BIODEGRADATION OF SYNTHETIC COMPONENTS IN METALWORKING FLUIDS

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CHAPTER 1

INTRODUCTION

Metalworking fluids are used extensively in the metalworking industries to perform two functions viz. the removal of heat from the metal surface and to minimize the friction at the metal site. Semi-synthetic metalworking fluids are chemical emulsions containing some oils, whereas the synthetic metalworking fluids contain no oil and are true solutions of complex organics in water. These metalworking fluids have successfully replaced the conventional oil in water emulsions. The addition of various chemical agents to the semi-synthetic and synthetic metalworking fluids renders them superior in terms of rust protection, stabilization, increased tool life, reduction of surface tension, and extreme pressure lubrication.

These fluids are required to serve for extended periods in the industry. Since bacterial growth can cause deterioration of fluid quality over time, it is controlled by addition of glycols, blending agents, humectants, and germicides. However, increased bio-resistance presents a considerable hardship in terms of disposal of the waste emulsions. The existing wastewater treatment processes for

oil in water emulsions are incompatible with the synthetic metalworking fluids because they contain little or no oil, but only water soluble organic compounds. Thus, their removal from the wastewater stream cannot be accomplished by the processes for removing oils. This leads to increased BOD and COD levels in the effluent. It, therefore, becomes imperative to either remove the non-biodegradable component of the used emulsion prior to disposal or to explore the feasibility of successfully performing biodegradation of the waste emulsion.

An experimental, laboratory study was undertaken to study the biodegradation of two different metalworking fluids, IRMCO 141 and IRMCO 156, obtained from the same company. The primary aim of the study was to determine the biodegradability of these fluids and the most favorable conditions for biodegradation. The present biodegradation studies also included the adaptation of mixed cultures. The experimental work was a follow up to that conducted by S. M. Lee at the Department of Chemical Engineering, Kansas State University, with thirteen different metalworking fluids(Lee, 1986).

Experiments were carried out in 500 ml Erlenmeyer flasks with heterogeneous microbial populations of sewage origin

and with metalworking fluids, each containing a synthetic medium as the inorganic nutrient source. The system parameters measured include biomass concentration in terms of the dry weight, chemical oxygen demand (COD), pH, and temperature. The experiments were continued for a period of over 30 days with the shake flasks maintained at 175 rpm.

Overview of Chapters

Chapter 2 of deals with the review of the literature available on the biodegradation of metalworking fluids. A brief discussion is included on the types of metalworking fluids, the technologies for waste metalworking fluid treatment and the importance of pH, oil concentration, inorganic nutrients and adaptation of the microbial populations, on biodegradation.

The experimental methods, analytical methods for data analysis and the results and discussions for the various shake flask experiments are given in chapter 3. The experimental data along with the results of regression analysis of the data are included in Appendix I.

Mass spectrometer analysis was carried out on the gas phase of the shake flasks used in the study of the effect of

dilution and inorganic nutrient composition on biodegradation. A brief discussion on the mass spectrometer equipment, the equations developed for the analysis of the data and the results obtained, are discussed in chapter 4. The data are tabulated in Appendix III.

The conclusions based on the biodegradation of samples of IRMCO 141 and IRMCO 156 are presented in chapter 5.

CHAPTER 2

LITERATURE REVIEW

2.1 Introduction

Nearly 6,000,000 tons of oil enters the environment each year. Only a small fraction of the oil, entering the environment, is altered by oxidation and photochemical reactions and the remaining is dispersed through volatilization, dissolved in water, buried in marine sediments, covered with ice or degraded by microorganisms. Although photooxidation and other abiotic mechanisms are important in terms of transforming the oil, they are not significant in terms of complete mineralization (breakdown of organics to carbon dioxide, water, and other inorganic chemicals). Only burning or microbial degradation can completely eliminate the oil from the environment (Brown, 1987).

Oil is a complex mixture of organics and inorganics depending on its source and the desired application. If an application involves lubrication then the base of the metalworking fluid is essentially non-aqueous with a viscosity greater than water (it may contain anti-corrosion

and extreme pressure additives). On the other hand if the primary application is cooling, then the metalworking fluid is basically water with additives for heavy duty operation and corrosion protection; in addition it may have antimicrobial agents as the presence of water promotes microbial contamination (Rossmore, 1985).

Organics present in the oil may be classified under three categories viz. biodegradable, persistent, and recalcitrant. Biodegradation implies the microbial transformation of an organic into a less toxic form. The term recalcitrance is defined as the inherent resistance of an organic compound to any degree of biodegradation. Persistence is defined to mean that a chemical fails to undergo biodegradation under a specified set of conditions, it may be biodegradable if the conditions were to be altered (Grady, 1985).

The main criterion for establishing the category of an organic compound is to determine the length of time the compound has been on earth. Most naturally occurring compounds have been present for a long time, and microbes have evolved that can initiate the mineralization of such compounds. Most of the chemicals exploited by the industry today closely resemble the naturally occurring chemicals and

these also are subject to biodegradation. However, there are certain other chemicals that are, both structurally and chemically, relatively new to the environment. These are classified as xenobiotics (foreign to the environment). Some of these may pose problems if they are toxic and tend to accumulate in the environment.

Oils have found a number of applications in industry. They have been used as electronic coolants, pump fluids, lubricants, damping fluids, greases, power transmission and hydraulic fluids, heat transfer fluids, heat pump fluids, and refrigeration fluids (Ranney, 1976). The largest source of the oil entering the environment, however, is the automobile engine. Other significant contributors to the waste oil are industrial lubricants, industrial engine oils, and oils for metalworking purposes (Potter, 1986).

Fluids have been used in the metalworking industries to perform lubrication and cooling of tools, permitting faster feeds of metals along with faster speeds, providing better surface finishes on metal, extending the life of the tool, and to carry off chips, fines and swarf (Rossmore, 1985). In the metalworking industry, after a period of usage the oil eventually gets contaminated with metal fines, water, and microorganisms and has to be discarded in nature. The

main components of the waste oils are heavy metals like lead, barium, cadmium, arsenic, chromium, and zinc; halogenated organics like PCBs and solvents (Potter, 1986). The waste oil along with its hazardous organic and inorganic components needs to be properly processed before it enters the environment.

2.2 Types of Metalworking Fluids

Several different types of metalworking fluids are used in the metal working industries. Based on their chemistry these fluids have been classified by the ASTM (American Society for Testing and Materials, 1978) under the following three categories (Rossmore, 1985);

i. Oil Emulsions
   - A. Water in oil-primarily deformation
   - B. Oil in water

ii. Chemical Solution
   - A. True solution
   - B. Colloidal solution

iii. Pre-formed emulsion, oil in water emulsion in concentrated form containing additives

The pictorial representations of these three types of oils are shown in figures 2.1 through 2.4 (Springborn, 1967).

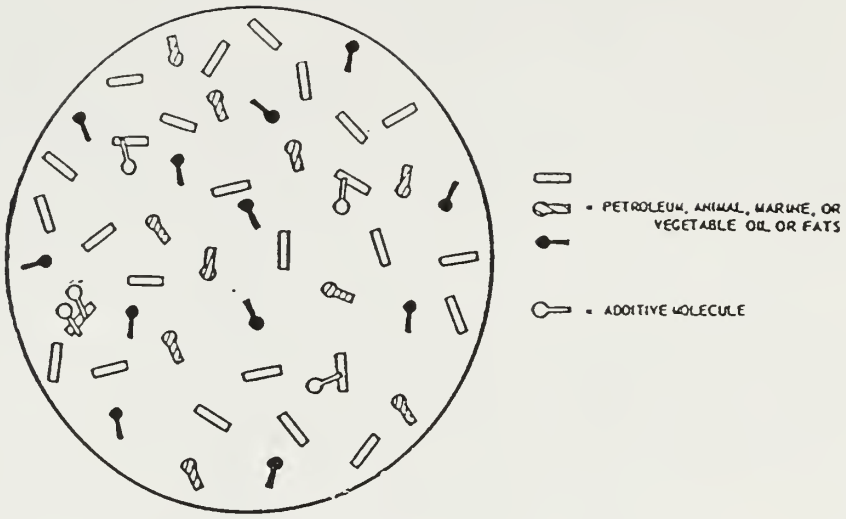

Figure 2.1. Pictorial representation of oil emulsions (Springborn, 1967).

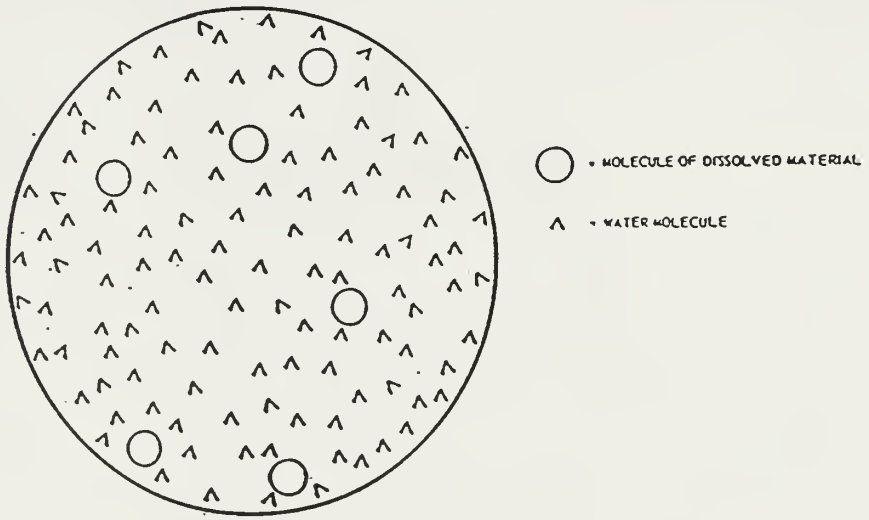

Figure 2.2. Pictorial representation of true solutions (Springborn, 1967).

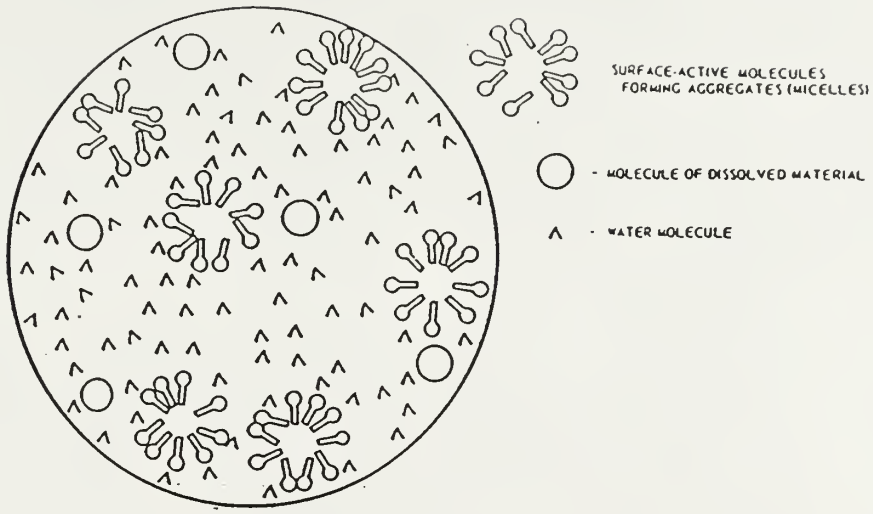

Figure 2.3. Pictorial representation of colloidal solutions (Springborn, 1967).

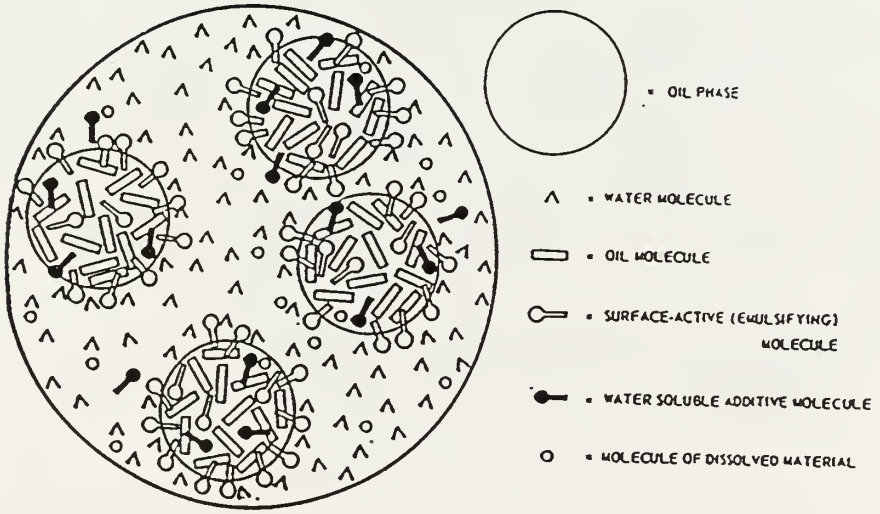

Figure 2.4. Pictorial representation of pre-formed emulsions (Springborn, 1967).

i. Oil emulsions : These are oils of petroleum, animal, marine, or vegetable origin or some combination of these. The addition of animal or vegetable fatty oils or esters produces emulsions with wonderful lubricating properties. An oil emulsion of petroleum origin may vary from naphthenic (saturated ring structures) to paraffinic (straight or branched chain hydrocarbons) depending on its origin and may include sulphonated derivatives (petroleum sulphonates) as emulsifiers. It may also have a broad viscosity range and can be used for various applications. The presence of additives like animal, marine or vegetable oil or inorganics like chlorine, sulphur or phosphorous improves the quality of the cutting fluid in terms of better wetting action, and better lubrication properties at high temperatures and high pressures.

ii. Chemical solutions or synthetic fluids : These are further subdivided as true solutions and colloidal solutions.

A. True solutions : They are transparent, colored fluids manufactured by dissolving inorganics and organics in water. The concentrated product may contain as much as 75% water by weight. The dissolved material is randomly dispersed in the solvent water molecules resulting in lower surface tension

than pure water. The advantage of having such a high water content is that they have a greater receptivity to a host of water-soluble additives like anti-corrosion and antimicrobial agents. Chemical agents present in chemical solutions are amines, nitrites, borates, and carboxylates for rust protection: nitrates, for stabilization: phosphates and borates for water softening; soaps and wetting agents for lubrication and reduction of surface tension; chlorine and sulphur for extreme pressure lubrication and glycols, blending agents, humectants, and germicides for retarding bacterial growth (Hunz, 1983). The discovery of nitrosamines in the metalworking fluids has resulted in a gradual reduction in the use of nitrites in these formulations (Spiegelhalder, 1984).

B. Colloidal solution : These contain additives that lower the surface tension and form colloidal aggregates among the surface active molecules. The other additives are the same as in true solutions.

iii. Pre-formed Emulsions or Semi-synthetic fluids : These are dilute oil-in-water emulsions containing 10% or more mineral oil. They are made by suspending droplets of mineral, paraffinic, or naphthenic base oils in water using emulsifying agents and other agents in order to limit the

size of the oil droplets between 0.0002 in. and 0.00008 in. in diameter, when blended with water. Water is the best possible coolant media because of its high specific heat, high thermal conductivity, and high heat of vaporization (Billet, 1979). Extreme pressure (EP) emulsions can be obtained by the addition of inorganics like sulphur, chlorine and phosphorous. The presence of a nutritionally rich oil phase dispersed in 10 to 100 times its volume in water provides an enormous interfacial area for the growth of microbial populations. Antimicrobial agents are required to prevent the spoilage of the emulsions (Hill, 1984). They differ from chemical fluids in having (Springborn, 1967):

(a) lower oil content (about 10% to 45% lower than chemical solutions, and

(b) higher proportion of emulsifying or surface active molecules than chemical solutions and with smaller oil droplets.

A new approach to classify the metalworking fluids is under consideration by the International Standards organization (ISO). This scheme would be based on the cooling and lubricating properties of the fluids with

factors like extreme pressure additives and corrosion inhibition extending the classification.

2.3 Treatment Technologies for Used Metalworking Fluids

Some of the most commonly employed schemes in the treatment of used metalworking fluids are recycle of used oil, biological treatment, and wet air oxidation(Potter, 1986).

i. Recycle of Used oil : Some of the technologies for recycling of waste oil include on-site filtration, distillation, chemical treatment, solvent treatment, and off-site recycle.

A. On-site Filtration : Several companies have designed and produced filtration systems that facilitate on-site purification of waste oil thereby reducing the amount of waste oil. These systems make use of thermal degradation and filtration. A direct method of dewatering soluble oil emulsions is ultrafiltration (Dick, 1982). A novel filter based on zirconia fibers has been designed and tested to control microbial contamination in aqueous metalworking coolants and lubricants. The laboratory evaluation and field trials have demonstrated that the filter is effective

in limiting the bacterial count at about 10^5 to 10^6 organisms/ml in a number of aqueous cutting fluids (Symes, et al., 1976).

B. Distillation : Distillation is used extensively in the oil recycle schemes. Both fractional distillation and flash distillation are employed to remove water and low molecular weight hydrocarbons. Vacuum distillation is also employed to volatilize the oil itself, and the oil is collected for later use in lubrication applications (Treybal, 1984).

C. Chemical Treatment : The oil is treated with chemicals to desolubilize metals and other fine contaminants. A variety of chemicals ranging from clay to sulphuric acid and diammonium phosphates are employed to remove metals from the oil. Emulsions are currently split into oil and water phases by treatment with mineral acid and alum (Harlow et al., 1981). The water phase can be dumped into sanitary waste after necessary pH adjustments. The oil can be recovered and sold to reclaimers that can bring it back to the specifications of the metalworking fluids.

Synthetic fluids have by design all the additives in the aqueous phase which may have BOD loadings greater than 10,000 mg/lit. This level should be reduced by biological treatment before discharging it into the sanitary waste.

D. Solvent Treatment : This treatment scheme may include three different stages. First, water may be removed by flash distillation or some other method. The next step is solvent extraction to remove the contaminants with the help of an appropriate solvent. Lastly, the oil is purified by removing the solids by filtration or distillation.

E. Off-Site Recycle : Several locations have large installations that offer off-site recycle of waste oil. The waste oil is subject to a number of steps including settling, dehydration, and several other thermal steps. The choice of resorting to on-site or off-site recycle is governed by the economics of the two schemes and also on the availability of the necessary treatment facilities.

ii. Biological Treatment : Biological degradation of waste material involves the breakdown of organic molecules by microorganisms that are either developed to attack specific organics or are natural populations of microbes. Microorganisms have been demonstrated to degrade both

aliphatic and aromatic hydrocarbons, along with synthetic organic chemicals such as chlorinated biphenyls.

Biodegradation can be subdivided into two categories depending on whether the degradation is carried out in the presence of oxygen or in its absence. The two categories are called aerobic and anaerobic biodegradation respectively.

Anaerobic biodegradation : Nitrate and sulphur are common electron acceptors for anaerobic respiration and methane, hydrogen sulphide, organic acids, and carbon dioxide are the common end products under fermentation conditions. It has been established that the best condition for thermophilic anaerobes to grow, from the point of view of temperature, pH, permeability, salinity, dissolved oxygen etc., is 5-6% NaCl at a neutral pH. However, anaerobes do not utilize organic compounds very efficiently, and the growth rates are extremely slow. Also, they are difficult to handle in the laboratory as extensive apparatus is required to eliminate the presence of oxygen that is toxic to the growth of anaerobes and analytical procedures have to be developed. Another problem with the use of anaerobes is that they do not produce surface active compounds like the kind produced by hydrocarbon degrading aerobes that are

important for reducing the viscosity and thereby causing the emulsification of the oil in excess water (Chakrabarty, 1985).

Aerobic biodegradation : In contrast to anaerobes, aerobes are a perfect choice for biodegradation of the waste oil. These have been known to degrade organics in the oil very efficiently to produce carbon dioxide, water, ammonia, nitrate and sulphate. They also produce surface active compounds that can reduce the interfacial tension between the oil water interface along with the viscosity of the emulsion thus making it easier for the oil to migrate easily in the water. Another advantage with the aerobes as opposed to the anaerobes is that they are more readily subject to genetic manipulation in order to produce more efficient strains. Their main shortcoming is that they need a continuous supply of oxygen in order to perform degradation of organics. The rate of degradation depends on several factors besides the type of oil and the choice of microorganisms as outlined below (Brown, 1987);

i. The presence of water and mineral salts.
ii. A nitrogen and phosphorous source.
iii. Free oxygen in the dissolved state with a concentration of at least 3-4 mg per mg of

saturated hydrocarbon for complete oxidation to carbon dioxide and water.

iv. Physical and chemical dispersion of oil in water to facilitate biodegradation.

v. An optimum temperature of 20-35 °C.

vi. Turbulent conditions in the fermentation broth as opposed to quiescent conditions.

vii. Low concentrations of organic matter in order to promote the growth of hydrocarbon consuming bacteria by providing the accessory growth factors.

viii. Low concentrations of oil because at low concentrations all components of the oil are degraded whereas at high concentrations only the more easily degraded components are attacked preferentially.

ix. Cytophagic (cell eating) protozoans and other invertebrates should be avoided as they can consume a large number of oil degrading microbes. Each protozoan can consume as many as 10^5 bacteria per day.

A number of technologies have been established to perform biodegradation of organics these include the

activated sludge process, aerated lagoons, trickling filters, rotating biological contactors, and fluidized-bed.

A. Activated Sludge Process : It is a continuous process where the incoming stream is mixed with activated biological sludge to form the mixed liquor. This mixed liquor is aerated and further agitated in an aeration tank. From the aeration tank the effluent flows into a sedimentation tank where the sludge is allowed to settle down and a part of this is recycled to mix with the incoming waste stream and the other part is processed before it is released (Bailey, 1977).

At the Oak Ridge Y-12 Plant, the activated sludge process is used to treat one million liters, per year of water based cutting fluids generated there(Taylor, 1983). A 110,000 L activated sludge reactor oxidizes an influent of 4 L/min containing 10,000 mg/L of total organic carbon (TOC) to levels below 1000 mg TOC/L. The reactor oxidizes an average of 38.6 kg of organic carbon per day with an efficiency of 90%. The aeration in the reactor is maintained at 100 kg of oxygen per day with the help of four floating aerators. The hydraulic retention time in the aeration basin is 29 days on the average. An adjacently placed 8000 L basin serves as a settling chamber. The

effluent from the reactor is discharged in a septic drainfield for further treatment (Taylor 1983).

B. Aerated Lagoons : Aerated Lagoons serve to treat dilute concentrations of industrial wastes. The microorganisms present include both aerobic and facultative organisms. Aerobic lagoons are deep basins that are maintained at high concentrations and are well mixed. The facultative lagoon combines aerobic and anaerobic digestion. The top portion is well agitated with a rich supply of oxygen and, therefore, promotes aerobic digestion. The lower portion is poor in oxygen supply and is relatively calmer. Anaerobic digestion takes place in this zone. The main advantage of a facultative lagoon is that it can facilitate the degradation of a vast range of compounds as it can combine both aerobic and anaerobic degradations.

C. Trickling Filters : This consists of a tank that is 3-15 ft. deep and is filled with stones or some other synthetic medium. Waste water is sprayed on this solid support media which promotes the formation of a bacterial film on the surface of the solid. This bacterial film degrades the incoming waste stream as it trickles down the depth of the tank. The waste stream is sprayed uniformly over the entire area of the tank (Bailey, 1977).

D. Rotating Biological Contactor : This scheme is also used to treat dilute industrial wastes. It consists of a series of flat, parallel disks that rotate in the waste water tank. The discs are covered with biological slime and they carry a film of waste water into the air where oxygen is available for aerobic biodegradation.

E. Fluidized-Bed Bioreactor : The fluidized bed bioreactor is a modification of the trickling filter process. It involves passing the waste stream at sufficiently high upward velocity to result in the expansion of the bed media, such as sand. On fluidization the bed provides a large surface area for biological growth and consequently a large area for biodegradation of the waste stream.

The Hydra-matic division, Ypsilanti, Michigan plant of General Motors, generates about 1890 cubic meters per day (500,000 gal/day) of waste water contaminated with synthetic and semi-synthetic metalworking fluids. The COD of the waste water stream is about 2000 mg/L, and the total Kjeldahl nitrogen (TKN) concentration is 120 mg/L. Before the waste stream can be discharged to the Ypsilanti community municipal wastewater plant, it need be brought to a COD level of 600 mg/L, BOD level of 300 mg/L, and TKN of

40 mg/L. Pilot plant data have been collected with a PVC fluidized-bed column containing silica sand; the column has a cross sectional area of 0.021 sq. m and a height of 3.658 m. It has been demonstrated that COD removal of the order of 70% can be obtained in-spite of having a large fraction of non-biodegradable components in the inlet stream (Sutton et al., 1985).

iii. Wet Air Oxidation : This process has been designed to destroy the hazardous organic components of the waste oil. This process is best suited to those kinds of oils that are too dilute to be incinerated economically and too concentrated (COD approximately 75,000 mg/L) in terms of toxicity to be biologically degraded in municipal waste water treatment plants. Waste with concentrations of 10,000 to 100,000 mg/L can be treated by this process. Wet air oxidation scheme employs a high temperature and high pressure process utilizing oxygen for oxidation of the hazardous compounds. The waste stream is mixed with incoming air at 350°C to 750°C and at pressures of 95,760 Pa to increase the dissolved oxygen and to prevent the volatiles from escaping. The oxidation reactions are exothermic and consequently raise the temperature of the stream. This process results in the oxidation of about 80% of the organics to carbon dioxide and water, and the

remaining 20% is converted to intermediate weight organic compounds, such as acetaldehyde, acetone, acids and methanol. Inorganic sulphates, ammonia or molecular nitrogen are also produced.

2.4. Disposal problems of synthetic metalworking fluids

There has been a rapid transition from the oil emulsions to the synthetic and semi-synthetic fluids in the metalworking industries in the last fifteen years or so. Today's water based metalworking lubricants have almost completely replaced the conventional oils because they can be fabricated to suit the needs of any kind of work environment. Some of the benefits of synthetic metalworking fluids over conventional oil emulsions are: increased productivity, tool life, and metalworking fluid life; reduction in scrap rate, oil mists and operating costs; and improvements in cleanliness of the plant. The purposeful addition of water to the matalworking operations has created an extremely favorable environment for a range of microorganisms. The synthetic fluids can thus cause occupational hazard, economic loss and environmental problems.

The chemical fluids are complex mixtures of chemical agents, designed to minimize friction at the metal surface, provide efficient cooling, protect against corrosion, maintain the stability of the emulsion, perform lubrication at elevated temperatures and pressures, curb microbial attack, and minimize the hazard to the worker and the environment. This rapid transition to a different kind of metalworking fluids has raised a very serious question of whether the disposal of waste oil is amenable to the existing facilities and technologies.

Most of the automotive metalworking industries have an oil based waste water treatment plant. These plants perform four kinds of operations, namely, removal of free oil, breaking of chemical emulsions, floation of oil flocs, and clarification. With the introduction of synthetic and semi-synthetic fluids there has been a serious difficulty with the existing waste water treatment systems. Even though it is possible to break the emulsion, the chemical usage and the sludge production goes up tremendously. The recovery of oil is reduced considerably and the quality of the recovered oil goes down resulting in increased operating costs. Recycle of oil is thus made difficult and most of the complex organics present in the waste oil go untreated and cause a much higher concentration of COD and BOD loading in

the effluent. This causes a serious concern as the effluent is too toxic to be accepted by the municipal treatment plants.

From the perspective of high initial costs of these fluids, they are required to serve for extended periods in the industry. It has been observed that bacterial growth is responsible for the deterioration of fluid quality over a period of time. An effort, therefore, has been made to use glycols, blending agents, humectants and germicides for the control of bacterial growth. Nevertheless, increased bio-resistance presents a considerable hardship in terms of disposal of the waste emulsions. It therefore becomes imperative to either remove the non-biodegradable component of the used emulsion prior to disposal or to explore the feasibility of successfully performing biodegradation of the waste emulsion.

2.5 Objectives of the present work

In the present work, an effort has been made to study the potential of biodegrading waste metalworking fluid belonging to the chemical solutions category as per the ASTM classification. In the studies conducted by Lee (1986), it has been demonstrated that 8 out of 13 samples selected are

readily biodegradable with mixed populations of sewage origin. Two samples, one that has been demonstrated to be readily biodegradable, and the other that was found to be not so readily biodegradable have been selected for further experimentation. These samples have been manufactured by International Manufacturing and Refining Company, Evanston, Illinois.

Both IRMCO 141 and IRMCO 156 contain hexahydro-1,3,5 tris (2 hydroxyethyl)-s-triazine as a preservative to perform both the biocidal and the biostatic functions. The structural formula of the preservative is shown in Figure 2.5. Table 2.1. shows the range of pH over which the preservative is effective.

The effect of a number of variables like pH, initial oil concentration, nutrient composition, adaptation, has been studied on the rate of biodegradation of the oil samples in terms of COD removal efficiencies.

i. Effect of pH on biodegradation : It is not sufficient for a metalworking fluid to be suitable for a particular metalworking operation. In addition to its lubrication and other useful properties, it must also be non-damaging to ferrous, non-ferrous, and polymer materials; harmless to the

operators; display correct foaming and wetting characteristics; permit the settling of small metallic particulates; resist the growth of microbial populations; be chemically stable, withstand filtering and centrifuging; and withstand chemical splitting so that it could be easily disposed (Becket, 1984). To keep the oil from bacterial contamination the more alkaline the solution the better but on the other hand a fluid of high alkalinity can easily cause dermatitis because of the normal acid (pH of 6.80) of the skin. It is thus impossible to incorporate all these properties into one blend of metalworking fluid and therefore an optimum of the properties has to be achieved. The above mentioned properties are a strong function of pH and therefore it becomes important to fix and control the pH of the metalworking fluid.

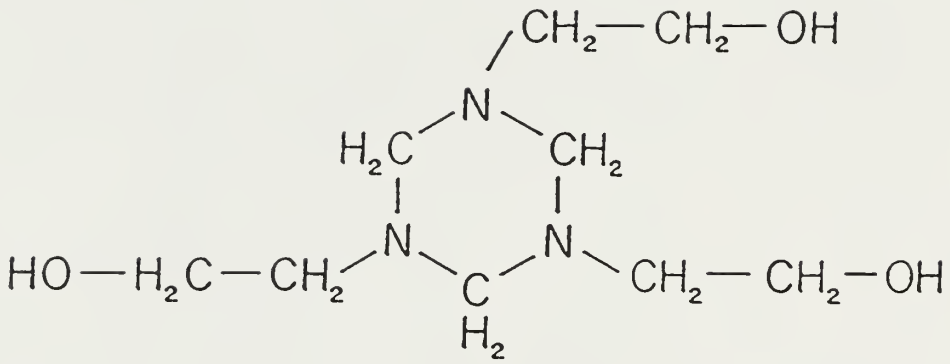

Figure 2.5. Structural formula of hexahydro-1,3,5 tris(2 hydroxy ethyl)-s-triazine.

After a period of use the fluid gets contaminated with microorganisms that can infiltrate in a variety of ways viz. airborne, from the machine parts, from the operator, and by contact with contaminated fluids. Bacteria feed on the cutting fluids and reduce their lubricating value and cause stain. Preservatives are added to the fluids to provide biocidal and biostatic characteristics. The biocidal and biostatic properties of a preservative are a strong function of the operating pH. After the fluid is discarded the pH assumes an even greater role as now the problem is the reverse of providing effective bio-retardation. It is now desired to reduce the biocidal and biostatic effects by manipulating the pH. The preservative should be able to curb both the bacterial and the fungus contamination (Boswell, 1976). Table 2.1 indicates the effectiveness of various preservatives with pH (Diehl, 1984).

Table 2.1. Range of efficiency and pH dependence of the preservatives (Diehl, 1984).

Preservative	Preferred pH range 4 5 6 7 8 9 10	Spectrum of activity Gram+	Gram-	Yeast	Mould
O-Formale	+ + + + +	+	+	-	-
Hexahydro-Triazine	+ + +	+	+	-	-
Bisoxazolidine	+ + + + +	+	+	+	+
N-Methylol-chloroacetamide	+ + + + +	+	+	+	+
N-Methylol-Ureas	+ + + +	+	+	-	-
Phenolateder	+ +	+	+	+	+
Isothiazolinone	+ + + +	+	+	+	+

+ : Excellent effectiveness.
- : Moderate effectiveness.

ii. Effect of initial oil concentration on biodegradation : This is yet another parameter that can significantly alter the rates of biodegradation. High concentrations of biocide can be extremely detrimental to the growth of microbial populations. The waste stream has to be diluted to a level where the effects of the biocide are insignificant. Care should also be taken so as not to over-dilute the waste stream as this would result in the waste of resources.

At low concentrations of waste fluids the organics promote the growth of microorganisms by providing the essential growth factors. Also, at low concentrations all the components are attacked evenly, whereas at high concentrations only the readily biodegradable fractions are degraded (Brown, 1987).

iii. Effect of inorganic nutrient composition : Choice of the supplemental nutrients in a biodegradation process is critical for effective degradation and mineralization of the synthetic chemicals. Nitrate and phosphate are known to be essential to the degradation process. An oil-degrading mixture known as Petrodez containing 20 hydrocarbonoclastic microorganisms and chemical nutrients principally compounds of nitrogen and phosphorous has been developed. Urine is

also known to be very stimulatory to microbes in metalworking fluids and deliberate addition of urine has been considered as a method of accelerating biodegradation of discarded fluids (Hill, 1984). A recently formulated nutrient media contains a solution of urea in brine, encapsulated in oleic acid as external phase, with laurylphosphate as co-surfactant (Brown, 1987).

Nurient media can also be formulated to enhance the process of adaptation. Adaptation of microorganisms to a recalcitrant chemical can be promoted by using chemicals that are structurally similar to the recalcitrant chemical. It can also be enhanced by providing a nurient media devoid of essential nutrients like nitrogen, sulphur and phosphorous that are present in the recalcitrant chemical. In the absence of essential nutrients the microorganisms are forced to attack the recalcitrant molecule for their nutrients.

iv. Effect of adaptation of mixed cultures : The capability of a cell to produce an enzyme that could degrade the components of the waste metalworking fluid depends on the genetic structure or the genome of the microorganism. The genome of the cell is not fixed and evolution or adaptation is possible. The basic mechanisms by which a

microbe can demonstrate some degree of plasticity in terms of its genetic structure is through gene mutation, recombination of genes, and gene amplification. The new chemicals introduced in the habitat of microorganisms may first resist biodegradation but then after a period of exposure may become vulnerable to the new genetic strains of the organisms. However, there can be two factors that can curb the adaptation process that leads to biodegradation. Firstly, the chemical may have characteristics that may place it above the thresholds of adaptive biodegradation. The other factor could be the presence of another readily biodegradable chemical that could retard the adaptive processes.

The likelihood of biological adaptation is a direct function of the increases in the number of genes and in the variety of combinations of the genes. The greater the number of genes the greater the probability of the presence of a gene that could code for enzyme responsible for the degradation of the chemical. Mutation (the appearance of mutant genes) is greatly favored by increased population densities. Multiplying the number of different combinations enhances the chances of bringing together enzyme systems that could complement each other's actions. This may not have been possible with individual enzyme systems.

Recombination is possible by the genetic exchange between individuals of different genotypes. Transfer of plasmid is a well established factor in bacterial adaptation (Lamanna, 1976).

Various kinds of microorganisms mainly aerobes are known to degrade oil. Mixed populations of microrganisms have been found to be better than pure strains of a particular kind of microorganism because the degradation of a compound involves several steps each of which is catalyzed by a different kind of enzyme. Also, the resistance of a mixed community of microorganisms to toxics is better than pure populations because there is a good chance that an organism that can detoxify the hazardous compound would be present. It is therefore better to have a mixed population in order to provide many different kinds of enzyme producing microbes.

Continuous culture techniques could be applied to the selection of organisms capable of degrading synthetic chemicals. Continuous culture can be useful to compare how pure or mixed cultures can affect population densities and the periodic selection of microbial populations in the presence and absence of the synthetic chemical, and under a range of nutrient compositions (Bailey, 1977).

CHAPTER 3

BIODEGRADATION OF METALWORKING FLUIDS

3.1 Introduction

Two synthetic metalworking fluid samples obtained from the International Refining & Manufacturing Company, Evanston, Illinois, were used to evaluate biodegradability under a variety of conditions. These two samples are labelled as IRMCO 141 and IRMCO 156. The composition of these fluid samples is given in Table 3.1 and Table 3.2. The two oil samples were used as the growth-limiting substrate in all the experiments.

The objective of the experiments was to evaluate the biodegradability of the afore mentioned oil samples. An effort was also made to enhance the rates and efficiencies of biodegradation by adjusting parameters like pH, initial oil concentration, inorganic nutrient composition and adaptation of the mixed cultures. The effect of lowering the initial pH of the oil solutions in the shake flasks, as compared to unaltered initial pH, was studied in Experiments 1 and 2. pH was controlled at six different values viz. 4, 5, 6, 7, 8 and 9, to study its effect on the COD removal

efficiencies. This was accomplished by performing Experiment 3. Effects of initial oil concentration and inorganic nutrient composition were evaluated through Experiment 4. The principal objective here was to determine the highest possible initial oil concentration that could be effectively degraded and see if the presence of urea could substantially improve the COD removal efficiencies.

Table 3.1. Composition of IRMCO 141, a synthetic metalworking fluid classified as a chemical solution.

Chemicals	% Wt.
Commercial 85 % Triethanolamine	18-24
C9-C12 Dicarboxylate Inhibitor	12-20
2-Amino- 2-Methyl - 1-Propanol	2- 4
Organic Ester	5- 9
Glycol	5- 9
Hexahydro-1,3,5 Tris (2 Hydroxyethyl)-S-Triazine	2
Poly Oxyethylene (Dimethyliminio) Ethylene Dichloride	0- 5
Defoamer (Non-Silicone Type)	< 1
Water	Balance

Density = 656 g/L

Table 3.2. Composition of IRMCO 156, a synthetic metalworking fluid classified as a chemical solution.

Chemicals	% Wt.
Commercial 85 % Triethanolamine	4-10
C9-C12 Dicarboxylate Inhibitor	2
Sodium Tetraborate	2
Polyglycol	10-20
Phosphate Ester	4- 8
Hexahydro-1,3,5 Tris (2 Hydroxyethyl)-S-Triazine	0.5
Nonionic Detergent	1
Modified Polyglycol	5
Water	Balance

Density = 624 g/L

3.2 Materials and Methods

3.2.1 Materials

Erlenmeyer flasks each with a volume of 500 ml., were used throughout the experimental work. These flasks were filled with 300 ml. of the fermentation solution at the start of the experiments. Aeration and agitation of the Erlenmeyer flasks were accomplished by shaking the flasks at the rate of 175 rpm in either a New Brunswick Scientific G24- Environmental Incubator Shaker or a Lab-line Instruments Orbit Environ-Shaker. The mouths of the shake flasks were left open to facilitate oxygen transfer. The temperature was maintained at 30°C in the shakers with the help of an in-built thermostat in the shaker system.

The scheme for conducting the experiments is described below.

Experiment 1. Biodegradation at the natural pH of the solution

Oil samples, IRMCO 141 and IRMCO 156, were diluted to 1% by taking 3 ml. of the samples in 297 ml. of solution. BOD dilution water was used as the inorganic nutrient source. This nutrient medium is labelled as C and its composition is listed in Table 3.5. The dilution water was prepared by dissolving the contents of the BOD nutrient buffer pillows manufactured by Hach Company, Loveland, Colorado, in tap water. The mineral composition of the tap water is listed in Table 3.6. The inoculum was obtained from the activated recycle sludge of the Wastewater Treatment Plant, Manhattan, Kansas. This inoculum was mixed with the biomass obtained from the previous preliminary experiments and stored at 20°C for 24 hrs. before being used in the shake flasks. The biomass from the previous experiments was collected by filtering the solution contained in the shake flasks. The filter was then washed to obtain the biomass that was mixed with a part of the filtrate and used as the adapted culture.

The solution in the shake flasks contained 3 ml. of the oil sample, 30 ml. of the inoculum, 100 ml. of nutrient solution and 167 ml. of the deionised water to make up 300 ml. at the start of the experiments. No initial pH adjustments were made in the shake flasks and the experiment was carried out for 17 days. Biomass, pH, COD, and temperature measurements were made each sampling day at regular intervals of time.

Experiment 2. Effect of lowering the initial pH

The objective of this experiment was to enhance the biodegradation by starting with a near neutral pH in the shake flasks in order to decrease the effect of the preservative, hexahydro-S-triazine, and to thereby promote bacterial growth. The pH in the two shake flasks was lowered to 7.3 for IRMCO 141 and 7.7 for IRMCO 156, by the addition of 0.1M sulphuric acid. The composition of inorganic nutrient media C is given in Table 3.5. The inoculum was a 30 ml. mixture of fresh seed microorganisms obtained from the waste water treatment plant and the adapted microorganisms obtained from experiment 1 in a 1:1 ratio. The composition of the solution in the shake flasks was the same as that used for experiment 1. Sampling

procedures similar to the ones employed in experiment 1 were repeated for this experiment.

Experiment 3. Effect of controlled pH

In order to get a much better understanding of the role of pH in biodegradation and its effect on the preservative action, another experiment was performed at six different pH values. Two sets of six shake flasks were taken for the two oil samples. The pH was controlled at 4, 5, 6, 7, 8, and 9 in the shake flasks. The nutrient composition in the shake flasks was the same as that used in experiments 1 and 2. The inoculum was a 50 ml. mixture of fresh inoculum obtained from the Wastewater Treatment Plant, Manhattan, Kansas, and the biomass from the shake flasks used in experiment 2 in a 1:1 ratio (employing the same procedure as used in Experiment 1). A higher concentration of biomass was taken at the start of the experiment so as to enhance the biodegradation rate. The solution in the shake flasks contained 3 ml. of the oil sample, 50 ml. of inoculum, 100 ml. of nutrient solution and 147 ml. of deionised water to make up 300 ml. at the start of the experiments. pH was controlled at each sampling day by the addition of 0.1 N sulphuric acid and/or 0.1 N sodium hydroxide.

Experiment 4. Effect of initial oil concentration and the inorganic nutrient media composition

This experiment was designed to study the effect of dilution and inorganic nutrient composition on the rate of biodegradation. Two sets of six shake flasks were taken respectively for the two oil samples. In shake flasks 1 through 4 the the concentration of the oil was taken as 0.5%, 1%, 2% and 5%. Nutrient B, of composition shown in Table 3.4 was used for these four shake flasks. Thirty ml. of inoculum obtained by mixing 15 ml. of the biomass from experiment 3 (pH 6.0 for IRMCO 141 and pH 7.7 for IRMCO 156) and 15 ml. of fresh inoculum obtained from the wastewater plant was used in this set of experiments. The solution in the four shake flasks contained 1.5, 3.0, 6.0, and 15 ml. of the oil sample, 30 ml. of inoculum, 100 ml. of nutrient solution and 168.5, 167, 164 and 155 ml. of deionised water in the four shake flasks respectively, to make up 300 ml. at the start of the experiments.

In shake flasks 5 and 6 the concentration of the oil was 0.5% but nutrient A and C of compositions shown in Tables 3.3 and 3.5 respectively were employed in preparing the two flasks. The inoculum was the same as that used in shake flasks 1 through 4. The solution in the two shake flasks

contained 1.5 ml. of the oil sample, 30 ml. of inoculum, 100 ml. of nutrient solution and 168.5 ml. of deionised water in the two shake flasks to make up 300 ml. at the start of the experiments.

Results obtained from shake flasks 1 through 4 were used to evaluate the effect of initial oil concentration on the rate of biodegradation, the nutrient composition being the same in all these flasks. Shake flasks results from flasks numbered 1, 5, 6 were used to determine the effect of nutrient composition as the oil concentration was kept constant at 0.5% in these experiments. The pH was maintained at 6.0 for IRMCO 141 and 7.0 for IRMCO 156.

Similar conditions were used for the other set of six shake flasks with the samples of IRMCO 156.

Table 3.3. Nutrient Composition A.

Component	Concentration (per L of tap water*)
$(NH_4)_2SO_4$	7.5 gm
Na_2HPO_4	1.5 gm
KH_2PO_4	3.5 gm
$MgSO_4.7H_2O$	0.5 gm
Nutrient Broth	2.0 gm
1. $FeSO_4.7H_2O$	$30 * 10^{-3}$ gm
2. $CaCl_2.2H_2O$	$60 * 10^{-3}$ gm
3. $CuSO_4.5H_2O$	$15 * 10^{-6}$ gm
4. $Na_2B_2O_7.10H_2O$	$30 * 10^{-6}$ gm
5. $MnCl_2.4H_2O$	$60 * 10^{-6}$ gm
6. $ZnSO_4.7H_2O$	$150 * 10^{-6}$ gm
7. $Na_2MoO_4.7H_2O$	$30 * 10^{-3}$ gm

* The composition of the tap water is listed in Table 3.6.

Table 3.4. Nutrient Composition B.

Component	Concentration (per L of tap water*)
$(NH_4)_2SO_4$	1.00 gm
$CO(NH_2)_2$	1.50 gm
KH_2PO_4	0.80 gm
$MgSO_4.7H_2O$	0.07 gm
$CaCl_2.2H_2O$	0.05 gm
$ZnSO_4.7H_2O$	0.02 gm
$MnSO_4.4H_2O$	0.02 gm
$CuSO_4.5H_2O$	0.005 gm
$FeSO_4.7H_2O$	0.10 gm
Na_2SO_4	1.00 gm

* The composition of the tap water is listed in Table 3.6.

Table 3.5. Nutrient Composition C.

Component	Concentration (per L of tap water*)
$MgSO_4.7H_2O$	22.50 mg
$CaCl_2$	27.50 mg
$FeCl_3.6H_2O$	0.25 mg
Na_2SO_3	1.57 mg
KH_2PO_4	8.50 mg
K_2HPO_4	21.75 mg
$Na_2HPO_4.7H_2O$	33.40 mg
NH_4Cl	1.70 mg

* The composition of the tap water is listed in Table 3.6.

Table 3.6. Composition of the Tap water.

Constituent	Concentration (per L)
Ca	28.00 mg
Mg	10.00 mg
Fe	0.23 mg
Mn	0.01 mg
Na	40.00 mg
Cl	47.00 mg
SO_4	79.00 mg
N	0.15 mg
F	1.10 mg
Total Hardness	111
Total Alkalinity	63
Total Phosphate	0.19 mg

The source of the data is Wastewater Treatment Plant, Manhattan, Kansas.

3.2.2 Methods

Analytical Procedures

Two basic problems were encountered while conducting trial runs before the start of actual experiments. The first one involved water evaporation from the shake flasks. The amount of evaporation per day was not constant and strongly depended upon the conditions prevailing in the surroundings. This caused the chemical oxygen demand (COD) concentration to fluctuate and resulted in erroneous measurements of the data. As much as 15 ml. of water was found to evaporate per day. This problem was overcome by weighing the shake flasks every sampling day. The loss in weight was compensated by the addition of distilled water to the shake flasks.

The second problem encountered was in the biomass measurement. Optical measurements could not be made because the samples were not homogeneous owing to the presence of flocculates of cells and other particulates. Also, it is known that optical density provides a reliable estimate of biological solids only for cases where the biomass concentration is no greater than 300 mg/lit. in dry weight terms. In the experiments conducted biomass concentrations

greater than 300 mg/lit were encountered. Dry weight analysis also presented some problems. The filtration equipment, Millipore 1225 Sampling Manifold, manufactured by Millipore Corporation, Bedford, MA, was used to filter the samples. This equipment necessitated the use of 0.45 μm pore size Millipore HA membrane filters to collect the biomass on filtration. The filter paper was dried in the oven for 24 hrs. at 105°C and later weighed to estimate the biomass concentration. The filter papers were however coated with a detergent that caused a loss in weight upon drying. This resulted in negative biomass measurements being made at various times. Pre-rinsing the filter papers to get rid of the detergent eliminated this problem.

The growth of biomass along the walls of the shake flasks also caused some difficulty in its measurement. The walls had to be scraped before withdrawing the samples from the shake flasks to rectify this error.

The filtrate collected from the biomass measurements was taken to determine the COD. EPA approved Hach system using pre-fabricated screw capped COD vials along with the reactor heater were used for COD measurements. Two ml. of the sample was added to the vial and was heated at 160°C for two hours in the reactor heater. On cooling the percent

transmittance was read on the Bausch & Lomb Spectronic 20 spectrophotometer, using 620 nm wavelength. The COD values were computed using Tables that correlated percent transmittance with the COD. pH measurements were made with a model 7 Corning pH meter.

The filtrate from the biomass measurement was also used in the HPLC analysis of the samples. The experimental set-up for HPLC analysis includes two columns, TSK 1000 PW and TSK 2000 PW, connected in series, in a Varian Model 5000 liquid chromatograph equipped with a Varian VARI-Chrm UV-Vis detector set at 283 nm, a Varian Model 9176 recorder, and an ISIS Auto sampler manufactured by ISCO. The results of the HPLC analysis could not be used in evaluation of biodegradability. For the oil sample IRMCO 141, there was no significant change in the peaks before and after biodegradation. For oil sample IRMCO 156, no peak was observed in the HPLC analysis. The Results of HPLC analysis are shown in Figure II.1 through Figure II.4 in Appendix II. Based on these observations HPLC analysis was not used in further experimentation.

Data Analysis Methods

The COD and biomass variation with time data, collected on various shake flask experiments, were plotted using SAS Graph techniques. Subsequent calculations were based on the first exponential growth phase that was observed in the growth curves of the biomass.

The biomass yield and specific growth rate can be assumed to remain constant during the exponential growth phase. Thus the specific growth rate can be determined using the expression ;

$$\mu m = \frac{1}{X}\frac{dX}{dt} \qquad (3\text{-}1)$$

The specific growth rate determined during the exponential growth phase by this expression is the maximum specific growth rate(μm). Substrate data along with the biomass data can also be used to estimate the biomass concentration based on the substrate form;

$$Z = X_o + Y_s (S_o - S) \qquad (3\text{-}2)$$

where Y_s is the biomass yield constant estimated by;

$$Y_s = \frac{(X - X_o)}{(S_o - S)} \qquad (3\text{-}3)$$

Thus, another estimate of maximum specific growth rate can be obtained by using the expression;

$$\mu m = \frac{1}{Z}\frac{dZ}{dt} \qquad (3\text{-}4)$$

Integration of equations (3-1) and (3-4) gives the following expressions, respectively;

$$\ln(X) = \mu m\ (t-t_o) + \ln(X_o) \qquad (3\text{-}5)$$

$$\ln(Z) = \mu m\ (t-t_o) + \ln(Z_o) \qquad (3\text{-}6)$$

Regression can be performed on the data to give two estimates of the maximum specific growth rate constant. In addition, all the data can be used simultaneously to give one combined estimate of the maximum specific growth rate constant.

3.3 Results and Discussion

Experiment 1. Biodegradation at the natural pH of the solution

Very little biodegradation was observed by the end of the 17 th day for the oil sample IRMCO 141. The high pH of 8.5 and 8.3 in the two samples permitted little bacterial growth. It is indicated in Table 2.1 that at pH between 8 and 10, hexahydro-s-triazine (preservative present in the oil samples) has excellent effectiveness.

The results obtained from this experiment are tabulated in Appendix A.

Experiment 2. Effect of lowering the initial pH

The reduction in the COD values for this experiment was significantly higher for IRMCO 141 than that for Experiment 1. For IRMCO 156, the increase in COD removal percentage was not very pronounced. The COD and biomass concentrations as functions of time are tabulated in Appendix A.

Table 3.7 illustrates how the two experiments compare in terms of the percentage COD removal for the two oil samples. Table 3.8 indicates the yield coefficients and the specific growth rates obtained by performing regression analysis on the data collected for the two experiments.

Table 3.7. Comparison of COD removal percentages for Experiment 1 and Experiment 2.

IRMCO 141

Expt. No.	pH	COD* Initial	COD* Final	% Removal
1	8.5	6798	6193	8.90
2	7.3	8294	4587	44.70

IRMCO 156

Expt. No.	pH	COD* Initial	COD* Final	% Removal
1	8.3	4587	2486	45.80
2	7.7	7623	3949	48.20

* COD concentration is in mg/L.

Table 3.8. Specific growth rate constant and the yield coefficient for Experiments 1 and 2 for biodegradation of IRMCO 141 and IRMCO 156.

IRMCO 141

	Specific growth rate(1/hr.)	
Expt. No.	1	2
Biomass Data	0.0061	0.0096
Substrate Data	0.0044	0.0092
Combined data	0.0052	0.0094
Yield	0.4029	0.1475

IRMCO 156

	Specific growth rate(1/hr.)	
Expt. No.	1	2
Biomass data	0.0084	0.0072
Substrate Data	0.0097	0.0059
Combined data	0.0090	0.0066
Yield	0.2373	0.6068

Experiment 3. Effect of controlled pH at six different values

The results obtained are shown in Figures 3.1 through 3.6 and the data for all the experiments are included in tables given in Appendix A. Maximum degradation was obtained at a pH of 6.0 for IRMCO 141. For IRMCO 156 at pH range between 5 and 8 uniform biodegradation was observed as evident from Figure 3.7. It is also observed that the COD removal percentage tends to go up at a pH beyond 7.0. This can probably be explained by the fact that the inoculum may have been acclimatized to an alkaline pH causing the percentages to go up at pH values higher than 7.0. Table 3.9 compares the COD removal percentages for the two oil samples at each pH. The specific growth rates and the yield coefficients calculated by performing regression analysis have been included in Table 3.10.

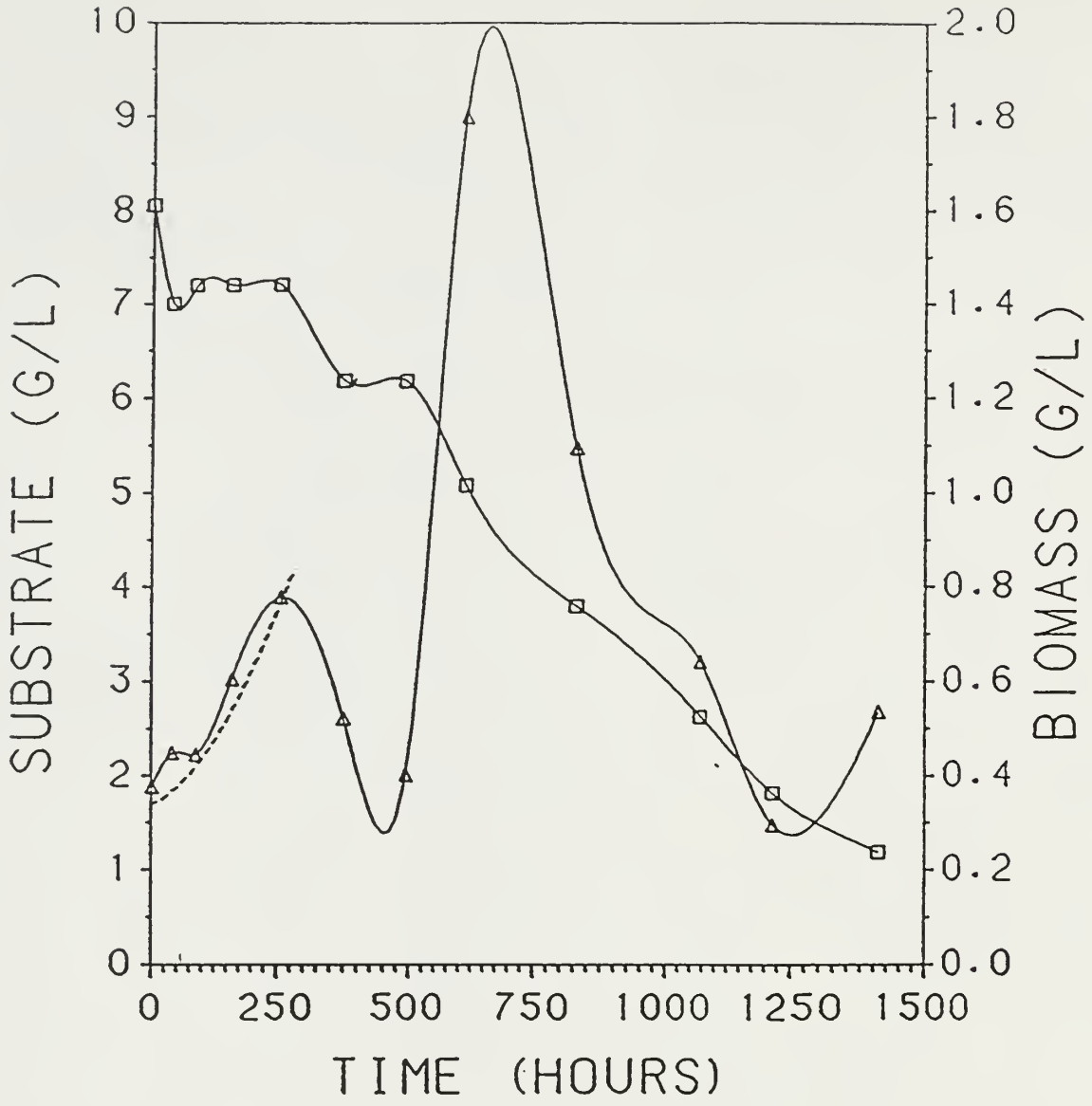

Figure 3.1 Biomass(Δ) and COD(□) variations with time for the mixed population of microorganisms growing on oil sample IRMCO 141 at a controlled pH of 5.0: dashed line represents the exponential growth model.

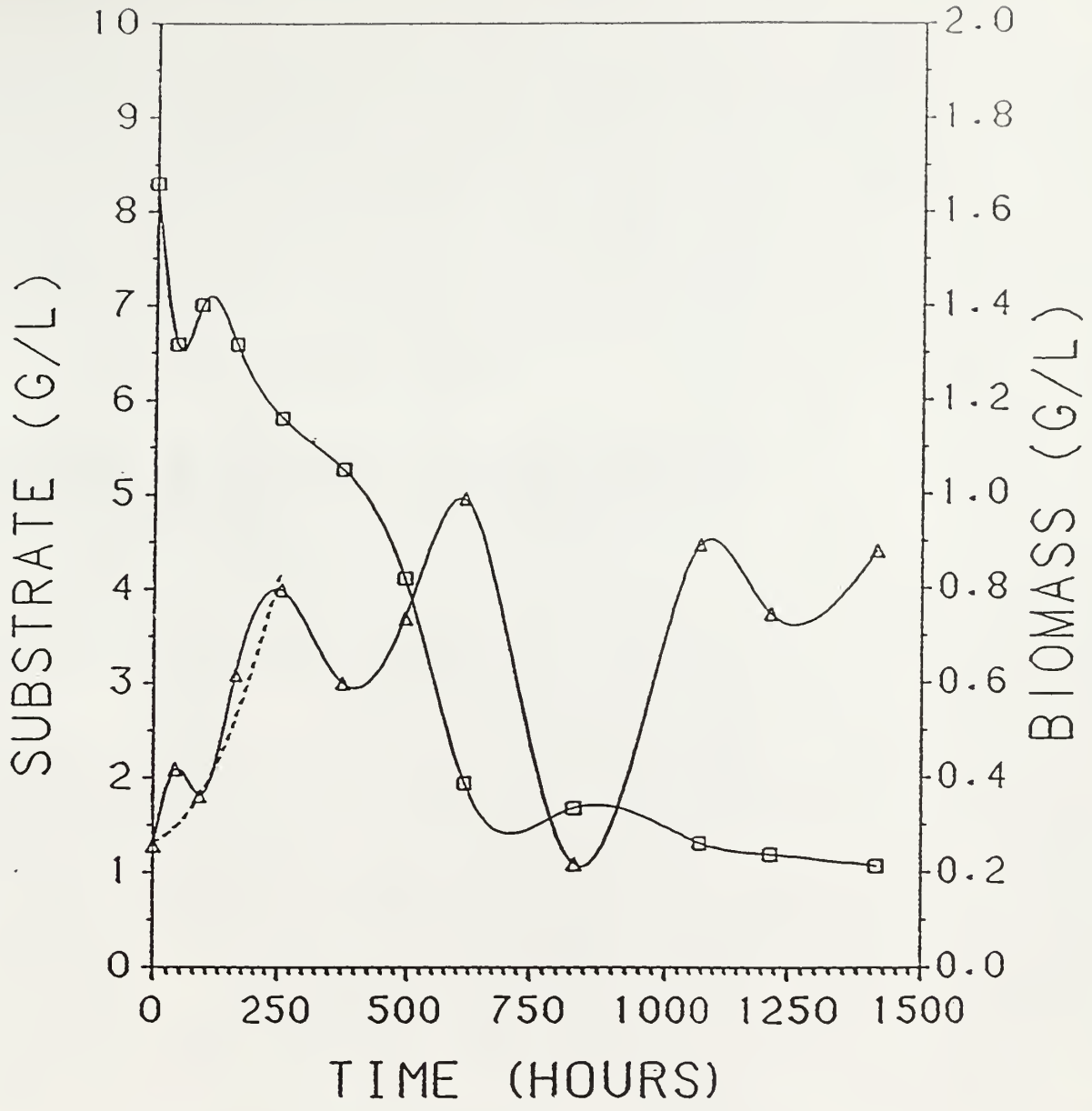

Figure 3.2 Biomass(Δ) and COD(□) variations with time for the mixed population of microorganisms growing on oil sample IRMCO 141 at a controlled pH of 6.0: dashed line represents the exponential growth model.

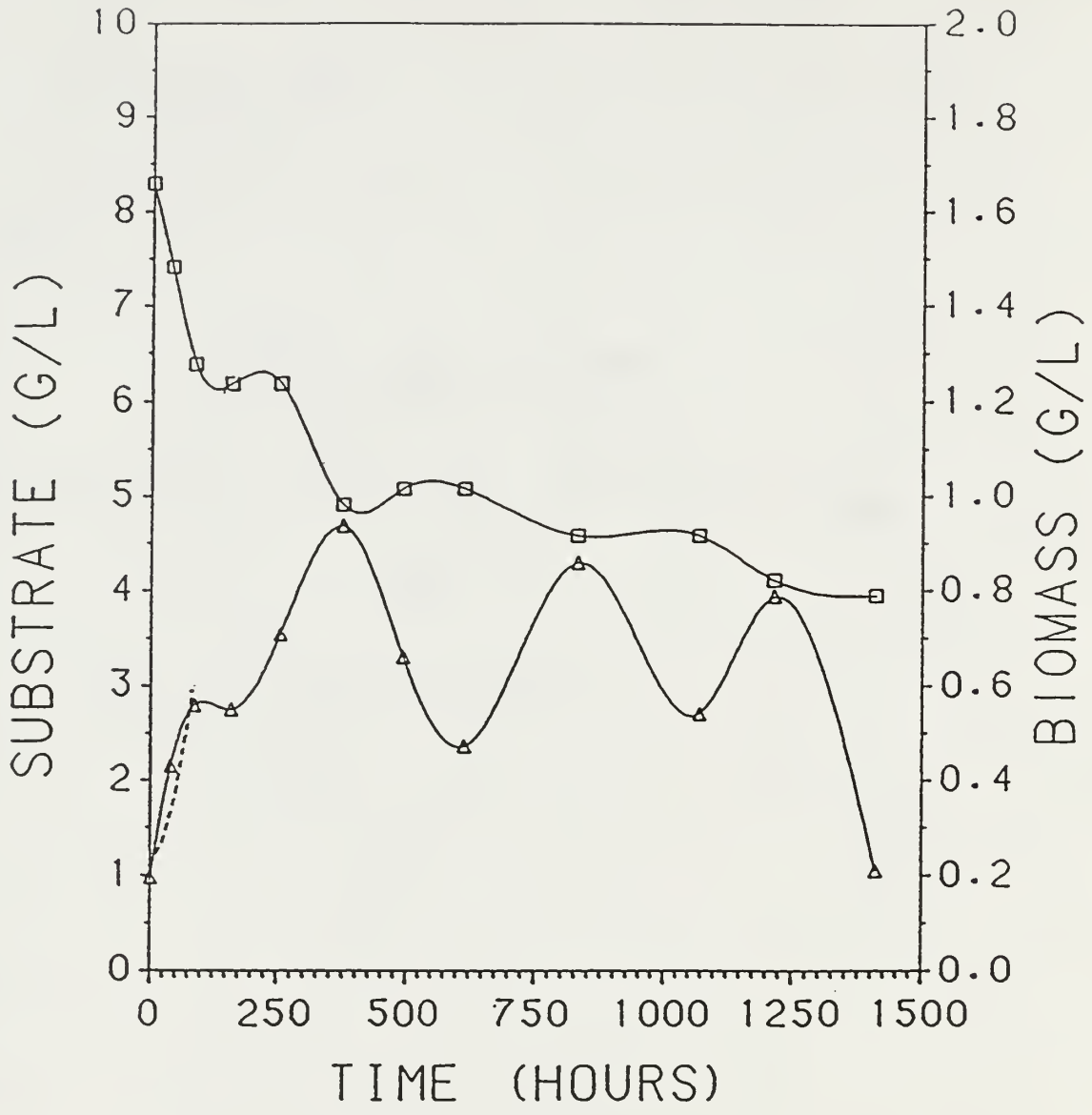

Figure 3.3 Biomass(Δ) and COD(□) variations with time for the mixed population of microorganisms growing on oil sample IRMCO 141 at a controlled pH of 8.0: dashed line represents the exponential growth model.

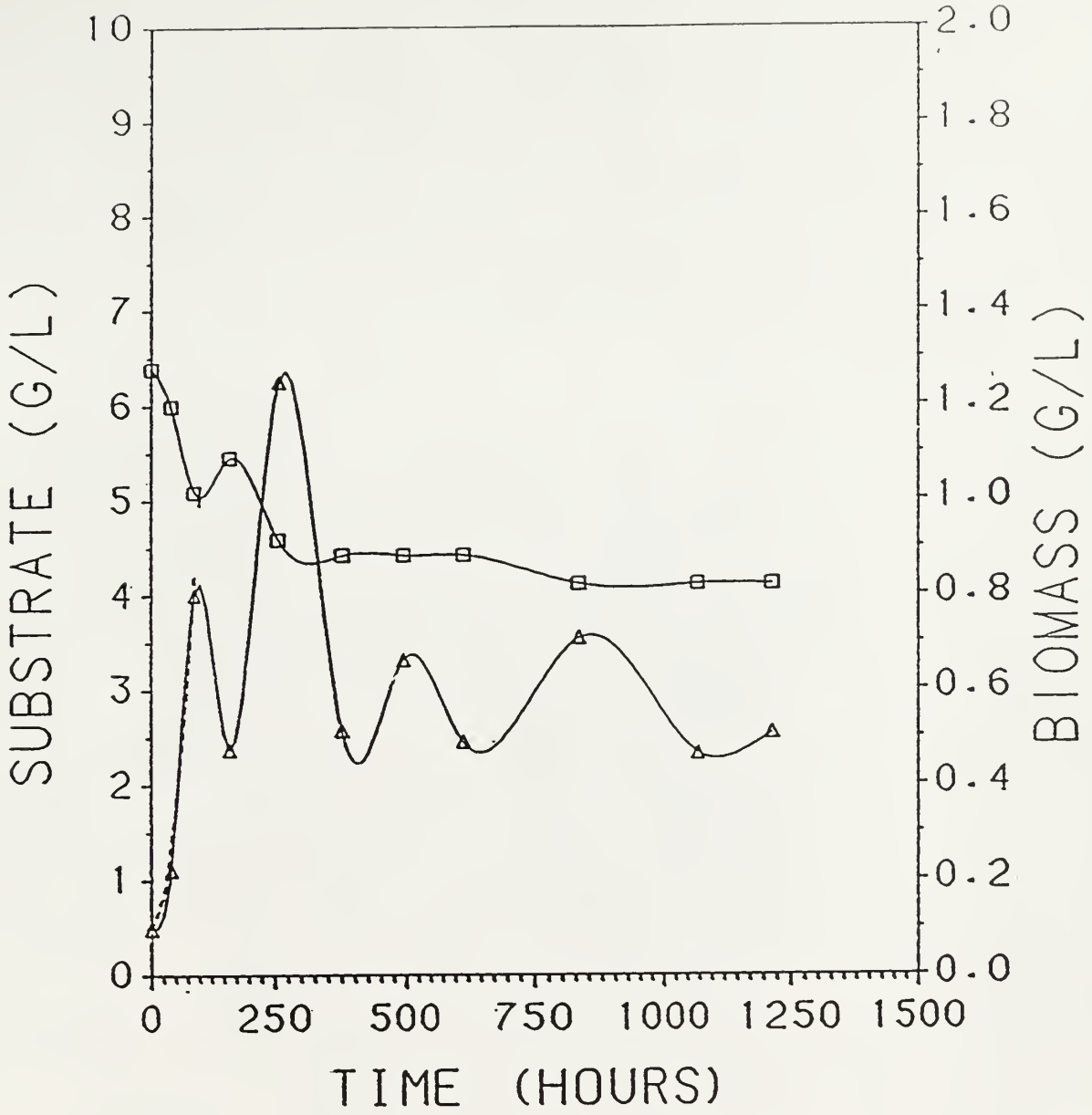

Figure 3.4 Biomass(Δ) and COD(□) variations with time for the mixed population of microorganisms growing on oil sample IRMCO 156 at a controlled pH of 7.0: dashed line represents the exponential growth model.

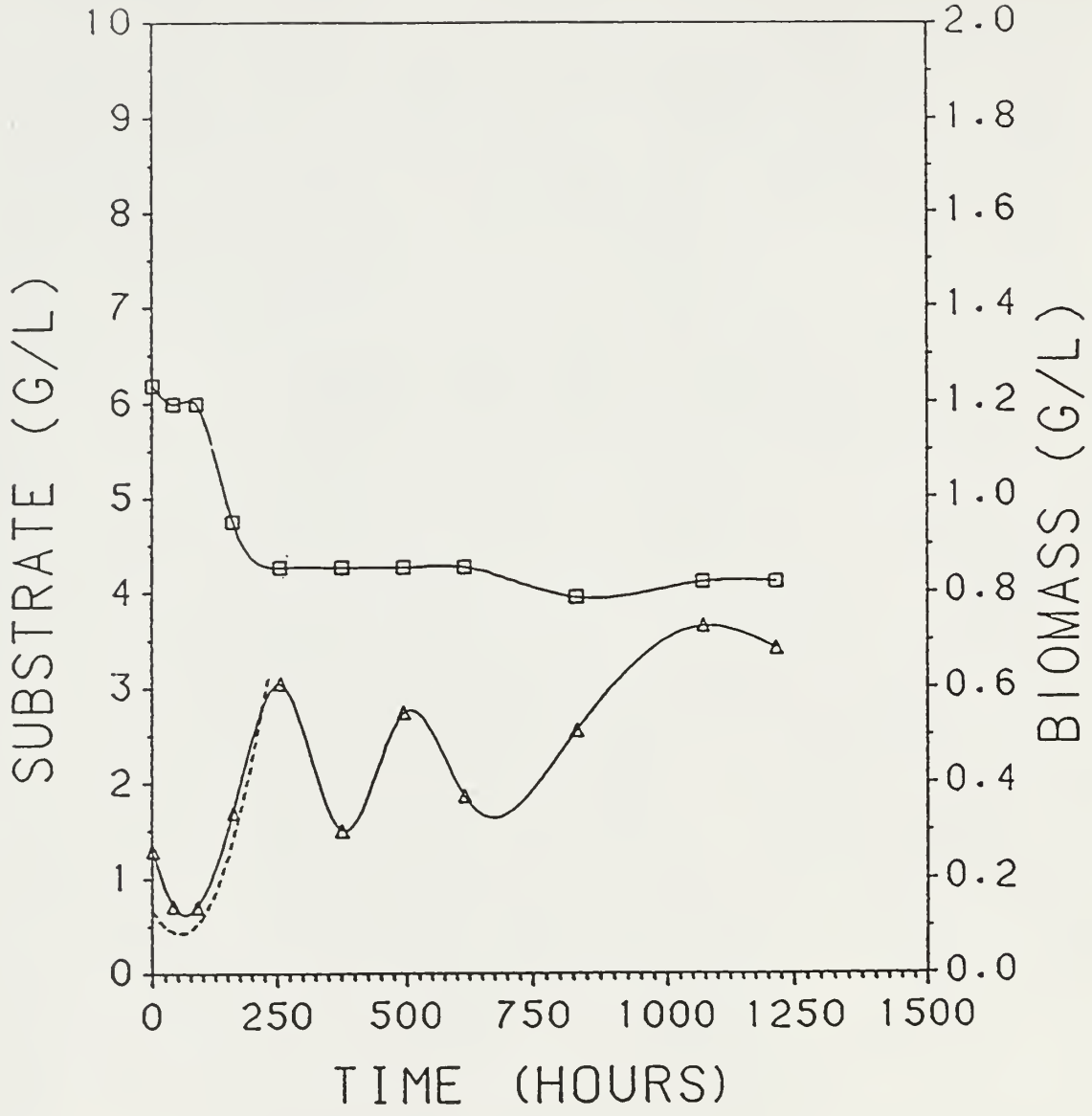

Figure 3.5 Biomass(Δ) and COD(□) variations with time for the mixed population of microorganisms growing on oil sample IRMCO 156 at a controlled pH of 8.0: dashed line represents the exponential growth model.

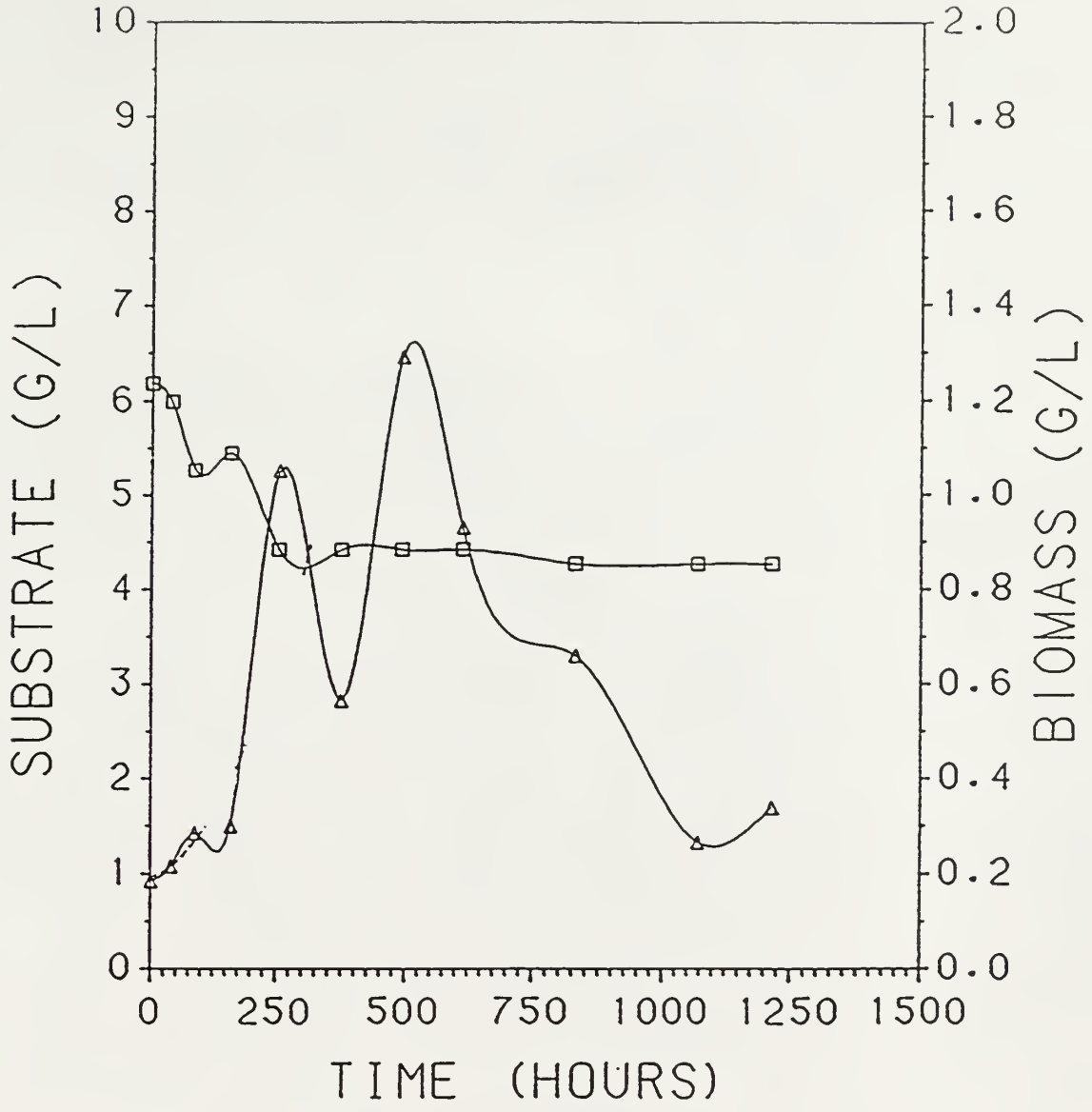

Figure 3.6 Biomass(Δ) and COD(□) variations with time for the mixed population of microorganisms growing on oil sample IRMCO 156 at a controlled pH of 9.0; dashed line represents the exponential growth model.

Table 3.9. Effect of pH on per cent COD reduction for IRMCO 141 and IRMCO 156.

IRMCO 141

pH	COD* Initial	COD* Final	% Removal
4	8294	5093	38.59
5	8063	1188	85.27
6	8294	1067	87.14
7	8294	4587	44.69
8	8294	3949	52.39
9	8294	1957	76.40

IRMCO 156

pH	COD* Initial	COD* Final	% Removal
4	6798	4587	32.52
5	6391	4114	35.63
6	6391	4114	35.63
7	6391	4114	35.63
8	6193	4114	33.57
9	6193	4268	31.08

* COD concentration is in mg/L.

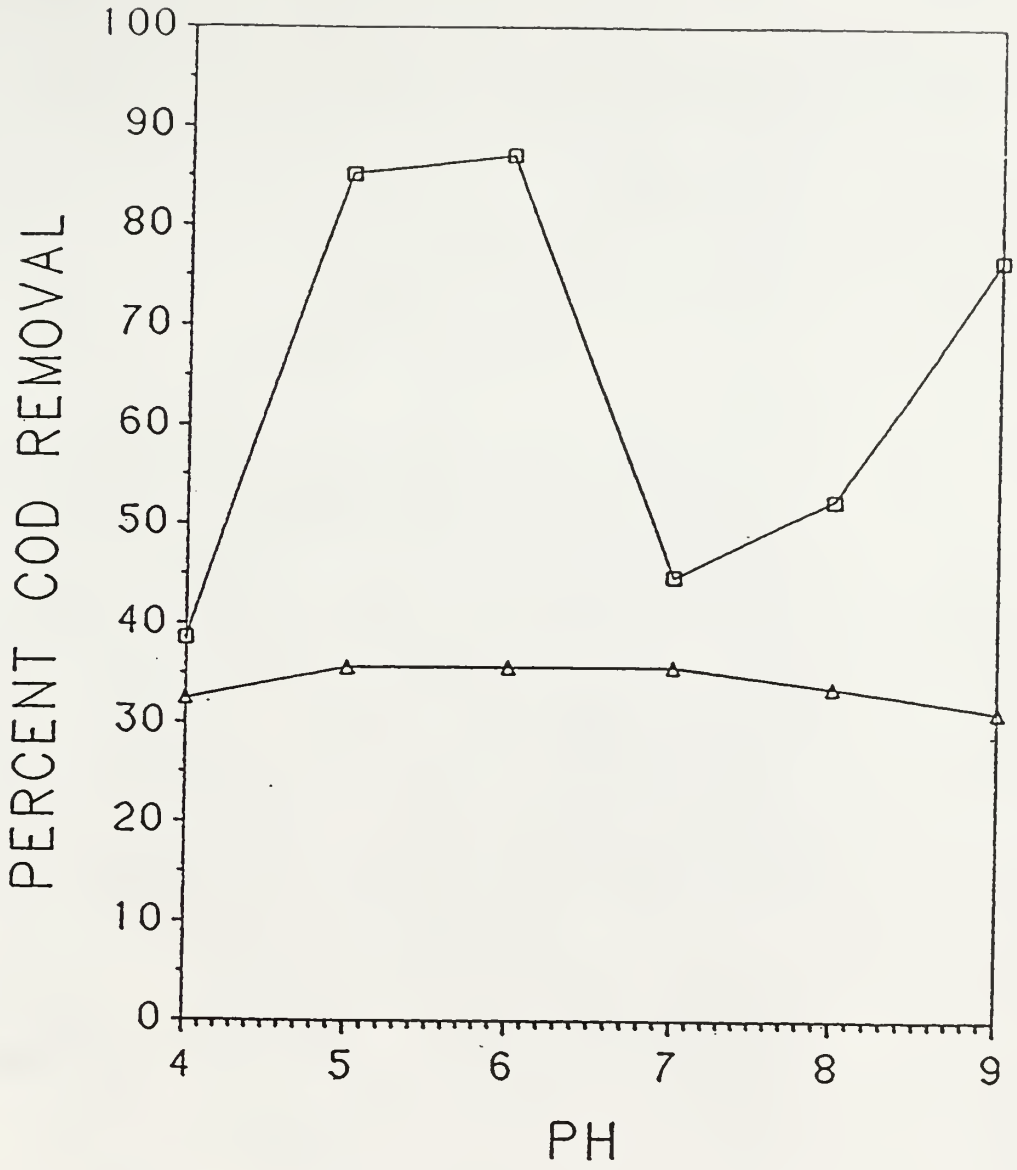

Figure 3.7. Percent COD removal at various pH values for mixed population growing on oil samples IRMCO 141(□) and IRMCO 156(Δ).

Table 3.10. Specific growth rates and yield coefficients for the two oil samples at each of the six values of pH.

IRMCO 141

pH	Specific growth rate (1/hr.)		
	Combined	Biomass	Substrate
4	0.0056	0.0056	-
5	0.0034	0.0033	0.0034
6	0.0047	0.0046	0.0047
7	0.0104	0.0105	0.0103
8	0.0118	0.0117	0.0118
9	0.0027	0.0026	0.0029

IRMCO 156

pH	Specific growth rate (1/hr.)		
	Combined	Biomass	Substrate
4	0.0016	0.0016	-
5	0.0027	0.0027	0.0027
6	0.0044	0.0043	0.0045
7	0.0239	0.0240	0.0237
8	0.0085	0.0086	0.0084
9	0.0049	0.0049	0.0049

Experiment 4. Effect of initial oil concentration and the inorganic nutrient media composition

The results obtained from this set of experiments are shown in Figures 3.8 through 3.11 and in Figure 4.1 and Figure 4.2. They are also been tabulated in Appendix A. Tables 3.11 and 3.13 list the percent COD removal for different dilutions and different inorganic nutrient compositions for the oil samples. Tables 3.12 and 3.14 list the yield coefficient and the specific growth rate estimates for the two oil samples for different dilutions and nutrient compositions.

From Figure 3.12 it is clear that the optimum initial concentration for maximizing the percent COD removal is 2% IRMCO 141 and 1% IRMCO 156, respectively. The probable cause of the sharp drop in the percent COD removal at higher dilutions may be due to the presence of the preservative hexahydro-s-triazine. At higher concentrations the biocidal effect of the preservative may be strong enough to disrupt the microbial populations and curb their growth.

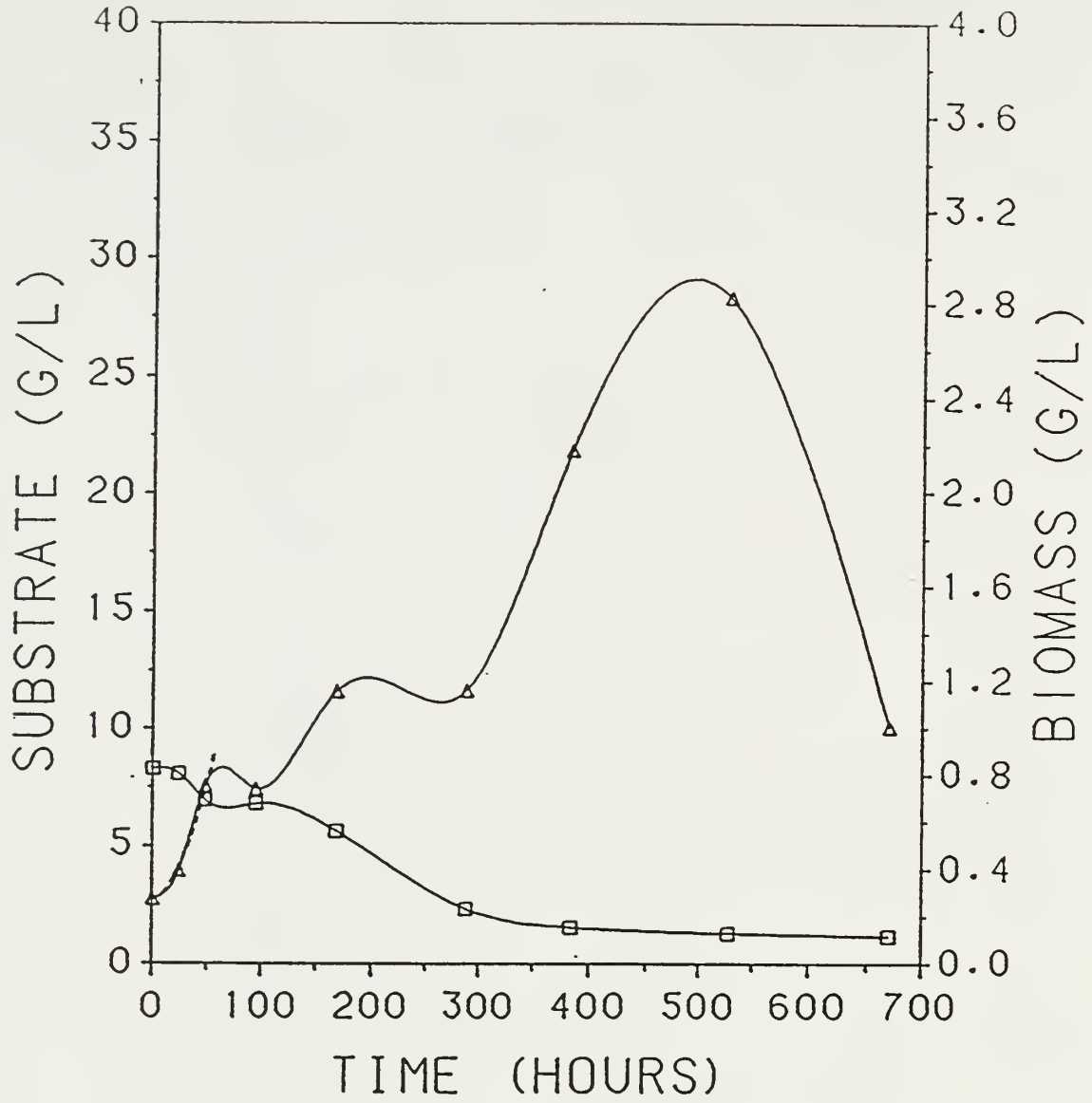

Figure 3.8 Biomass(Δ) and COD(□) variations with time for the mixed population of microorganisms growing on oil sample IRMCO 141 at an initial concentration of 1.0% and with inorganic nutrient media composition B: dashed line represents the exponential growth model.

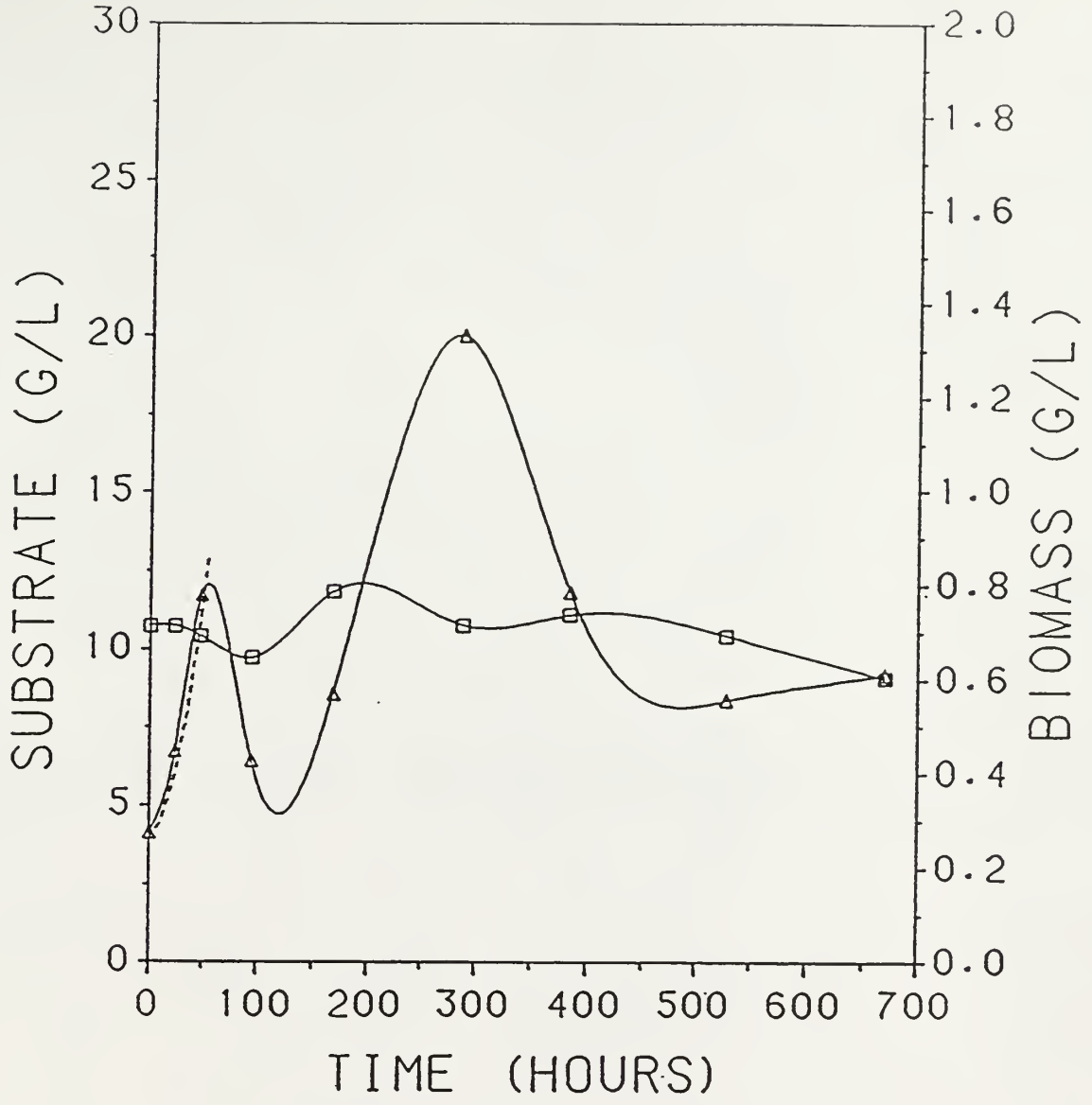

Figure 3.9 Biomass(Δ) and COD(□) variations with time for the mixed population of microorganisms growing on oil sample IRMCO 141 at an initial concentration of 2.0% and with inorganic nutrient media composition B: dashed line represents the exponential growth model.

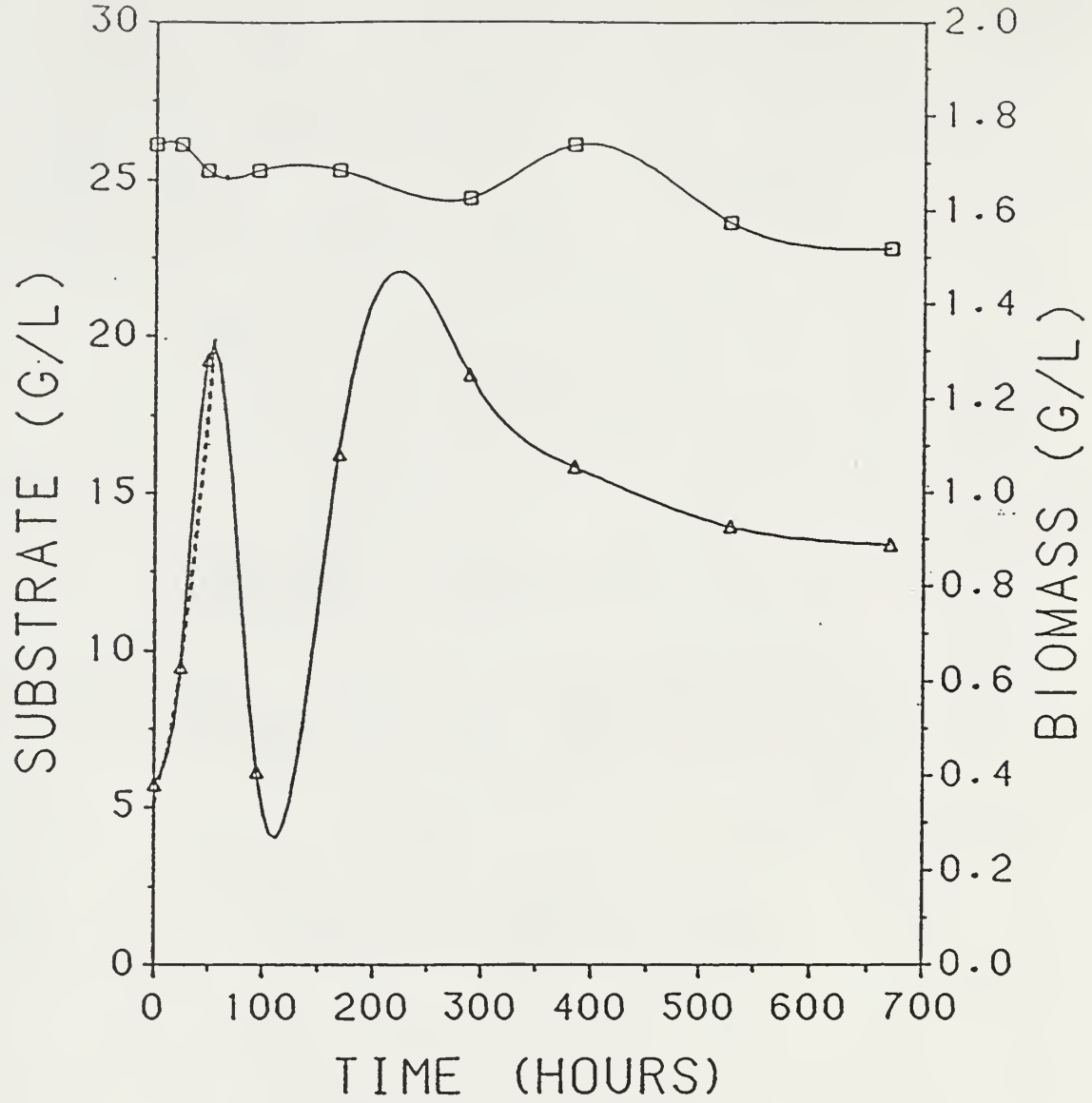

Figure 3.10 Biomass(Δ) and COD(□) variations with time for the mixed population of microorganisms growing on oil sample IRMCO 141 at an initial concentration of 5.0% and with inorganic nutrient media composition B: dashed line represents the exponential growth model.

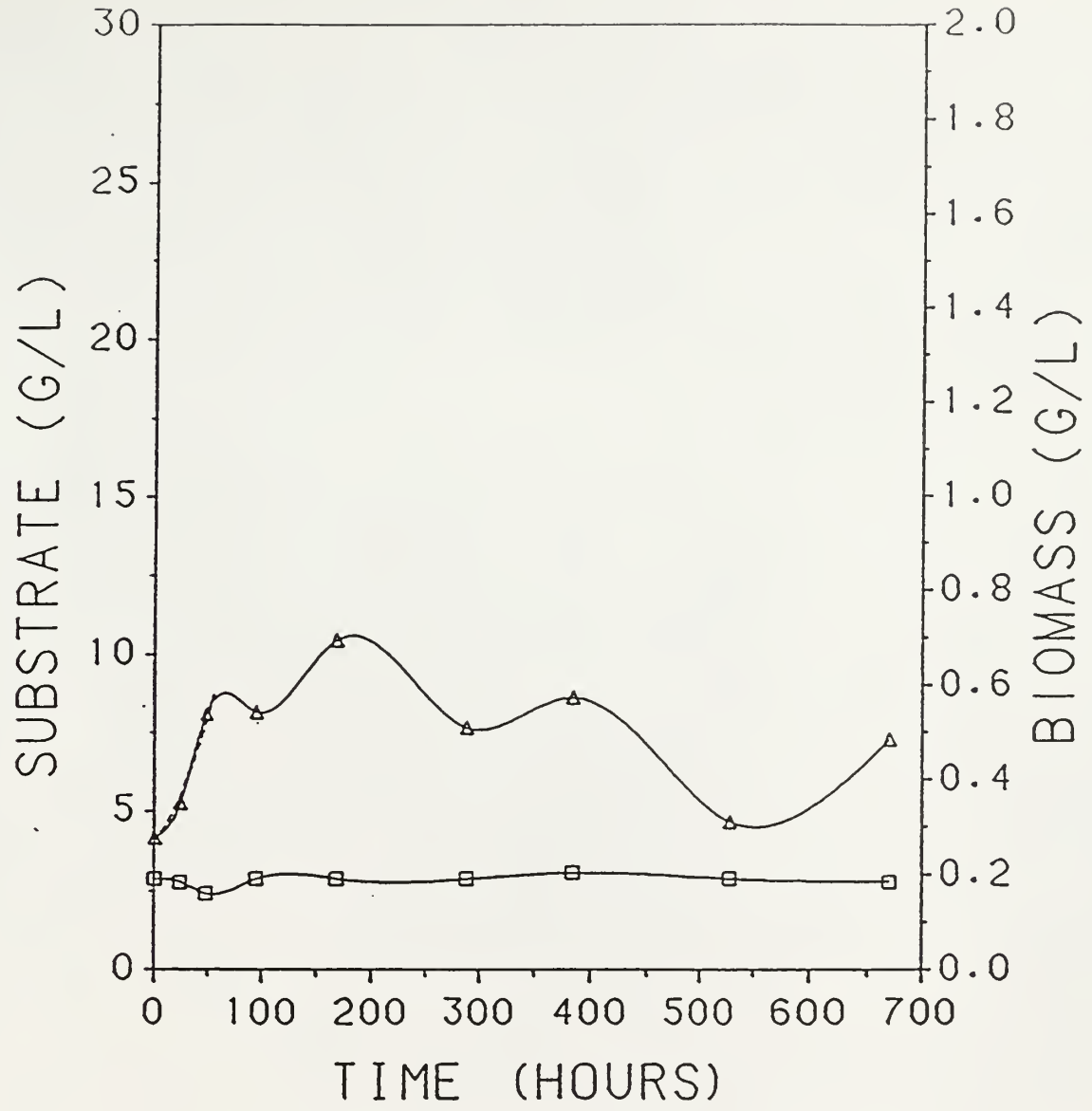

Figure 3.11 Biomass(Δ) and COD(□) variations with time for the mixed population of microorganisms growing on oil sample IRMCO 156 at an initial concentration of 2.0% and with inorganic nutrient media composition B: dashed line represents the exponential growth model.

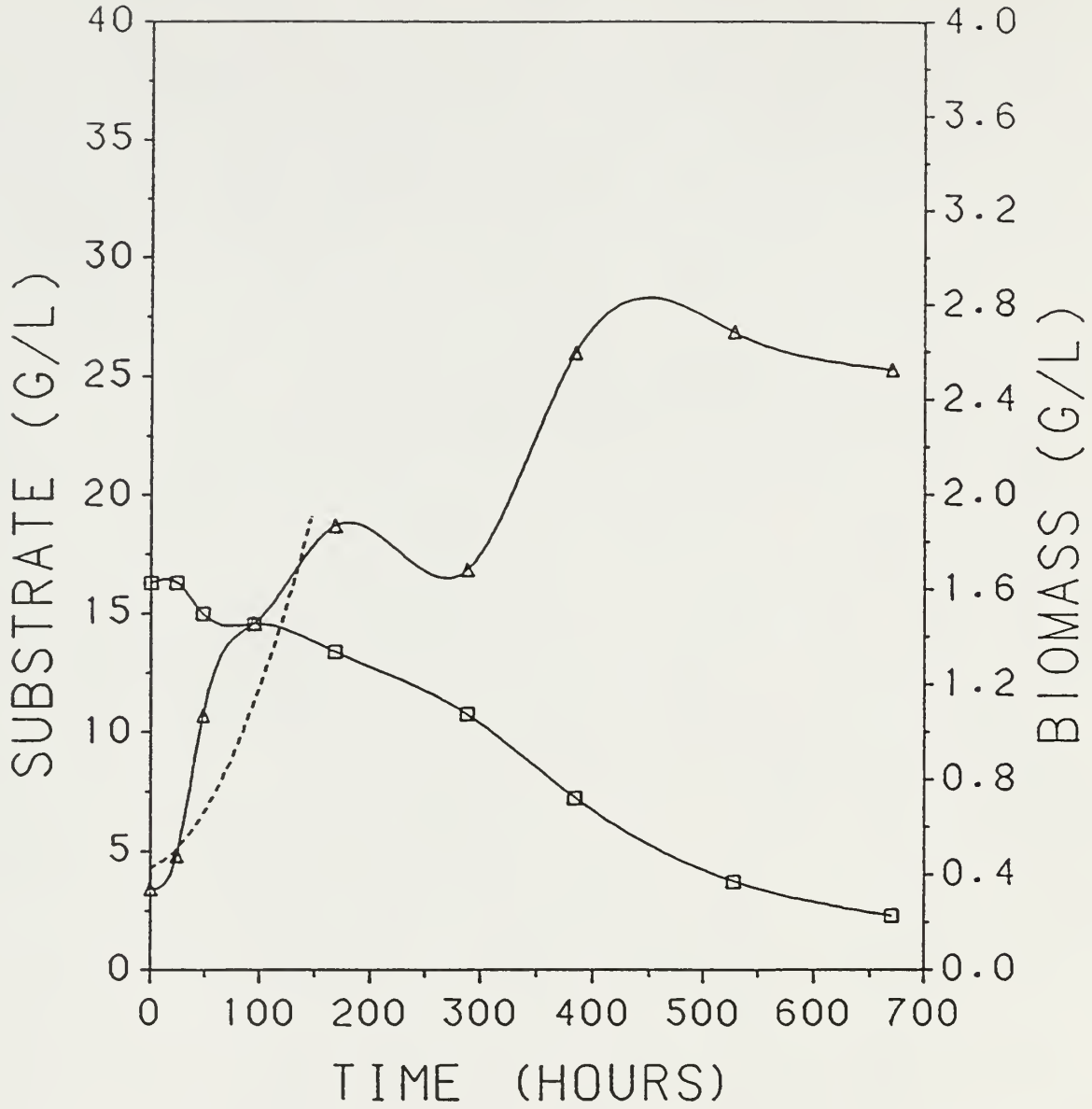

Figure 3.12 Biomass(Δ) and COD(□) variations with time for the mixed population of microorganisms growing on oil sample IRMCO 156 at an initial concentration of 5.0% and with inorganic nutrient media composition B: dashed line represents the exponential growth model.

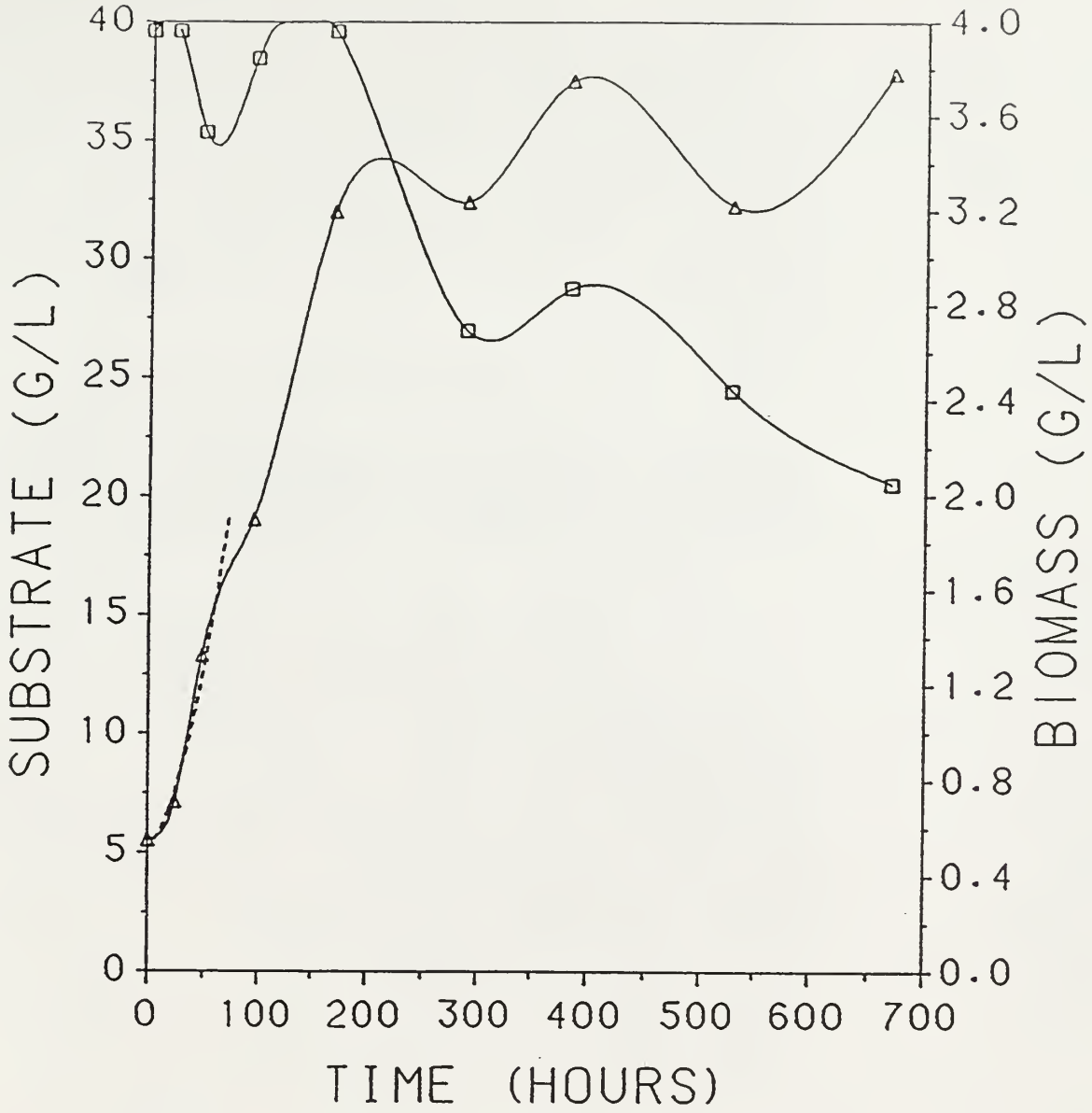

Figure 3.13 Biomass(Δ) and COD(□) variations with time for the mixed population of microorganisms growing on oil sample IRMCO 156 at an initial concentration of 0.5% and with inorganic nutrient media composition A: dashed line represents the exponential growth model.

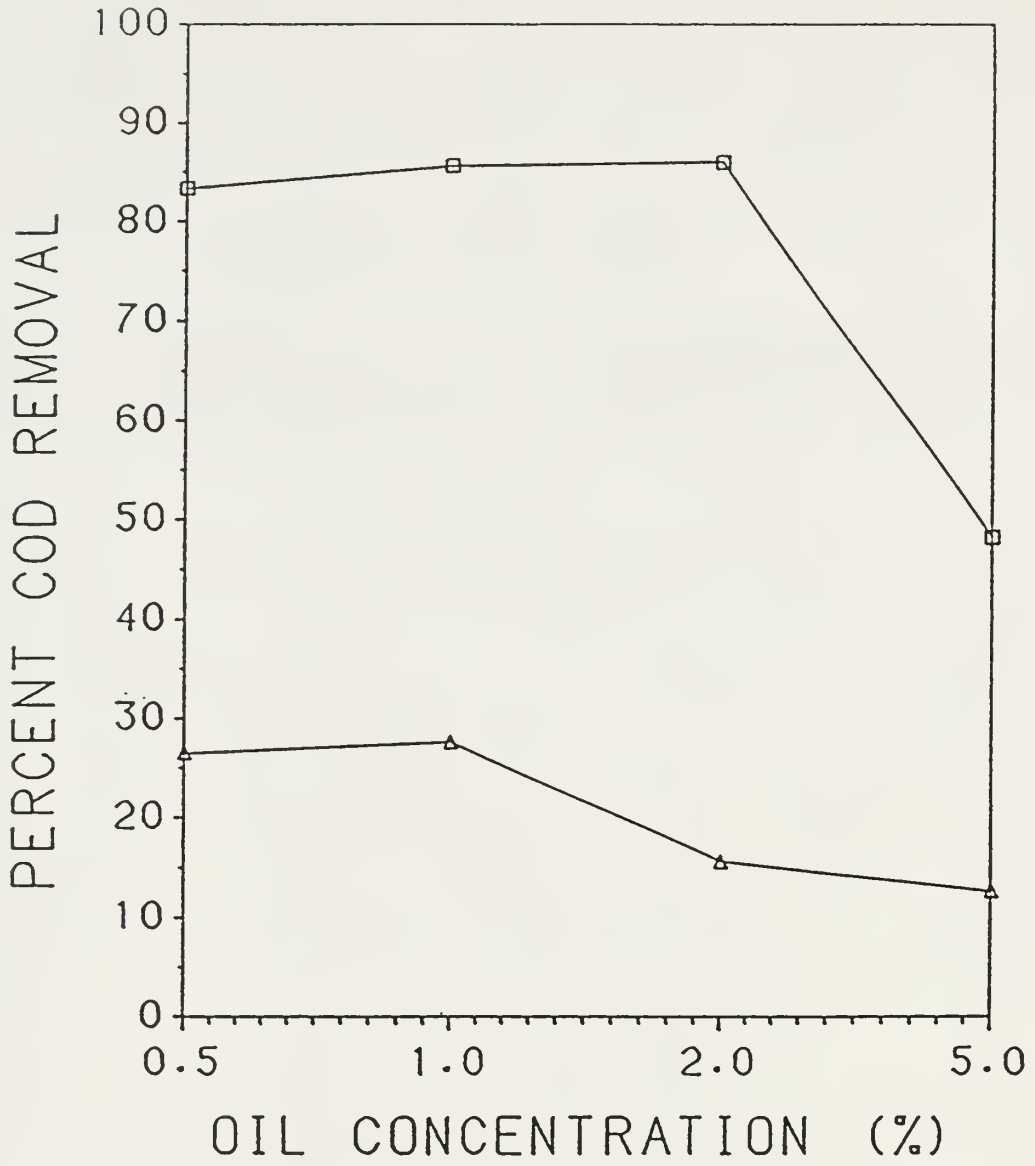

Figure 3.14 Percent COD removal at various initial concentrations for the mixed population of microorganisms growing on oil samples IRMCO 141(□) and IRMCO 156(Δ).

Table 3.11. Percent COD removal at different dilutions for the two oil samples and with inorganic nutrient B.

IRMCO 141

Volume % IRMCO 141	COD* Initial	COD* Final	% Removal
0.5%	4278	714	83.31
1.0%	8294	1188	85.68
2.0%	16296	2268	86.08
5.0%	39576	20502	48.20

IRMCO 156

Volume % IRMCO 156	COD* Initial	COD* Final	% Removal
0.5%	3168	2328	26.52
1.0%	5456	3949	27.62
2.0%	10752	9072	15.63
5.0%	26112	22797	12.70

* COD concentration is in mg/L.

Table 3.12. Specific growth rate constant for the two oil samples at different dilutions with B as the inorganic nutrient media.

IRMCO 141

	Specific growth rate (1/hr.)			
Volume % IRMCO 141	0.5%	1.0%	2.0%	5.0%
Biomass Data	0.0094	0.0210	0.0100	0.0183
Substrate Data	0.0110	0.0210	0.0106	0.0183
Combined Data	0.0102	0.0210	0.0103	0.0183
Yield	0.4091	0.3473	0.5242	0.1833

IRMCO 156

	Specific growth rate (1/hr.)			
Volume % IRMCO 156	0.5%	1.0%	2.0%	5.0%
Biomass Data	0.0043	0.0072	0.0217	0.0253
Substrate Data	0.0041	0.0072	0.0217	0.0253
Combined Data	0.0042	0.0072	0.0217	0.0253
Yield	1.1633	0.2700	1.5060	1.1054

Nutrient B appears to be the best nutrient composition for the two oil samples. This inference is evident from Table 3.13 which shows the percent COD removal for the three nutrient compositions A, B, and C listed in Tables 3.3 through 3.5. It has been speculated that the presence of urea in the fermentation broth aids the COD removal(Hill, 1984). This belief is strengthened by observing that only nutrient B contained urea in its formulation and for both the oil samples with nutrient B there was a higher percentage of COD reduction.

Table 3.13. Percent COD removal at different inorganic nutrient compositions for both oil samples at an oil concentration of 0.5%.

IRMCO 141

Nutrient	COD* Initial	COD* Final	% Removal
A	4158	714	82.83
B	4278	714	83.31
C	4158	1309	68.52

IRMCO 156

Dilution	COD* Initial	COD* Final	% Removal
A	2874	2778	3.34
B	3168	2328	26.52
C	3072	2328	24.22

* COD concentration is in mg/L.

Table 3.14. Specific growth rate estimates for IRMCO 141 oil sample at different inorganic nutrient compositions at oil concentration of 0.5%.

IRMCO 141

	Specific growth rate (1/hr.)		
Nutrient Media	A	B	C
Biomass Data	0.0125	0.0094	0.0160
Substrate Data	0.0134	0.0110	0.0160
Combined Data	0.0130	0.0102	0.0160
Yield	0.7414	0.4091	0.2081

IRMCO 156

	Specific growth rate (1/hr.)		
Nutrient Media	A	B	C
Biomass Data	0.0138	0.0043	0.0134
Substrate Data	0.0138	0.0041	0.0181
Combined Data	0.0138	0.0042	0.0158
Yield	0.5671	1.1633	0.5455

CHAPTER 4

APPLICATION OF MASS SPECTROMETER IN BIODEGRADATION STUDIES

4.1 Introduction

In order to design and operate a bioreactor, meaningful data must be collected by conducting experiments at laboratory or pilot-plant scale. Performing biological experiments is usually a very expensive operation and therefore it is desirable to get as much useful information as possible out of each experiment. The experiments are rather slow and need controlled conditions for their success. It therefore becomes necessary to continuously monitor and control the various operating parameters. Batch sampling techniques employing chemical and physical measurements can be used in extremely slow processes where ample time is available for the analysis of the samples. However, it is highly desirable to devise measurement techniques that are capable of quick analysis of the samples in order to facilitate better control and understanding of the biological process.

On-line measurements provide faster and easier analysis of the process and aid in the rapid collection of the data

thereby minimizing the operating time and costs. Also, if the process is regarded as a black-box, i.e. if no mechanistic model exists for the process, then the parameters to be controlled in the experimentation must be directly measured. Very often the most useful parameters in a biological experiment is the rate of substrate utilization and/or the rate of product generation. An estimate of these parameters can be easily made if the other accompanied gaseous components like, oxygen, carbon dioxide, methane, and ammonia can be assayed. A mass spectrometer can play an immensely important role in the analysis of the gases in a fermentation process.

4.2 Mass spectrometer

The basic principles of mass spectrometry (MS) have been known for a long time. Quantitative mass spectrometry, however, has evolved not so long ago and has been made possible by the advances in instrumentation and computerized data processing. The main components of an MS equipment are sample introduction, vacuum, ionization, mass separation, detection, MS-control and a data acquisition interface.

Sample introduction : The gas sample inlet to the system is through a 1 meter long, inert fused silica capillary with

an inside diameter of 50 microns. The small diameter and a large length are required to decrease the sample gas pressure from atmospheric to about 10^{-6} Torr (pressure in the ionization chamber). The pressure drop is created by employing a rotary vacuum pump. The advantage of using a capillary is that the gas travels through in a continuous laminar flow. Also, if a portion of the capillary gets clogged at the inlet, it can be snapped away and the capillary can be used again. One end of the capillary is inserted in the fermentation reactor and the other end is sealed to the MS equipment using a graphitized vespel ferrule. The ferrule is tightened to avoid any air leaks.

Vacuum : The vacuum required in the analyzer section is usually below 10^{-5}mbar. Above this pressure the ions will not survive as a high pressure would cause collision with other molecules resulting in undesirable reactions. At a vacuum below 10^{-5}, the mean free path of particles is sufficiently long and the number of collisions sufficiently small to result in satisfactory analysis.

Ionization : Although a range of methods have been devised for ionizing the gas molecules the electron impact ionization is normally the overwhelming choice because of

its stability in operation over a period of time. A cathode source within the ionization chamber maintained at high vacuum is used to generate electrons of high energies. Fragmentation of large molecules is typically the most formidable problem encountered by employing electron impact ionization. The degree of fragmentation can be lowered by employing soft ionization techniques that use lower energies to ionize the molecules. It is highly desirable to get a single peak from a particular molecule instead of getting a number of peaks that result from its fragmentation.

Mass separation : Quadrupole mass separation and the magnetic scanning mass separation are the two most widely employed methods for mass separation. Quadrupole mass spectrometers employ four parallel rods arranged to lie in the four corners of a square and serve as mass filter. Diagonally opposite rods are connected to radio frequency and direct current voltages. One pair of rods is out of phase by π radians with regards to the other pair. At a given ratio of the radio frequency to the direct current only ions with a specific mass to charge ratio would avoid collision with the rods and pass through following a complicated path. The magnetic instruments on the other hand rely on the magnetic strength to deflect ions of a particular mass to charge ratio. By changing either the

magnetic field strength or the operating voltage, a particular m/z ion can be focussed on to the exit slit and subsequently measured(Odham, G., et al. 1984; Lloyd, D. et al., 1985).

A Dycor Quadrupole Gas analyzer model no. M100 (Ametek, Pittsburg, Pennsylvania) was used in the gas phase study as an integral part of the mass spectrometer equipment.

Detection : The ions are detected by reading the electric current produced by the ions that are collected on a plate or a Faraday cup. The current may be amplified by using a electrometer amplifier (Heinzle, E., 1987). The mass spectrometer equipment used in the experimental studies had an electrometer connected directly to the analyzer head in order to obtain the best signal to noise ratio. The Faraday cup was used to collect ions and to thereby register the current.

MS control : The basic reason for employing MS control is to control the mass scan and the gain of ion amplification. Microcomputers are the most recent means used to perform these two operations. Selection of the molecules (with specific mass numbers) to be studied during

the course of an experiment can be monitored very easily in a quadrupole type instrument by adjusting the DC voltage.

Data acquisition interface : The mass spectrometer was interfaced with a Zenith Electronics Corporation Model 120/240 computer. The software package included Lotus Measure and Lotus 123. This was used to permanently store the data collected on each of the experiments. Digital signals from the mass spectrometer were converted from partial pressures to mole fractions by the computer program developed for this purpose. Twelve sampling channels were available based on the molecular weight. The channels were set to 2, 4, 16, 18, 20, 28, 32, 40, 44, and 56 molecular weights.

4.3 Data analysis equations

The equations employed to evaluate the rate of oxygen uptake (OUR) and the carbon dioxide production rate (CPR) for a batch fermentation from the mass spectrometer data include a liquid phase balance on the gas species 'i'

$$V_l \frac{dC_{li}}{dt} = k_{la} (C^*_{li} - C_{li}) V_l + r_i X V_l \qquad (4\text{-}1)$$

and a gas phase balance

$$V_g \frac{dC_{gi}}{dt} = F(x_{oi} - x_i) - k_{la} (C^*_{li} - C_{li}) V_l \qquad (4\text{-}2)$$

Adding equations (4-1) and (4-2) gives

$$V_l \frac{dC_{li}}{dt} + V_g \frac{dC_{gi}}{dt} = F(x_{oi} - x_i) + r_i X V_l \qquad (4\text{-}3)$$

The gas and liquid phase equilibrium concentrations are related through Henry's law

$$C_{li}^* = H_i x_i P_{tot} \qquad (4\text{-}4)$$

and the ideal gas law

$$C_{gi} = \frac{P_{tot} x_i}{RT} \qquad (4\text{-}5)$$

When the rate of biodegradation is extremely slow and the mass transfer driving force is relatively constant and

$$\frac{dC_{li}^{*}}{dt} = \frac{dC_{li}}{dt} \qquad (4\text{-}6)$$

can be assumed.

Differentiating equation (4-5)

$$\frac{dC_{gi}}{dt} = \frac{P_{tot}}{RT} \frac{dx_i}{dt} \qquad (4\text{-}7)$$

From equations (4-4) and (4-6)

$$\frac{dC_{li}}{dt} = H_i P_{tot} \frac{dx_i}{dt} \qquad (4\text{-}8)$$

From equations (4-3), (4-7) and (4-8)

$$\left(V_g \frac{P_{tot}}{RT} + V_l H_i P_{tot}\right) \frac{dx_i}{dt} = F(x_{oi} - x_i) + r_i \text{ X } V_l \qquad (4\text{-}9)$$

The term 'r_i X V_l' is either the oxygen uptake rate (OUR) or the carbon dioxide production rate (CPR) depending on whether the subscript 'i' is oxygen or carbon dioxide.

Let

$$(V_g \frac{P_{tot}}{RT} + V_l H_i P_{tot}) = n_{oi}$$

and

$$R_i = r_i \times V_l$$

Thus,

$$n_{oi} \frac{dx_i}{dt} + F (x_i - x_{oi}) = r_i \times V_l \qquad (4\text{-}10)$$

$$n_{oO2} \frac{dx_{O2}}{dt} + F (x_{O2} - x_{oO2}) = - OUR \qquad (4\text{-}10\ A)$$

$$n_{oCO2} \frac{dx_{CO2}}{dt} + F (x_{CO2} - x_{oCO2}) = CPR \qquad (4\text{-}10\ B)$$

Solving these differential equations with the initial condition at $t = 0$, $x_i = x_{oi}$, we obtain

$$x_i - x_{oi} = \frac{R_i}{F} (1 - \exp(- \frac{F\ t}{n_{oi}})) \qquad (4\text{-}11)$$

Let

$$\theta_1 = \frac{R_i}{F} \qquad (4\text{-}12)$$

and

$$\theta_2 = \frac{F}{n_{oi}} \qquad (4\text{-}13)$$

Following equations are used to estimate the values of biomass energetic yields and the specific growth rate constant (Ferrer, et al., 1979)

$$\text{RQ (Respiratory Quotient)} = \frac{Q_{CO2}}{Q_{O2}} = \frac{CPR}{OUR} \qquad (4\text{-}14)$$

Q_i = Rate of uptake or production of 'i' $(\frac{\text{gmoles}}{\text{hr gm DW}})$

Also,

$$\eta \text{ (Biomass Energetic Yield)} = \frac{1 - 1/4\ \gamma_s\ RQ}{\gamma_s/\ \gamma_b - 1/4\ \gamma_s\ RQ} \qquad (4\text{-}15)$$

$$\mu \text{ (Specific Growth Rate Const.)} = \frac{48\ Q_{O2}}{\gamma_b\ \sigma_b\ (1/\eta - 1)} \qquad (4\text{-}16)$$

γ_b (Biomass Reductance Degree) = 4.29

γ_s (Substrate Reductance Degree) = 5.00 (For IRMCO 141)

σ_b (Weight Fraction Carbon in Biomass) = 0.462

4.4 Application in shake flask experiments

The mass spectrometer was used to collect data on the variation of gas phase composition with time for the shake flask experiments performed to study the effect of dilution and nutrient composition on biodegradation(Experiment No. 4). The two shake flasks selected were with the oil concentration of 2.0% and 5.0%. The shake flasks were tightly capped with a rubber stopper and a needle was inserted into the shake flask through the stopper. The fused silica capillary was then introduced into the shake flask through the needle. The entire system was made air-tight with the help of Parafilm "M". The Mass Spectrometer was turned on and data was collected on the Zenith computer at regular intervals of time. The probe was inserted at about 500 hrs. from the start of the experiments for IRMCO 141 at 2.0% initial oil concentration and at 550 hours for IRMCO 141 at 5.0% initial oil concentration. At this point

in time most of the COD removal had taken place for both the oil samples and the COD concentration was beginning to attain steady state value.

The objective here was to ascertain if the carbon dioxide production rates and the oxygen uptake rates had indeed dropped to insignificant values. The value of the specific growth rate constant was also calculated from the equations detailed above to get a comparison between the value at this stage of the experiment and the maximum specific growth rate constant as estimated in chapter 3, Table 3.12. The variation of carbon dioxide and oxygen with time is shown in Figures 4.1 and 4.2 for two different initial oil concentrations respectively. The mole fraction data has been tabulated in Appendix III.

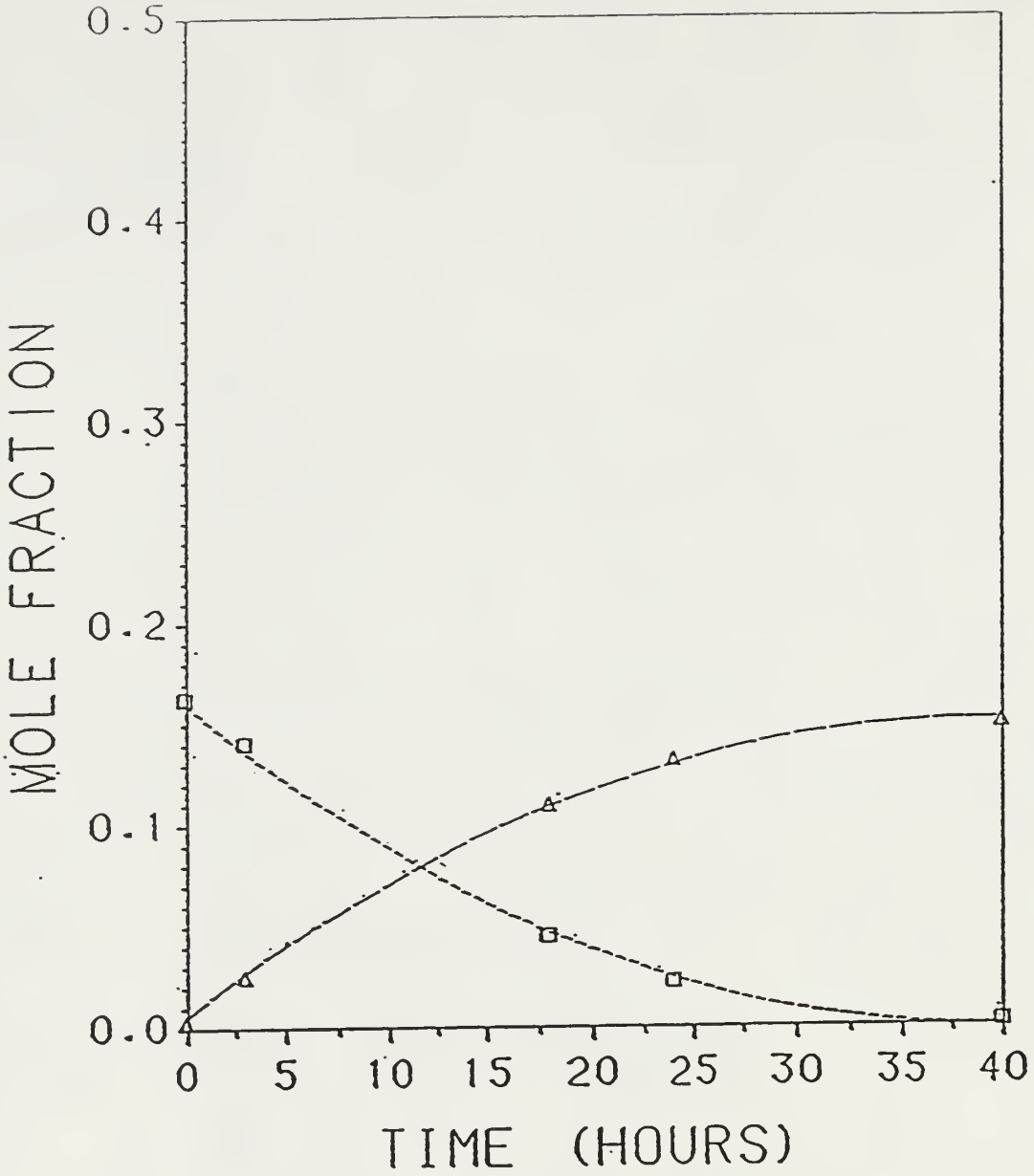

Figure 4.1 Carbon dioxide(Δ) and Oxygen(□) variations with time in the gas phase for the mixed population of microorganisms growing on oil sample IRMCO 141 at an initial oil concentration of 2.0%: dotted line indicates exponential regression of the data.

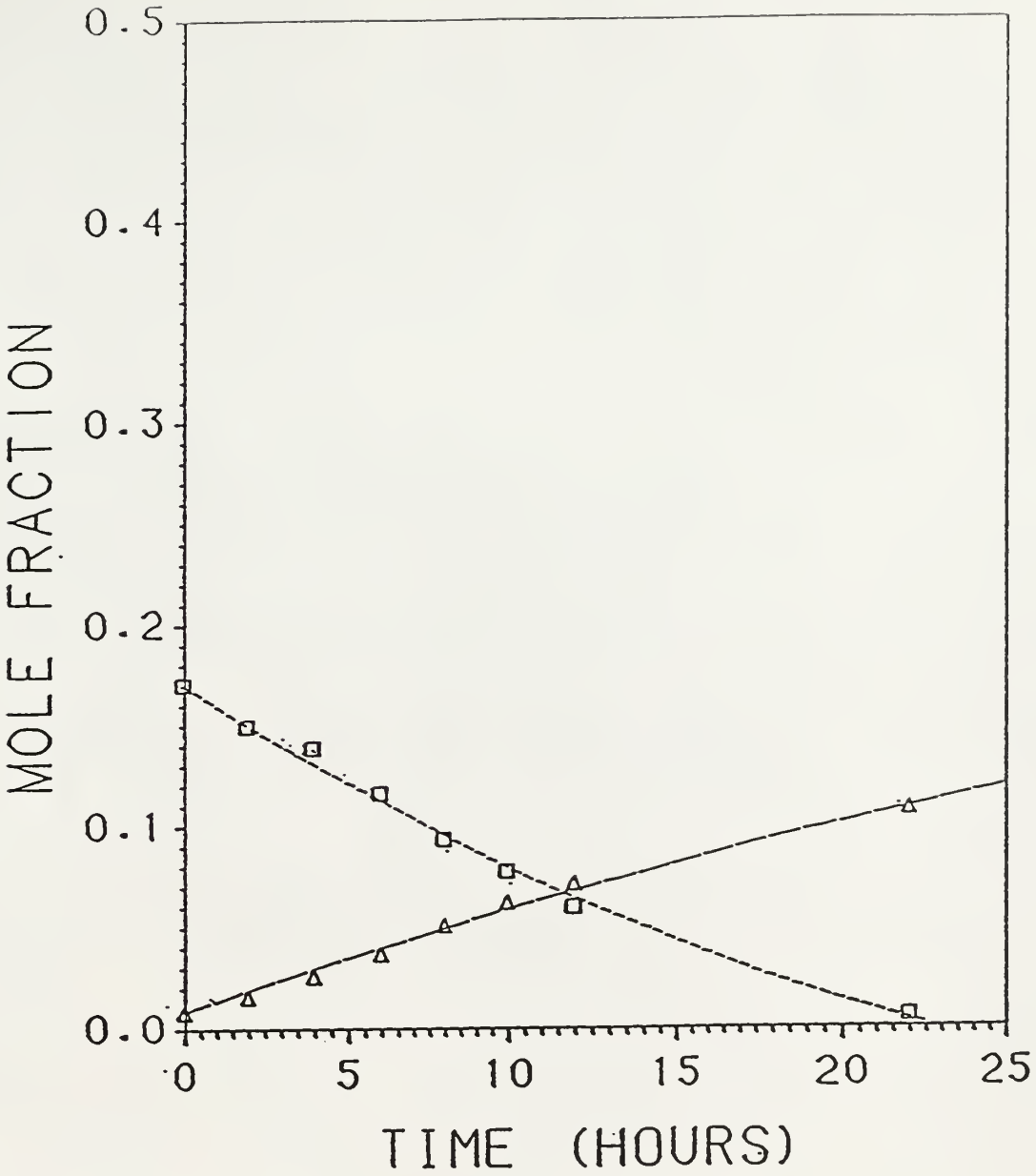

Figure 4.2 Carbon dioxide(Δ) and Oxygen(□) variations with time in the gas phase for the mixed population of microorganisms growing on oil sample IRMCO 141 at an initial oil concentration of 5.0%: dotted line indicates exponential regression of the data.

On linearizing equation (4-11) by performing Taylor series expansion and then conducting linear regression on the data we get the following results for the OUR and the CPR.

For IRMCO 141 at 2.0 % initial oil concentration, the oxygen data yields the estimates θ_1 = -0.181 and θ_2 = 0.058. For F = 1.8 ml/hr, OUR = 0.00033 g mol/hr. For the carbon dioxide data θ_1 = 0.164 and θ_2 = 0.059. For F = 1.8 ml/hr, CPR = 0.00030 g mol/hr.

For IRMCO 141 at 5.0 % initial oil concentration the parameter estimates based on oxygen data are θ_1 = -0.322 and θ_2 = 0.033. For F = 1.8 ml/hr, OUR = 0.00058 g mol/hr. For the carbon dioxide time data θ_1 = 0.304 and θ_2 = 0.018. For F = 1.8 ml/hr, CPR = 0.00055 g mol/hr.

4.5 Results and discussion

From equations (4-14), (4-15) and (4-16) for IRMCO 141 at 2.0 % initial oil concentration, the biomass energetic yield, η, and specific growth rate, μ, are estimated to be η = 0.368 and μ = 0.00044 (1/hr). From the biomass measurements, the value of μm = 0.01 (1/hr) has been estimated (See Table I.18, App. I).

For IRMCO 141 at 5.0 % initial oil concentration η = 0.253 and μ = 0.00034 (1/hr). From the biomass measurements, the value of μm = 0.02 (1/hr) (See Table I.19, App. I).

The values of OUR and the CPR along with the information on the specific growth rate constants (μ) indicated that the biodegradation rate had indeed decreased to extremely low values for both the shake flasks at the point in time where the mass spectrometer readings were taken.

CHAPTER 5

CONCLUSIONS

From the various experiments conducted on the biodegradation of the two metalworking fluid samples viz. IRMCO 141 and IRMCO 156, the following conclusions have been drawn.

1. Multiple lag, exponential growth and death phases are common occurrence with microbial populations of mixed origin. There is an antagonistic relationship between bacteria, fungi and protozoans.

2. Adaptation of the microbial mixed population reduces the lag phase but is of little significance in terms of the percentage COD reduction of the oil samples.

3. Metalworking fluids are designed to minimize bacterial contamination while they are in use in the industry. The problem of their biodegradation as wastes is just the very reverse, i.e., to provide a condition where there are no inhibitory factors detrimental to microbial biodegradation. The pH appears to be an extremely important factor as the biocidal effect of the preservatives that are

added to the oil samples appears to be a very strong function of the operating pH.

4. The presence of the preservative hexahydro-s-triazine was felt at pH above 8.0, and little biodegradation was observed at an operating pH greater than 8.0 for both the oil samples.

5. The optimum pH for maximum biodegradation of the oil sample IRMCO 141 was 6.0. For oil sample IRMCO 156 there was a range of pH between 5.0 and 8.0 at which the maximum biodegradation was observed.

6. Dilution of the oil sample is important in the optimization of the biodegradation and the economics of the process. The optimum concentration of IRMCO 141 is 2% and for IRMCO 156 it is 1%.

7. Urea as an additive to the nutrient media has beneficial results of reducing the COD of oil samples as compared to the synthetic media devoid of urea.

8. Mass spectrometer analysis can give a valuable insight into the rate of biodegradation by accurately predicting the intake of nutrients like oxygen and outflow of products such as carbon dioxide, ammonia, and methane. From the data collected, it could be predicted that the rates of degradation had gone down to extremely low values for the oil sample IRMCO 141 at an initial concentration of 2.0% and 5.0% after about 500 hours of operation.

9. HPLC analysis could not be adopted of for the oil samples as no peaks were observed in the experiments conducted.

10. Continuous culture and fed batch techniques were found to be of little practical use because of the extremely slow biodegradation rates involved.

APPENDIX I

EXPERIMENTAL DATA AND REGRESSION OUTPUT ON SHAKE FLASK EXPERIMENTS

Table I.1 Experimental data and regression output for Experiment 1 for 1% IRMCO 141 oil sample at initial pH of 8.5 with C as nutrient media. (Jun. 27 - July 13, 1987)

Experimental Data:

Sampling Time(HRS)	pH	Temp. (°C)	Biomass (G/L)	COD (G/L)
0	8.5	28	0.100	6.798
22	8.4	30	0.076	6.798
69	8.5	30	0.096	6.193
117	8.7	30	0.136	6.193
214	8.6	30	0.240	6.391
282	8.4	30	0.248	6.391
377	8.6	30	0.390	6.193

Biomass Yield Coefficient = 0.403

Regression Output:
$\ln(X) = \mu m * t + \ln(Xo)$

	Combined	Biomass	Substrate
Intercept (ln(Xo))	-2.381	-2.727	-2.035
Std Err of Y Est	0.512	0.031	0.712
R Squared	0.413	0.997	0.278
No. of Observations	8	4	4
Degrees of Freedom	6	2	2
Slope (μm, 1/hr.)	0.00524	0.00608	0.00440
Std Err of Coef.	0.00255	0.00022	0.00501

Table I.2 Experimental data and regression output for Experiment 1 for 1% IRMCO 156 oil sample at initial pH of 8.3 with C as nutrient media. (Jun. 27 - July 13, 1987)

Experimental Data:

Sampling Time(HRS)	pH	Temp. (°C)	Biomass (G/L)	COD (G/L)
0	8.3	28	0.070	4.587
22	8.4	30	0.096	4.422
69	8.3	30	0.080	3.949
117	8.5	30	0.178	3.959
214	8.1	30	0.294	3.047
282	8.1	30	0.180	3.487
377	6.3	30	0.550	2.486

Biomass Yield Coefficient = 0.237

Regression Output:
$\ln(X) = \mu m * t + \ln(Xo)$

	Combined	Biomass	Substrate
Intercept (ln(Xo))	-3.170	-2.949	-3.391
Std Err of Y Est	0.295	0.296	0.369
R Squared	0.837	0.899	0.882
No. of Observations	6	3	3
Degrees of Freedom	4	1	1
Slope (μm, 1/hr.)	0.00905	0.00842	0.00967
Std Err of Coef.	0.00199	0.00283	0.00354

Table I.3 Experimental data and regression output for Experiment 2 for 1% IRMCO 141 oil sample at initial pH of 7.3 with C as nutrient media. (July 23 - Aug. 23, 1987)

Experimental Data:

Sampling Time(HRS)	pH	Temp. (°C)	Biomass (G/L)	COD (G/L)
0	7.3	30	0.268	8.294
22	7.3	30	0.260	7.843
49	7.6	30	0.422	7.250
71	7.6	30	0.316	7.414
91	7.6	30	0.408	7.623
139	7.6	30	0.344	7.250
190	7.5	30	0.618	5.808
258	7.1	30	0.312	4.587
330	7.1	30	0.570	4.587
430	7.4	30	0.394	4.752
545	6.8	30	0.420	4.587
737	7.1	30	0.318	4.587

Biomass Yield Coefficient = 0.148

Regression Output:
$\ln(X) = \mu m * t + \ln(Xo)$

	Combined	Biomass	Substrate
Intercept (ln(Xo))	-1.356	-1.402	-1.310
Std Err of Y Est	0.109	0.191	0.015
R Squared	0.819	0.753	0.998
No. of Observations	6	3	3
Degrees of Freedom	4	1	1
Slope (μm, 1/hr.)	0.00942	0.00959	0.00924
Std Err of Coef.	0.00222	0.00550	0.00042

Table I.4 Experimental data and regression output for Experiment 2 for 1% IRMCO 156 oil sample at initial pH of 7.7 with C as nutrient media. (July 23 - Aug. 23, 1987)

Experimental Data:

Sampling Time(HRS)	pH	Temp. (°C)	Biomass (G/L)	COD (G/L)
0	7.7	30	0.242	7.623
22	7.4	30	0.200	5.632
49	7.2	30	0.262	5.995
71	7.0	30	0.254	5.269
91	6.6	30	0.506	5.632
139	7.4	30	0.400	5.456
190	7.6	30	0.734	4.752
258	7.1	30	0.264	4.268
330	6.0	30	0.266	4.268
430	6.2	30	0.254	3.795
545	5.7	30	0.146	4.114
737	6.2	30	0.152	3.949

Biomass Yield Coefficient = 0.607

Regression Output:
$\ln(x) = \mu m * t + \ln(Xo)$

	Combined	Biomass	Substrate
Intercept (ln(Xo))	-1.507	-1.712	-1.303
Std Err of Y Est	0.344	0.233	0.417
R Squared	0.581	0.820	0.492
No. of Observations	12	6	6
Degrees of Freedom	10	4	4
Slope (μm, 1/hr.)	0.00657	0.00720	0.00594
Std Err of Coef.	0.00176	0.00169	0.00302

Table I.5 Experimental data and regression output for Experiment 3 for 1.0% IRMCO 141 oil sample at pH of 4.0 with C as nutrient media. (Sept. 14 - Nov. 13, 1987)

Experimental Data:

Sampling Time(HRS)	pH	Temp. (°C)	Biomass (G/L)	COD (G/L)
0	4.1	30	0.488	8.294
40	4.2	30	0.322	8.063
88	4.0	30	0.472	8.063
161	4.3	30	0.646	8.063
256	4.1	30	0.558	8.063
375	4.0	30	0.408	8.294
494	4.0	30	0.868	7.843
615	4.0	30	0.260	8.063
831	4.1	30	1.070	7.414
1071	4.2	30	0.238	7.205
1215	4.2	30	0.548	6.391
1409	4.2	30	0.382	5.093

Biomass Yield Coefficient = -

Regression Output:
$\ln(X) = \mu m * t + \ln(Xo)$

	Combined	Biomass	Substrate
Intercept (ln(Xo))	-	-1.316	-
Std Err of Y Est	-	0.086	-
R Squared	-	0.969	-
No. of Observations	-	3	-
Degrees of Freedom	-	1	-
Slope (μm, 1/hr)	-	0.00563	-
Std Err of Coef.	-	0.00100	-

Table I.6 Experimental data and regression output for Experiment 3 for 1.0% IRMCO 141 oil sample at pH = 5.0 with C as nutrient media. (Sept. 14 - Nov. 13, 1987)

Experimental Data:

Sampling Time(HRS)	pH	Temp. (°C)	Biomass (G/L)	COD (G/L)
0	5.1	30	0.376	8.063
40	5.2	30	0.448	7.007
88	5.4	30	0.446	7.205
161	5.5	30	0.606	7.205
256	5.2	30	0.778	7.007
375	5.1	30	0.522	6.193
494	5.1	30	0.402	6.193
615	5.1	30	1.798	5.093
831	5.0	30	1.096	3.795
1071	5.0	30	0.642	2.629
1215	5.0	30	0.295	1.815
1409	5.2	30	0.536	1.188

Biomass Yield Coefficient = 1.677

Regression Output:
$\ln(X) = \mu m * t + \ln(Xo)$

	Combined	Biomass	Substrate
Intrcept (ln(Xo))	-1.136	-1.072	-1.201
Std Err of Y Est	0.120	0.053	0.197
R Squared	0.848	0.982	0.812
No. of Observations	6	3	3
Degrees of Freedom	4	1	1
Slope (μm, 1/hr)	0.00336	0.00328	0.00344
Std Err of Coef.	0.00071	0.00044	0.00165

Table I.7 Experimental data and regression output for Experiment 3 for 1.0% IRMCO 141 oil sample at pH = 6.0 with C as nutrient media. (Sept. 14 - Nov. 13, 1987)

Experimental Data:

Sampling Time(HRS)	pH	Temp. (°C)	Biomass (G/L)	COD (G/L)
0	6.0	30	0.256	8.294
40	6.0	30	0.418	6.589
88	6.0	30	0.362	7.007
161	6.0	30	0.618	6.589
256	6.1	30	0.798	5.808
375	6.0	30	0.600	5.269
494	6.1	30	0.738	4.114
615	6.1	30	0.992	1.947
831	6.3	30	0.218	1.683
1071	6.0	30	0.894	1.309
1215	6.0	30	0.746	1.188
1409	6.0	30	0.880	1.067

Biomass Yield Coefficient = 0.364

Regression Output:
$\ln(X) = \mu m * t + \ln(Xo)$

	Combined	Biomass	Substrate
Intercept (ln(Xo))	-1.388	-1.350	-1.427
Std Err of Y Est	0.087	0.156	0.006
R Squared	0.953	0.925	1.000
No. of Observations	6	3	3
Degrees of Freedom	4	1	1
Slope (μm, 1/hr)	0.00465	0.00461	0.00470
Std Err of Coef.	0.00051	0.00131	0.00005

Table I.8 Experimental data and regression output for Experiment 3 for 1.0% IRMCO 141 oil sample at pH = 7.0 with C as nutrient media. (Sept. 14 - Nov. 13, 1987)

Experimental Data:

Sampling Time(HRS)	pH	Temp. (°C)	Biomass (G/L)	COD (G/L)
0	7.2	30	0.232	8.294
40	7.1	30	0.370	7.623
88	7.0	30	0.498	6.589
161	7.0	30	1.318	6.395
256	7.0	30	0.832	5.995
375	7.0	30	0.496	5.456
494	7.2	30	0.830	5.269
615	6.9	30	0.308	5.456
831	7.1	30	0.574	4.752
1071	7.0	30	0.232	4.587
1215	7.1	30	0.474	4.587
1409	7.0	30	0.408	4.587

Biomass Yield Coefficient = 0.572

Regression Output:
$\ln(X) = \mu m * t + \ln(Xo)$

	Combined	Biomass	Substrate
Intercept (ln(Xo))	-1.298	-1.478	-1.117
Std Err of Y Est	0.334	0.120	0.445
R Squared	0.824	0.982	0.795
No. of Observations	8	4	4
Degrees of Freedom	6	2	2
Slope (μm, 1/hr)	0.01042	0.01051	0.01034
Std Err of Coef.	0.00197	0.00100	0.00371

Table I.9 Experimental data and regression output for Experiment 3 for 1.0% IRMCO 141 oil sample at pH = 8.0 with C as nutrient media. (Sept. 14 - Nov. 13, 1987)

Experimental Data:

Sampling Time(HRS)	pH	Temp. (°C)	Biomass (G/L)	COD (G/L)
0	8.0	30	0.196	8.294
40	8.0	30	0.430	7.414
88	8.0	30	0.560	6.391
161	8.0	30	0.550	6.193
256	8.0	30	0.708	6.193
375	8.0	30	0.938	4.917
494	8.0	30	0.662	5.093
615	8.1	30	0.472	5.093
831	8.0	30	0.860	4.587
1071	8.0	30	0.542	4.587
1215	8.0	30	0.788	4.114
1409	8.0	30	0.210	3.949

Biomass Yield Coefficient = 0.191

Regression Output:
$\ln(X) = \mu m * t + \ln(Xo)$

	Combined	Biomass	Substrate
Intercept (ln(Xo))	-1.548	-1.518	-1.578
Std Err of Y Est	0.143	0.252	0.116
R Squared	0.930	0.894	0.976
No. of Observations	6	3	3
Degrees of Freedom	4	1	1
Slope (μm, 1/hr)	0.01177	0.01172	0.01183
Std Err of Coef.	0.00162	0.00404	0.00187

Table I.10 Experimental data and regression output for Experiment 3 for 1.0% IRMCO 141 oil sample at pH = 9.0 with C as nutrient media. (Sept. 14 - Nov. 13, 1987)

Experimental Data:

Sampling Time(HRS)	pH	Temp. (°C)	Biomass (G/L)	COD (G/L)
0	9.0	30	0.248	8.294
40	9.0	30	0.082	7.843
88	9.0	30	0.122	8.294
161	9.0	30	0.416	7.414
256	9.1	30	0.388	7.623
375	9.0	30	0.412	8.083
494	9.0	30	0.500	6.391
615	9.0	30	0.364	6.391
831	9.0	30	0.482	5.456
1071	9.0	30	0.694	3.333
1215	9.0	30	1.984	2.343
1409	9.0	30	1.612	1.957

Biomass Yield Coefficient = 0.400

Regression Output:
$\ln(X) = \mu m * t + \ln(Xo)$

	Combined	Biomass	Substrate
Intercept (ln(Xo))	-2.747	-2.747	-2.748
Std Err of Y Est	0.289	0.368	0.106
R Squared	0.862	0.836	0.987
No. of Observations	8	4	4
Degrees of Freedom	6	2	2
Slope (μm, 1/hr)	0.00273	0.00256	0.00290
Std Err of Coef.	0.00045	0.00080	0.00023

Table I.11 Experimental data and regression output for Experiment 3 for 1.0% IRMCO 156 oil sample at pH = 4.0 with C as nutrient media. (Sept. 14 - Nov. 5, 1987)

Experimental Data:

Sampling Time(HRS)	pH	Temp. (°C)	Biomass (G/L)	COD (G/L)
0	4.0	30	0.182	6.798
40	4.0	30	0.208	6.391
88	4.1	30	0.206	6.391
161	4.0	30	0.212	6.798
256	4.1	30	0.240	6.391
375	4.2	30	0.200	5.995
494	4.2	30	0.238	5.995
615	4.1	30	0.152	5.995
831	4.2	30	0.286	5.093
1071	4.0	30	0.324	4.587
1215	4.2	30	0.512	4.587

Biomass Yield Coefficient = 0.122

Regression Output:
$\ln(X) = \mu m * t + \ln(Xo)$

	Combined	Biomass	Substrate
Intercept (ln(Xo))	-2.813	-2.796	-2.830
Std Err of Y Est	0.136	0.223	0.152
R Squared	0.883	0.849	0.924
No. of Observations	6	3	3
Degrees of Freedom	4	1	1
Slope (μm, 1/hr)	0.00164	0.00164	0.00165
Std Err of Coef.	0.00030	0.00069	0.00047

Table I.12 Experimental data and regression output for Experiment 3 for 1.0% IRMCO 156 oil sample at pH = 5.0 with C as nutrient media. (Sept. 14 - Nov. 5, 1987)

Experimental Data:

Sampling Time(HRS)	pH	Temp. (°C)	Biomass (G/L)	COD (G/L)
0	5.2	30	0.204	6.391
40	5.0	30	0.224	6.193
88	5.1	30	0.200	6.193
161	5.2	30	0.200	5.995
256	5.4	30	0.290	5.632
375	5.1	30	0.358	4.587
494	5.1	30	0.202	4.422
615	5.1	30	0.314	4.422
831	5.0	30	0.258	3.949
1071	5.2	30	0.340	4.114
1215	5.3	30	0.360	4.114

Biomass Yield Coefficient = 0.112

Regression Output:
$\ln(X) = \mu m * t + \ln(Xo)$

	Combined	Biomass	Substrate
Intercept (ln(Xo))	-2.039	-1.999	-2.079
Std Err of Y Est	0.067	0.092	0.060
R Squared	0.950	0.951	0.980
No. of Observations	6	3	3
Degrees of Freedom	4	1	1
Slope (μm, 1/hr)	0.00271	0.00268	0.00275
Std Err of Coef.	0.00031	0.00061	0.00039

Table I.13 Experimental data and regression output for Experiment 3 for 1.0% IRMCO 156 oil sample at pH = 6.0 with C as nutrient media. (Sept. 14 - Nov. 5, 1987)

Experimental Data:

Sampling Time(HRS)	pH	Temp. (°C)	Biomass (G/L)	COD (G/L)
0	6.0	30	0.218	6.391
40	6.4	30	0.310	6.193
88	6.0	30	0.392	5.808
161	6.1	30	0.448	4.587
256	6.1	30	0.432	4.422
375	6.3	30	0.338	4.422
494	6.2	30	0.258	4.422
615	6.2	30	0.230	4.268
831	6.0	30	0.326	4.268
1071	6.2	30	0.420	3.949
1215	6.3	30	0.614	4.114

Biomass Yield Coefficient = 0.127

Regression Output:
$\ln(X) = \mu m * t + \ln(X)$

	Combined	Biomass	Substrate
Interept (ln(Xo))	-1.494	-1.419	-1.569
Std Err of Y Est	0.113	0.129	0.062
R Squared	0.879	0.889	0.975
No. of Observations	8	4	4
Degrees of Freedom	6	2	2
Slope (μm, 1/hr)	0.00441	0.00430	0.00452
Std Err of Coef.	0.00067	0.00108	0.00052

Table I.14 Experimental data and regression output for Experiment 3 for 1.0% IRMCO 156 oil sample at pH = 7.0 with C as nutrient media. (Sept. 14 - Nov. 5, 1987)

Experimental Data:

Sampling Time(HRS)	pH	Temp. (°C)	Biomass (G/L)	COD (G/L)
0	7.0	30	0.098	6.391
40	7.1	30	0.222	5.995
88	7.2	30	0.802	5.093
161	7.2	30	0.474	5.456
256	7.5	30	1.250	4.587
375	7.3	30	0.514	4.422
494	7.1	30	0.664	4.422
615	7.4	30	0.490	4.422
831	7.0	30	0.708	4.114
1071	7.0	30	0.466	4.114
1215	7.1	30	0.508	4.114

Biomass Yield Coefficient = 0.542

Regression Output:
$\ln(X) = \mu m * t + \ln(X)$

	Combined	Biomass	Substrate
Intercept (ln(Xo))	-2.311	-2.373	-2.248
Std Err of Y Est	0.123	0.112	0.167
R Squared	0.987	0.994	0.987
No. of Observations	6	3	3
Degrees of Freedom	4	1	1
Slope (μm, 1/hr)	0.02386	0.02398	0.02375
Std Err of Coef.	0.00139	0.00180	0.00268

Table I.15 Experimental data and regression output for Experiment 3 for 1.0% IRMCO 156 oil sample at pH = 8.0 with C as nutrient media. (Sept. 14 - Nov. 5, 1987)

Experimental Data:

Sampling Time(HRS)	pH	Temp. (°C)	Biomass (G/L)	COD (G/L)
0	8.1	30	0.258	6.193
40	8.1	30	0.144	5.995
88	8.1	30	0.142	5.995
161	8.1	30	0.338	4.752
256	8.1	30	0.610	4.268
375	8.3	30	0.300	4.268
494	8.3	30	0.548	4.268
615	8.2	30	0.372	4.268
831	8.1	30	0.510	3.949
1071	8.1	30	0.730	4.114
1215	8.2	30	0.684	4.114

Biomass Yield Coefficient = 0.271

Regression Output:
$\ln(X) = \mu m * t + \ln(X)$

	Combined	Biomass	Substrate
Intercept (ln(Xo))	-2.544	-2.617	-2.471
Std Err of Y Est	0.265	0.190	0.474
R Squared	0.878	0.966	0.816
No. of Observations	6	3	3
Degrees of Freedom	4	1	1
Slope (μm, 1/hr)	0.00847	0.00856	0.00838
Std Err of Coef.	0.00157	0.00160	0.00398

Table I.16 Experimental data and regression output for Experiment 3 for 1.0% IRMCO 156 oil sample at pH = 9.0 with C as nutrient media. (Sept. 14 - Nov. 5, 1987)

Experimental Data:

Sampling Time(HRS)	pH	Temp. (°C)	Biomass (G/L)	COD (G/L)
0	9.0	30	0.186	6.193
40	9.0	30	0.216	5.995
88	9.0	30	0.286	5.269
161	9.0	30	0.300	5.456
256	9.0	30	1.052	4.422
375	9.1	30	0.566	4.422
494	9.1	30	1.292	4.422
615	9.1	30	0.930	4.422
831	9.0	30	0.660	4.268
1071	9.0	30	0.266	4.268
1215	9.0	30	0.338	4.268

Biomass Yield Coefficient = 0.108

Regression Output:
$\ln(X) = \mu m * t + \ln(X)$

	Combined	Biomass	Substrate
Intercept (ln(Xo))	-1.706	-1.699	-1.713
Std Err of Y Est	0.041	0.038	0.071
R Squared	0.966	0.985	0.950
No. of Observations	6	3	3
Degrees of Freedom	4	1	1
Slope (μm, 1/hr)	0.00493	0.00492	0.00495
Std Err of Coef.	0.00046	0.00060	0.00113

Table I.17 Experimental data and regression output for Experiment 4 for 0.5% IRMCO 141 oil sample at pH = 6.0 with B as nutrient media. (Nov. 17 - Dec. 16, 1987)

Experimental Data:

Sampling Time(HRS)	Initial pH	Final pH	Temp. (°C)	Biomass (G/L)	COD (G/L)
0	6.2	6.2	26	0.488	4.278
24	6.7	6.1	29	0.322	4.278
48	7.4	6.0	30	0.472	4.398
95	6.5	6.0	30	0.646	3.486
168	5.9	6.0	30	0.558	3.486
288	4.4	6.0	30	0.408	0.846
384	8.1	6.3	30	0.868	0.780
528	5.9	6.0	30	0.260	0.648
670	5.8	6.0	30	1.070	0.714

Biomass Yield Coefficient = 0.409

Regression Output:
$\ln(x) = \mu m * t + \ln(Xo)$

	Combined	Biomass	Substrate
Intercept (ln(Xo))	-1.432	-1.295	-1.568
Std Err of Y Est	0.207	0.118	0.322
R Squared	0.760	0.943	0.753
No. of Observations	6	3	3
Degrees of Freedom	4	1	1
Slope (μm, 1/hr)	0.01018	0.00937	0.01098
Std Err of Coef.	0.00286	0.00231	0.00630

Table I.18 Experimental data and regression output for Experiment 4 for 1.0% IRMCO 141 oil sample at pH = 6.0 with B as nutrient media. (Nov. 17 - Dec. 16, 1987)

Experimental Data:

Sampling Time(HRS)	Initial pH	Final pH	Temp. (°C)	Biomass (G/L)	COD (G/L)
0	6.1	6.1	26	0.276	8.294
24	6.3	6.3	29	0.394	8.063
48	7.4	6.0	30	0.756	6.912
95	6.1	6.1	30	0.742	6.789
168	5.8	6.0	30	1.160	5.632
288	3.7	6.0	30	1.164	2.343
384	7.9	6.0	30	2.184	1.551
528	6.3	6.3	30	2.827	1.309
670	5.9	6.0	30	1.005	1.188

Biomass Yield Coefficient = 0.347

Regression Output:
$\ln(X) = \mu m * t + \ln(Xo)$

	Combined	Biomass	Substrate
Intercept (ln(Xo))	-1.353	-1.337	-1.370
Std Err of Y Est	0.120	0.121	0.203
R Squared	0.946	0.972	0.925
No. of Observations	6	3	3
Degrees of Freedom	4	1	1
Slope (μm, 1/hr)	0.02099	0.02099	0.02099
Std Err of Coef.	0.00250	0.00356	0.00598

Table I.19 Experimental data and regression output for Experiment 4 for 2.0% IRMCO 141 oil sample at pH = 6.0 with B as nutrient media. (Nov. 17 - Dec. 16, 1987)

Experimental Data:

Sampling Time(HRS)	Initial pH	Final pH	Temp. (°C)	Biomass (G/L)	COD (G/L)
0	6.1	6.1	26	0.340	16.296
24	6.3	6.3	29	0.480	16.296
48	7.1	6.2	30	1.068	14.973
95	6.3	6.3	30	1.456	14.553
168	5.9	6.0	30	1.870	13.377
288	6.0	6.0	30	1.683	10.752
384	6.0	6.0	30	2.600	7.245
528	5.0	6.1	30	2.685	3.717
670	5.6	6.1	30	2.528	2.268

Biomass Yield Coefficient = 0.524

Regression Output:
$\ln(X) = \mu m * t + \ln(Xo)$

	Combined	Biomass	Substrate
Intercept (ln(Xo))	-0.891	-0.817	-0.965
Std Err of Y Est	0.328	0.351	0.391
R Squared	0.813	0.826	0.813
No. of Observations	10	5	5
Degrees of Freedom	8	3	3
Slope (μm, 1/hr)	0.01029	0.00997	0.01061
Std Err of Coef.	0.00174	0.00264	0.00294

Table I.20 Experimental data and regression output for Experiment 4 for 5.0% IRMCO 141 oil sample at pH = 6.0 with B as nutrient media. (Nov. 17 - Dec. 16, 1987)

Experimental Data:

Sampling Time(HRS)	Initial pH	Final pH	Temp. (°C)	Biomass (G/L)	COD (G/L)
0	6.0	6.0	26	0.552	39.576
24	6.2	6.2	29	0.712	39.576
48	6.3	6.3	30	1.328	35.343
95	6.8	6.3	30	1.902	38.454
168	6.2	6.2	30	3.194	39.576
288	6.1	6.1	30	3.237	26.928
384	8.1	6.0	30	3.753	28.713
528	4.3	6.1	30	3.222	24.429
670	3.8	6.1	30	3.780	20.502

Biomass Yield Coefficient = 0.183

Regression Output:
$\ln(X) = \mu m * t + \ln(Xo)$

	Combined	Biomass	Substrate
Intercept (ln(Xo))	-0.698	-0.656	-0.741
Std Err of Y Est	0.201	0.151	0.358
R Squared	0.826	0.944	0.750
No. of Observations	6	3	3
Degrees of Freedom	4	1	1
Slope (μm, 1/hr)	0.01829	0.01829	0.01829
Std Err of Coef.	0.00419	0.00444	0.01056

Table I.21 Experimental data and regression output for Experiment 4 for 0.5% IRMCO 141 oil sample at pH = 6.0 with A as nutrient media. (Nov. 17 - Dec. 16, 1987)

Experimental Data:

Sampling Time(HRS)	Initial pH	Final pH	Temp. (°C)	Biomass (G/L)	COD (G/L)
0	6.1	6.1	26	0.288	4.158
24	6.2	6.2	29	0.538	3.930
48	6.0	6.0	30	0.672	3.378
95	5.9	6.0	30	1.022	3.168
168	5.8	6.0	30	1.160	1.062
288	5.8	6.0	30	0.954	0.714
384	7.3	6.1	30	0.906	0.846
528	4.9	6.0	30	0.707	0.648
670	5.5	6.0	30	0.815	0.714

Biomass Yield Coefficient = 0.741

Regression Output:
$\ln(X) = \mu m * t + \ln(Xo)$

	Combined	Biomass	Substrate
Intercept (ln(Xo))	-1.089	-1.082	-1.097
Std Err of Y Est	0.188	0.183	0.266
R Squared	0.886	0.920	0.862
No. of Observations	8	4	4
Degrees of Freedom	6	2	2
Slope (μm, 1/hr)	0.01295	0.01250	0.01341
Std Err of Coef.	0.00189	0.00260	0.00379

Table I.22 Experimental data and regression output for Experiment 4 for 0.5% IRMCO 141 oil sample at pH = 6.0 with C as nutrient media. (Nov. 17 - Dec. 16, 1987)

Experimental Data:

Sampling Time(HRS)	Initial pH	Final pH	Temp. (°C)	Biomass (G/L)	COD (G/L)
0	6.2	6.2	26	0.178	4.158
24	6.4	6.1	29	0.294	4.044
48	6.3	6.3	30	0.384	3.168
95	6.2	6.2	30	0.384	3.378
168	6.2	6.2	30	0.640	2.874
288	6.4	6.3	30	0.574	2.778
384	6.3	6.3	30	1.234	1.430
528	6.3	6.3	30	2.077	1.309
670	6.0	6.0	30	0.441	1.309

Biomass Yield Coefficient = 0.208

Regression Output:
$\ln(X) = \mu m * t + \ln(Xo)$

	Combined	Biomass	Substrate
Intercept (ln(Xo))	-1.750	-1.687	-1.812
Std Err of Y Est	0.139	0.096	0.212
R Squared	0.884	0.970	0.868
No. of Observations	6	3	3
Degrees of Freedom	4	1	1
Slope (μm, 1/hr)	0.01602	0.01602	0.01602
Std Err of Coef.	0.00290	0.00282	0.00624

Table I.23 Experimental data and regression output for Experiment 4 for 0.5% IRMCO 156 oil sample at pH = 7.0 with B as nutrient media. (Nov. 17 - Dec. 16, 1987)

Experimental Data:

Sampling Time(HRS)	Initial pH	Final pH	Temp. (°C)	Biomass (G/L)	COD (G/L)
0	7.0	7.0	26	0.192	3.168
24	7.2	7.2	29	0.364	3.270
48	6.3	7.0	30	0.284	3.072
95	5.5	7.0	30	0.262	3.270
168	5.6	7.0	30	0.360	3.072
288	5.6	7.0	30	0.604	2.976
384	7.0	7.0	30	0.374	2.592
528	5.5	7.0	30	0.401	2.154
670	5.0	7.0	30	0.395	2.328

Biomass Yield Coefficient = 1.163

Regression Output:
$\ln(x) = \mu m * t + \ln(Xo)$

	Combined	Biomass	Substrate
Intercept (ln(Xo))	-1.674	-1.750	-1.598
Std Err of Y Est	0.143	0.001	0.255
R Squared	0.891	1.000	0.829
No. of Observations	6	3	3
Degrees of Freedom	4	1	1
Slope (μm, 1/hr)	0.00420	0.00433	0.00407
Std Err of Coef.	0.00073	0.00001	0.00185

Table I.24 Experimental data and regression output for Experiment 4 for 1.0% IRMCO 156 oil sample at pH = 7.0 with B as nutrient media. (Nov. 17 - Dec. 16, 1987)

Experimental Data:

Sampling Time(HRS)	Initial pH	Final pH	Temp. (°C)	Biomass (G/L)	COD (G/L)
0	7.1	7.1	26	0.238	5.456
24	7.2	7.2	29	0.306	5.456
48	6.5	7.0	30	0.336	5.093
95	5.5	7.0	30	0.324	5.456
168	5.6	7.0	30	0.452	5.632
288	5.7	7.1	30	0.454	5.093
384	7.6	7.0	30	0.414	5.632
528	7.4	7.0	30	0.535	3.795
670	5.0	7.3	30	0.347	3.949

Biomass Yield Coefficient = 0.270

Regression Output:
$\ln(X) = \mu m * t + \ln(Xo)$

	Combined	Biomass	Substrate
Intercept (ln(Xo))	-1.451	-1.409	-1.493
Std Err of Y Est	0.093	0.064	0.141
R Squared	0.775	0.935	0.750
No. of Observations	6	3	3
Degrees of Freedom	4	1	1
Slope (μm, 1/hr)	0.00718	0.00718	0.00718
Std Err of Coef.	0.00193	0.00190	0.00415

Table I.25 Experimental data and regression output for Experiment 4 for 2.0% IRMCO 156 oil sample at pH = 7.0 with B as nutrient media. (Nov. 17 - Dec. 16, 1987)

Experimental Data:

Sampling Time(HRS)	Initial pH	Final pH	Temp. (°C)	Biomass (G/L)	COD (G/L)
0	7.2	7.2	26	0.276	10.752
24	7.1	7.1	29	0.448	10.752
48	6.6	7.0	30	0.782	10.416
95	5.6	7.0	30	0.428	9.723
168	5.7	7.1	30	0.570	11.823
288	5.6	7.1	30	1.333	10.752
384	8.0	7.0	30	0.787	11.088
528	6.1	7.0	30	0.558	10.416
670	6.1	7.0	30	0.612	9.072

Biomass Yield Coefficient = 1.506

Regression Output:
$Ln(X) = \mu m * t + ln(Xo)$

	Combined	Biomass	Substrate
Intercept (ln(Xo))	-1.380	-1.299	-1.461
Std Err of Y Est	0.235	0.030	0.425
R Squared	0.831	0.998	0.750
No. of Observations	6	3	3
Degrees of Freedom	4	1	1
Slope (μm, 1/hr)	0.02170	0.02170	0.02170
Std Err of Coef.	0.00489	0.00087	0.01253

Table I.26 Experimental data and regression output for Experiment 4 for 5.0% IRMCO 156 oil sample at pH = 7.0 with B as nutrient media. (Nov. 17 - Dec. 16, 1987)

Experimental Data:

Sampling Time(HRS)	Initial pH	Final pH	Temp. (°C)	Biomass (G/L)	COD (G/L)
0	7.2	7.2	26	0.382	26.112
24	7.2	7.0	29	0.632	26.112
48	6.8	7.0	30	1.284	25.296
95	6.4	7.0	30	0.408	25.296
168	5.8	7.0	30	1.084	25.296
288	5.7	7.0	30	1.253	24.429
384	7.9	7.0	30	1.057	26.112
528	5.8	7.0	30	0.928	23.613
670	6.6	7.0	30	0.888	22.797

Biomass Yield Coefficient = 1.105

Regression Output:
$\ln(X) = \mu m * t + \ln(Xo)$

	Combined	Biomass	Substrate
Intercept (ln(Xo))	-1.080	-0.997	-1.164
Std Err of Y Est	0.271	0.084	0.495
R Squared	0.833	0.991	0.750
No. of Observations	6	3	3
Degrees of Freedom	4	1	1
Slope (μm, 1/hr)	0.02526	0.02526	0.02526
Std Err of Coef.	0.00565	0.00247	0.01458

Table I.27 Experimental data and regression output for Experiment 4 for 0.5% IRMCO 156 oil sample at pH = 7.0 with A as nutrient media. (Nov. 17 - Dec. 16, 1987)

Experimental Data:

Sampling Time(HRS)	Initial pH	Final pH	Temp. (°C)	Biomass (G/L)	COD (G/L)
0	7.1	7.1	26	0.278	2.874
24	6.2	7.0	29	0.350	2.778
48	6.5	7.0	30	0.540	2.412
95	5.8	7.0	30	0.544	2.874
168	5.5	7.0	30	0.696	2.874
288	5.6	7.1	30	0.510	2.874
384	7.6	7.0	30	0.574	3.072
528	5.8	7.0	30	0.311	2.874
670	6.3	7.0	30	0.485	2.778

Biomass Yield Coefficient = 0.567

Regression Output:
$\ln(X) = \mu m * t + \ln(Xo)$

	Combined	Biomass	Substrate
Intercept (ln(Xo))	-1.323	-1.314	-1.331
Std Err of Y Est	0.076	0.083	0.125
R Squared	0.950	0.970	0.934
No. of Observations	6	3	3
Degrees of Freedom	4	1	1
Slope (μm, 1/hr)	0.01383	0.01383	0.01383
Std Err of Coef.	0.00158	0.00245	0.00368

Table I.28 Experimental data and regression output for Experiment 4 for 0.5% IRMCO 156 oil sample at pH = 7.0 with C as nutrient media. (Nov. 17 - Dec. 16, 1987)

Experimental Data:

Sampling Time(HRS)	Initial pH	Final pH	Temp. (°C)	Biomass (G/L)	COD (G/L)
0	7.0	7.0	26	0.124	3.072
24	7.3	7.3	29	0.202	3.168
48	7.0	7.0	30	0.176	2.592
95	6.5	7.3	30	0.484	2.412
168	6.8	7.0	30	0.340	2.412
288	5.8	7.0	30	0.412	2.244
384	7.3	7.3	30	0.340	2.154
528	6.6	7.0	30	0.415	2.070
670	5.3	7.3	30	0.225	2.328

Biomass Yield Coefficient = 0.545

Regression Output:
$\ln(X) = \mu m * t + \ln(Xo)$

	Combined	Biomass	Substrate
Intercept (ln(Xo))	-2.227	-2.096	-2.357
Std Err of Y Est	0.420	0.246	0.662
R Squared	0.698	0.880	0.649
No. of Observations	8	4	4
Degrees of Freedom	6	2	2
Slope (μm, 1/hr)	0.01575	0.01339	0.01811
Std Err of Coef.	0.00423	0.00350	0.00943

APPENDIX II

CHROMATOGRAMS OF HPLC ANALYSIS

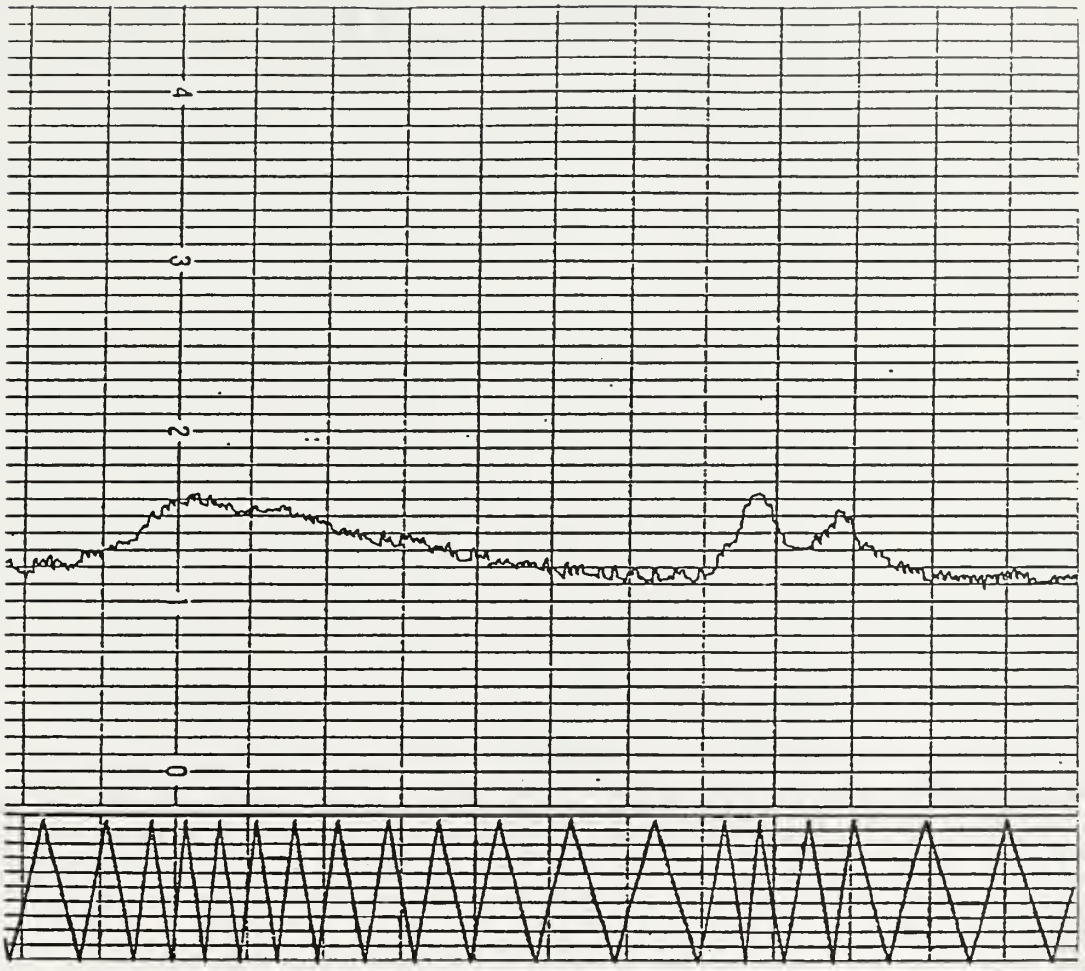

Figure II.1 Result of HPLC analysis before biodegradation for oil sample IRMCO 141 at initial pH of 7.3.

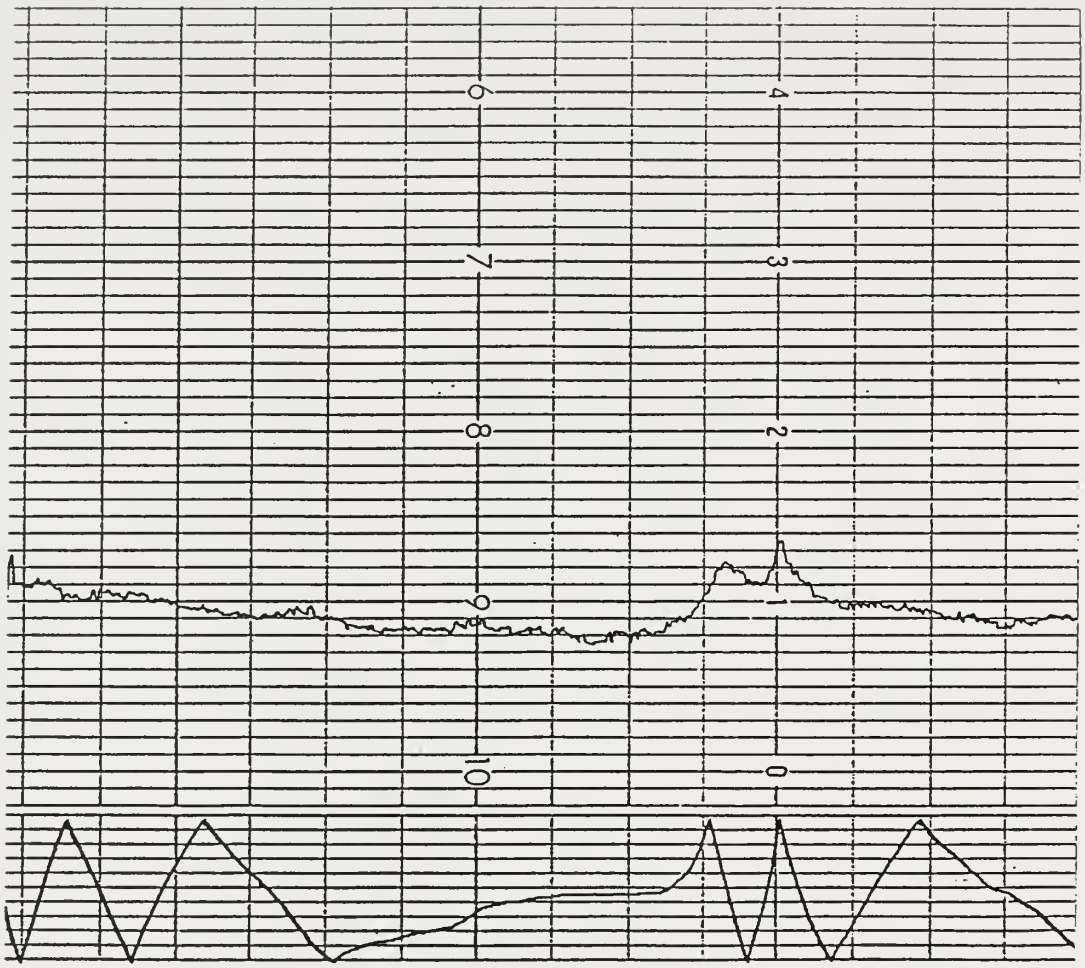

Figure II.2 Result of HPLC analysis after biodegradation for oil sample IRMCO 141 at initial pH of 7.3.

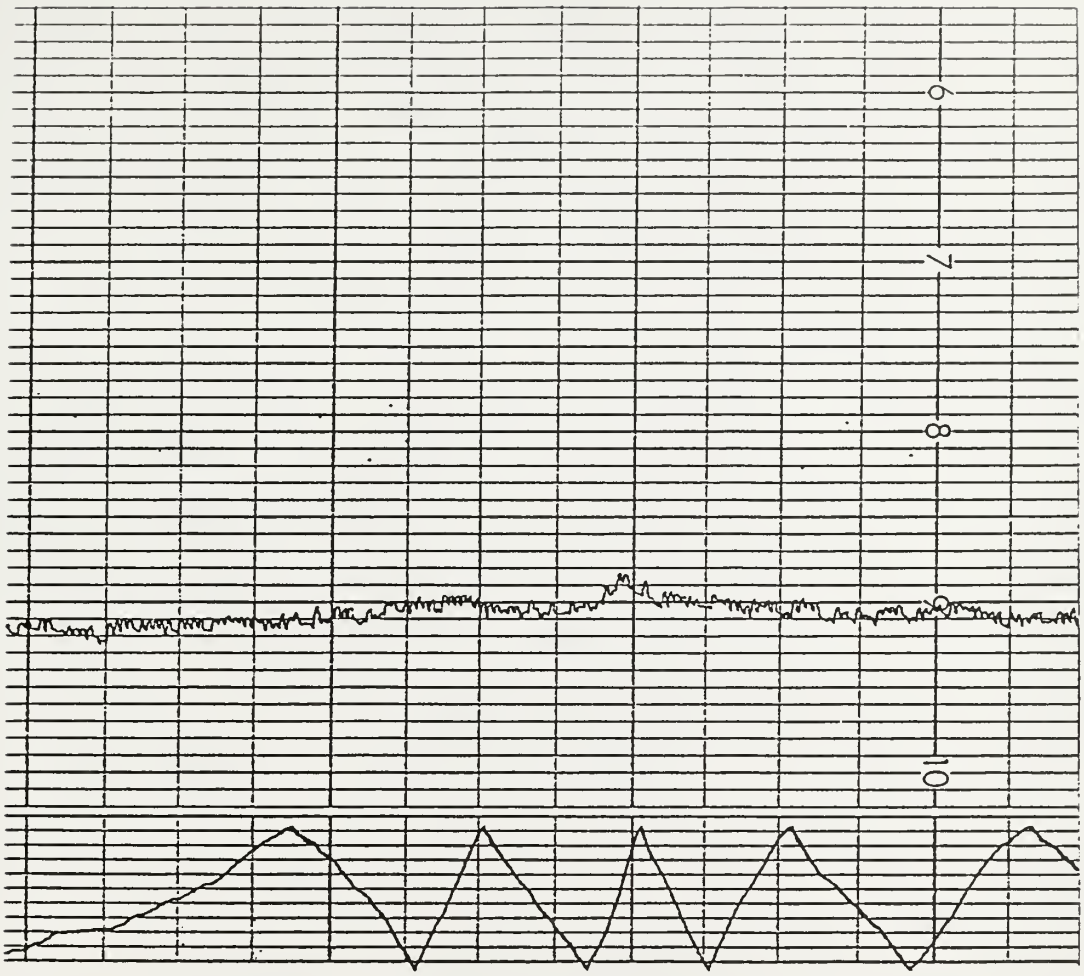

Figure II.3 Result of HPLC analysis before biodegradation for oil sample IRMCO 156 at initial pH of 7.7.

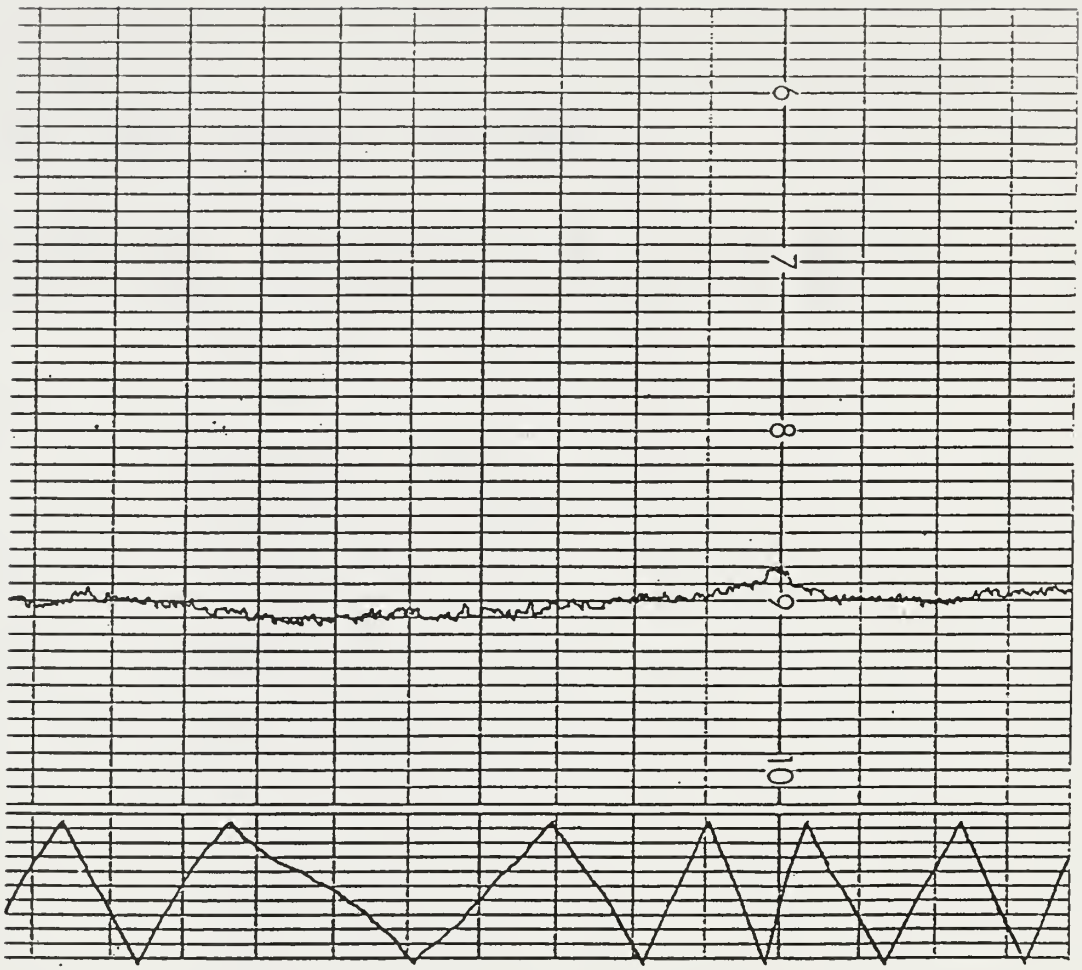

Figure II.4 Result of HPLC analysis after biodegradation for oil sample IRMCO 156 at initial pH of 7.7.

APPENDIX III

EXPERIMENTAL DATA ON MASS SPECTROMETER

Table III.1 Variation of the gas phase concentration of carbon dioxide and oxygen for IRMCO 141 at an initial oil concentration of 2.0%, 500 hours after the start of Experiment 4.

Time (hrs)	Mole Fraction	
	Oxygen	Carbon dioxide
0.000	0.163	0.003
3.000	0.141	0.025
18.000	0.045	0.110
24.000	0.023	0.132
40.000	0.002	0.149

Table III.2 Variation of the gas phase concentration of carbon dioxide and oxygen for IRMCO 141 at an initial oil concentration of 5.0%, 550 hours after the start of Experiment 4.

Time (hrs)	Mole Fraction Oxygen	Carbon dioxide
0.000	0.171	0.009
2.000	0.150	0.016
4.000	0.139	0.026
6.000	0.116	0.037
8.000	0.094	0.051
10.000	0.078	0.062
12.000	0.060	0.071
22.000	0.006	0.108

APPENDIX IV

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REFERENCES

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APPENDIX V

NOMENCLATURE

NOMENCLATURE

C	concentration (mol/L)
CPR	carbon dioxide production rate (mol/sec)
F	flow rate of gas (ml/hr)
H	Henry coefficient (mol/m^3 /Pa)
n	total moles of species 'i'
k_1a	volumetric mass transfer coefficient (1/sec)
OUR	oxygen uptake rate (mol/sec)
P	total pressure (Pascal)
R	gas law constant (Pa m^3 K)
RQ	respiratory quotient
S	substrate concentration (mol/L)
t	time (sec, hr)
T	temperature (K)
V	volume (m^3)
x	mole fraction
X	biomass concentration (g/L)
μ	specific growth rate (1/hr)
μm	maximum specific growth rate constant (1/hr)
η	biomass energetic yield
γ	reductance degree
Y	biomass yield
Z	biomass concentration based on substrate

Subscripts and Superscripts

g	refers to the gas phase
l	refers to the liquid phase
*	refers to equilibrium value
i	refers to species 'i'
o	refers to the initial condition
tot	total
b	biomass
s	substrate

BIODEGRADATION OF SYNTHETIC COMPONENTS IN METALWORKING FLUIDS

BY

AYUSH GUPTA

B.Tech. Indian Institute of Technology, 1986
New Delhi, India

AN ABSTRACT OF MASTER'S THESIS

submitted in partial fulfillment of the
requirements for the degree

MASTER OF SCIENCE

Department of Chemical Engineering

KANSAS STATE UNIVERSITY
Manhattan, Kansas

1988

ABSTRACT

With the emergence of synthetic and semisynthetic fluids in the metalworking industries, there is great concern whether the waste is compatible with the existing wastewater treatment technology. Chemical emulsions by design are required to serve for extended periods of time in the metalworking industry, and various preservatives are added to retard microbial contamination. This results in a two fold problem. Firstly, most of the preservatives are xenobiotic and are hard to degrade. Secondly, they prevent microbial action that is necessary in the biological treatment of the waste fluids.

An experimental study was undertaken to evaluate the effect of pH, adaptation, oil concentration and inorganic nutrient media composition on the biodegradability of two synthetic metalworking fluid samples viz. IRMCO 141 and IRMCO 156 which contain hexahydro-1,3,5 tris(2 hydroxy ethyl)-s-triazine as the preservative. Experiments were carried out in batch operation with 500 ml Erlenmeyer flasks. The inoculum was of a mixed origin obtained from the waste water treatment plant, and the degradation studies were carried out for about 30 days.

The value of pH appears to be an extremely important parameter in altering the effectiveness of the preservative and enhancing the biological growth. The optimum values of pH are identified as 6.0 and 7.0 for biodegradation of IRMCO 141 and IRMCO 156, respectively. The optimum initial concentration of metalworking fluid was found to be about 1% from the experiments conducted. Urea as a supplement to the inorganic nutrient media performed consistently well in both the samples.