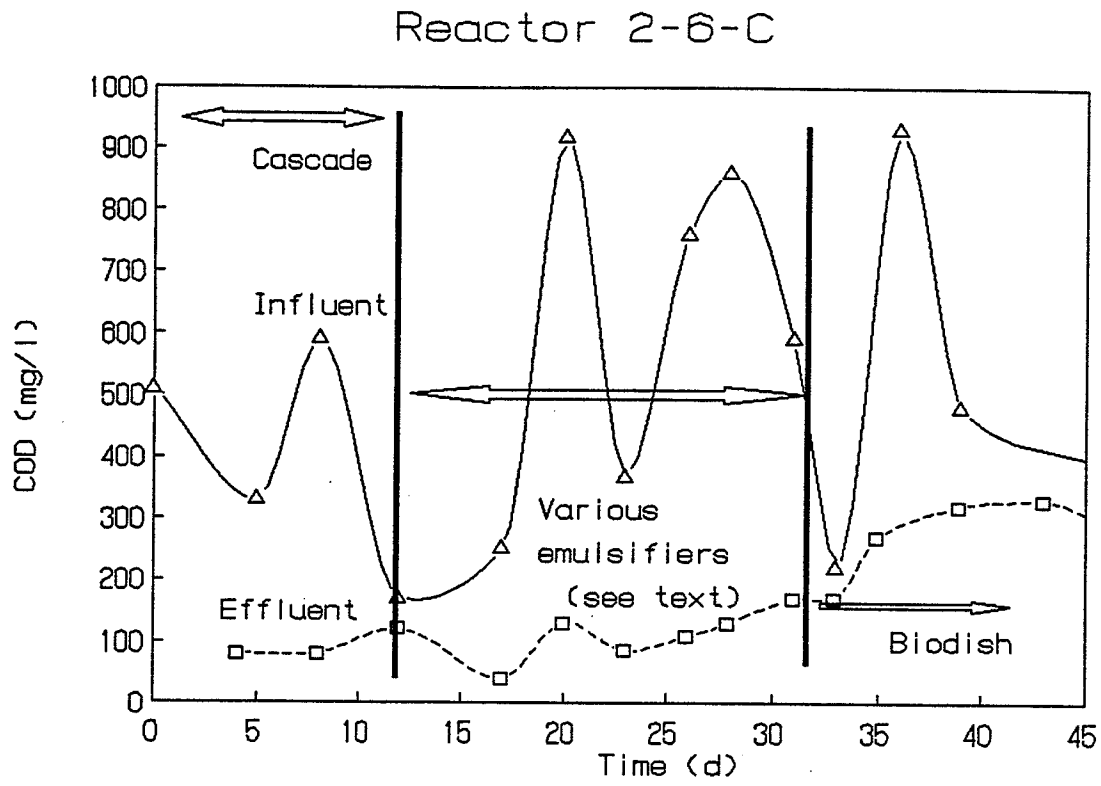
* Temperature 38 °C

TABLE A-29 CONTINUED:...

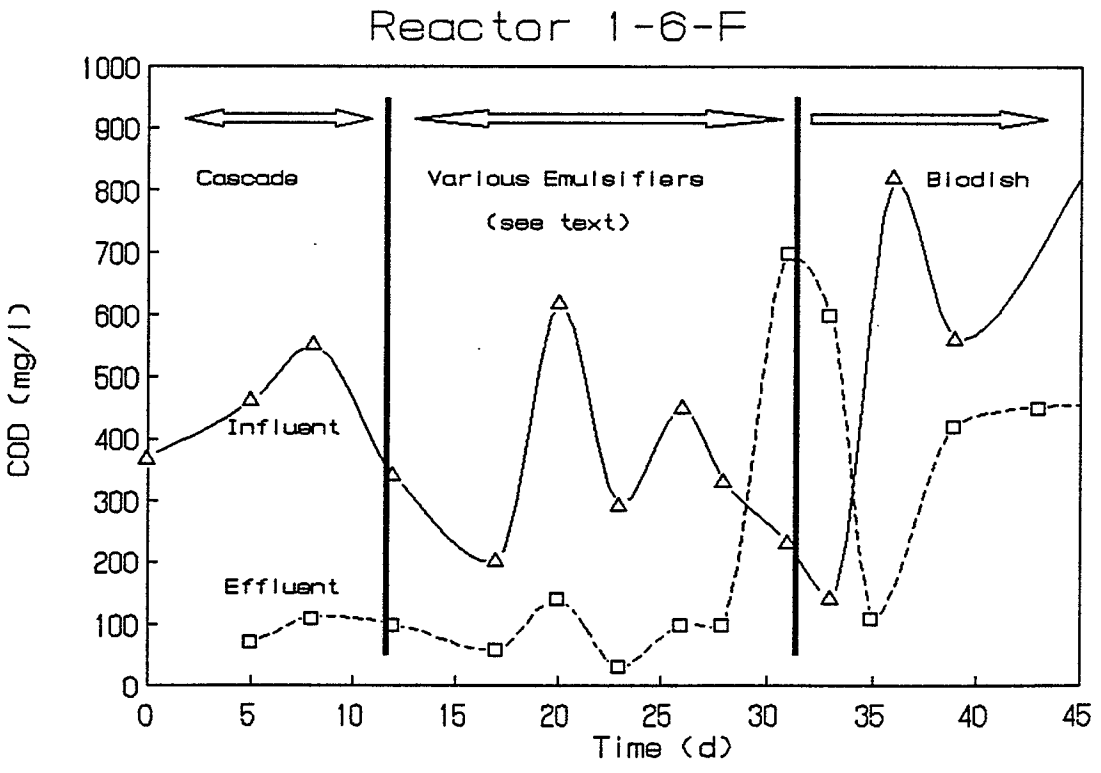
Day	A	B	C	D	E	F	G	H
45	200	260	280	210	310	560	190	340
46	200	200	310	180	290	450	140	260
46.04	270	410	390	330	310	870	290	700
47	140	170	340	190	230	430	140	290



GRAPH A-37: COD (mg/l) vs TIME - PHASE I - REACTOR 1-O-A



GRAPH A-38: COD (mg/l) vs TIME - PHASE I - REACTOR 2-6-C



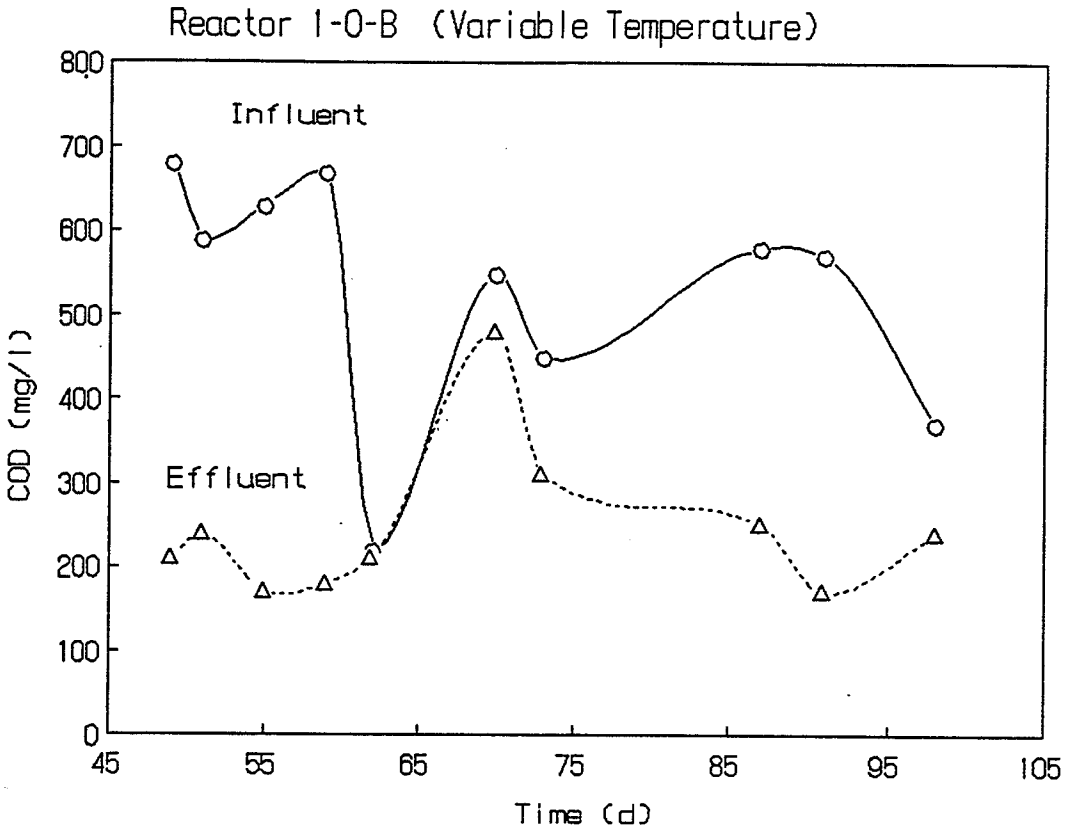
GRAPH A-39: COD (mg/l) vs TIME - PHASE I - REACTOR 1-6-F

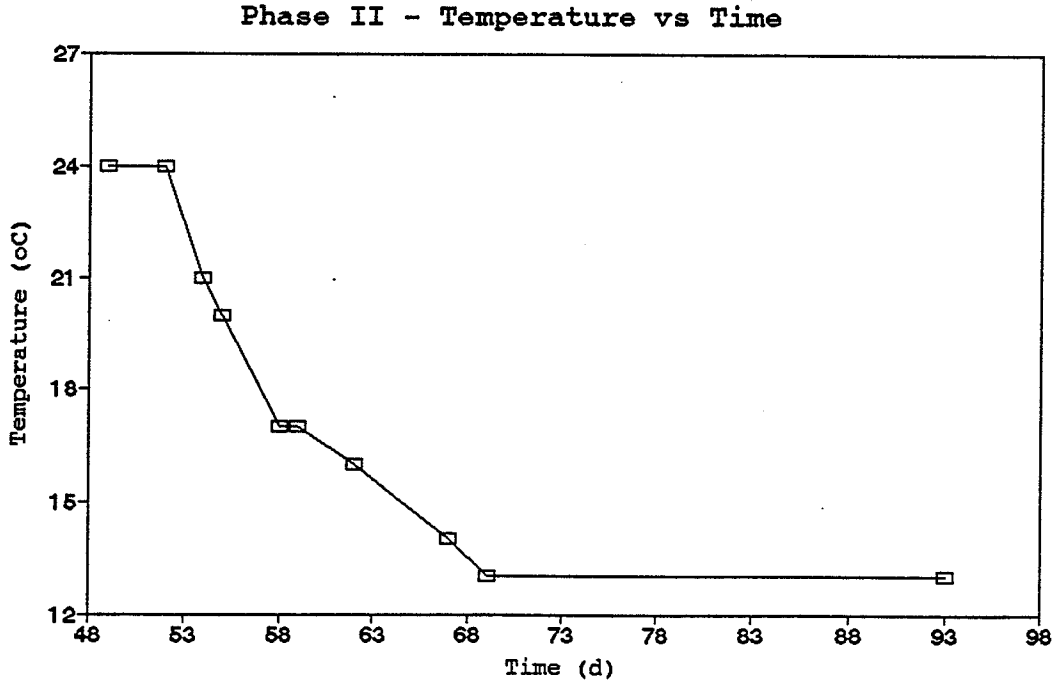
TABLE A-30: COD (mg/l) - PHASE II

Day	B	C	F	S
49	210	280	610	650
49.04	680	940	630	920
51	240	250	590	820
51.04	590	380	610	710
55	170	250	940	520
55.04	630	940	-	-
56	-	-	720	500
58	-	-	700	450
58.04	-	-	940	850
59	180	230	640	410
59.04	670	950	-	-
61	-	-	610	450
61.04	-	-	510	460
62	210	510	480	370
62.04	220	450	-	-
65	-	-	500	460
65.04	-	-	710	620
67	460	990	490	710
69	-	-	460	630
69.04	-	-	490	630
70	480	990	-	-
70.04	550	940	-	-
72	-	-	530	650
72.04	-	-	540	480
73	310	950	-	-

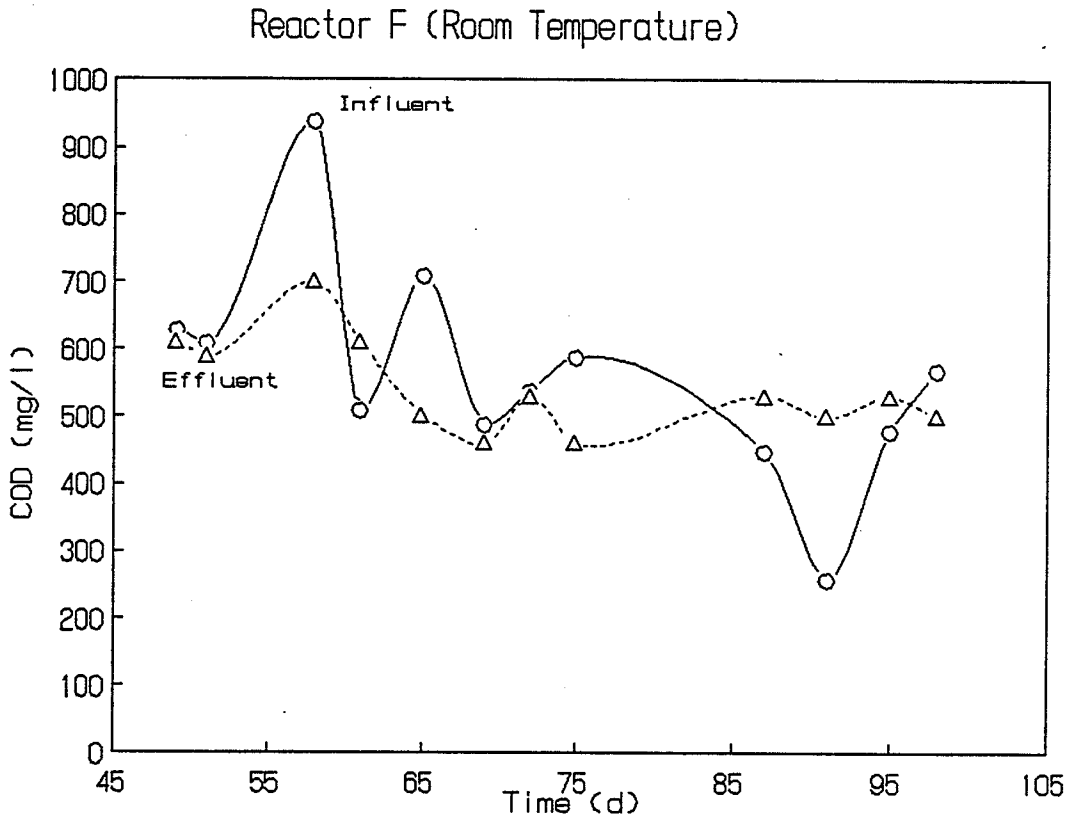
TABLE A-30 CONTINUED:....

Day	B	C	F	S
73.04	450	880	-	-
75	-	-	460	440
75.04	-	-	590	430
87	250	500	530	310
87.04	580	830	450	310
91	170	450	500	300
91.04	570	440	260	250
95	-	-	530	310
95.04	-	-	480	460
98	240	250	500	260
98.04	370	500	570	340





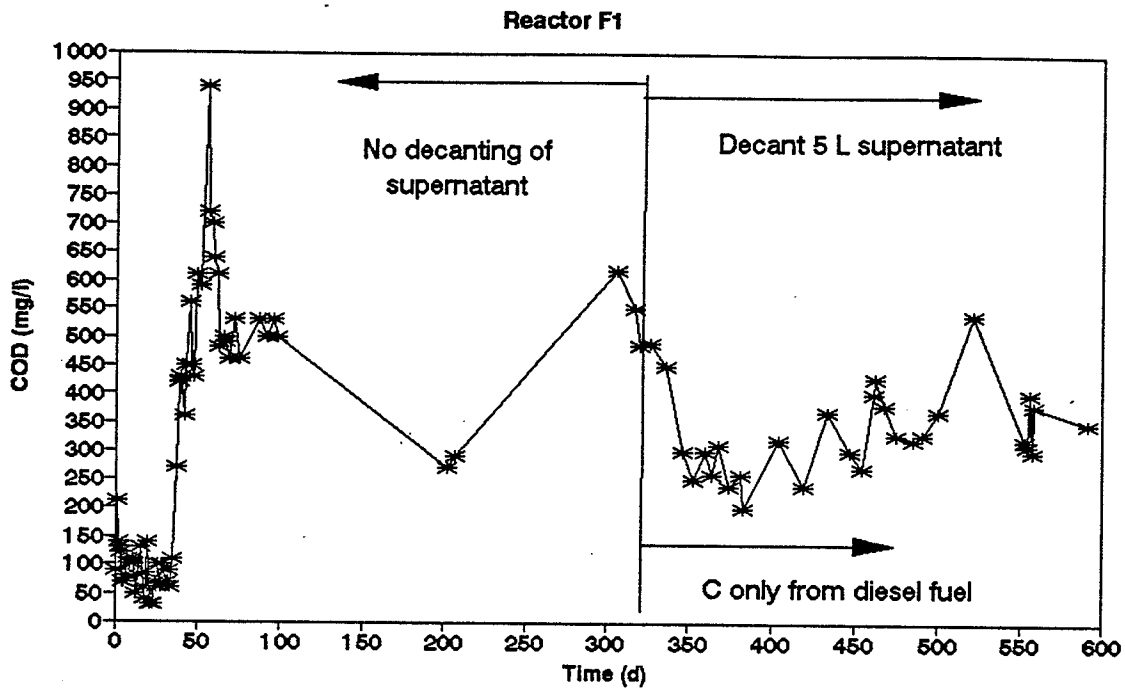
GRAPH A-40: COD (mg/l) vs TIME - PHASE II - REACTOR B



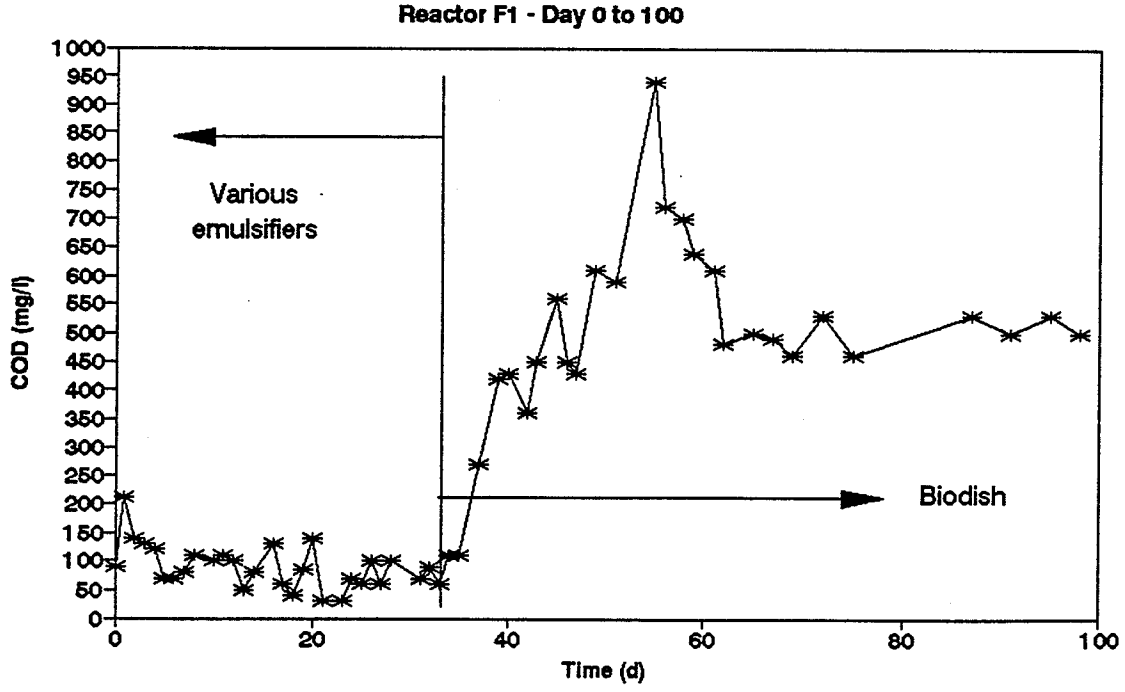
GRAPH A-41: COD (mg/l) vs TIME - PHASE II - REACTOR F

TABLE A-31: EFFLUENT CHEMICAL OXYGEN DEMAND - PHASE III

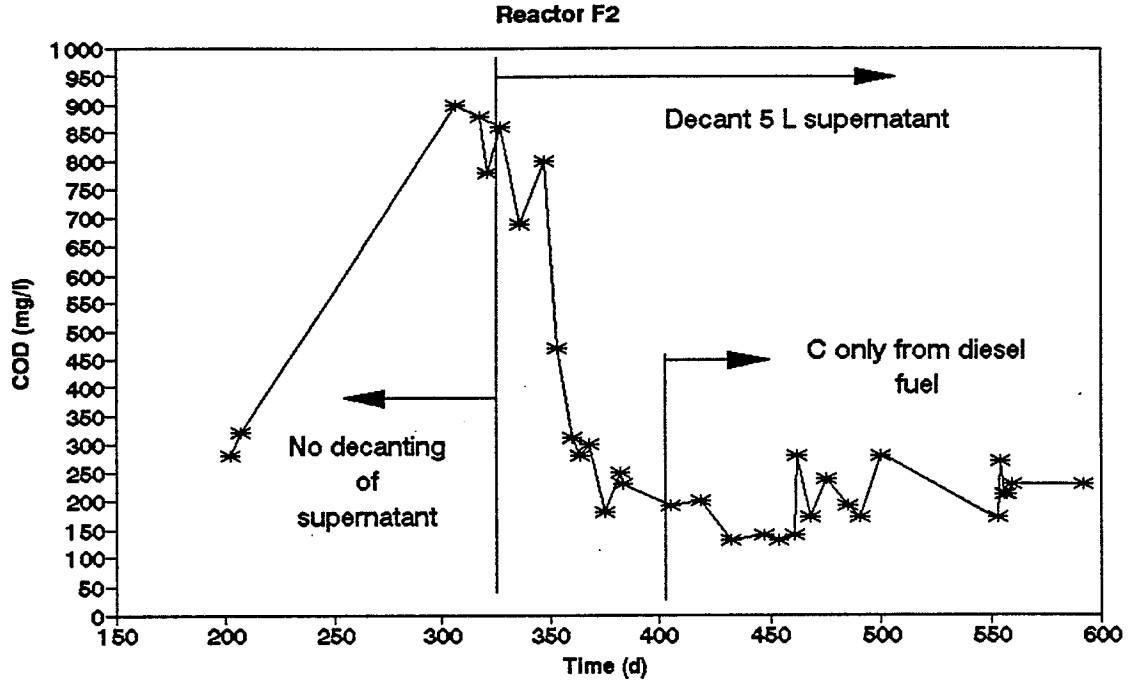
Date	Day	F1 (mg/l)	F2 (mg/l)	F3 (mg/l)	S (mg/l)	Influent (mg/l)
February 13	202	270	280	260	280	
18	207	290	320	260		3200
May 27	306	620	900	860		
June 8	318	550	880	700	530	1600
11	321	485	780	570	575	
17	327	490	860	575	530	
26	336	450	690	450	520	
July 7	347	300	800	500	450	
13	353	250	470	450	420	
20	360	300	310	520	360	
24	364	260	280	470	280	
28	368	310	300	410	270	
August 4	375	240	180	390	200	4000
11	382	260	250	370	190	
12	383	200	230	210	160	
September 3	405	320	190	280	240	
17	419	240	200	370	200	
October 1	433	370	130	300	180	
15	447	300	140	260	180	
22	454	270	130	260	240	
29	461	400	140	380	340	
30	462	430	280	340	410	
November 5	468	380	170	280	530	
12	475	330	240	240	460	
22	485	320	190	210	270	
27	490	330	170	240	210	
December 7	500	370	280	310	260	
28	521	540		320	520	
January 29	553	320	170	240	500	
30	554	310	270	170	460	
February 1	556	400	210	210	620	
2	557	300	210	260	760	
4	559	380	230	280	760	
March 19	592	350	230	310	620	



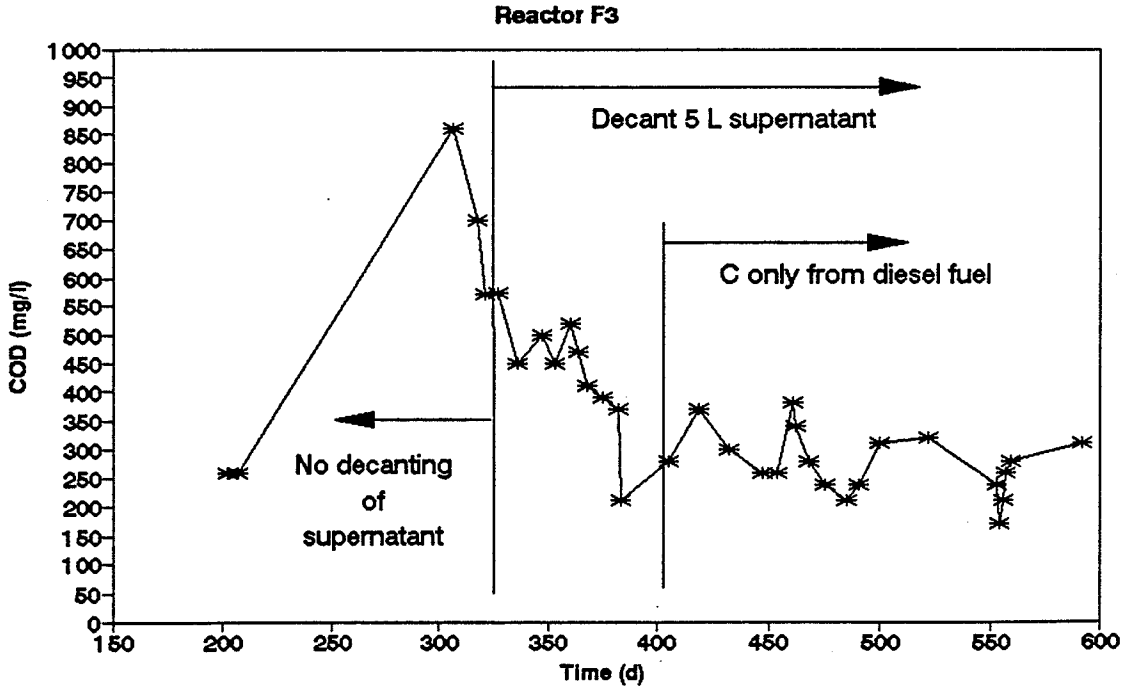
GRAPH A-42: COD (mg/l) vs TIME - PHASE III - DAY 0-600



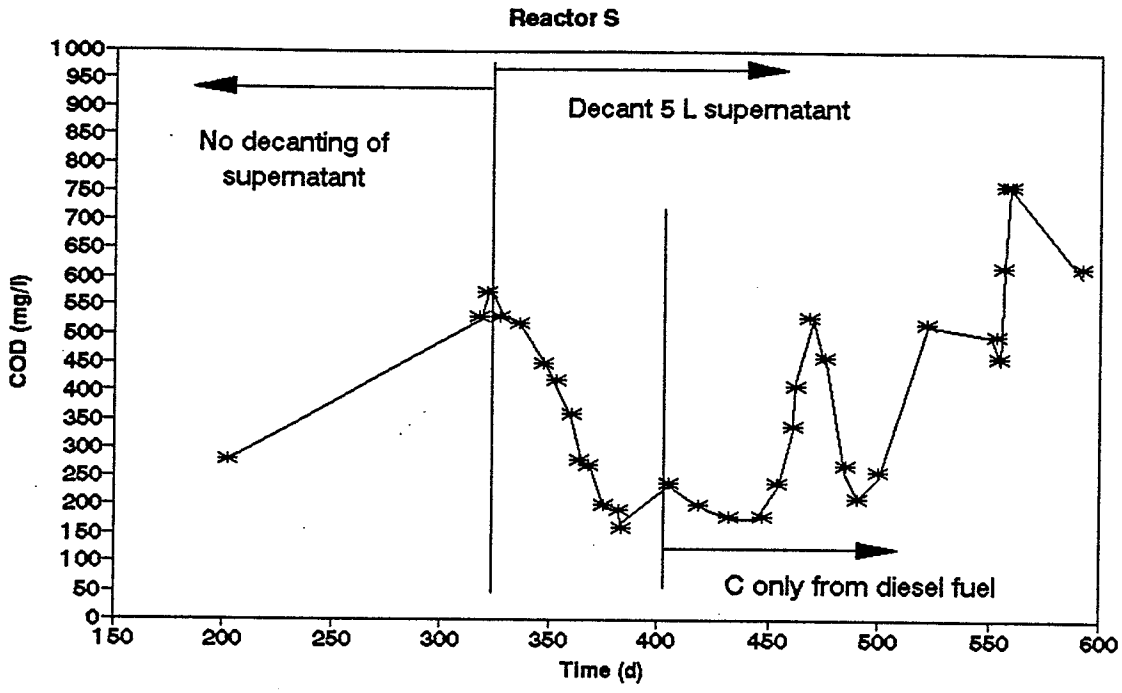
GRAPH A-43: COD (mg/l) vs TIME - PHASE III - DAY 0-100



GRAPH A-44: COD (mg/l) vs TIME - PHASE III - DAY 0-600



GRAPH A-45: COD (mg/l) vs TIME - PHASE III - DAY 0-600



GRAPH A-46: COD (mg/l) vs TIME - PHASE III - DAY 0-600

TABLE A-32: PERCENT COD REMOVAL - PHASE I

Day	1-0 A	1-0 B	2-6 C	2-6 D	1-6 E	1-6 F	1-6 G	1-6 H
1	0		62.7			43.2		
2	30.4		74.5			62.2		
3	71.7		72.5			64.9		
4	89.1		84.3			67.6		
5	-		-			81.0		
6	55.6	28.4	75.8	76.5	71.4	84.8		
7	52.7	40.3	78.8	61.8	78.6	82.6		
8	52.7	52.2	-	79.4	66.7	76.1		
10	84.2	66.7	69.5	78.9	71.4	81.8		
11	85.5	68.9	79.7	87.7	78.6	78.6		
12	86.8	73.3	79.7	85.9	78.6	81.8		
13	59	83.8	23.5	81.8	79.2	85.3	61.5	68.8
17	68.2	86.5	76.5	84.8	83.3	82.4	76.9	87.5
18	67.4	63.0	64.0	50.0	73.1	80.0	41.7	47.6
20	73.9	56.5	48.0	33.3	61.5	30.0	70.8	38.0
21	89.0	86.0	95.7	90.0	89.4	95.0	91.5	90.9
23	89.0	84.2	90.8	91.4	81.9	95.0	88.1	84.1
24	79.6	84.3	64.9	76.4	68.9	75.9	65.6	48.1
27	82.9	76.5	90.8	94.5	75.8	86.7	80.9	81.7
28	80.3	65.4	82.9	85.7	75.8	77.8	82.4	68.3
31	83.3	70.9	80.2	83.5	79.7	78.8	74.6	62.3
32	58.8	60.9	67.8	45.5	84.1	60.9	58.2	52.7
33	41.2	59.6	71.2	51.5	84.1	73.9	62.7	70.3
37	72.6	28.6	64.5	75.9	75.8	67.1	58.3	71.2

TABLE A-33: MICROBIOLOGICAL EXAMINATION

Day	Reactor	
0	1-0-B	- nothing visible
	2-6-C	- nothing visible
19	1-0-B	- many ciliated protozoa, 8 on one floc
	2-6-C	- few ciliated protozoa, algae
24	1-0-B	- many ciliated protozoa
	2-6-C	- many ciliated protozoa
30 - 90	B,C,F,S	- monitored regularly, with activity noted at all times
98	B,C	- less activity and presence of filamentous bacteria
	F	- rotifer observed for the first time
100 - 200	F1,F2,F3,S	- monitored regularly, with activity
227	F1,F2,F3,S	- no visible activity
230	F1	- no activity
	F2,F3,S	- many small protozoa
264	F1,F2,F3,S	- large protozoa observed for the first time
297	F1,F2,F3,S	- very active, small & large protozoa, rotifers
318	F3	- sludge worm observed for the first time
320 - 400	F1,F2,F3,S	- active, thriving, mixed-population
406	F1,F2,F3,S	- flagellated protozoa, paramecium
423	F1,F2,F3,S	- vorticella (stalked protozoa)
425 - 475	F1,F2,F3,S	- some algae observed, very large protozoa, all reactors very active

APPENDIX B: BAKERS NARROWS RESULTS

TABLE B-1: FEED SCHEDULE

Day	Oil (ml)	Biodish (g)	Dex/Gl. Acid (ml)	N mg	P mg
July 5	f.d.	-	-	-	-
7	f.d.	-	-	-	-
8	f.d.	-			-
9	0.5	1	10.0	14	2.0
13	0.5	1	10.0	14	2.0
20	0.5	1	10.0	14	2.0
24	0.5	1	10.0	14	2.0
28	0.5	1	10.0	14	2.0
31	0.5	1	10.0	14	2.0
Aug. 4	0.5	1	10.0	14	4.0
7	0.5	1	10.0	28	4.0
11	0.5	1	10.0	28	4.0
14	0.5	1	10.0	28	4.0
18	0.5	1	10.0	28	4.0
27	0.5	1	10.0	28	4.0
Sept. 4	0.5	1	10.0	14	0
11	0.5	1	10.0	14	0
17	0.5	1	10.0	14	2.0
24	0.5	1	10.0	0	2.0
Oct. 1	0.5	-	10.0	-	2.0
9	0.5	1	-	14	2.0
15	0.5	1	10.0	14	2.0
22	0.5	1	10.0	14	2.0
29	0.5	1	10.0	14	2.0

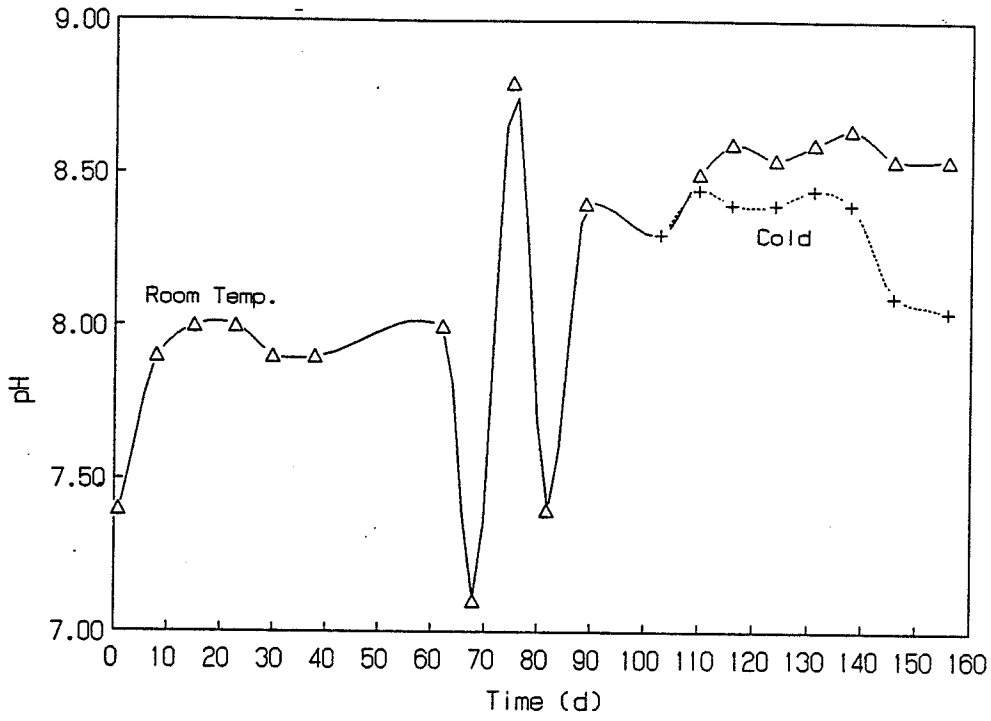
* f.d. = few drops
** Dextrose/Glutamic Acid
*** 6 capfuls of Biodish is approximately equal to 1 g

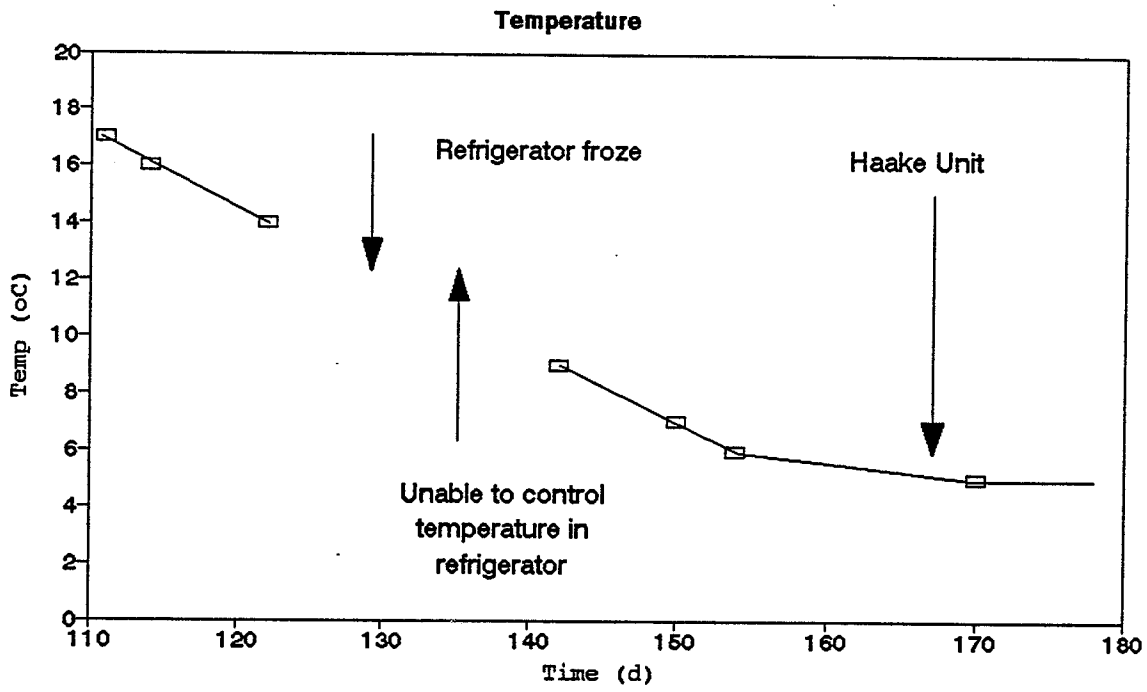
TABLE B-1 CONTINUED:....

Day	Oil (ml)	Biodish (g)	Dex/Gl. Acid (ml)	N mg	P mg
Nov. 5	0.5	1	0	30	4.0
12	0.5	1	0	30	4.0
19	0.5	1	0	30	4.0
26	0.5	1	0	30	4.0
Dec. 3	0.5	1	0	30	4.0
14	0.5	1	0	30	4.0
21	0.5	1	0	30	4.0
Jan. 6	0.5	1	0	30	4.0
19	0.5	0	0	30	4.0
28	0.5	0	0	30	4.0
Feb. 4	0.5	0	0	30	4.0
11	0.5	0	0	30	4.0
19	0.5	0	0	40	4.0

TABLE B-2: pH

Date	Day	pH	
		Room Temp.	Cold
July 6	1	7.40	-
13	8	7.90	-
20	15	8.00	-
28	23	8.00	-
August 4	30	7.90	-
11	38	7.90	-
September 4	62	8.00	-
10	68	7.10	-
17	75	8.80	-
24	82	7.40	-
October 1	89	8.40	-
15	103	8.30	8.30
22	110	8.50	8.45
28	116	8.60	8.40
November 5	124	8.55	8.40
12	131	8.60	8.45
19	138	8.65	8.40
27	146	8.55	8.10
December 7	156	8.55	8.05

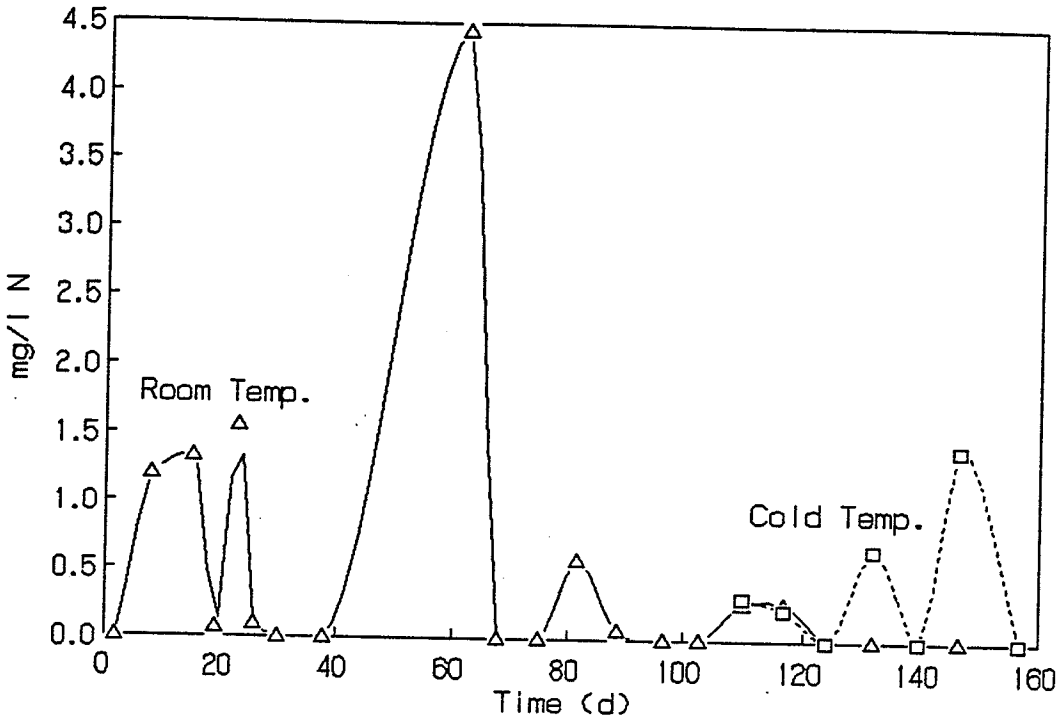


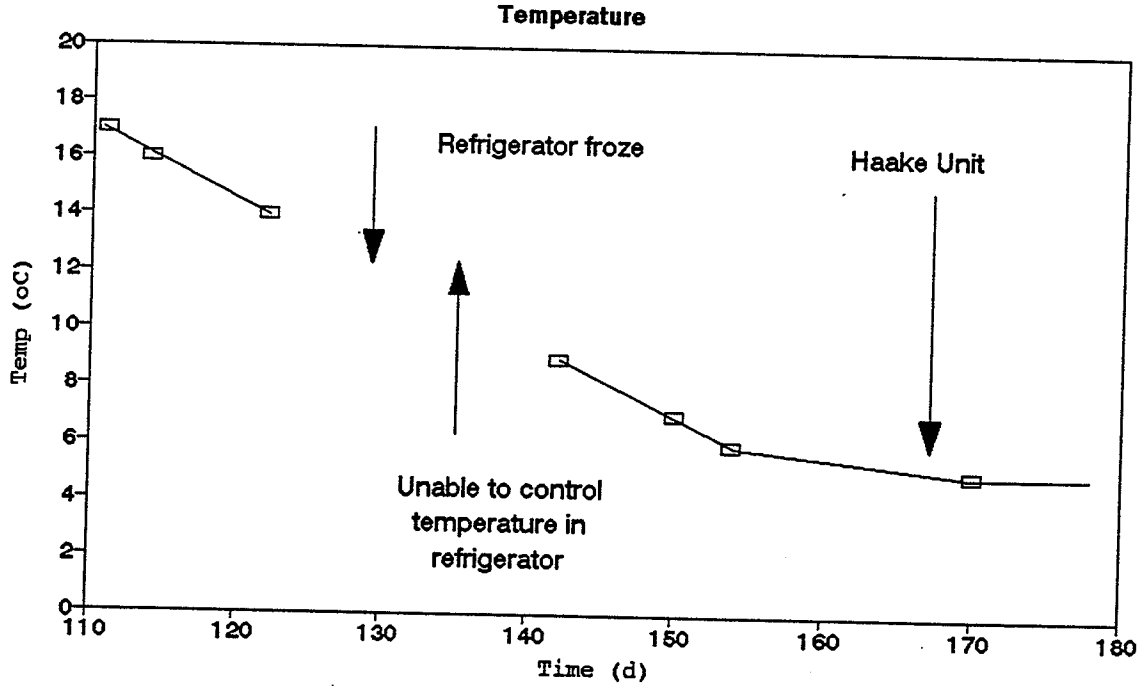


GRAPH B-1: pH vs TIME AT TWO TEMPERATURES, BN10

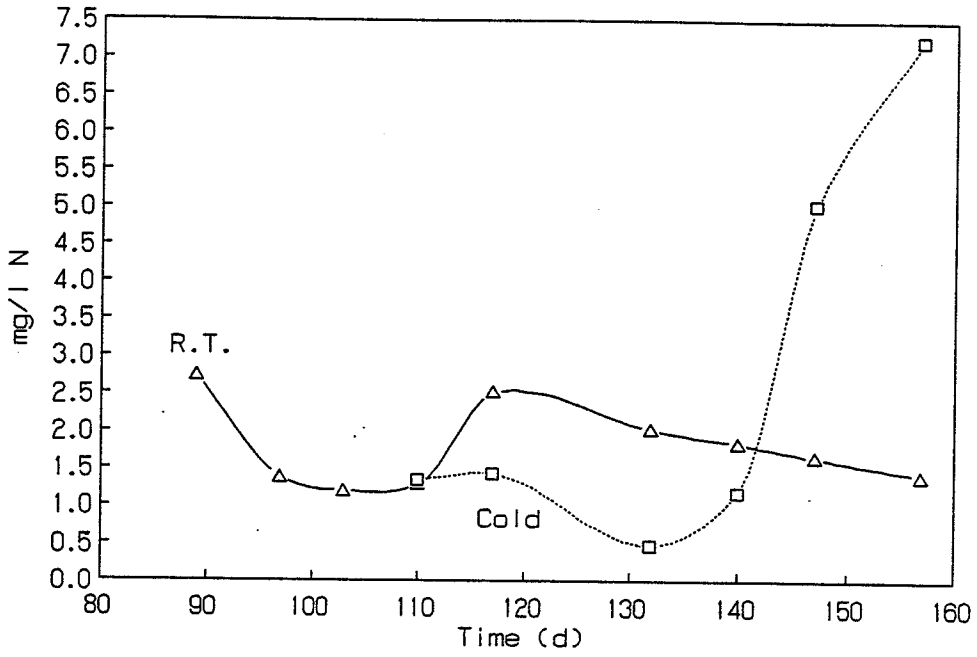
TABLE B-3: NITROGEN ANALYSIS

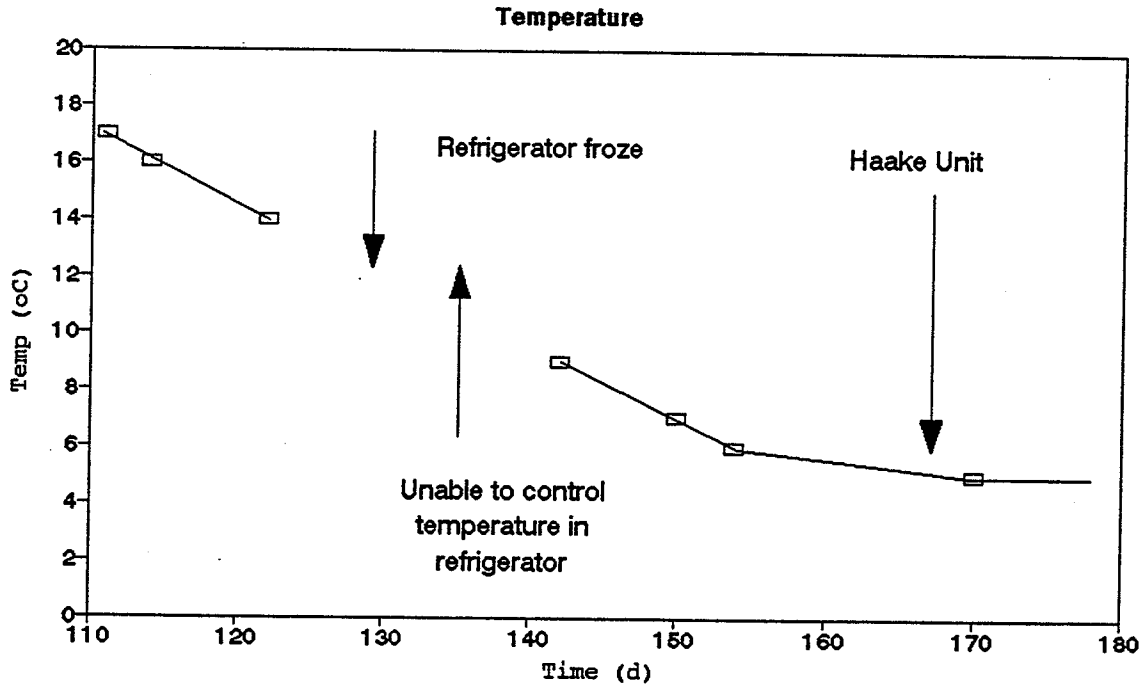
Date	Day	Ammonia mg/l N		TKN mg/l N		NO_2/NO_3 mg/l N	
		R. T.	Cold	R.T.	Cold	R.T.	Cold
July 7	2	0	-	4.34	-	0	
13	8	1.19	-	-	-	0	
20	15	1.33	-	-	-	-	
24	19	0.07	-	-	-	-	
28	23	1.55	-	-	-	-	
31	26	0.10	-	-	-	-	
Aug. 4	30	0	-	-	-	0.1	
11	38	0	-	-	-	0	
Sept. 4	62	4.45	-	-	-	0	
10	68	0	-	-	-	0	
17	75	0	-	-	-	-	
24	82	0.58	-	-	-	13	-
Oct 1	89	0.06	-	2.73	-	> 2.5	-
9	97	0	-	1.37	-	0	-
15	103	0	-	1.19	-	0.05	-
22	110	0.27	0.31	1.28	1.35	0.05	0.05
29	117	0.27	0.22	2.51	1.43	0.3	0
Nov. 5	124	0	0	-	-	0	0
12	132	0	0.67	2.02	0.48	0.9	0
20	140	0	0	1.83	1.19	-	-
27	147	0	1.42	1.65	5.03	-	-
Dec. 7	157	0	3.62	1.41	7.25	-	-





GRAPH B-2: AMMONIA (mg/l) vs TIME AT TWO TEMPERATURES, BN10

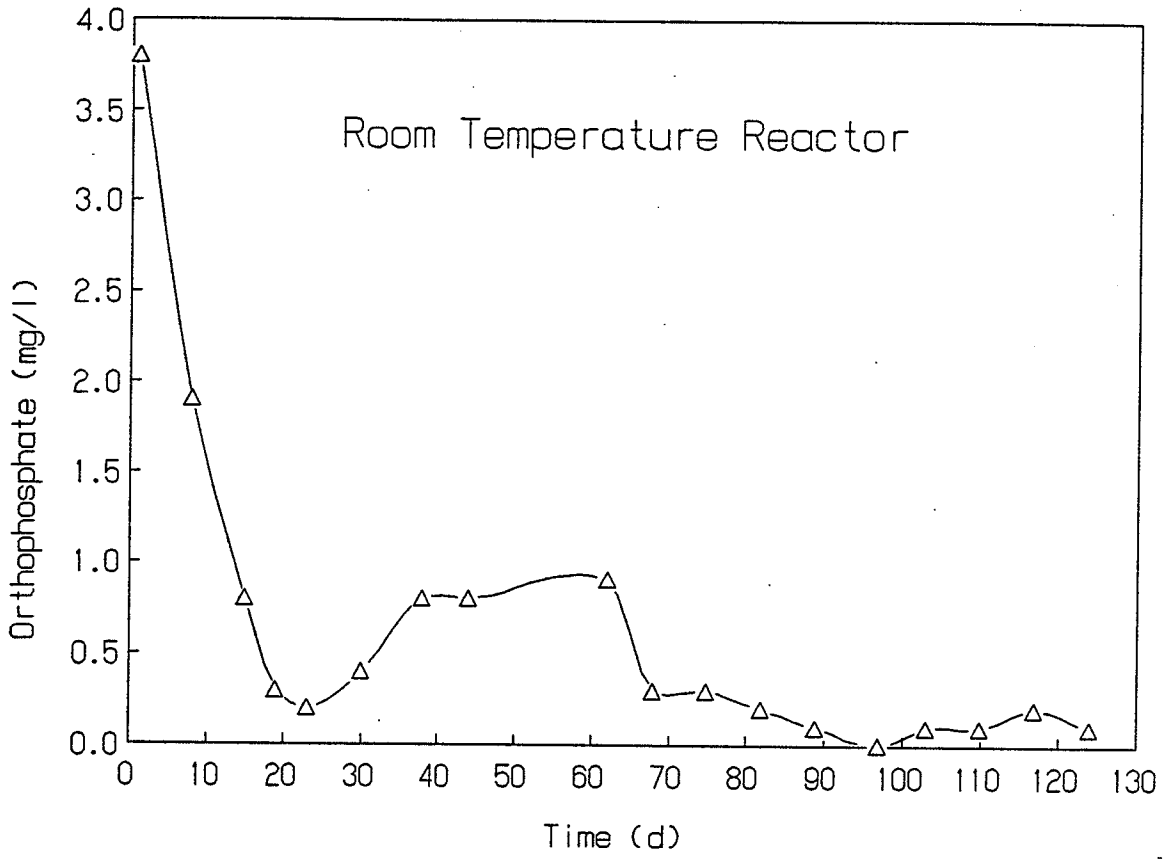




GRAPH B-3: TKN (mg/l) vs TIME AT TWO TEMPERATURES, BN10

TABLE B-4: PHOSPHATE ANALYSIS

Date	Day	Orthophosphate (mg/L)		Total Phosphate (mg/L)
		Room Temp.	Cold	
July 6	1	3.8	-	-
13	8	1.9	-	1.1
20	15	0.8	-	0.4
24	19	0.3	-	-
28	23	0.2	-	-
August 4	30	0.4	-	-
11	38	0.8	-	-
17	44	0.8	-	-
September 4	62	0.9	-	-
10	68	0.3	-	-
17	75	0.3	-	-
24	82	0.2	-	-
October 1	89	0.1	-	-
9	97	0.0	-	-
15	103	0.1	-	-
22	110	0.1	0.1	-
29	117	0.2	0.0	-
November 5	124	0.1	0.1	-

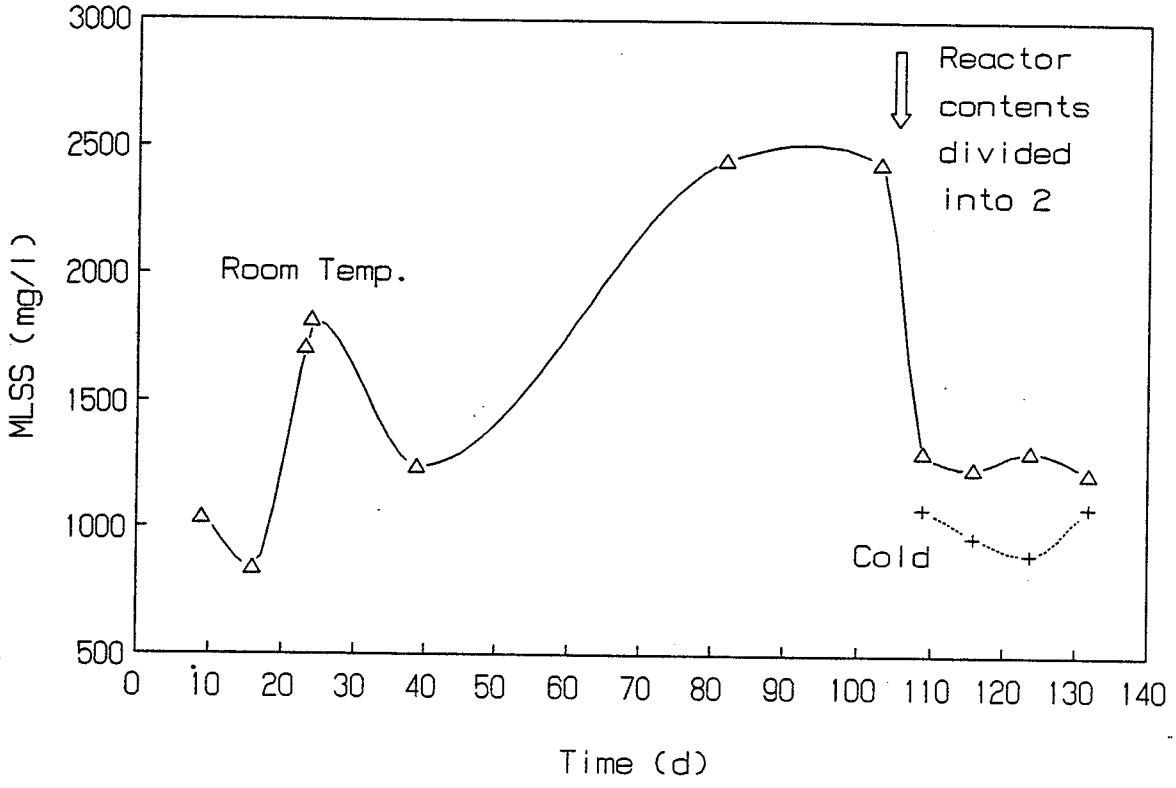


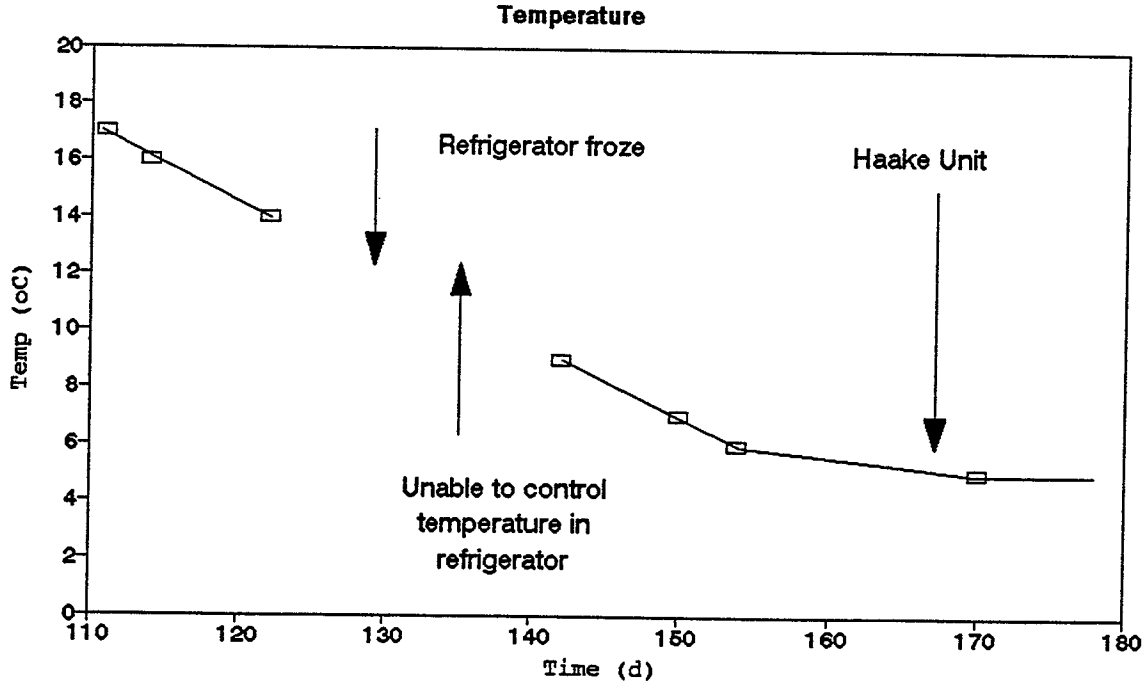
GRAPH B-4: ORTHOPHOSPHATE vs TIME AT ROOM TEMPERATURE, BN10

TABLE B-5: SOLIDS

Date	Day	MLSS * (mg/L)	
		Room Temp.	Cold
July 14	9	1040	-
21	16	840	-
28 (before feed)	23	1710	-
29 (after feed)	24	1820	-
August 12	39	1240	-
September 24	82	2450	-
October 15	103	2440	-
21	109	1300	1080
28	116	1240	970
November 5	124	1310	900
12	132	1220	1090

* Mixed Liquor Suspended Solids





GRAPH B-5: SOLIDS (MLSS) vs TIME AT TWO TEMPERATURES, BN10

TABLE B-6: TOC, COD OF FEED

	COD (mg/l)	TOC (mg/l)
0.5 ml oil + 1 g Biodish	400	255.5/172.1/177.5
1 g Biodish	200	103.9/78.1/81.8
1.0 ml oil + 1 g Biodish	-	279.6
2 g Biodish	-	169.4

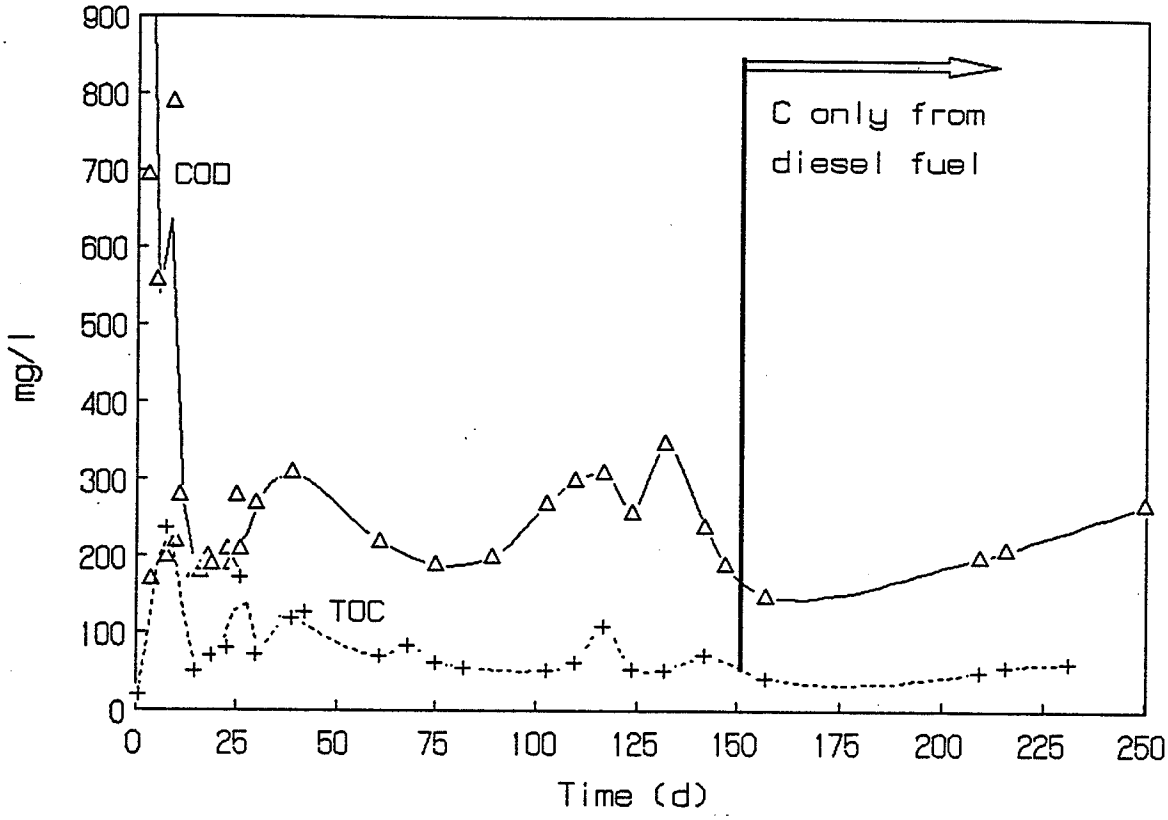
TABLE B-7: TOC, COD

Date	Day	TOC * (mg/l)	COD ** (mg/l)
July 6	1	21.69	-
9	4	-	170
10	5	-	560
13	8	238.3 +	200
14	9	-	790
15	10	-	220
16	11	-	280
20	15	50.35	180
21	16	-	180
22	17	-	190
23	18	-	200
24	19	71.36	190
28	23	81.06	210
29	24	-	190
30	25	-	280
31	26	173.3	210
August 4	30	73.70	270
11	38	123.5	310
12	39	120.4	310
September 3	61	71.25	220
10	68	84.98	-
17	75	64.13	190
24	82	57.20	***

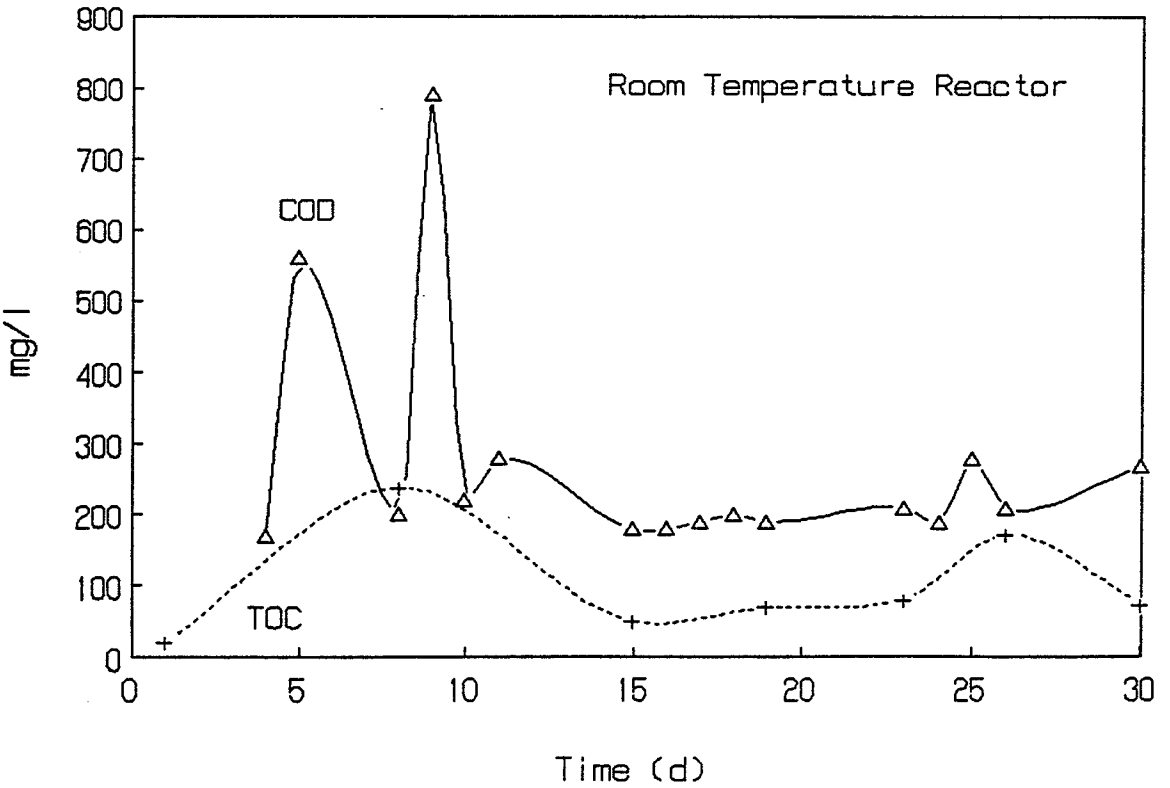
* Total Organic Carbon
** Chemical Oxygen Demand
*** Data Lost
\+ Data in doubt

TABLE B-7 CONTINUED:....

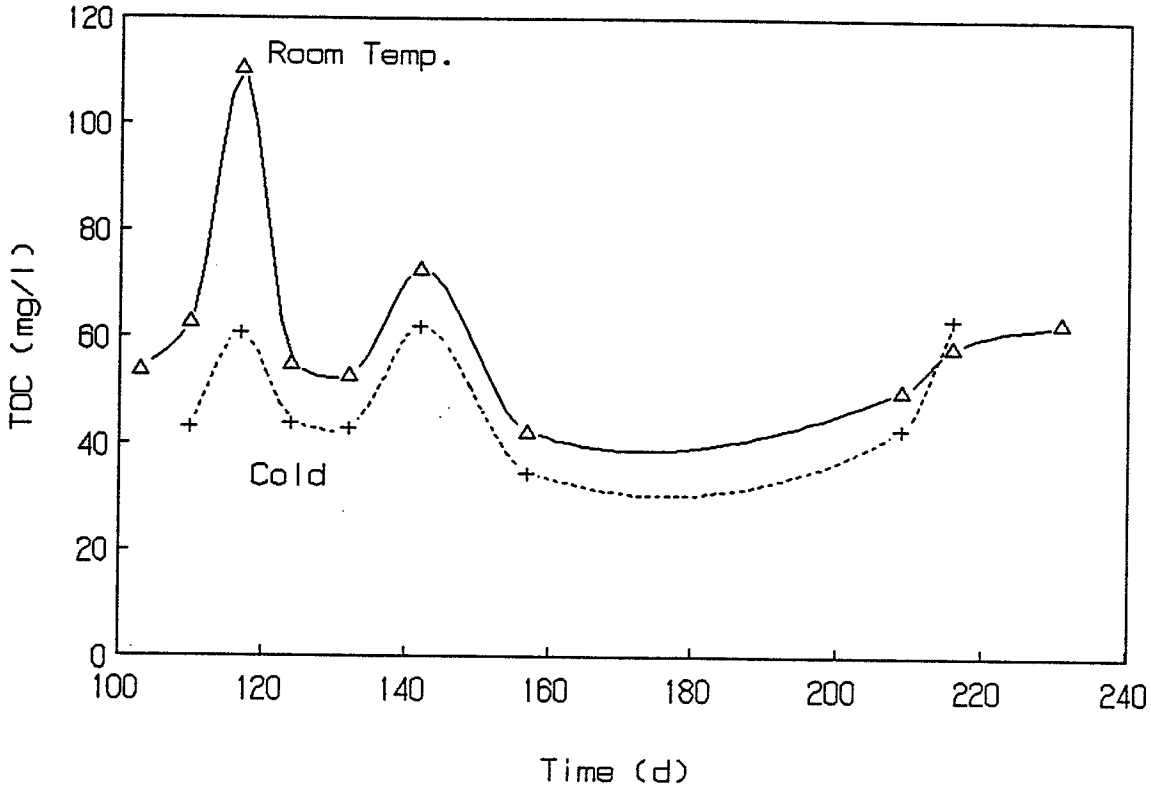
Date	Day	TOC * (mg/l)		COD ** (mg/l)	
		Room Temp.	Cold	Room Temp.	Cold
October 1	89		-	200	-
15	103	53.9	-	270	-
22	110	62.7	43.3	300	140
29	117	110.4	61.1	310	190
November 5	124	55.2	44.3	260	170
12	132	52.9	43.0	350	280
22	142	72.9	62.2	240	230
27	147	-	-	190	340
December 7	157	42.4	34.7	150	240
January 28	209	50.3	43.0	200	450
February 4	216	58.6	63.7	210	240
19	231	63.0	-		
March 9	250			270	770

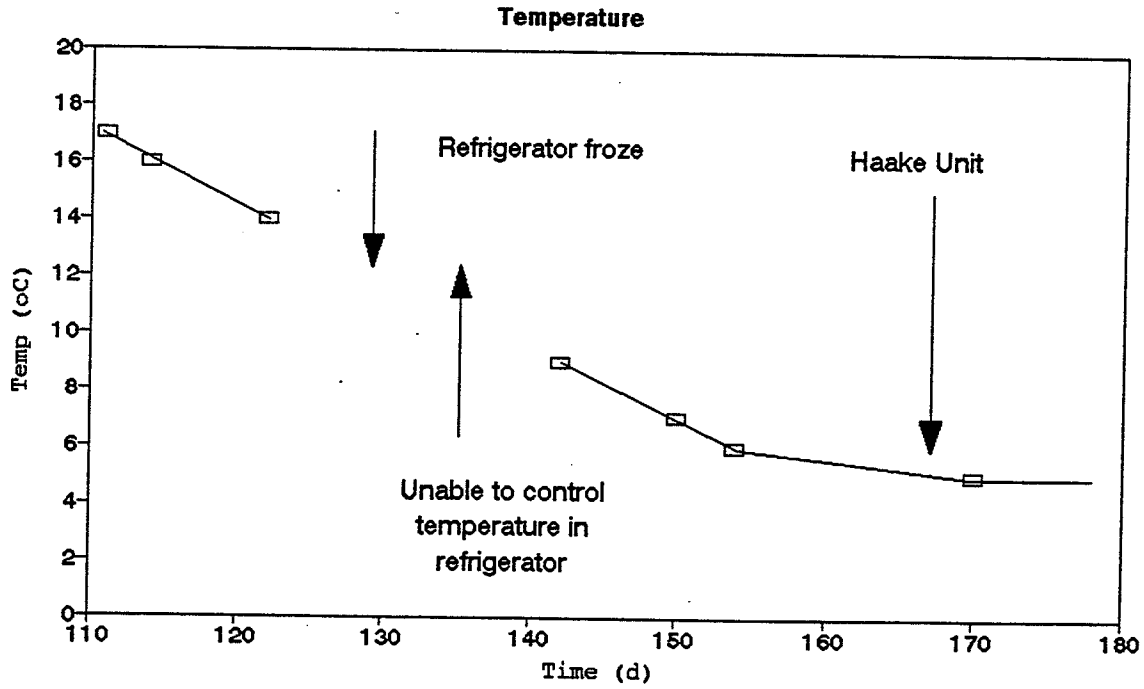


GRAPH B-6: COD AND TOC (mg/l) vs TIME (ROOM TEMPERATURE)

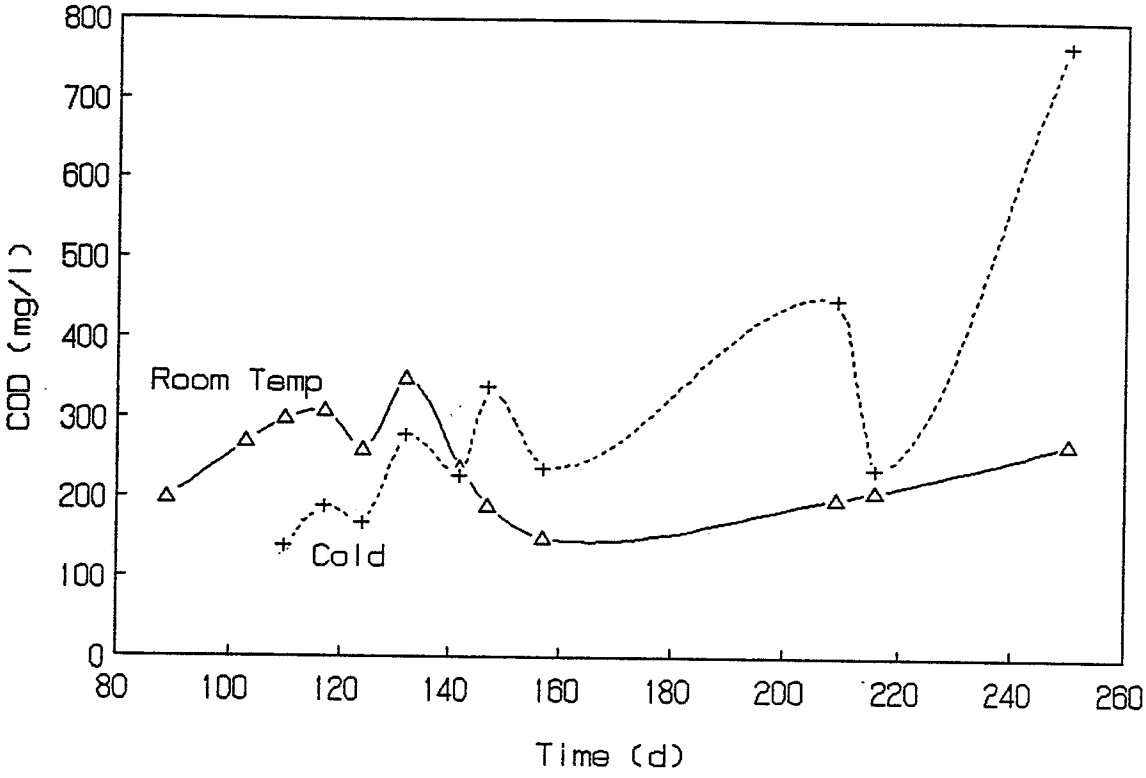


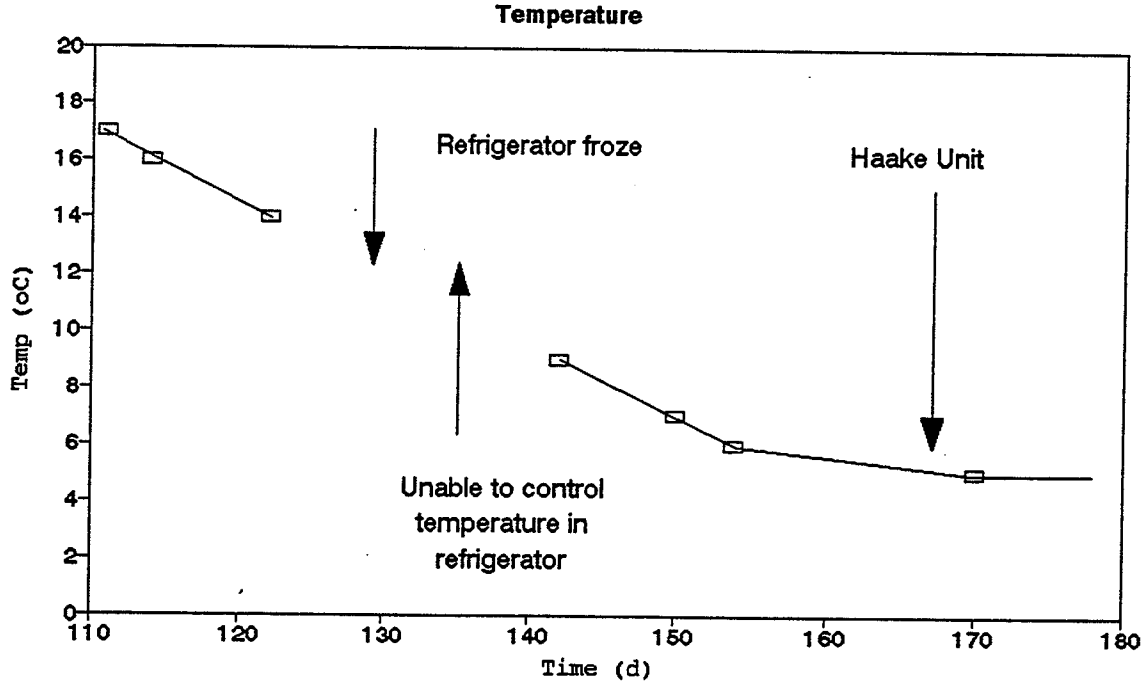
GRAPH B-7: COD AND TOC (mg/l) vs TIME - DAY 0 TO 30 - (22 °C)





GRAPH B-8: TOC (mg/l) vs TIME AT TWO TEMPERATURES, BN10





GRAPH B-9: COD (mg/l) vs TIME AT TWO TEMPERATURES, BN10

TABLE B-8: MICROSCOPIC EXAMINATION

Date	Microscopic Examination	
	Room Temperature	Cold
July 6	- nothing	
14	- no sludge; some small movements	
21	- some sludge; sme very small protozoa	
August 13	- moderate number small protozoa	
September 4	- some paramecium; some small protozoa	
21	- many small protozoa; rotifers; large protozoa	
October 1	- many small protozoa; rotifers	
15	- some small protozoa; a few rotifers	
28	- very active, 25 to 35 per view, small protozoa, moving quickly	- few movements; 5 to 10 per view; active small protozoa
November 5	- small protozoa; filamentous (few); rotifers (very few)	- some small protozoa, quite active; filamentous
12	- some small protozoa; rotifer; filamentous	- small active protozoa; 15 to 20 per view; nematode(?)

APPENDIX C: PUKATAWAGAN RESULTS

TABLE C-1: FEED SCHEDULE FOR REACTORS A, B, C

Day	Oil (ml)	Biodish (g)	Dex/Gl.Acid * (ml)	N (mg/l)	P (mg/l)
July 5	f.d. *	-	-	-	-
7	f.d. *	-	-	-	-
8	f.d. *	-	-	-	-
9	0.5	1	10.0	7.0	1.0
13	0.5	1	10.0	7.0	1.0
20	0.5	1	10.0	7.0	1.0
24	0.5	1	10.0	7.0	1.0
28	0.5	1	10.0	7.0	1.0
31 **	0.5	1	10.0	7.0	1.0
Aug. 4	0.5	1	10.0	7.0	2.0
7	0.5	1	10.0	14.0	2.0
11	0.5	1	10.0	14.0	2.0
14	0.5	1	10.0	14.0	2.0
18	0.5	1	10.0	14.0	2.0
27	0.5	1	10.0	14.0	2.0
Sept.4	0.5	1	10.0	7.0	0

* f.d. = few drops

** Dextrose/Glutamic Acid

TABLE C-2: REACTOR A

Date	Ammonia N (mg/l)	pH	MLSS * (mg/l)	Orthophosphate (mg/l)
July 6	-	7.55	-	2.2
7	0	-	-	-
13	1.72	7.75	-	0.6
14	-	-	520	-
20	0.44	7.95	-	0.1
21	-	-	400	-
24	0	-	-	0.2
28	0	8.10	670	0
29	-	-	620	-
31	0	-	-	-
Aug. 4	0	8.05	-	0
11	1.33	7.90	510	0
17	-	-	-	0.2
Sept. 4	1.54	8.10	-	2.5
10	0.38	7.20	-	0.5

* MLSS = Mixed Liquor Suspended Solids

TABLE C-3: REACTOR B

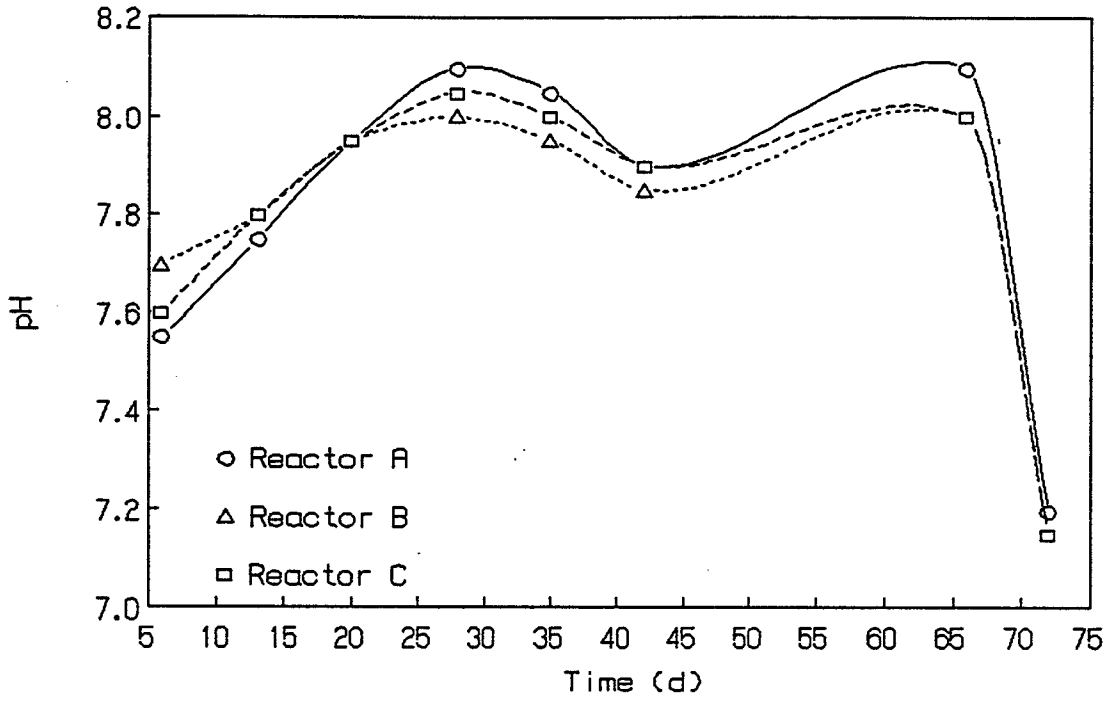
Date	Ammonia N (mg/l)	pH	MLSS * (mg/l)	Orthophosphate (mg/l)
July 6	-	7.70	-	1.7
7	0	-	-	-
13	2.69	7.80	-	0.5
14	-	-	380	-
20	1.77	7.95	-	0.4
21	-	-	370	-
24	0	-	-	0
28	0.77	8.00	720	0.1
29	-	-	650	-
31	0	-	-	-
Aug. 4	0	7.95	-	0
11	2.30	7.85	920	0
17	-	-	-	0.1
Sept. 4	1.14	8.00	-	1.5
10	0.14	7.15	-	0.2

* MLSS = Mixed Liquor Suspended Solids

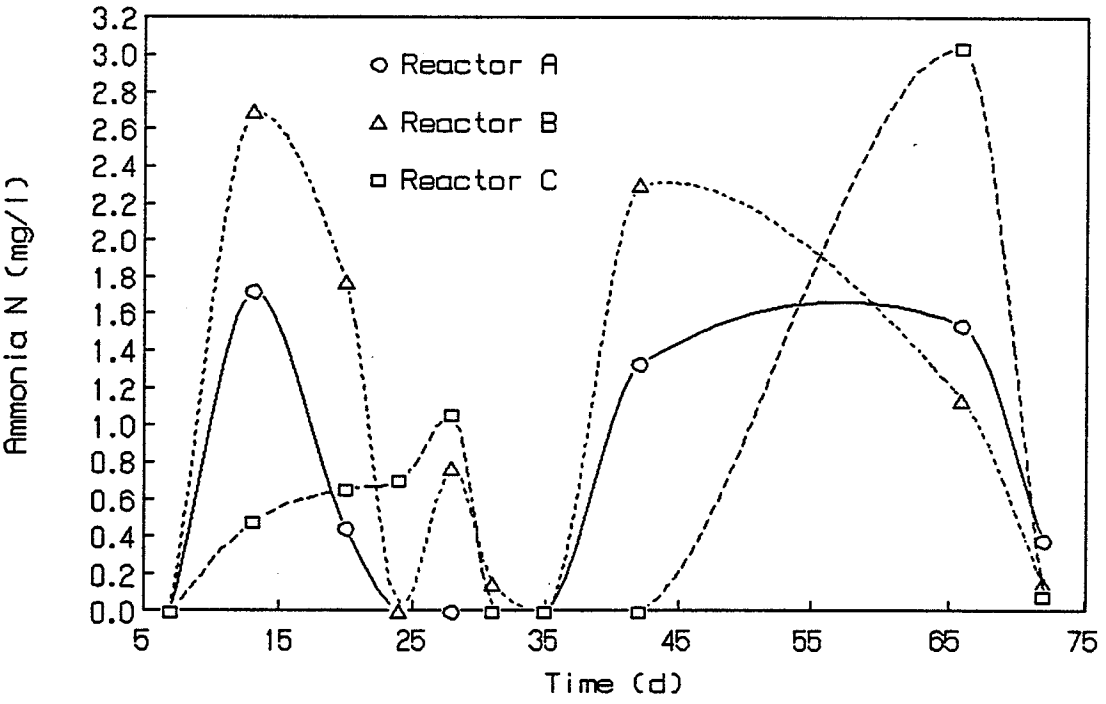
TABLE C-4: REACTOR C

Date	Ammonia N (mg/l)	pH	MLSS * (mg/l)	Orthophosphate (mg/l)
July 6	-	7.60	-	0.8
7	0	-	-	-
13	0.485	7.80	-	0.3
14	-	-	250	-
20	0.66	7.95	-	< 0.1
21	-	-	190	-
24	0.7	-	-	0
28	1.06	8.05	330	0.1
29	-	-	340	-
31	0	-	-	-
Aug. 4	0	8.00	-	0.1
11	0	7.90	520	0
17	-	-	-	0
Sept. 4	3.04	8.00	-	0.6
10	0.08	7.15	-	0

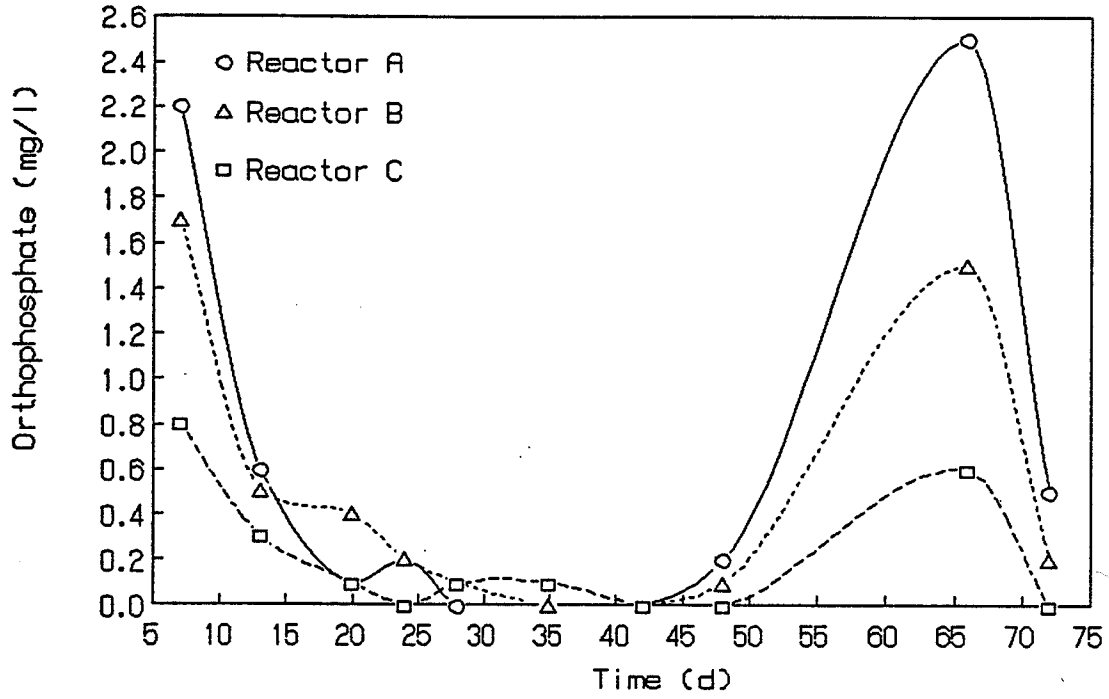
* MLSS = Mixed Liquor Suspended Solids



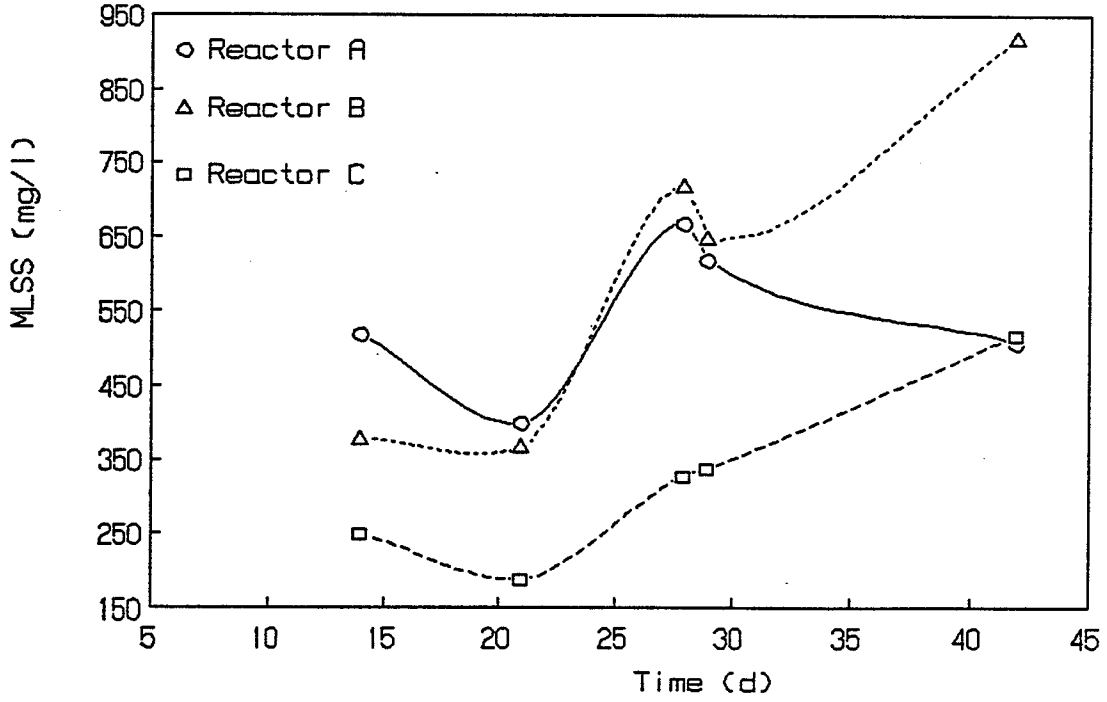
GRAPH C-1: pH vs Time for Reactor A, B, C



GRAPH C-2: Ammonia N vs Time for Reactors A, B, C



GRAPH C-3: Orthophosphate vs Time for Reactors A, B, C



GRAPH C-4: MLSS vs Time for Reactors A,B,C

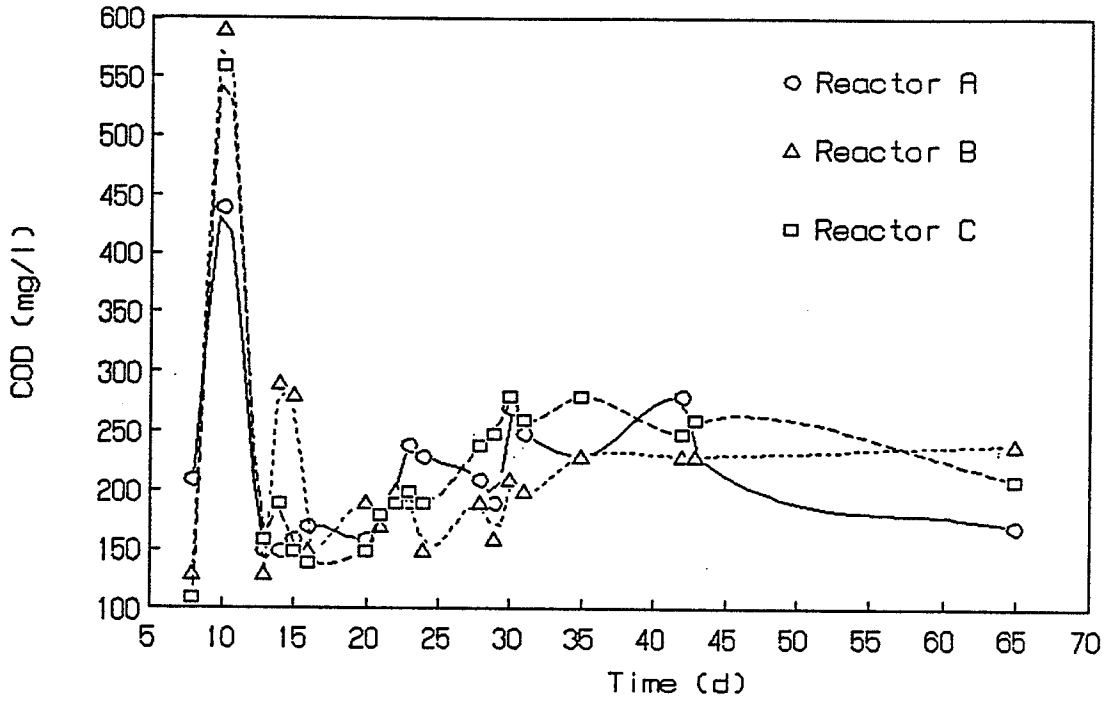
TABLE C-5: EFFLUENT COD AND TOC FOR REACTORS A, B, C

Date	COD (mg/l)			TOC (mg/l)		
	A	B	C	A	B	C
July 6	-	-	-	18.16	12.74	17.39
8	210	130	110	-	-	-
9 (Feed)						
10	440	590	560	-	-	-
13	150	130	160	-	-	-
13 (Feed)						
14	150	290	190	-	-	-
15	160	280	150	-	-	-
16	170	150	140	-	-	-
20	160	190	150	51.39	52.30	51.01
20 (Feed)						
21	180	170	180	-	-	-
22	200	200	190	-	-	-
23	240	190	200	-	-	-
24	230	150	190	79.95	65.23	64.88
24 (Feed)						
28	210	190	240	80.31	73.84	88.99
28 (Feed)						
29	190	160	250	50.73 *	46.52 *	76.55
30	270	210	280	-	-	-
31	250	200	260	82.07	57.64	90.33
31 (Feed)						
Aug.4	230	230	280	83.10	80.50	88.11
4 (Feed)						
7 (Feed)						
11	280	230	250	84.06	88.22	103.9

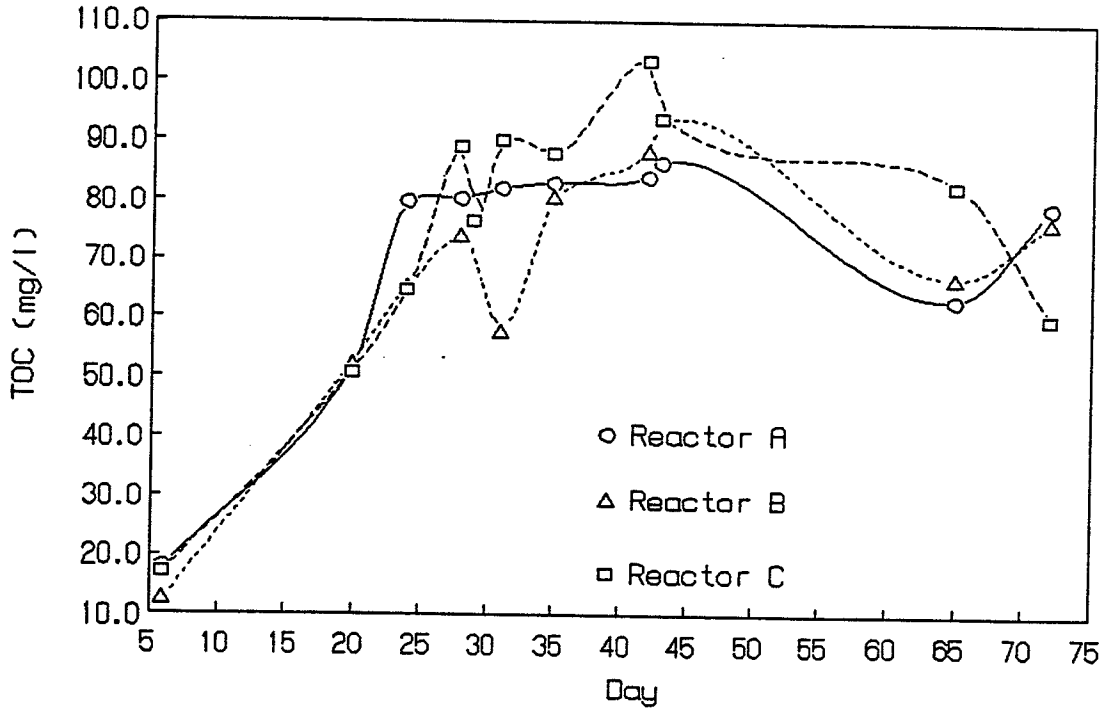
TABLE C-5 CONTINUED:....

Date	COD (mg/l)			TOC (mg/l)		
	A	B	C	A	B	C
11 (Feed)						
12	230	230	260	86.63	93.52	94.01
14 (Feed)						
18 (Feed)						
27 (Feed)						
Sept. 3	170	240	210	63.27	66.98	82.76
4 (Feed)						
10	**	**	**	79.45	76.67	60.29

* filtered sample
** sample lost



GRAPH C-5: Chemical Oxygen Demand vs Time for Reactors A,B,C



GRAPH C-6: Total Organic Carbon (Supernatant) vs Time

TABLE C-6: MICROBIOLOGY ANAYLSIS

Date	Reactor	Microbiology Examination
6	A	- nothing visible
	B	- nothing visible
	C	- nothing visible
14	A	- large ciliated protozoa, 2 per view - some small protozoa
	B	- very few small protozoa, very little sludge
	C	- some small protozoa, very little sludge
21	A	- many large protozoa, 2 per view - some small protozoa
	B	- many small protozoa, few large protozoa
	C	- many small protozoa, few large protozoa
29	A	- stalked protozoa - small protozoa, 20 per view - large protozoa, 2 per view - filamentous bacteria ?
	B	- many small and large protozoa, very active
	C	- some small and some large protozoa

DEXTROSE

$CH_2OH(CHOH)_4CHO$ .....M.W. 180.16 mg

6 C
6 O
12 H

$+ 6\ O_2 \text{ ------> } 6\ CO_2 + 6\ H_2O$

Weight of C = 12

So, in 180.16 mg Dextrose there is 6 C * 12 = 72 mg C

In a 7.5 g/L concentration of dextrose there is:

180.16 mg --------> 72 mg C
7500 mg --------> x_1 mg C

x_1 = 2997 mg C

GLUTAMIC ACID

$HO_2CCH_2CH_2CH(NH_2)CO_2H$.....M.W. 147.14 mg

5 C
4 O
9 H
1 N

$+ O_2 \text{ ------> } CO_2 + H_2O + NH_3$

Weight of C = 12
Weight of N = 14

So, in 147.14 mg Glutamic acid there is 5 C * 12 = 60 mg C and
1 N * 14 = 14 mg N

In a 7.5 g/L concentration of Glutamic Acid there is:

147.14 mg ------> 60 mg C
147.14 mg ------> 14 mg N
7500 mg -------> x_2 mg C
-------> y mg N

x_2 = 3058 mg C

y = 713.6 mg N

Therefore, 7.5 ml dextrose/glutamic acid contains:

$x_1 + x_2 = 2997 + 3058 = 6055$ mg C and

$y = 713.6$ mg N

In 10 ml of 7.5 g/L dextrose/glutamic acid there is:

60 mg C and 7.136 mg N

CARBON: NITROGEN: PHOSPHATE (100:7:1)

CARBON

0.5 ml Diesel Oil .
3 capfuls Cascade..... TOC ~ 215 mg/L 53.75%

Reactor 1-6 after 24 hour
aeration from initial trial..... TOC ~ 43 mg/L 10.75%

20 ml Dextrose/Glutamic Acid..... TOC ~ 155 mg/L 38.75%

~ 400 mg/L C 100%

NITROGEN

20 ml Dextrose/Glutamic Acid..... 14 mg N/L

Ammonium Chloride..... NH_4Cl .....M.W. 53.49 mg

53.49 mg has 14 mg N
1000 mg N has 3820.7 mg NH_4Cl

Therefore 3.82 g NH_4Cl in one liter makes 1000 mg/L.

Need 28-14 = 14 mg N supplemental addition.

14 ml of 1000 mg/L N = 14 mg N

PHOSPHATE

0.5 ml Diesel Oil
3 capfuls Cascade..... Total P ~ 4.0 mg/L

No supplemental phosphate required initially.