BIOLOGICAL DEGRADATION OF THE PHENOXY ACID HERBICIDE 2,4-DICHLOROPHENOXYACETIC ACID (2,4-D)

By

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A Thesis

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ABSTRACT

A bench-scale study was performed to investigate the biodegradation potential of a phenoxy acid herbicide (2,4-dichlorophenoxyacetic acid or 2,4-D) under various experimental conditions. This study included two main phases. During the first phase, the effect of hydraulic retention time (HRT) and the presence or absence of supplemental substrate was explored using sequencing batch reactors (SBRs). In the second phase, batch reactors were used to investigate the effect of temperature, pH, and seed type on 2,4-D degradation.

In the beginning, two identical two-liter liquid volume SBR units were employed. SBR 1 was fed with a mixture of phenol and 2,4-D and SBR 2 with a mixture of dextrose and 2,4-D. A long acclimation period (over 80 days) was observed in both SBR 1 and SBR 2 before the first signs of 2,4-D degradation were recorded. In order to achieve a sustainable 2,4-D degradation pattern, it was found that, the initial substrate concentration, biomass concentration, and HRT have to be carefully selected.

After achieving steady state operation, an additional SBR unit was introduced (SBR 3) identical to the other two with 2,4-D as the sole source of carbon and energy. All three systems were able to degrade more than 95% of the 2,4-D concentrations applied at an HRT range of 12 to 48 hrs. Even though the use of supplemental substrate resulted in lower 2,4-D-specific removal rates, better system stability in terms of good settling characteristics and superior floc

formation was achieved in the corresponding reactors. Furthermore, it was found that throughout the SBR phase, the dextrose-fed system (SBR 2) experienced a 42 % lower 2,4-D specific removal rate relative to the phenol-fed system (SBR 1). Generally, supplemental substrate removal was completed before the onset of 2,4-D degradation. However, at high supplemental substrate concentrations, there was evidence of concurrent utilization of the 2,4-D and the corresponding supplemental substrate. No detectable abiotic losses were observed during the study.

The batch experiments were conducted using conical flasks with an active liquid volume of 100 mL. It was observed that pH control was more critical at temperatures higher than ambient (22°C) and less critical at 10 °C. The increase in temperature from 10 to 30°C resulted in a dramatic increase of 2,4-D-specific-removal rates in all reactors. Regarding the use of different inocula, 2,4-D degraders were able to accelerate remarkably the degradation process as compare to mixed cultures. In contrast to the generally observed pattern of sequential substrate utilization, a clear pattern of concurrent substrate utilization occurred at 10°C using mix culture and at 22°C using 2,4-D degrading cultures.

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This Thesis is dedicated to my father Mr. Harminder Singh Mangat and my mother Gurnam Kaur Mangat

Chapter 1

INTRODUCTION

Pesticides have introduced certain benefits as well as certain costs to modern society. The balance of costs and benefits is largely a function of the existing social, economic, and environmental conditions. The use of pesticides involves management of many risks which may vary from one situation to another. Such risks include the negative impact of pesticides on public health and the ecological system. Like in most nations, Canadian agriculture's drive to boost productivity has resulted in the widespread usage of chemicals. The Canadian Prairie region is an important agricultural area. Due to the large quantities used, area affected, and potential hazards, pesticides must be ranked high in priority for the development of improved waste disposal practices. As part of the national environmental improvement strategies, Canadian regulatory agencies have been actively involved in developing plans for reducing the negative impact of pesticide use. One step in this direction has been the creation of a pest management promotion office, which sets targets and guidelines for pesticide use. This office also coordinates research, implementation, and education on ecologically sound pest management strategies (Agriculture Canada, 1990).

A serious concern is pesticide pollution due to wastewater discharges from formulation units or manufacturing plants, surface runoff, leaching, and other point sources and non-point sources. The non-target contamination by

agrochemicals, such as surface and ground water contamination, has raised public attention towards their potentially destructive nature and has elicited considerable research on developing technologies to remove these toxic chemicals from the environment.

One of the most common agrochemicals used is 2,4-dichlorophenoxyacetic acid (2,4-D), which belongs to the phenoxy herbicide group. Due to widespread usage, this group of herbicides has been at the forefront of pesticide-related research for several decades. 2,4-D has been widely used to control broad-leaved weeds in both terrestrial and aquatic ecosystems (NRCC, 1978; Ware, 1989). In Canada, 2,4-D was first registered for use to control the aquatic weed Myriophyllum spicatum (Eurasian milfoil) (NRCC, 1978). Historically, 2,4-D was a major component (about 50%) of the product Agent Orange used extensively during the Vietnam war. The association of 2,4-D with Agent Orange, had also prompted a considerable attention towards it.

2,4-D contamination of aquatic systems via several routes such as aerial drift, surface runoff, and leaching has been detected in various parts of Canada (Currie and Williamson, 1995; Elefsiniotis and Mangat, 1996; Fisheries and Oceans, 1992). For instance, a study performed in 1977 revealed that up to 30 μg/L 2,4-D was detected in the Red River in Winnipeg, Manitoba. This was largely attributed to urban runoff (Chacko and Gummer, 1980). Overall, 2,4-D has been frequently detected in most water bodies in Manitoba mainly as a result of surface runoff from target areas, such as lawns in urban areas and agricultural

fields. In this province, another very common source of 2,4-D contamination is liquid waste from empty pesticide containers. Annual empty container collection and processing results in concentrated waste of 2,4-D among other pesticides (Yee and Omiuck, 1989). 2,4-D degradation in terrestrial environments may be rapid, whereas prevalent conditions in aquatic systems may not favor such an accelerated degradation.

An economical and effective method is needed to dispose of such a waste on a continuous basis. To detoxify the waste, the compounds of concern must be altered by any combination of physical, chemical, or biological processes. Although commonly practiced in Manitoba, concentration of pesticide waste and incineration are not considered to be effective alternatives. Concentration affords a slurry of highly concentrated waste, while during incineration some pesticides or their byproducts are merely displaced from the liquid- to the vapor-phase. Physico-chemical methods, such as activated carbon adsorption, incineration, and radiation treatment, are usually expensive, and require sophisticated equipment and trained personnel. However, biodegradation has been a very promising option. In this case, unlike the adsorption processes, there is no secondary waste produced which requires disposal. In addition, biodegradation usually results in harmless final products.

A number of research reports have indicated the potential for 2,4-D biodegradation by both mixed and pure cultures under aerobic conditions (Hill et al., 1986; Orhon et al., 1989; Steenson and Walker, 1956). The application of a continuously-running system for 2,4-D-contaminated wastewater has not been

explored in detail in the past. There is limited information regarding the degradation of 2,4-D in commonly used biological treatment processes such as activated sludge or trickling filters. Removal of 2,4-D was poor (about 25%) in an activated sludge unit treating municipal waste water (Hill et al., 1986). It is apparent that there is lack of information regarding the degradation of concentrated pesticide liquid wastes, particularly in simple systems such as sequencing batch reactors (SBR).

The purpose of this study was to investigate the biodegradation behavior of 2,4-D liquid waste. Emphasis was laid on the applicability of SBRs for continuous treatment operation. This study also focused on the impact of an operational parameter, namely hydraulic retention time (HRT) and various environmental parameters (i.e. presence or absence of supplemental substrate, temperature, pH, and seed type) on the biodegradation potential of mixed microbial cultures.

Chapter 2

LITERATURE REVIEW

2.1 Pesticide Behavior in the Environment

In order to effectively plan protection and remediation of both ground- and surface-water resources, knowledge of transport and fate of pesticides in terrestrial subsurface environments is essential. This plan includes: 1) prediction of the time of arrival and concentration of the released contaminant at a receptor; 2) installation of a monitoring system; and 3) design of safe and cost effective strategies for remediation of the waste (EPA, 1993). To attain the first goal of prediction of movement, knowledge of various aspects of translocation is required.

The tendency of anthropogenic chemicals to dissipate from their target area and thus contaminate natural systems, is a very common scenario. Runoff, drift, and leaching play a significant role during pesticide mobility. Generally, the persistence of a compound in the environment is an inverse function of the potential for degradation via physical, chemical, biological, and photocatalytic processes. Typically, loss of a chemical from the target area in natural systems is biphasic; a short period of rapid dissipation is followed by a longer period of contaminant release (Beck et al., 1995).

2.1.1 Translocation

Translocation involves the carrying away of a molecule between air, surface and ground water, organisms, aquatic sediments, and soil without changing its molecular structure. The mobility of pesticides in the aquatic/terrestrial environment has been well documented in the literature (Krawchuck and Webster, 1987; Maguire, 1991; Beck et al. , 1992). Parameters such as solubility in water (C_w), soil sorption coefficient (K_d), octanol-water partitioning (K_{ow}), and bioconcentration factor (BCF) are essential in assessing the potential for mobility and bioconcentration of released pesticides (Wood et al., 1984). Apart from the above mentioned parameters, agricultural practices may play a crucial role in pesticide mobility in the soil. For example, an Ontario study revealed a 63% variation in atrazine concentration in surface runoff as a result of using different tillage practices (Gaynor et al., 1995). Dissipation of pesticides in the soil, water, and air is influenced by volatilization, movement into/within the aquatic system, and partition into the solid phase.

Volatilization involves transportation of pesticides from the solid or aqueous phase to the vapor phase and redeposition (by the reverse mechanism). This phenomenon is more pronounced around surfaces exposed to the atmosphere and can result in long-range transport and redeposition. Some of the physical and chemical factors which effect dissolution into and volatilization from the aqueous phase are vapor pressure, solubility, temperature, turbulence within the two phases, concentration and its profile with depth, Henry's Law constant, and diffusion coefficient. In field conditions, several of these interacting

factors may affect the particular pesticide behavior. Simulation of these conditions in the laboratory may not be possible. Generally, laboratory measurements of such data may not reflect the true pesticide behavior in the environment. However, pesticides with high vapor pressure and low water solubility, are more susceptible to volatilize. Figure 2.1 illustrates the relationship between vapor pressure and concentration (Sudo and Kunimatsu, 1992). It can be clearly seen that high vapor pressure results in low pesticide concentration in the soil. Generally, pesticides exhibiting vapor pressure greater than 10^{-5} mm Hg at 25°C volatilize easily (CCME, 1994). Similarly, atrazine, one of the top five pesticides used in Canada, has a Henry's law constant of 2.48×10^{-9} atm m^3/mol, which suggests that volatilization does not play a major role on atrazine dissipation (Soloman et al., 1996).

Movement within the aquatic system of a soluble pesticide is governed by the rates of diffusion within the water body and by the flow characteristics of water itself (convection or advection phenomena). Molecular diffusion is a function of the concentration gradient, and it is usually slower in surface water than in groundwater or sediment interstitial spaces. This process can be described by Fick's first law (CCME, 1994):

$$F = -D \frac{dc}{dl} \qquad [2.1]$$

Where: F = mass flux of chemical ($M/L^2 T$)

D = diffusion coefficient (L^2/T)

dc/dl = concentration gradient

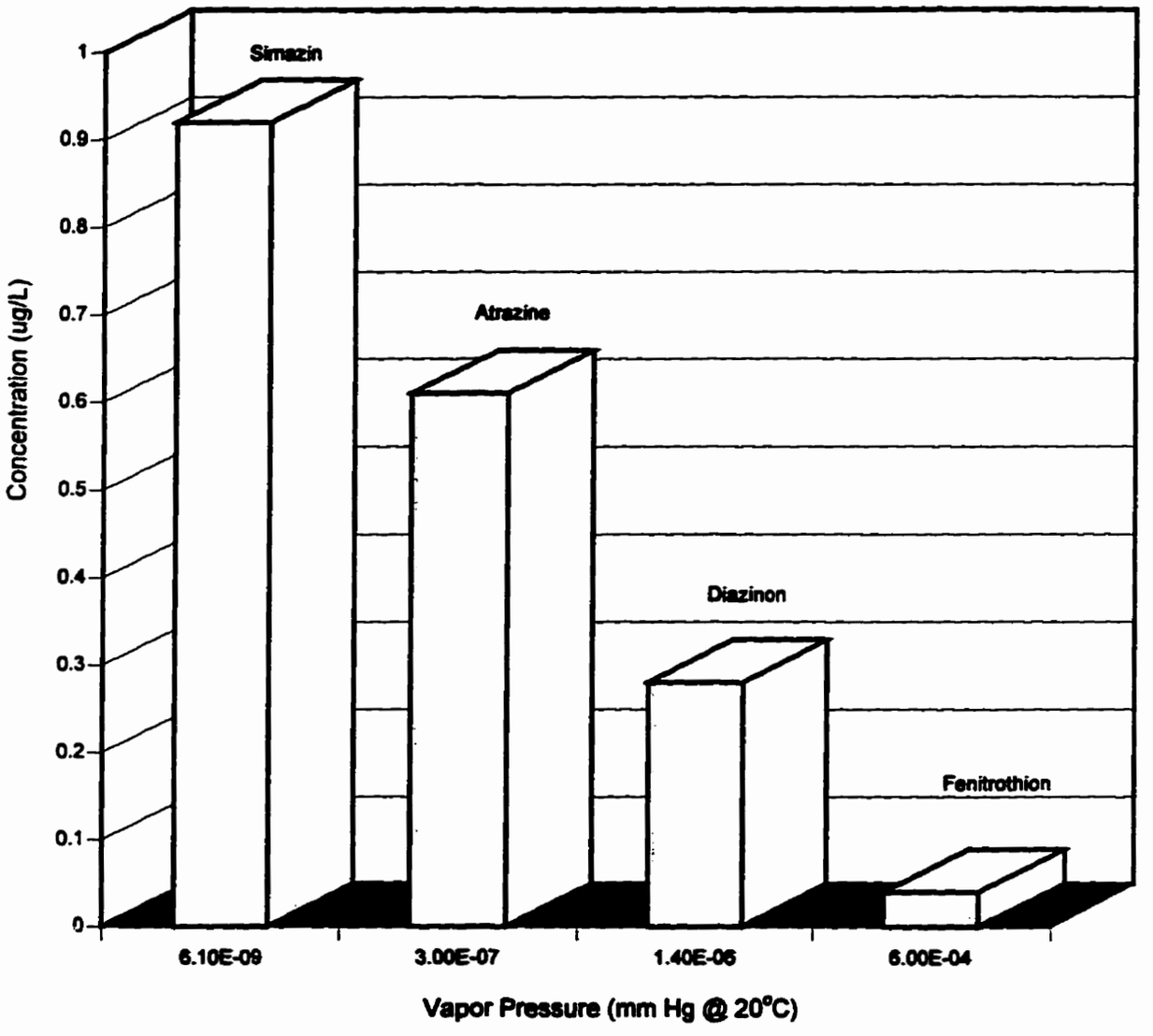


Figure 2.1 Relationship Between Vapor Pressure and Concentration of Variety of Pesticides in the Soil

Transport of a soluble compound throughout a water system, such as lakes or rivers, is governed by thermal, gravitational, or nearly frictionless flows, as well as by turbulence caused by nonhomogeneities at the boundaries of the aqueous phase. In rivers, eddy dispersion, created by friction between the water and the bottom of the river, is a significant factor for the movement of contaminants (Larson and Weber, 1994).

Pesticides with water solubility greater than 10 mg/L move primarily in the aqueous phase, while less soluble compounds move mainly by sorption to eroding soil particles (Wauchope, 1978). There are exceptions, however, such as the herbicide paraquat which, despite its high solubility in water, can be readily taken up by hydrophilic sorption in clays due to strong electrostatic interactions (Larson and Weber, 1994).

Partition into the solid phase is a function of **sorption**, or the transfer of molecules from solution into the solid phase such as soil or sediments. The reverse process is termed desorption. The relationship between the uptake of a compound and the partition coefficient, K_p, (defined as the ratio of concentration or activity between an organic solvent and a particular substance) largely determines its environmental behavior. Compounds with high K_{ow} values (octanol/water partition coefficient) are readily adsorbed by natural sediments. Hoffman and Matheson (1987) reported that compounds with $K_{ow}>10$ do not significantly adsorb. For soil and sediments with varied organic matter content, the concept of K_{oc} (organic carbon partition coefficient) has been introduced, which assumes that only organic carbon is active in the sorption

process. Data for many herbicides have confirmed the applicability of this concept. For instance, the K_{oc} values for atrazine, ranging from 54 to 258, signify that atrazine is moderately to highly mobile (Soloman et al., 1996). This may explain the frequent occurrence of atrazine in surface and subsurface water in Quebec and Ontario (Fisher et al., 1995; Maguire and Tkacz, 1993; Muir et al., 1987).

Parameters	Value /Range
Water solubility	Greater than 10 µg/L
K_d(Sorption Coefficient)	Less than 10 mL/g
K_{oc}	Less than 50 mL/g
Henry's law constant	Less than 10^{-3} atm m^3 / mol
Hydrolysis half-life	Greater than 175 days
Photolysis half-life	Greater than 7 days
Field-dissipation half-life	Greater than 30 days

Table 2.1 Properties of Pesticides Related to Water Contamination Potential

Table 2.1 summarizes the physical and chemical properties of pesticides and the potential for surface and subsurface water contamination. Deviations from the values included enhance the potential for contamination (CCME, 1994; Smith and Tillotson, 1992).

Recent studies have attempted to correlate the occurrence of pesticides with other water quality parameters such as salinity and nitrate-

nitrogen concentration. In the Prairie Region, several lakes with low salinity levels exhibited high concentrations of commonly used pesticides, as illustrated in Figure 2.2 (Donald and Syrgiannis, 1995). In Atlantic Canada, there was found to be a tendency for wells with pesticides to have elevated nitrate-nitrogen concentrations (Briggins and Moerman, 1995).

2. 1. 2 Fate of Pesticides

In general there are six processes that can influence the fate of chemical compounds applied to the soil system and sediment system: 1) solubilization; 2) sorption by soil mineral and organic matter; 3) degradation by soil microorganisms; 4) photodecomposition; 5) volatalization; and 6) Plant uptake. Pesticides may be subjected to three chemical reactions in natural or controlled environments. These mechanisms are hydrolysis, reduction, and oxidation. It is generally recognized that reductive processes are more prominent than oxidative processes in transforming, degrading, and mineralizing many environmental contaminants.

Hydrolysis involves reaction of an organic compounds with water resulting in the formation of a new covalent bond with OH^- and cleavage of the covalent bond with X (the leaving group).

$$RX+H_2O \longrightarrow ROH + X^- + H^+ \qquad [2.2]$$

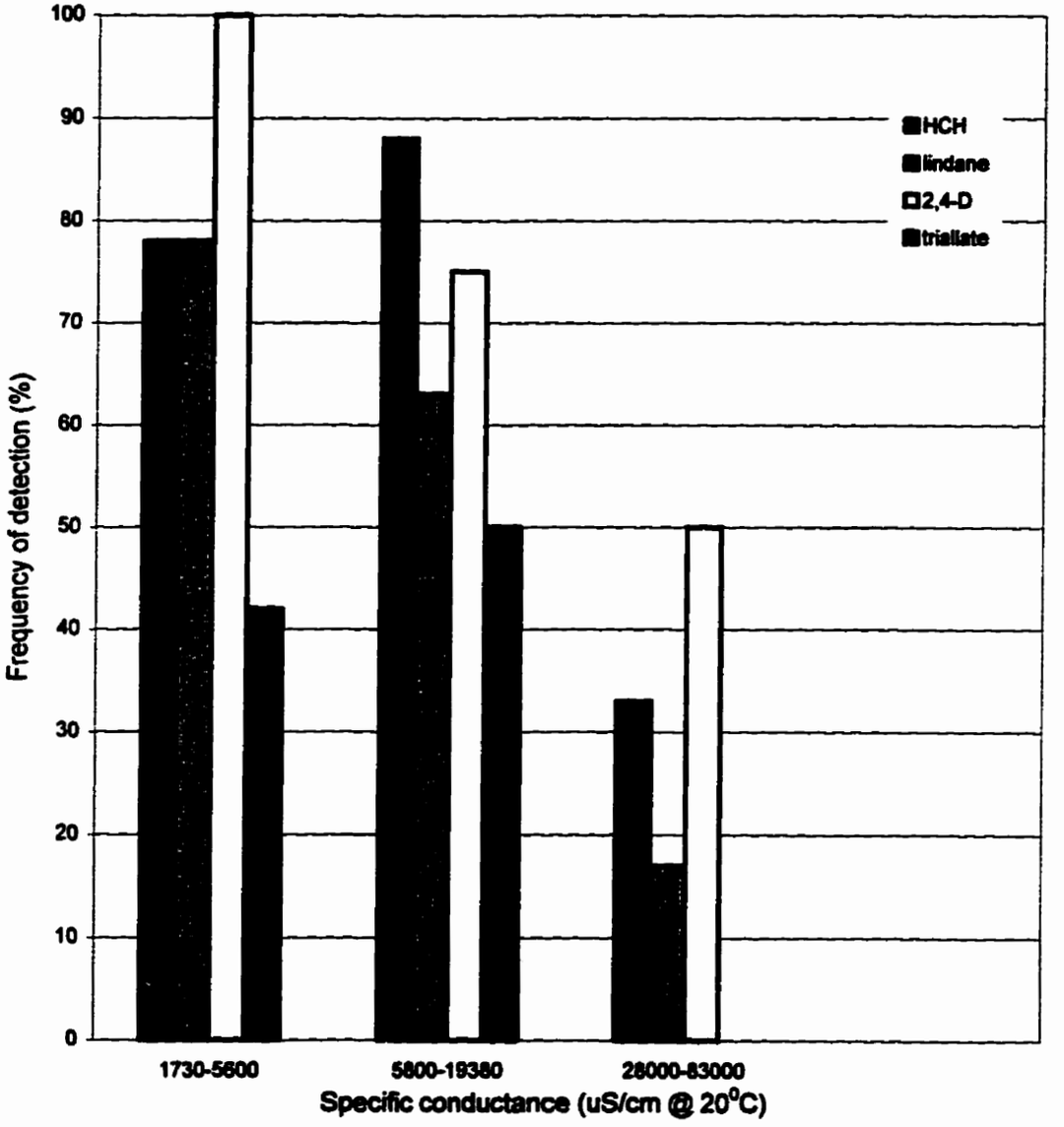


Figure 2. 2 Salinity vs. Frequency of Detection of Pesticides (Donald and Syrgiannis, 1995)

This process is prevalent in rivers, streams, lakes, oceans, biological treatment systems, groundwater systems, and the aqueous microenvironment associated with soils and sediments (Larson and Weber, 1994).

Reduction is defined as a gain of electrons. There is transfer of electrons from an electron donor (reductant) to an electron acceptor (oxidant). The oxidant is usually a pesticide. The source of electrons in this case is not oxygen but abiotic reduced compounds reagents such as hydrogen sulfide (H_2S), minerals, reducing agents such as iron porphyrins, and microbial coenzymes. Reduction may occur by a combination of abiotic and biotic processes. Abiotic processes usually occur at a constant rate, whereas, biotic processes display a lag period. Natural organic matter also exhibits a crucial role in reduction processes. Some common pesticide reduction reactions in natural environments are dehalogenation to alkanes, nitro reduction to the corresponding amine, and sulfone reduction to sulfoxide or sulfide (Webster, 1994).

Oxidation encompasses loss of electrons. There are various sources of oxidants. The availability of a particular source depends on the environmental conditions. Hydroxyl radical ($OH^{\bullet}$) is a prominent oxidant in the gas phase or during combustion, but not in soils. Oxygen is present near the surface of water bodies. Combustion / incineration are thermal oxidation processes frequently applied to dispose pesticide contaminated wastes. High pressure and elevated temperature in the presence of oxygen accomplish significant destruction of pesticide wastes.

2. 1. 2. A Physical and Chemical Parameters Affecting Reactions in Natural Waters

The intrinsic reactivity of the labile functional group of a specific pesticide is the first factor that should be considered when studying transformations of pesticides in natural waters. The reaction rate constant is a function of this reactivity. In fact, the rate constant often describes the velocity of the rate-determining step of the reaction in which bond cleavage or bond formation take place. The activity of the most important species influence the transformation of pesticides. The major environmental parameters which affect these transformations are briefly described in the following paragraphs.

The effect of pH is of paramount importance in transformation kinetics. Other parameters such as buffering and general acid-base catalysis are also critical, since hydronium ion (H_3O^+) and hydroxide ion (OH^-) greatly affect transformation kinetics. Hydrogen ion activity affects transformation kinetics in two distinct ways. The most common is in acid-base-mediated hydrolysis reactions. Hydrolysis-susceptible functional groups such as epoxide, organophosphorus esters, carboxylic acid esters, carbamates, and urea undergo hydrolysis via different mechanisms depending upon acidic, basic, or neutral conditions. A typical base-catalyzed rate equation is given below (EPA,1989) :

$$(-dp/dt) = k\,(P)\,(OH^-) \qquad [2.3]$$

Where: k = second order rate constant (1/mol. sec)

P = pesticide concentration (mg/L)

OH^- = hydroxide ion concentration (mol/L)

Acid-catalyzed reaction processes would be expected to accelerate in sediment-water environments at pH values above the zero point of charge (ZPC). For example, the methyl ester of 2,4-D has been observed to hydrolyze faster in a sediment-water environment at a pH value above the expected ZPC for sediment-organic matter environment (NRCC, 1978).

Metal ions can effect the hydrolysis mechanisms. For example, the hydrolysis of pesticide chlorpyrifos is enhanced in the presence of Ca^{+2}, Mg^{+2}, Fe^{+2},Cu^{+2}. Similarly, parathion hydrolysis is greatly effected by the presence of Ca^{+2}, Cu^{+2} (Webster, 1994).

Organic matter in soil systems may also play an important role in the transformation of pesticides. Organic matter may exist in the form of polymeric, singly-bound to soil, or dissolved and suspended particulates. Soil-bound organic matter significantly enhances the solubility of some aromatic hydrocarbons and quinones by charge-transfer interactions (Larson and Weber, 1994). Stevenson (1972) has pointed out that the soil humus may serve as a sink for intermediate products of 2,4-D decomposition. Due to negligible concentration of humic acid in natural waters, dissolved and suspended organic matter mainly affect abiotic transformations of pesticides (Chiou et al., 1986; Khan, 1974). For example, the presence of fulvic acid has been shown to accelerate the hydrolysis of atrazine in water (Chiou et al. , 1986). Also, Perdue and Wolfe (1982) observed that dissolved organic matter initiated the basic hydrolysis of the octyl ester of 2,4-D.

Often, the rate of pesticide transformation increases with increasing temperature. The effect of the temperature on the rate is described by the Arrhenius equation:

$$k=Ae^{(-E_a/RT)} \qquad [2.4]$$

Where: E_a = activation energy (cal/k.mol)

R = gas constant (cal/k.mol)

T = temperature (^{0}K)

A = preexponential factor constant

The redox state in the water column of deep lakes and reservoirs is likely to vary with depth and may be defined in terms of the hypolimnion and the epilimnion (Linsley, et al., 1982). In the hypolimnion, the water is nearly anoxic and reducing conditions exist. In the epilimnion, O_2 is present and aerobic conditions prevail.

2. 1. 2. B Photo-decomposition

Photochemical transformations play a significant role in the degradation of pesticides in the air, water, soil, and other surfaces exposed to the atmosphere. A pesticide can undergo direct or indirect photo-transformation reactions. Direct photo-transformation involves absorption of energy by the molecule which eventually reaches an excited state. There are two mutually

exclusive possibilities for the compound at this excited state: chemical transformation or deactivation to the initial state. On the other hand, three types of indirect photo-transformation may occur: 1) sensitized; 2) photo-induced (where a molecule other than the target organic compound absorbs light and transfer its energy to the target species); 3) photosensitized (where additional photo-initiated generation of free radicals in solution takes place). Degradation of the herbicide heptachlor in the presence of acetone at 300 nm is a representative example of indirect photo-degradation (Webster, 1994).

Often the mechanism of photo-incorporation (where pesticides permanently incorporate into soil or organic matter) affects the environmental behavior of pesticides. Particularly, several polychlorobenzenes have been found to incorporate into humic monomers such as phenol, benzoic acid,, vanillic acid, and syringic acid (Choudhry and Webster, 1985).

2. 1. 2. C Microbial Degradation

Biological processes have long formed the basis for the treatment of wastewater. A principle of microbial infallibility as expounded in the late nineteenth century, says that "whatever man makes microbes can degrade" (Zitrides, 1983). Decades of continuous growth of information in the fields of microbial genetics, biochemistry and molecular biology have expanded the potential for applications in the environmental engineering realm.

There are indications that the natural construction of a multi-step degradation pathway occurs through plasmid transfer (i.e. have the required genes encoded for degrading the selected compound). Usually plasmids that do not confer a selective advantage are lost in a non selective environment over certain time period (i.e., lag period) (Boyle, 1989). The time period involved in such natural gene modifications can be termed as acclimatization period. Acclimatization of microbial cultures to various xenobiotics usually includes resumption of resting cells to growing cells and synthesis of the enzymes required for biodegradation. Generally, the initial biomass concentration (specialized in target compound degradation) is directly related to degradation potential. Rittman (1987) demonstrated that bacterial mass and substrate utilization declined to negligible quantities at low substrate concentrations and insufficient enzyme synthesis. The effect of low biomass/substrate (glucose) concentration on acclimation has been also shown by Chudoba (1990).

Basically, four processes are involved in the microbial transformation of pesticides (Richards and Shieh, 1986) :

1. Mineralization or Metabolism, in which pesticides serve as substrate for growth;
2. Cometabolism, in which pesticides are transformed by metabolic reactions where growth is sustained using unrelated substrate (co-metabolite);
3. Polymerization or conjugation, in which pesticide molecules are linked together or with naturally-occurring compounds;

4. Accumulation, in which pesticides are accumulated in or on the body of the microorganisms.

Complete mineralization of a particular compound is a growth-related process. Breakdown of a compound results in assimilation of some of the substrate carbon. This assimilated carbon, along with energy, can be used for biosynthesis and formation of cellular components. The requirements of carbon for the growth of active biomass dictates the need for complete or partial substrate breakdown. This type of conversion can be toxic to some organisms and non-toxic to others (Oh and Tuovinen, 1991).

The aromatic structure of a chemical has a profound effect on biodegradability. Specifically, the aromatic ring cleavage mechanism depends upon the type, size, number, and position of the subsequent groups already present in the molecule. These characteristics determine the recalcitrant nature of pesticides (Boyle, 1989). Monosubstituted benzene rings are almost always hydroxylated at the ortho and para positions (Ghosal et al. , 1985). Woodcock et al. (1957) have studied the influence of halogenated substituents on microbial hydroxylation of the aromatic ring. It is mainly restricted to para hydroxylation. As the number of halogen atoms increases, the interference on the ring cleavage increases as well. For example, the herbicide 2,4,5-T is much more resistant to microbial attack due to the extra chlorine atom as opposse to 2,4-D, which has only two chlorine atoms at the ortho and para positions (Yadav and Reddy, 1993). Similarly, it has been observed that the presence of nitro and amino

substituents on the ring render the compound more recalcitrant to microbial attack (Balajee and Mahadevan, 1990).

Halogenated pesticides may undergo various reactions such as dehydrohalogenation, reductive dehydrohalogenation, and halogen migration with the help of microbial enzymes. A classic example of halogen migration of 2,4-D has been observed in plants (Webster, 1994).

Biodegradable compounds can be grouped into two categories according to the amount of energy captured by bacteria during biotransformation (primary and secondary substrates). In one scenario, cells may grow by consuming the primary substrate, while the secondary substrate supplies no or negligible energy for cell synthesis; consequently, cell growth is not feasible when only the secondary substrate is transformed. A secondary substrate totally incapable of supplying energy or carbon for cell growth is termed cometabolite (Alexander, 1981; Saez and Rittmann, 1991). Generally a mosaic of catabolic genes affects the biodegradability of a specific compound. The competition between the growth substrate and the cometabolic substrate for a specific enzyme may significantly deter the degradation rates (Chang and Cohen, 1995). In cases where the natural substrate structure has similarities with the introduced compound, the existing enzymes may be employed to breakdown the introduced compound. Under such conditions, the kinetic rates can be enhanced by increasing enzyme production. On the contrary, novel enzyme activities may be derived from preexisting genes modification (Ghosal et al. , 1985).

Some of the important physical, chemical, and biological factors that affect biodegradability are grouped in Table 2. 2 (Melcer and Bridle, 1985). The role of some of these parameters on biodegradation of a substance becomes significant when the substance of interest occurs at low concentrations in the medium (Richards and Shieh, 1986).

Physical Properties	Chemical Properties	Biological Properties
Molecular Weight	pH range	Enzyme Specificity
Polymerization	Temperature	Habitat Selection
Aromaticity	Dissolved Carbon	Plasmid Encoding
Halogen Grouping	Solubility	Competition
Xenobiotic Origin	Dissolved Oxygen	Inhibition
	Nutrients	Population density
		Toxicity

Table 2. 2 Some Physical, Chemical, and Biological Properties Effecting Biodegradation (adapted from Richards and Shieh, 1986)

2. 2 2,4-Dichlorophenoxyacetic Acid (2,4 - D) Overview

The herbicide 2,4-D belongs to the phenoxy acid herbicide group. In addition to 2,4-D, this group includes 2,4,5-trichlorophenoxyacetic acid (2,4,5-T), 2,4-dichlorophenoxypropionic acid (dichlorprop), 2-(2methyl-4chloro) phenoxypropionic acid (mecoprop), and [4-(chloro-o-tolyl)oxy]-acetic acid (MCPA). Chemically they are phenoxy carboxylic acids with various substituents. As growth regulators, these herbicides have been very effective for weed eradication, which has resulted in a sharp increase of formulations available. 2,4-

D usage is particularly high in the prairie region. According to 1987 to 1989 data it has been the number one pesticide used in this region with an approximate application of 3000,000 kg active ingredient per annum (Agriculture Manitoba, 1991).

2. 2. 1 Physical Chemical Properties of 2,4-D

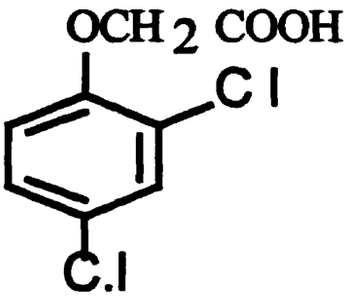


Figure 2.3 Structure of 2,4-D Molecule

The 2,4-D is a white crystalline salt. It possesses an aromatic ring to which are attached two chlorine atoms at the ortho and para positions as shown in Figure 2.3. This herbicide behaves as an organic anion in aqueous solutions. The solubility of 2,4-D in water is 0.9 g/L (Verschueren, 1983). The low solubility in water of the parent compound can be altered by using appropriate salt forms. For example, the sodium, potassium, and amine salts of 2,4-D are highly soluble in water. However, there is a controversy about the low solubility of calcium and magnesium salts in water. 2,4-D is far more soluble in other liquid solvents such as ethanol, ether, and acetone. The solubility values in these three solvents are 13 g/,L 8.5 g/L, and 12.7 g/L, respectively. The melting point of 2,4-D is 140.5°C. A vapor pressure of $8 \cdot 10^{-6}$ mm Hg suggests a low volatilization potential (CCME, 1994). The polarity of a compound is directly related to the sorption into solid surfaces, which also dictates the mobility in the environment and the bioconcentration potential. 2,4-D with a K_{ow} (octanol/water) value in the 2.81-

20.0 range possesses no major bioconcentration threat. On the other hand, DDT with a K_{ow} of about 10^6 exhibits a very high bioconcentration potential (WHO, 1984). The K_{ow} and C_w (aqueous solubility) are closely related by the following equation:

$$\ln K_{ow} = 7.494 - \ln C_w \qquad [2.5]$$

Synthesis of 2,4-D commonly involves the reaction of phenol with chloroacetic acid in an alkaline aqueous medium at a 10-12 pH range and temperature of about 105°C. Another method includes chlorination of phenoxyacetic acid or its esters (NRCC, 1978).

2. 2. 2 Mode of Action

The effect of phenoxy acid herbicides on broadleaf plants is auxin- (a growth hormone) like. 2,4-D acts as growth regulator by increasing cell division and RNA synthesis. This causes uncontrolled and abnormal growth responses. This abnormal growth disrupts normal plant functions and eventually plant death occurs (Ware, 1989).

2. 2. 3 Environmental Fate of 2,4-D

2,4-D may reach both surface and subsurface water by several routes including runoff, erosion, leaching, spray drift, improper disposal of containers,

and accidental spills. The mobility (translocation) and fate after its release is briefly outlined below. Fate studies explore a variety of interrelated factors such as the degradation of a compound or its persistence, and formation of potentially harmful metabolites that may be destructive to the ecological system. Low degradation potential can result in high persistence values for a given compound. For example, Yadav and Reddy (1993) have demonstrated that low pH and temperature deterred the degradation potential of 2,4-D, which resulted in longer persistence. In addition, 2,4-D degradation biproducts can also exhibit toxicological properties in the environment. The production of the antibiotic protoanemonin during 2,4-D breakdown by one of the bacterial species is a typical example (Young, Personal Communication). As far as degradation is concerned it may occur via photochemical, chemical, and biological processes, or any combination of these.

2. 2. 3. A Environmental Properties

The persistence of 2,4-D in the environment depends upon concentration, formulation, soil-water content, pH, temperature, application frequency, type of microbial flora available in the system, and other factors (Stott et al., 1983). Movement of 2,4-D to non target areas can occur through several routes such as spray drift during application, post application losses due to leaching, and surface runoff. In one study, a combination of ester formulation and aerial spray drift resulted in 60% losses of the applied pesticide from the target area (NRCC,1978). The drift losses can be minimized with suitable

additives, mechanical adjustments, and favorable meteorological factors including wind velocity, relative humidity, and temperature. A number of studies have indicated low evaporative losses of various 2,4-D formulations from different surfaces (Anderson et al. , 1952; Vernetti and Freed, 1963). The phenomenon of adsorption / desorption directly or indirectly influences herbicide availability, mobility, and degradation. The acidic nature of phenoxy acid herbicides is due to the carboxyl group, which ionizes readily in aqueous solutions. In the pH range of 4 to 9, adsorption onto the negatively charged soil particles is not extensive. Usually, 2,4-D adsorption is correlated to the soil organic matter content and has no correlation to soil clay content (Hamaker et al., 1966; Grover, 1973; Liu and Cibes-Viade, 1973). The relative strength of adsorption of 2,4-D onto different media (surfaces) is in following order (Grover and Smith, 1974):

Activated charcoal > Anion exchange resin > Peat Moss > Cellulose triacetate > Cellulose powder > Wheat Straw > Silica Gel > Montmorillonite

Another important parameter i.e. diffusion can be qualitatively defined as the movement of a herbicide from an area of higher concentration to an area of lower concentration. The coefficient of diffusion can be correlated to soil organic matter content adsorption, pore size, and temperature. The value of diffusion coefficient for 2,4-D in a variety of soils has been calculated in the range of 0.6×10^{-6} to 4.3×10^{-6} cm^2 / s (NRCC, 1978).

2,4-D, along with its group member MCPA, are placed in class 4 (relatively mobile) chemical list. Surfactants greatly increase the 2,4-D mobility in soil. High 2,4-D movement was observed in sandy and mineral soils with high organic content and clay soils (NRCC, 1978). This study also reported that 2,4-D moved only 5 to 8 cm depth in the soil. Acid "formulations" are known to move deeper relative to other formulations (Young, 1984). 2,4-D has been included in the leaching compound list compiled by EPA. This information is supported by studies in Canada which have also indicated leaching potential (NRCC, 1978).

In general, ester formulations have a higher runoff potential than amine salts (Barnett et al. , 1967). However, in soil systems, ester and amine forms are converted to acids, which subsequently degrade. In aquatic systems rapid hydrolysis can lead to photochemical degradation. Although, the 2,4-D detection frequency is high in the prairie region, the concentrations detected are often very low. Several recent comprehensive studies have found a 20-30% detection frequency for 2,4-D in the prairie region (Currie and Williamson, 1995). These detection frequencies were mainly due to the runoff during the spring snow melt off season (Muir and Grifth, 1987). Overall, during the last 10 years, the concentrations detected in various rivers have been in the range of 0.03 - 7.7 μg/L, whereas, the Canadian fresh water guideline limit is 4 μg/L (Beck et al., 1992; Currie and Williamson, 1995; CWG,1995; Muir and Grifth, 1987; Therrien-Richards and Williamson, 1987). A summary of maximum detection concentrations in Manitoba surface-waters is presented in Table 2.3.

Another concentrated source of 2,4-D liquid waste is empty pesticide containers (Chapter 1). A study has revealed that 2,4-D and MCPA have been detected at higher concentrations in container residues and rinsates in comparison to other pesticides commonly used in Manitoba (Table 2.4, Yee and Omiucke,1989) .

Location	Max. Concentration (μg/L)	Reference
Boyne River	0.26	(Beck et al., 1992)
Ochre River	0.45	(Muir & Grift, 1987)
Red River	0.20	(Unpub. Data)
Stephenfield Reservoir	0.97	(Beck et al., 1992)
La Salle and Assiniboine Rivers	3.98	(Therrien-Richards& Williamson, 1987)

Table 2.3 Summary of Maximum Detection Concentrations of 2,4-D in Manitoba

2. 2. 3. B Toxicological Properties

2,4-D can be classified as a moderately toxic compound on the basis of its lethal dose (LD_{50}) value, which is in the 100 to 1000 mg/kg for various animals (Verschueren, 1983). The toxicity of chlorophenoxy compounds is generally complicated due to the contamination with polychlorodibenzo-p-dioxin (PCDD). For example, herbicide 2,4,5-T (due its high levels of dioxin

contamination) has been completely phased-out in Canada . If impurities are kept aside, the issue of environmental impact of phenoxy herbicides does not vary appreciably from compound to compound. Ester, alkali, and amine salt formulations are generally more susceptible to PCDD contamination than the pure acid form. Trace amounts of PCDD have been found in ester and amine products, however, 2,4-D "technical grade acids" are usually free from PCDD. According to a study in Canada, out of 26 amine samples tested, 8 were positive for PCDD in the range of 5 to 500 ppb. All but 1 of 21 ester samples were also PCDD positive (EPA, 1987).

According to the National Library of Medicine (1992) there have been over 200 independent studies that link 2,4-D with various diseases. This general-use chemical is known to produce skin and eye irritation problems. Prolonged breathing of 2,4-D causes coughing, burning, dizziness, and temporary loss of muscle coordination. The 2,4- D is readily absorbed through the skin and lungs in mammals and is excreted through urine. There have been indications of reproductive problems with moderate exposures. However, in a mutagenic effect study, 2,4-D cultured with human cells showed a potential for mutational changes (Schlop et al., 1990). Several studies have linked 2,4-D exposure to cancer in humans (Hoar et al., 1986; Zahm, et al., 1990).

The Veterans Legal Services Project of Washington D.C. reviewed 285 published studies of human exposure to phenoxy acid herbicides and dioxins. It was found that these compounds caused specific cancers, skin and liver disorders, reproductive and developmental disorders, low sperm counts,

spontaneous abortions in the wives of the affected individuals, increased birth defects affecting the skin, nerves, heart, and kidneys. The study concluded that there was "sound scientific evidence" of an association between the 2-4,D and a whole host of other diseases including cancer of the kidneys, testicles, stomach, colon, prostate, liver, as well as psychological effects, immune system disorders, gastrointestinal ulcers, and altered lipid metabolism (EPA, 1994).

Pesticide (Common name)	Metal Container Maximum Conc. (mg/L)	Plastic Container Maximum Conc. (mg/L)	Leachate (mg/L)		
			C[1]	RP[2]	UP[3]
2,4-D/MCPA	11,361	225	1.0	5.0	51
Poast	33	61	<1.0	<1.0	
Roundup	10	10	<1.0	<1.0	9.0
Treflan	164	19	<1.0	<1.0	<1.0
Avadex	620	10	<1.0	<1.0	<1.0
Hoegrass	1527	10	<1.0	<1.0	<1.0
Banvel	78	42	2.0	1.0	1.0
Avenge	650	88	<1.0	1.0	2.0
Buctril	1358	251	<1.0	<1.0	<1.0

[1]Cardbord [2]Rinsed Plastic [3]Unrinsed Plastic

Table 2.4 Maximum Pesticide Concentrations Detected During Container Collection and Processing Operation in Manitoba

(Adapted from Yee and Omiucke, 1989)

2. 2. 4 Degradation

Once a particular pesticide is released into the environment several physical, chemical, and biological factors can influence its behavior (CCME, 1994). As mentioned earlier the phenoxy herbicides are frequently formulated as amine/alkali metal salt or ester forms which undergo rapid chemical transformation to the corresponding acid, which facilitates the process of microbial degradation (Smith, 1972). Like other pesticides, 2,4-D is also amenable to photochemical decomposition. A brief description of chemical, photochemical, and biological degradation processes is given below.

2. 2. 4. A Chemical Degradation

Although phenoxy herbicides have been known to degrade predominantly via biological processes in natural systems, two main chemical reactions have also been recognized. These are hydrolysis of ester into respective acids and amine salts. Hydrolysis reactions have been shown to be dependent upon biotic factors (presence of aquatic life), temperature, and pH of the water body. In soil environments, apart from pH and temperature, soil moisture has also a profound effect on chemical degradation (NRCC, 1978).

2. 2. 4. B Photochemical Degradation

In order to generate photoinduced transformation of the phenoxy herbicides, 280-290 nm ultraviolet radiation (UV) wavelengths are required.

Sunlight falling on the earth's surface is composed of wavelengths greater than 280 nm, which facilitate the naturally-occurring photochemical degradation of phenoxy acids. Typical photolysis pathways for 2,4-D are shown in Figure 2. 4 (NRCC, 1978).

The number of chlorine atoms on the molecule affects the photochemical breakdown of a compound. Substitution at the ortho and meta positions renders the chemical more photochemically reactive than at the para position (Boule et al., 1982). Important factors in photodecomposition are solubility of herbicides in the plant cuticle, moisture content, nature of the reaction surface, and phytotoxicity of transformation products (Crosby, 1976). Photolysis rates for 2,4-D were observed to be enhanced with the addition of adjuvants (ingredients that improve the properties of a pesticide formulation). In addition, in the aqueous environment pH has an important effect on 2,4-D photochemical degradation. At alkaline pH conditions (pH 9), enhanced photodegradation has been observed (NRCC, 1978).

Photosensitized degradation of 2,4-D is an important phenomenon (Webster, 1994). Riboflavin has been observed to be an effective sensitizer for the photocatalytic breakdown of 2,4-D in aqueous solutions (Hansen and Buchholtz, 1952). It was also found that the rate of 2,4-D-methyl ester photolysis was increased in the presence of various surfactants (Hautala, 1978).

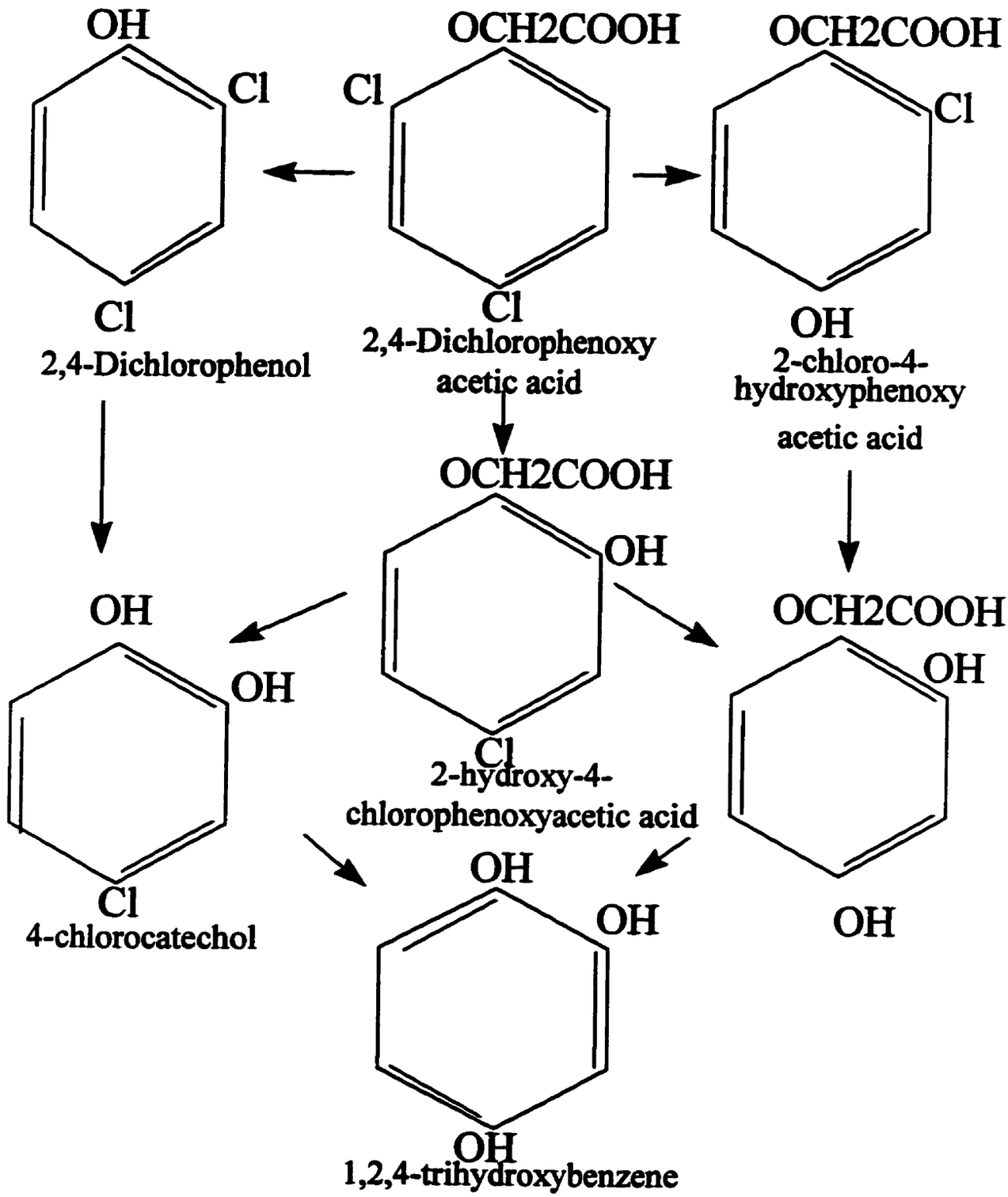


Figure 2. 4 Photolysis of 2,4-D (Adapted from Crosby and Tutass, 1966)

2. 2. 4.C Microbial degradation

Since the first evidence of microbial degradation of 2,4-D was conclusively demonstrated by Audus (1964), a number of studies have further explored this subject. Degradation studies were mainly conducted in batch-type experiments. The focus of these studies was to investigate the degradation behavior under different environmental conditions (such as dissolved oxygen, temperature), type of microbial species, and cross adaptation (biomass adapted on one carbon source employed to utilize other carbon sources). Generally 2,4-D degradation is fast and effective under aerobic conditions, whereas, there is limited success under anaerobic conditions (NRCC, 1978; Liu et al., 1981) Dissolved oxygen below 1mg/L may be rate-limiting for the aerobic biodegradation of 2,4-D or any other aromatic compound. It has been observed that oxygen concentration above 2 mg/L did not change 2,4-D degradation rates (Shaler and Klecka, 1986).. Temperature has also a profound effect on the 2,4-D degradation. A maximum specific growth rate of bacterial species on 2,4-D was reported at a temperature of 25^{0}C (Tyler and Finn, 1974). Also, Smith et al.(1994) have demonstrated that 20-30^{0}C range is an optimum temperature range for 2,4-D degradation. The same authors reported that at 35 ^{0}C the rate of breakdown was 40-50% lower than that of optimum value. However, during a compost study, 2,4-D has been shown to be mineralized by microbial consortia even at 55°C(Fogarty and Tuovinen, 1991).

The depression in bacterial ability to degrade 2,4-D at higher

temperatures (Smith et al, 1994) can be explained on the basis that specific bacterial species were used in this study. Some 2,4-D-degrading bacterial enzymes are heat-labile in nature, which lose their activity at high temperatures (Loos et al.,1967).

Usually, microbes adapted to growth on one phenoxy acid herbicide can degrade a number of other phenoxy acid herbicides (Kilpi, 1980). Microbiological degradation studies using pure cultures have shown that several microbial species are capable of degrading 2,4-D after proper acclimation. Representative species are *Pseudomonas, Arthrobacter, Flavobacterium, Achromobacter,* Actinomycetes (Evans et al., 1971; Greer et al., 1990).

On the other hand, numerous studies have explored in detail microbiological and biological aspects of 2,4-D degradation using mixed cultures. As a result, a plethora of information exists on degradation pathways, specific enzyme involvement, and type of bacterial species involved in the degradation process. It should be noted that a series of interacting parameters such as sequential, synergistic, and cometabolic transformations play a significant role in the degradation of xenobiotic compounds. It has been demonstrated that mixed cultures are more robust than pure cultures towards breakdown of complex organic compounds. For example, when a mixture of 2,4-D and 2,4,5-T was exposed to two specific strains (Pseudomonas cepacia and Alcaligenes eutrophus) in a liquid medium, these strains were unable to completely degrade the compounds. However, in a separate experimental when mixture of several species was employed to degrade a mixture of both of the above herbicides, a

complete degradation was achieved. The inability of two specific strains to degrade a herbicide mixture was attributed to the accumulation of intermediate breakdown products in the liquid medium (Haughland et al., 1990; Lappin et al., 1985). In addition, continuously-operating and mixed-culture studies provide a more realistic approximation of the real field conditions.

Generally, when 2,4-D is subject to biodegradation, after an initial lag period, an accelerated degradation has been observed (Steenson and Walker, 1956). The acclimation period required, is partly attributed to the lack of an adequate population of specialized 2,4-D degraders. Greer et al (1990) demonstrated that the lag time prior to the onset of 2,4-D degradation and the total time required for degradation were linearly related to the starting degrader population density and initial 2,4-D concentration. Similarly, in another study, under similar operating conditions, increasing concentrations of 2,4-D prolonged the acclimation period (Orhan et al. ,1989). Direct correlation of concentration and length of lag phase is also evident in a study by Parker and Doxtader (1982), using fine sandy loam soil. The threshold values at which further measurable degradation is not possible is above the 5000 mg/L range (Yadav and Reddy, 1993). Several batch studies have indicated an optimum concentration of around 500 mg/L (Clarkson et al., 1993; Kilpi, 1980).

In a microbial consortium, biodegradation is successful when an adequate amount of enzymes capable of metabolizing the target chemicals are naturally synthesized. A study has indicated that such enzyme production persisted as long as there was availability of 2,4-D in the system. Such capability

of 2,4-D breakdown existed even after one year of non exposure to the chemical (Smith et al., 1994). However, genetic tools can be employed to artificially introduce such enzyme-producing genes into non-degrading species and hence transforming them into 2,4-D degraders (Haugland et al., 1990). On the other hand, natural spontaneous mutations also result in the formation of genes required for new enzyme production (Lackmann et al., 1980).

The previously mentioned concept of biphasic degradation (Section 2.1) has also been observed in one of the 2,4-D degradation studies. This study involved a 2,4-D concentration of 5 mg/L in malt extract medium. Minimal percent mineralization was experienced until the 6th day and followed by a gradual percent increase between day 6 and 30, and a peak afterwards. This behavior was mainly attributed to metabolic switch from the breakdown of the target compound to that of intermediates.

Often, the concurrent presence of two substrates in a system may alter the overall biodegradation pattern (Oh and Tuovinen, 1991). The presence of cosubstrates may affect the extent of the degradation as well as the degradation rates, or may inhibit degradation of the target substrate. Microbial consortia that can metabolize two particular compounds separately have often been shown to poorly degrade a mixture of these compounds. This could be attributed to misrouting of the two types of compounds in catabolic pathways and such activity leads to the formation of toxic and dead-end intermediates and ultimately to the death of the organisms (Haugland et al., 1990). Conversely, additional substrates may enhance cell growth or adaptability for the target

compound in mixed culture conditions. Both scenarios, shown in Figure 2.5, could be the result of changes in NAD(P)H and oxygenase enzyme synthesis or/and use (Chang and Cohen, 1995).On the other hand, two or more substrates present in a culture may be sequentially utilized. The second substrate can be degraded only after the depletion of the preferred compound (McTernan and Periera, 1991).

For a mixed culture grown on 2,4-D and glucose, it was observed that although both substrates were used concurrently, the presence of one substrate had a detrimental effect on the rate of utilization of the other substrate (Papanastasiou, 1982). An interesting observation on the specificity of enzymes for a particular compound has been cited by Oh and Tuovinen (1992). During this study, biomass grown on 2,4-D could not degrade methylchlorophenoxy propionoic acid (MCPP). Whereas, biomass grown on MCPP successfully removed 2,4-D and partially degraded MCPP in mixed substrate conditions. On the other hand, due to structural analogies, a culture grown in the presence of sodium acetate exhibited higher 2,4-D removal rates than these grown in its absence (McTernan and Pereira, 1991). In support of the above observation, the presence of yeast extract facilitated the complete degradation of 2,4-D (Oh and Tuvinen, 1991). Regarding the effect of compounds having a simple molecular structure on MCPA (structurally related to 2,4-D) degradation, it was observed that repeated exposure to glucose extended the acclimation time (Kilpi, 1980). This suggests that either the cells grown on glucose had to induce the enzymes

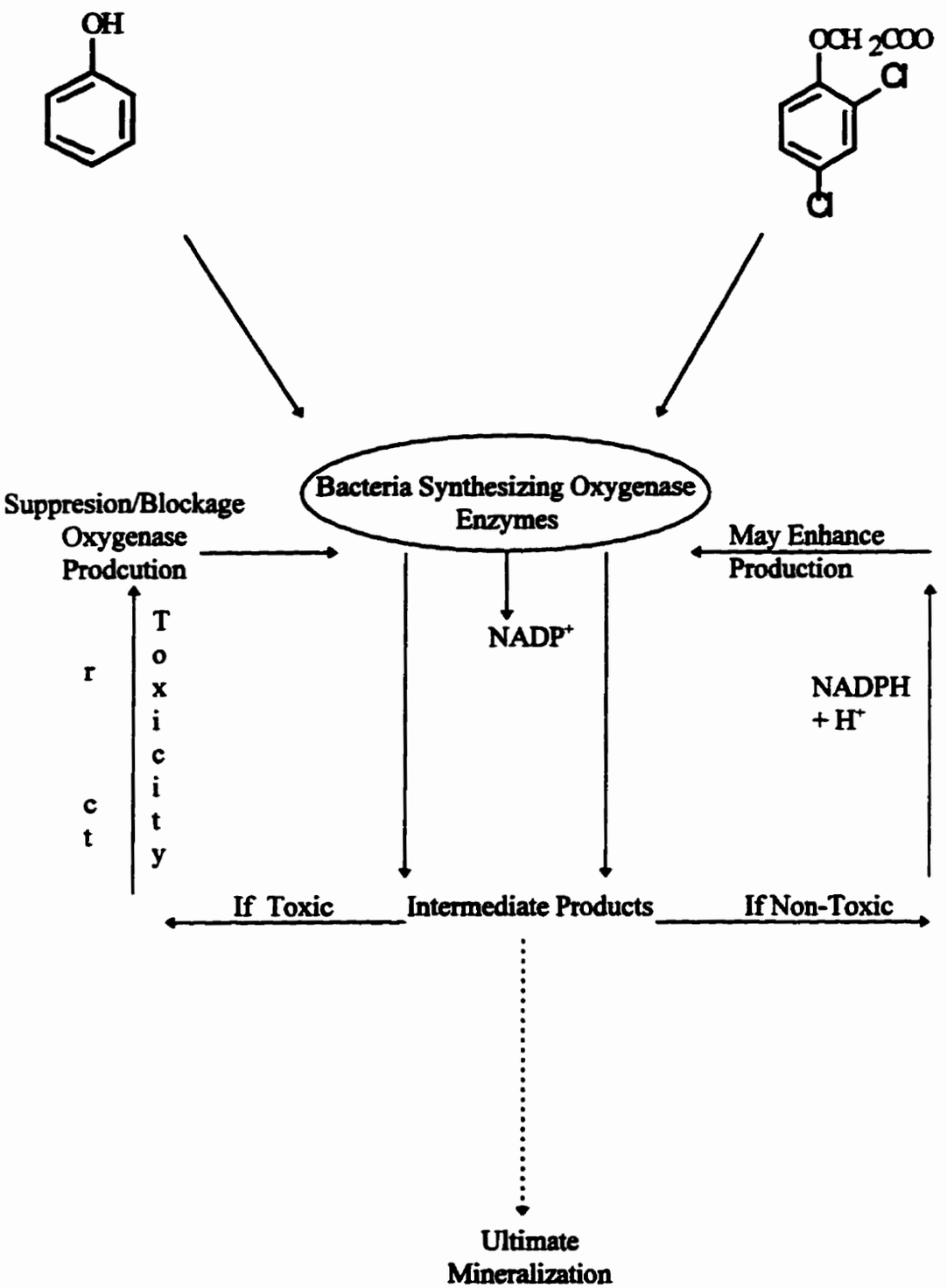


Figure 2. 5 Substrate and Cosubstrate Suppression/Enhancement Mechanism (adapted from Chang and Cohen, 1995)

needed for growth on MCPA or there had been a change in the relative amounts of bacterial strains in the populations.

As stated earlier, the simplicity or the complexity of the chemical structure has a profound effect on degradation (Alexender, 1981). The presence of chlorine atoms at ortho and para positions in 2,4-D is not known to inhibit the breakdown potential. Studies have indicated the existence of an ether-oxygen aliphatic side-chain cleavage mechanism in Arthobacter species to form glyoxylate. Figure 2.6 elucidates that the released glyoxylate undergoes condensation with decarboxylation and incorporation of ammonia to form alanine.

The 2,4-D breakdown pathways are species dependent. Pseudomonas, Alcaligenes, Flavobacterium, and Arthrobacter species transform 2,4-D into 2,4-dichlorophenol (2,4-DCP) with the application of the enzyme alphaketoglutarate dioxygenase. Supporting this information, Hausinger and Fukumori (1995) observed that in Alcaligenes eutrophus 2,4-D monoxygenase was not the first enzyme but dioxygenase was the one which initiated the process. This is a hydroxylation reaction. Repeated hydroxylation results in the formation of 3,5-dichlorocatechol, which leads to ortho cleavage of the ring structure. The chloromuconate cycloisomerase enzyme facilitates the release of the para positioned chlorine atom. Later, maleylacetate reductase releases the second chlorine atom (from the ortho position). This release of chlorine in ionic form profoundly effects the pH of the system (Oh and Tuovinen, 1990). Finally,

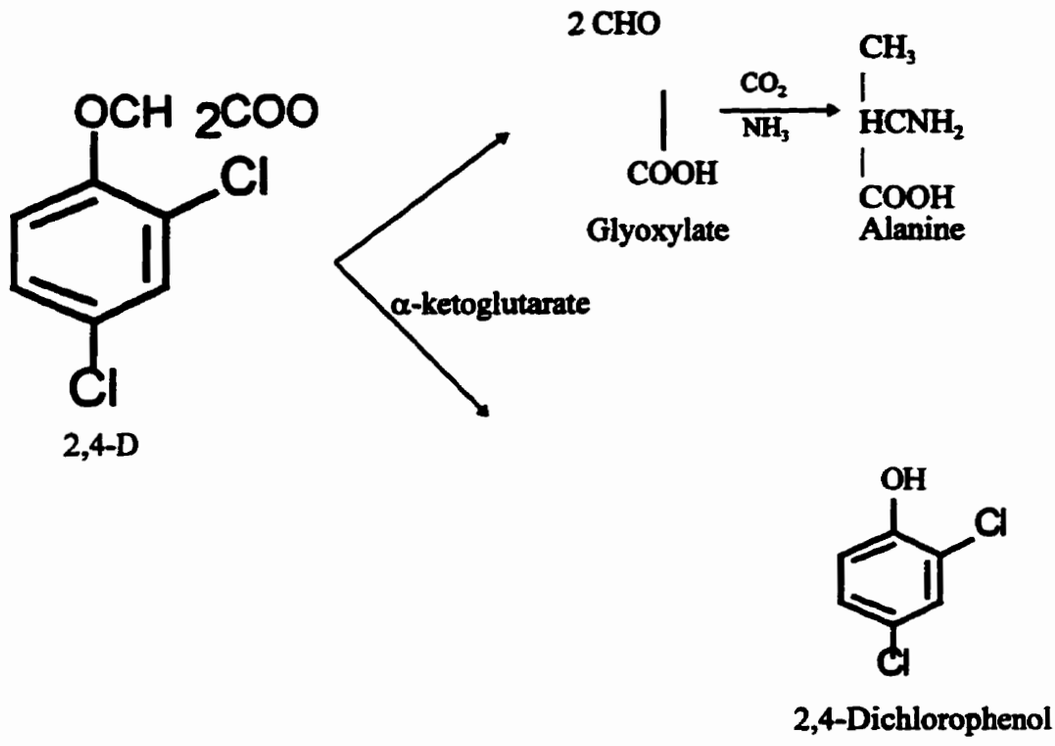


Figure 2. 6 Mechanism of Side Chain Dissociation During 2,4-D Breakdown (adapted from NRCC, 1978)

as depicted in Figure 2.7, the 3-oxodipate formed leads into the Tri-carboxylic acid (TCA) cycle which results in carbon availability for assimilatory purposes.

The pathway for 2,4-D degradation for *Pseudomonas* species is similar to that for *Arthrobacter* species, except for the presence of a few additional metabolic products. The degradation pattern in *Azotobacter chrococcum* is very different from the above cases, where formation of chlorophenol as a first step and pyruvate as last step are important variations (Evan et al., 1971).

2. 3 Analytical Determination of 2,4-D

Various environmental matrices (soil, plants, water/wastewater etc.) pose different challenges for phenoxy residue analysis. In most of the previous studies, 2,4-D has been detected mainly through indirect methods such as chloride assays or colorimetric analysis (Lappin et al, 1985; Rathore and Khan, 1987). Such techniques are of questionable accuracy. A brief description of some useful techniques is provided below.

2. 3. 1 Hydrolysis / Pyrolysis Techniques

The breakdown of the side chain of phenoxyacetic acid, when heated at 150°C for two minutes in the presence of sulphuric acid is the basis of several

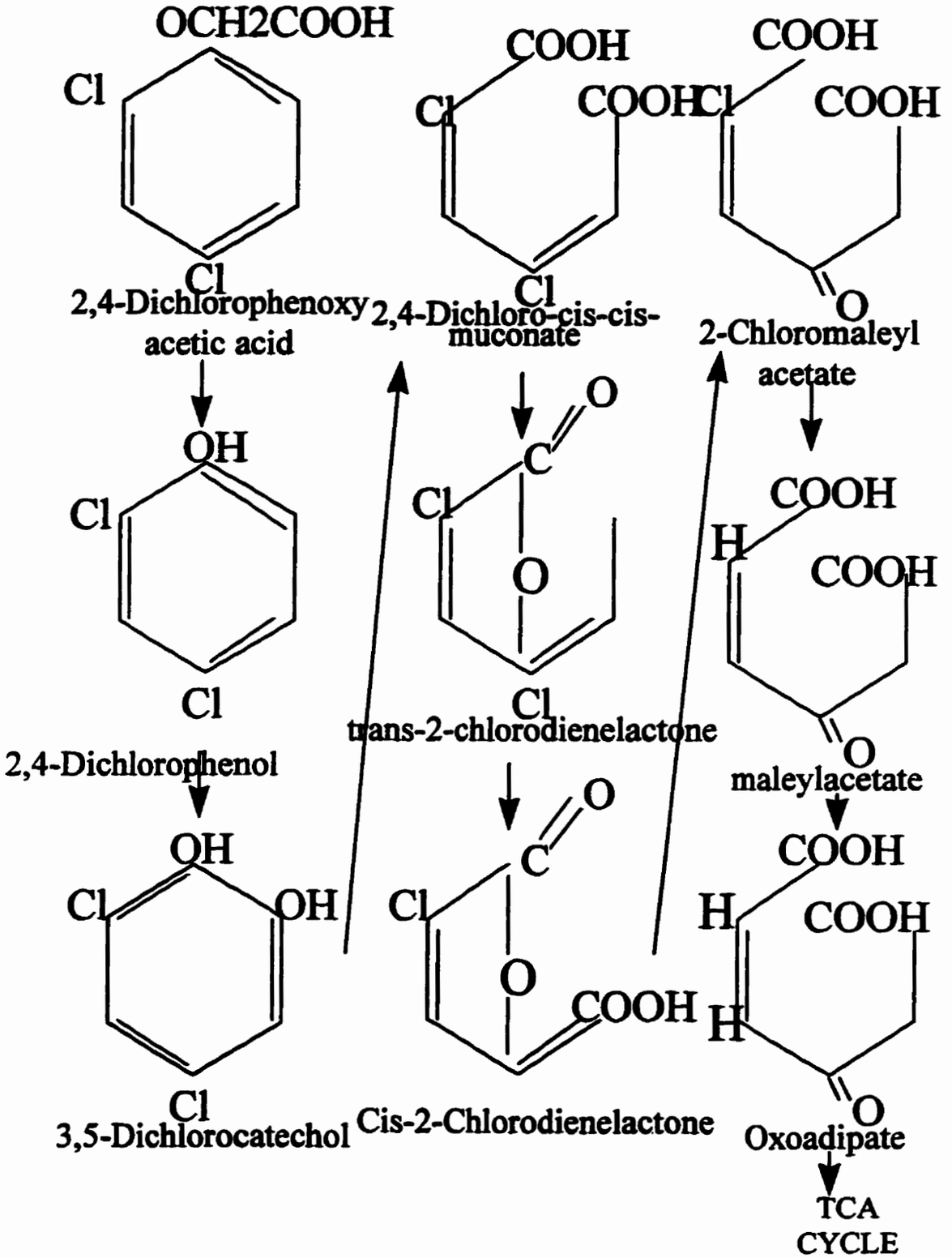


Figure 2.7 Biodegradation Pathway of 2,4-D (Adapted from Evans et al., 1971)

semiquantitative colorimetric procedures. The 2,4-D is hydrolysed/pyrolysed to produce formaldehyde. To avoid the contamination hindrance in the proper coloration development, often the vapor form of aldehyde is collected separately (NRCC, 1978).

2. 3. 2 HPLC (High Pressure Liquid Chromatography) Technology

Due to the heat sensitivity of 2,4-D, often HPLC is preferred over Gas Chromatography. 2,4-D's strong absorbance in the 210-290 range of ultraviolet region facilitates detection. Cappiello et al. (1995) have improved the liquid chromatography technique to increase the sensitivity of the equipment for very low levels of detection. Improvements includes minimization of decomposition and adsorption in the instrument system, which have enabled to acquire a 0.1-1 μg/l range of detection.

2. 3. 3 Gas Chromatography

Esterification (defined as ester formation using an agent such as diazomethane, borontrifluoride-methanol, or dimethyl sulphate) of the phenoxy compounds is an important step in gas chromatogarphy for increased sensitivity, specially in soil samples.

The ether bond of phenoxyacetic acid is readily broken down by concentrated hydrogen iodide or pyridine hydrochloride to yield the

corresponding phenol. The pyridine hydrochloride cleavage has been used in quantitative analytical methods for phenoxy acetic acid herbicides. The phenolic product is being determined by colorimetry or gas chromatography (NRCC, 1978).

Nitration (addition of N atoms) of the aromatic nucleus of 2,4-D yields mainly 2,4-dichloro-5-nitrophenoxyacetic acid and traces of 2,4-dichloro-6-nitrophenoxyacetic acid. These nitro derivatives can be detected on thin plates. The presence of chlorine in the molecule helps the detection of phenoxy herbicides on paper and thin layer chromatography (NRCC, 1978).

2. 4 Waste Water Treatment Approaches

Waste streams from 2,4-D manufacturing industries can carry concentrations up to 500 mg/L (Tyler and Fin, 1974). It is important that pesticide waste disposal methods be developed to treat such high concentrations. Conventional physicochemical treatment processes such as coagulation, filtration, etc. can not achieve effective removal (Clarkson et al., 1993). Minimization of waste by rinsate and spraying on treatable areas is not always a reasonable and effective means for rinsate disposal (Monsanto, Personal Communication). However, the need for practical, inexpensive, and cost effective methods for small volumes of highly concentrated pesticide waste is imperative. A variety of potential technologies suitable for pesticide waste disposal have been evaluated, but very few satisfactory methods are available to pesticide users. For 2,4-D contaminated wastewater, the principal removal processes are

hydrolysis, oxidation, adsorption, and microbial degradation (Clarkson et al., 1993; Dennis and Kobylinski, 1983; Pignatello and Baehr, 1994).

2. 4. 1 Physical and Chemical Treatment

A variety of physical and chemical treatment techniques can be applied to treat 2,4-D pesticide-laden wastewater. A list of such processes has been provided below (Table 2.5).

To convert a liquid waste to a solid waste, sorption plays a significant role. The availability of commercially simplified and confined adsorption techniques has resulted in wide-spread acceptance of adsorption for the treatment of toxic wastes (Ahmed and Ram, 1992; Martin and Fernandes, 1992). One such application of a commercially packaged adsorption system (CARBOLATOR®) for 2,4-D removal from wastewater resulted in 95% removal of the initially applied 100 mg/L concentration. This removal was achieved in approximately 21 hrs of operation (Dennis and Kobylinski, 1983). Even though, the use of adsorbing material such as peat moss and activated carbon results in significant removal, yet associated operational expenses in terms of spent carbon/peat regeneration or disposal are high (Couillard, 1994). Overall, the practice of conversion of a liquid waste into a solid waste is not an environmentally sustainable solution.

Chemical treatment using a variety of methods such as reverse osmosis and chemical oxidation may offer certain advantages over other degradation processes (Hindin et al., 1969; Leigh et al., 1969). Ease of treatment and faster

or more consistent degradation rates are the factors which influenced the choice of chemical degradation. Use of chemical oxidants such as Fentons' reagent, H_2O_2 etc. for pesticide destruction may be effective but certainly costly methods (Pignatello and Baehr, 1994). One such approach can be the use of ferrous-ligand complexes. These complexes are capable of catalyzing the degradation of 2,4-D by H_2O_2 in water at pH value of 6 (Sun and Pignatello, 1993). These studies have explored the use of Fenton's type reaction of hydrogen peroxide with Fe compound, where OH^- is potentially useful for degrading organic contaminant in soils. Even in the presence of substantial natural soil organic matter, fast and effective 2,4-D removal was observed

Physical Treatment	Chemical Treatment
Storage & Recycling	UV-Ozonation
Activated Carbon Adsorption	Fentons Reaction
Peat Adsorption	Wet-Air Oxidation
Reverse Osmosis	Chemical Reduction
Solvent Extraction	Microwave Plasma Destruction

Table 2.5 Physical and Chemical Treatment Options for 2,4-D-Laden Wastewater

2. 4. 2 Biological Treatment

Involvement of bacterial species has become an important part of

wastewater treatment operations. The first step in this direction is to provide a constant source of bacteria to degrade specific compounds or compound classes. The second step is to enhance the specific degrading activities and develop an understanding of the major variables controlling the microbial populations. Manipulating these variables (e.g., temperature, nutrient addition, organic loading) along with a careful choice of reactor design has been shown to be successful in establishing high specific microbial activities.

The following discussion focuses on aerobic treatment of liquid wastes. Biological reactor systems fall into two general categories 1) suspended-growth systems; 2) attached growth (immobilized-cell or fixed film) systems. The basic concept is the same in both cases, that is to bring the biomass in contact with 1) organic compounds (substrate or cosubstrate) as a source of energy, 2) an electron acceptor (oxygen), and 3) other appropriate nutrients for microbial growth. Suspended growth systems have been further classified into two main types: continuous flow (parallel or series) and semi-continuous batch operation. The use of the activated sludge system or related suspended growth systems for the treatment of 2,4-D-contaminated wastewater has been limited and unsuccessful. For instance, mixed microbial consortia have resulted in only 7 to 25% 2,4-D removal in activated sludge system (Hill et al., 1986). Other typical examples of suspended growth systems include lagoons and stabilization ponds. The microbial immobilization technology involves the attachment of bacteria to inert bio-carriers in a reactor designed to support a high level of chemical-degrading activity by the immobilized microorganisms. Some of the simplest and oldest configurations of attached growth systems are trickling filters, rotating

biological contactors and submerged fixed film reactors. Other immobilized-cell systems include fluidized beds, and sparged packed beds (Tchobanoglous, 1992). Immobilized-cells systems configured as fluidized bed reactors (FBRs) offer technical advantages for the biotreatment of concentrated waste streams, volatile chemicals and toxic chemicals (Puhakka and Järvinen, 1992). In FBRs, the biocarrier bed is fluidized by the liquid recycled within the reactor. Since chemical wastes are injected into the recycled liquid, toxic chemicals are immediately diluted and microorganisms in the FBR are more resistant to direct chemical toxicity than in many conventional treatment systems. In addition, since the FBRs are usually oxygenated by supplying oxygen into the recycle loop, a high level of microbial activity may be supported with minimal air stripping of volatile chemicals.

In packed bed reactors, inert supports such as diatomaceous earth, hollow fibers or porous silica serve as biocarriers for microbial attachment and growth. These systems are oxygenated by compressed air, are simple to operate in a compact area, and may maintain microbial selection much easier. However, diffusion kinetics can be slow and high levels of microbial growth from concentrated wastewater can cause operational problems due to channeling (Cooper, 1991). A representative example of such technology for 2,4-D wastewater treatment used magnetite to facilitate the bacterial attachment. This attempt resulted in 81% removal of the applied 50 mg/L concentration (McRae, 1985).

One study involving a biofilm reactor for 2,4-D contaminated waste treatment used genetically engineered strains. The concentrations applied were in the range of 200 to 800 mg/L. The system was successful in removing as high as 99% of 2,4-D (Clarkson et al., 1993). The same authors have reported the advantage of mixed cultures over pure cultures due to the robust and adaptable nature against changing influent characteristics.

Overall SBR systems have been used successfully to treat organic compounds. Particularly, in industrial-type operations, with a requirement of high liquid waste concentrations and space limitation (i.e. small-scale treatment system), the use of SBRs has been very effective. Sequencing batch reactor technology has been considered as an attractive alternative to conventional biological wastewater treatment systems, mainly because of its simplicity of operation, flexibility, and cost effectiveness for small-scale treatment facilities (Irvine and Ketchum, 1989; Kikkeri and Virarghavan, 1988). SBR is a time-oriented suspended growth process with waste flow, air/nutrient additions, and contact time determined by a defined operational strategy. A single tank (or a tank sequence) is used in a regime of FILL, REACT, SETTLE, DRAW, and IDLE periods. Cycle manipulation (e.g., air on/off through FILL and REACT or periodic nutrient additions) can create a unique microbial ecology for treating specific wastewater (Ng and Tan, 1990).

The nature of this system is such that it can endure fluctuating concentrations of toxicants in the influent and hence it can sustain adverse conditions with minimum impact. Such conditions are unavoidable during industrial

waste generation. No information was found in the literature regarding the application of SBR systems for treating 2,4-D waste. Consequently, one component of the study was to evaluate removal performance of 2,4-D in SBRs. In particular, the system's response was explored at various HRTs and changing feed conditions.

In conclusion, the future of biological treatment for pesticide waste is very promising. There is an immense need for developing certain treatment technologies to deal with the rather complex formulations of pesticides. The application of such important new information in the engineering field will certainly revolutionize the treatment approach applied for pesticide wastes.

Chapter 3

RESEARCH OBJECTIVES

As discussed in the previous chapter much of the information available on biodegradation of 2,4-D has been obtained using pure or mixed cultures in batch studies. These studies have focused principally on the basic microbiological and biochemical aspects of 2,4-D degradation. In many industrial operations, there is a requirement for stable continuously-running processes for the treatment of concentrated liquid waste. However, the few reported attempts to treat 2,4-D liquid waste in continuously-running suspended growth systems have achieved limited success. Therefore, it is an apparent need for further investigation on the feasibility of suspended growth treatment systems for 2,4-D removal.

To fulfill the above need, this study has attempted to focus primarily on the application of SBR technology. Overall the following two phases were incorporated:

1. During the first phase, the efficiency and stability of SBR operation was explored by studying the effect of the following parameters on the biodegradation pattern of 2,4-D:

   - Need for acclimation
   - Hydraulic retention time (HRT)

- Presence and absence of supplemental substrate (glucose or phenol)

- Variation of initial feed concentrations (target compound or supplemental substrate)

2. During the second phase of the experiment, batch cultures were used. This was done to optimize the overall time required (both phases were conducted concurrently) and to further explore certain basic aspects of 2,4-D degradation. The effect of following parameters on 2,4-D degradation was investigated:

   - Temperature

   - pH

   - Different types of seed (inocula)

Chapter 4

EXPERIMENTAL SETUP AND PROCEDURES

4. 1 Experimental Setup and Operation

4. 1. 1 SBR System Setup and Operation

Continuous operation of waste treatment was conducted in three identical laboratory-scale sequencing batch reactors (Figure 4.1). Each reactor used was made of 6 mm-thick Plexiglas cylinders with an internal diameter of 100 mm. The operating liquid volume was two liters. Feeding, decanting, and wasting were controlled by appropriately-calibrated peristaltic pumps. Mixing was provided by magnetic stirrers. Aeration was supplied through submerged diffusers placed at the bottom of the reactors. All SBRs were running on the same reaction, settling, decanting, and filling periods at any given HRT (Table 4.1). All operations were controlled by optional battery-operated timers.

At the end of each reaction period, the biomass was allowed to settle. Then, after decanting one liter of the supernatant, the reactors were replenished with an equal amount of fresh feed. Throughout the study, the temperature was fixed at 22°C ± 1. The nominal solids retention time (SRT) was 20 days and was controlled by wasting an appropriate volume of biomass just before the end of

Figure 4.1 Typical View of the SBR Set-up

the react period. This biomass wastage was conducted once a day during the morning cycle. There was no attempt to control the pH, which was in the 6.5-7.0 range during the reaction period. The liquid losses from the reactor due to evaporation etc. were insignificant in all systems. A schematic of the reactor setup is shown in Figure 4.2.

Parameters	HRT Variation			
	48 hrs	24 hrs	16 hrs	12 hrs
Fill Period	15 min	15 min	15 min	15 min
React Period	22 hrs	10 hrs	6 hrs	4.75 hrs
Settle Period	1.50 hrs	1.50 hrs	45 min	45 min
Draw Period	5 min	5 min	5 min	15 min
Idle Period	10 min	10 min	10 min	0

Table 4.1 Operating Conditions for SBRs

In biodegradation studies, the percent depletion of organic feed may be used to represent the efficiency of biomass for degrading xenobiotic compounds. The percent removal is calculated as follows:

% Removal = (influent conc.)-(effluent conc.)/(influent conc.) X 100

% Remaining = 100 - % Removal

4. 1. 2 SBR Biomass and Feed Characteristics

SBR 1 was seeded by harvesting biomass, used to degrade

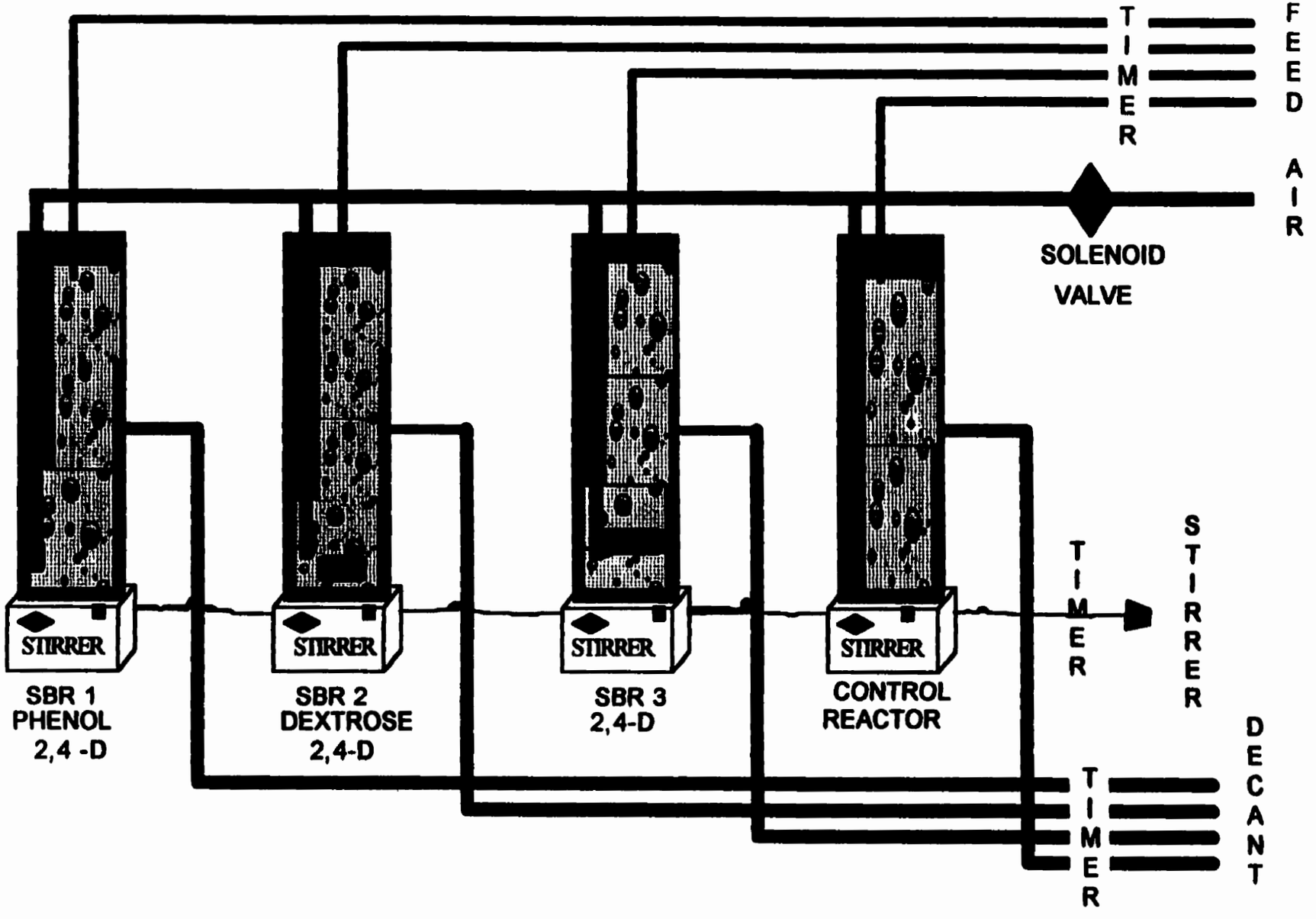


Figure 4. 2 Schematic of SBR system used in this study

chlorophenols in previous experiments conducted at the Environmental Engineering Laboratory, University of Manitoba (Basu, 1995). The source of seed in SBR 2 was mixed cultures collected from an activated sludge system at the North End Water Pollution Control Center (NEWPCC) in Winnipeg, MB. SBR 3 was seeded using a mixture from SBR 2 and fresh activated sludge from the above mentioned wastewater treatment plant.

The reactors were fed using synthetic feed comprised of phenol and 2,4-D (SBR 1), dextrose and 2,4-D (SBR 2), and 2,4-D as a sole carbon source (SBR 3 and SBR 4). The selection of phenol in SBR 1 was based on the fact that there is a structural relationship between the 2,4-D and phenol. In addition, this type of seed has been previously degrading phenols (Basu, 1995). Whereas, SBR 2 was decided to be fed with dextrose as a supplemental substrate. Dextrose (an isomer of glucose) is an easily degradable carbon source and has been frequently used in wastewater treatment studies (Hickman and Novak, 1984). To facilitate 2,4-D salt dissolution, 0.05 N NaOH was used. Sufficient amounts of NH_4Cl, and KH_2PO_4 were added to the feed solution, based on a 100:20:5 ratio of carbon, nitrogen, and phosphorus, respectively. Tap water was used to provide the essential micronutrients.

During preliminary run (no 2,4-D addition), 2,4-D was introduced at a concentration of 40 mg/L in the feed line (acclimation 1, Table 4.2). Afterwards, this concentration was increased to 100 mg/L (acclimation 2, Table 4.2), which was held constant throughout the study, except in SBR 3 during the last phase

Operation Stage	SBR 1		SBR 2	
	2,4-D (mg/L)	Phenol (mg/L)	2,4-D (mg/L)	Dextrose (mg/L)
Preliminary Run	0	100 (76.6)	0	185 (76.6)
Acclimation 1	40 (17.4)*	100 (76.6)	40 (17.4)	185 (74)
Acclimation 2	100 (43.4)	100 (76.6)	100 (43.4)	185 (74)
Normal	100 (43.4)	200 (153.2)	100 (43.4)	388 (155.2)
S 1	100 (43.4)	0	100 (43.4)	0
S 2	100 (43.4)	407 (311.7)	100 (43.4)	775 (310)
S 3	100 (43.4)	680 (520.9)	100 (43.4)	1350 (540)

*The values inside the parentheses represent equivalent SOC values

Table 4.2 Feed Variations in SBR 1 and SBR 2

Feed Pattern	2,4-D (mg/L)	SOC (mg/L)
Normal	100	44
T 1	200	88
T 2	300	130

Table 4.3 Feed Variations in SBR 3

of the experiment, where higher concentrations were applied (Stages T1 and T2, Table 4.3). SBRs 1 and 2 received an equivalent amount of organic feed, in terms of SOC. The deviations from the " normal feed " pattern of supplemental substrates are indicated by the S1 to S3 stages (Table 4.2). The selected 2,4-D concentration is well beyond the detected range in contaminated natural water systems, but it reflects a typical concentration in industrial waste streams.

4. 1. 3 Batch Systems Setup and Operation

Batch experiments were conducted throughout the study using biomass from the SBRs. Conical glass flasks, with total volume of 250 mL were used (Figure 4.3). These flasks had a narrow mouth for effective plugging. The reactors were plugged with cotton and were only opened during sample withdrawal. Regular pH control was also practiced. Aseptic techniques were applied to prevent contamination.

Similar to the SBR units, the 2,4-D concentration was fixed at 100 mg/L concentration in all reactors. The supplemental substrate SOC concentration varied from 50 to 200 mg/L as indicated in Table 4.4. All reactors were duplicated. Two additional reactors having 2,4-D as the only source of carbon were designated as 2,4-D. Another reactor again having only 2,4-D as carbon source was installed as a control reactor to observe any potential abiotic losses of 2,4-D. Altogether fifteen different reactors were used for each experiment. The flasks were placed in a shaker to provide enough mixing and

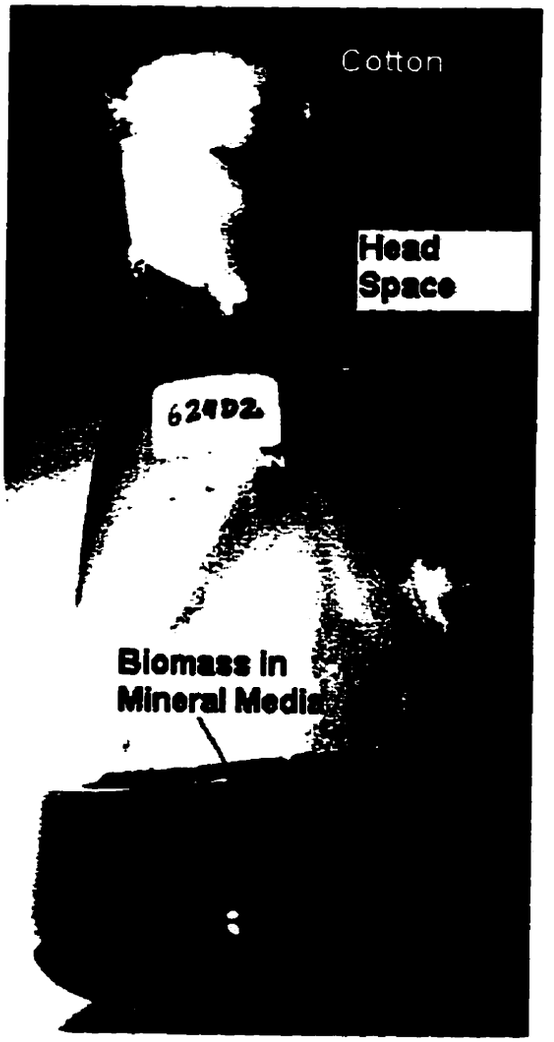


Figure 4.3 Typical View of the Batch Culture Flask

aeration to maintain proper aerobic conditions. Sampling frequency was adjusted based on the percent removal rate, i.e. slow/accelerated degradation. Apart from temperature variation, in the range of 10 to 30°C, all other conditions (such as reactor operation, mixing, biomass concentrations etc.) were fixed during the temperature study. Similarly, for the pH effect study, mixed-cultures were used. pH adjustment in batch cultures was done using a 0.1 N NaoH or 0.1 N H_2SO_4 solutions to keep pH within 6.5 to 8.0 range.

Supplemental Substrate SOC (mg/L)	Phenol Reactors		Dextrose Reactors	
	Reactor Designation	Phenol (mg/L)	Reactor Designation	Dextrose (mg/L)
50	P50	65	D50	125
100	P100	135	D100	250
200	P200	270	D200	500

Table 4. 4 Reactor Set-up for pH, Temperature, and Seed Studies

4. 1. 4 Batch Reactor Biomass and Feed Characteristics

Following the list provided in Table 4. 5, a mineral medium was prepared for use during the batch studies. Each reactor received 90 mL of this mineral medium, 5 mL of biomass, and 5 mL of the 2,4-D solution to provide a concentration of 100 mg/L 2,4-D and a biomass concentration of 75 mg/L (Tyler and Fin, 1974).

Constituents	Concentration (mg/L)
Na_2HPO_4	7.6
KH_2PO_4	3.6
NH_4Cl	0.5
$MgSO_4$	0.1
$CaCl_2.H_2O$	0.02
FeCl	0.01
$ZnSO_4$	0.005
Na_2MoO_4	0.002

Table 4. 5 Ingredient List for Basal Salt Medium

4. 1. 5 Biomass Preparation for Seeding Batch Reactors

Harvesting of bacterial cells from the operating SBRs (SBR 1, 2, and 3) was done by withdrawing adequate amount (with the help of a pipette) of mixed liquor to obtain equal concentrations of all three types of biomass. A mixture from three different sources (SBR 1 to 3) or 2,4-D degrading biomass (SBR 3) was poured into a cylinder and allowed to settle. After discarding the clear supernatant, biomass was re-suspended into the basal media (described earlier). The suspension was transferred into centrifuge tubes, and centrifuged for 10 min at 4000 RPM using a Damon/IEC Division Centrifuge, Model HN - S (Needham Hts., Massachusetts). After centrifugation the supernatant was discarded

carefully with the help of a specially designed syringe. The biomass was re-suspended into basal media and the same procedure was repeated once again. Finally equal volumes of this solution were used to seed the reactors. Seed material was dispensed with the help of automatic pipettes.

4. 2 Analytical Methods

4. 2. 1 2,4-D and Phenol Analysis

2,4-D and its intermediate metabolic products were analyzed by high pressure liquid chromatography (HPLC), due to the heat-sensitive nature of the phenoxy compounds (Cappiello et. al, 1995). Phenol was also determined concurrently. Samples were filtered through disposable Nylon Acrodisc R13mm HPLC certified 0.45 μm syringe filters. Filtered 20 μl samples were then injected into a Millipore-Waters HPLC system. This system was equipped with a Lambda Max 481 LC spectrophotometer at 215 nm wavelength. A Hamilton® PRP-1 column (Hamilton Co. , Reno, Nevada) was eluted with a mixture of acetonitrile (100%) and trifluroacetic acid (TFA 0.1%) (50:50 v/v) at a flow rate of 2 mL/min. The areas, retention times, and UV spectra of the sample peaks were compared with those of the known standards prepared in acetonitrile and distilled water (1:1 ratio). The lowest detection limit of this method has been 0.5 mg/L.

4. 2. 2 Dextrose Analysis

Dextrose analysis was also performed by HPLC. This particular setup was

equipped with a Waters 501 pump. A mobile phase of 0.02% H_2SO_4 delivered the 20 µl sample through a Biorad organic acid analysis column (Biorad Co., Richmond, California), which was heated at 65° C. The final response was detected by a Biorad refractive index monitor model 1750A. This response was integrated through an HP3396A integrator. The peaks were compared with the known standards, prepared with distilled water.

4. 2. 3 Soluble Organic Carbon (SOC) Analysis

For soluble organic carbon (SOC) analysis, a drop of phosphoric acid was added to approximately 3 mL of sample volume which was filtered through 0.45 µm pore size syringe filters. The samples were purged with pure oxygen for 3.5 min before injecting into a Dohrmann DC80 TOC analyzer. After calibration of the equipment with a known standard, the samples were run in a similar manner.

4. 2. 4 Mixed-Liquor Suspended Solids (MLVSS) Concentration Analysis

Mixed-liquor suspended solids (MLVSS) were determined during the first hour of the reaction period. The gravimetric method followed is outlined in Standard Methods (sections 2540 D, 2540 E, and 2540 F, respectively) (APHA, 1992).

4. 2. 5 pH and Temperature Measurement

The pH meter used in this study was digital pH meter by Fisher Scientific, Acumet 50 pH meter. Temperature measurements were conducted with a standard thermometer.

4. 3 Reagents

The following analytical reagents were used: 1) 2,4-dichlorophenoxy acetic acid (2,4-D), Analytical grade, Sigma Chemical Co. ; 2) Solvent Acetonitrile (CH_3CN), HPLC grade, E. M. Science; 3) Buffer solution of Trifluroacetic acid, analytical grade, Sigma Chemical Co.

Chapter 5

RESULTS AND DISCUSSION

5. 1 Preliminary Run

In order to establish a viable biomass and minimize any potential toxic effects due to the presence of 2,4-D, SBR 1 and 2 were initially fed with the corresponding supplemental substrate only (Table 4.2) and operated at an HRT 16 hrs. Complete removal of phenol and dextrose was observed throughout the duration (approximately 2 months) of the preliminary run.

In this preliminary run, SBR 1 phenol concentration was step-increased till it reached the required 100 mg/L level. This was done to avoid any toxic effects of phenol on the biomass. Due to the non-toxic nature of dextrose, no such substrate step increase procedure was performed in SBR 2. Eventually, SBR 1 and 2 were fed equal amounts of SOC, which corresponded to different concentrations of phenol and dextrose (Table 4.2). SBR 1 and SBR 2 systems were operated for a period of more than two months, at the above conditions. Reactor stability was judged by the complete removal of phenol or dextrose along with a stable biomass concentration of 700 and 400 mg/L MLVSS, in SBR 1 and SBR 2 respectively. In a previous study Orhon et al. (1989), a similar range of MLVSS concentration (about 600-700 mg/L) was employed for 2,4-D degradation.

5. 2 Acclimation

At this stage (acclimation 1) 2,4-D was introduced in both systems at a 40 mg/L concentration. Theoretically, in both SBRs, supplemental substrate and target compound together contributed to approximately 90 mg/L SOC (Table 4.2).

A track study was performed to assess the reactor's behavior just after the introduction of 40 mg/L 2,4-D into the feed line. First day of monitoring indicated a dilution effect in SOC values both in SBR 1 and 2, resulting in values near the detection limit (12-20 mg/L) of the SOC analyzer (Figure 5.1). In the event that the total SOC contributed by phenol or dextrose was fully depleted, the residual amount in the effluent should not have exceeded 8.7 mg/L (half of the 2,4-D related SOC in the feed line value, mainly due to the dilution factor). Due to the background SOC and the possible presence of other soluble products, generally, the lowest concentration detected in the effluent was in the 12-20 mg/L SOC range. Therefore, it is hard to predict whether all the residual SOC in SBR 1 and SBR 2 was actually associated with undepleted dextrose or phenol. However, in SBR 2 the residual SOC was still above the masked levels. Evidently, there was still some undepleted SOC contributed by dextrose in SBR 2 system.

A Follow-up track study, three days after the initial 2,4-D introduction, revealed that there was a decrease in SOC percent removal in SBR 2 particularly (Figure 5.2). This decreasing trend in SBR 2 was evident for the next 6 days of operation till it reached its lowest value on day 10 (Figure 5.3). Both system appeared to recover within 20 days after the 2,4-D addition.

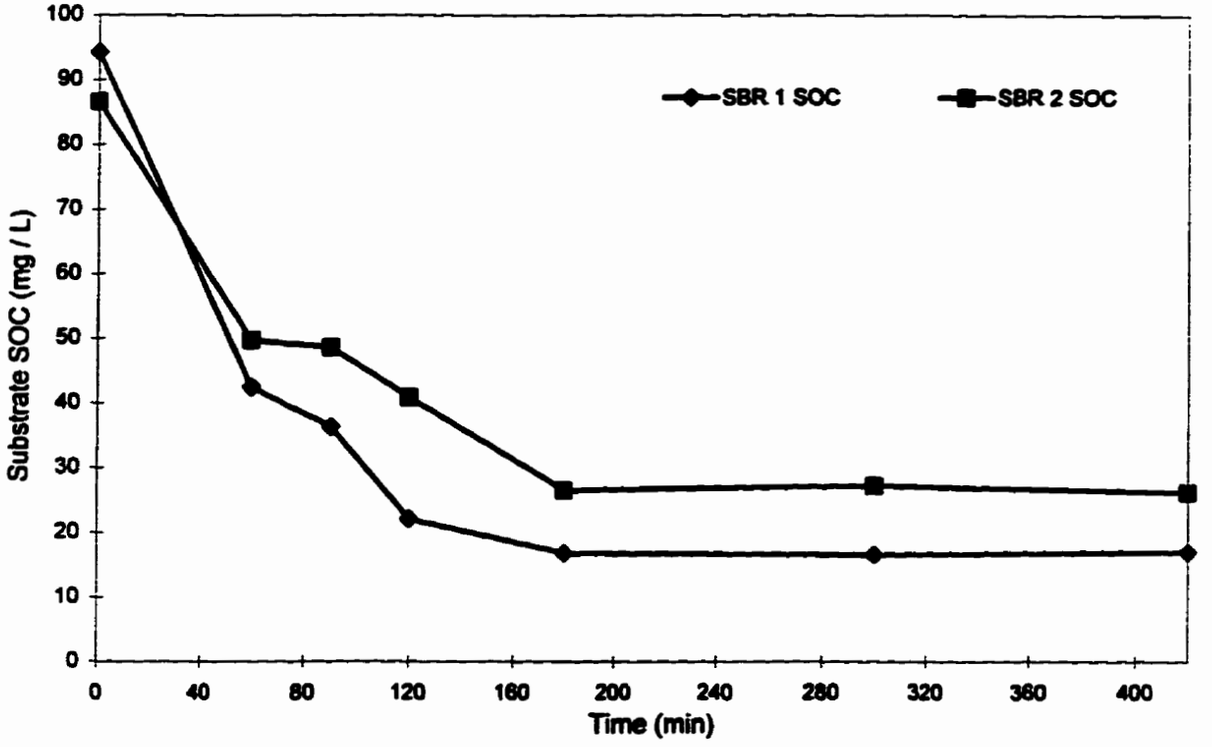


Figure 5.1. SOC Depletion Pattern on Day 1 after 2,4-D introduction

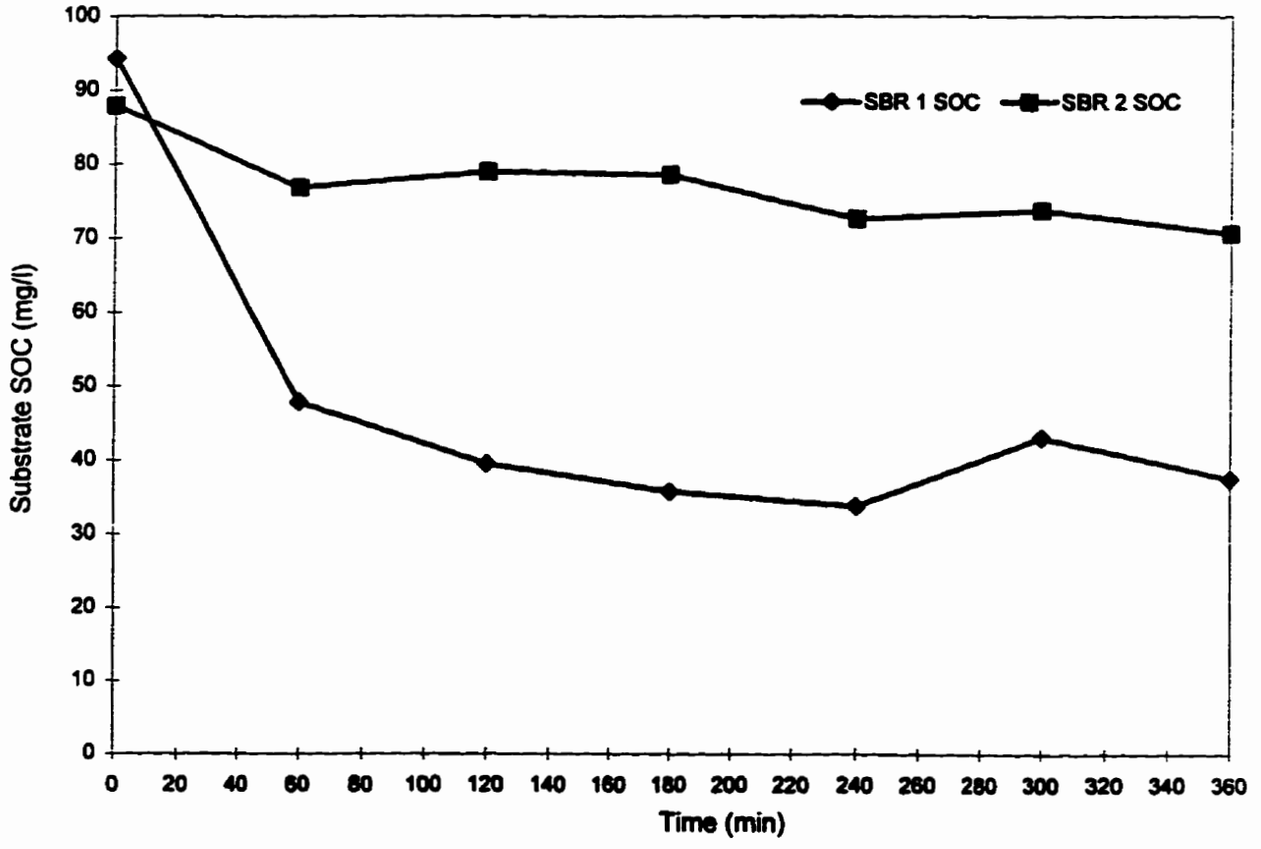


Figure 5. 2 SOC Depletion Pattern on Day 3 after the 2,4-D introduction

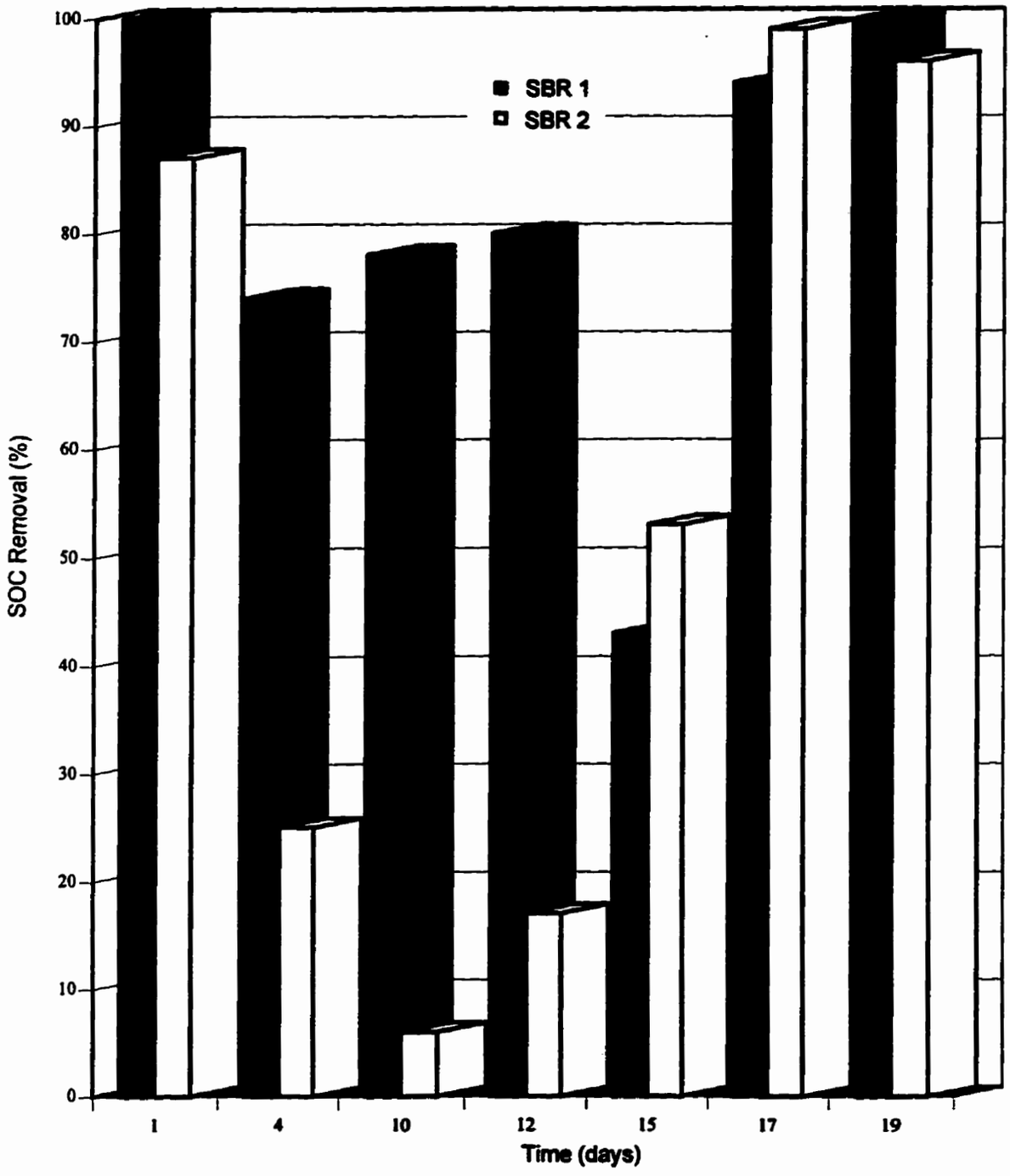


Figure 5.3 Inhibitory Effect of 2,4-D Addition on Supplemental Substrate SOC Removal in SBR 1 and 2

Further analysis indicated an upward trend in percent SOC removal in both SBRs with more pronounced one in SBR2 (Figure 5.4). The average SOC removal efficiency for this period (day 20 to day 80 after 2,4-D addition) was in the range of 50 to 55% in both reactors. Typical SOC degradation profiles, plotted in Figures 5.5 to 5.7, indicate a consistent trend through out the acclimation period.

It is evident from the above observations that 2,4-D even at 40 mg/L concentration, may have a negative impact on dextrose degradation. Even though such inhibitory effects were a short-term phenomenon, the severity of inhibition (5% removal of the influent SOC at the worst case scenario) warrants an attention regarding the initial 2,4-D concentration selection. Related observations have been also recorded in the literature, where 2,4-D has been shown to induce such inhibitory effects on supplemental substrate utilization. For instance, a chemostate system capable of utilizing succinate (an intermediate product of 2,4-D breakdown) indicated severe reduction in the specific uptake rates of this compound (Daugherty and Karel, 1994). However, no information on the effect of 2,4-D on dextrose depletion was found. Orhon et al. (1989) reported that biodegradation of bacto nutrient was severely inhibited by the addition of 200-600 mg/l 2,4-D in a continuously-fed chemostat, employing 2,4-D acclimated biomass. A study by Hickman and Novak (1984) reported that the dextrose SUR rate can be inhibited severely by the addition of chloroorganics into an activated sludge system.

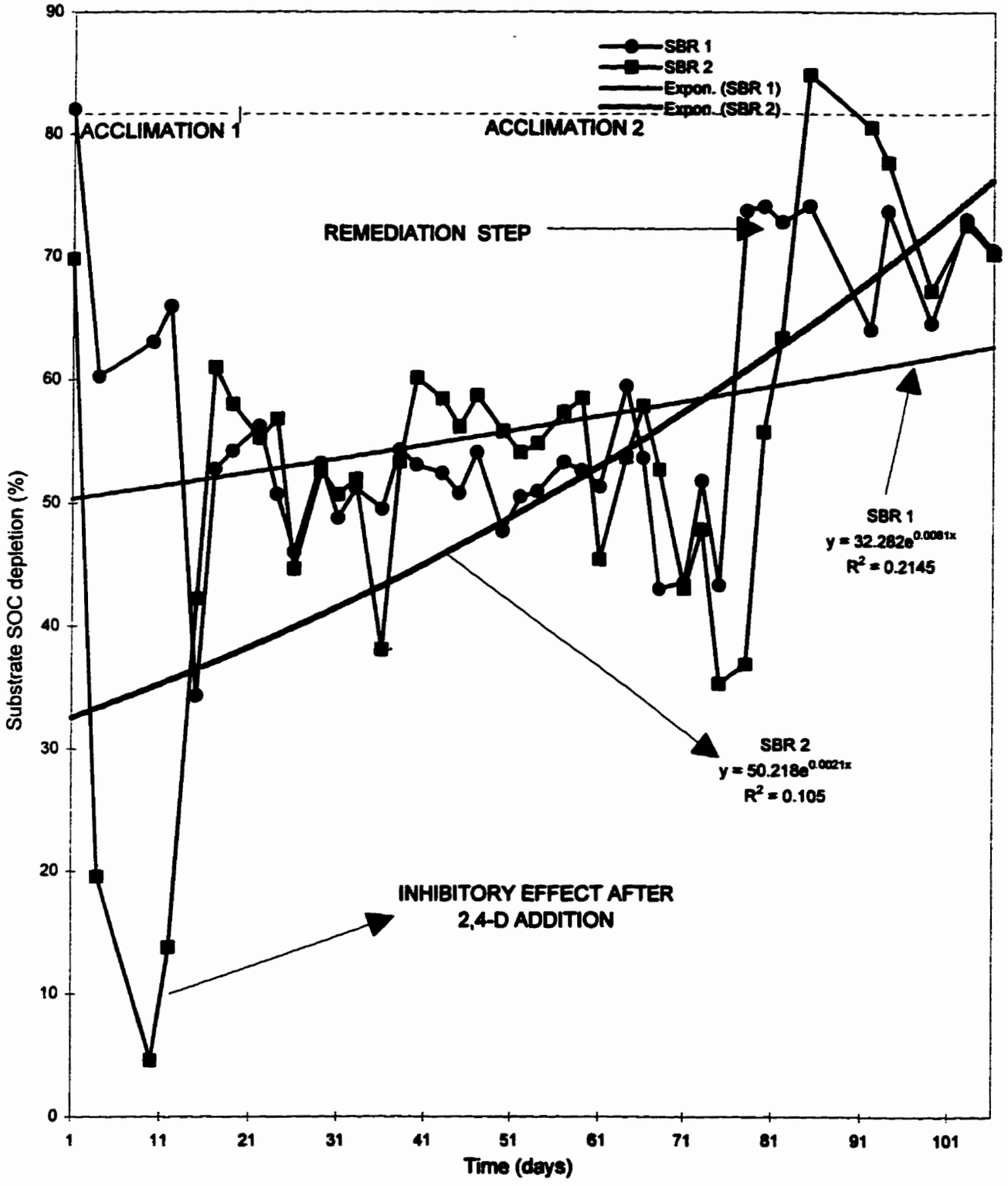


Figure 5. 4 SOC Profile Highlighting % Depletion During Acclimation Period in SBR1 and SBR 2

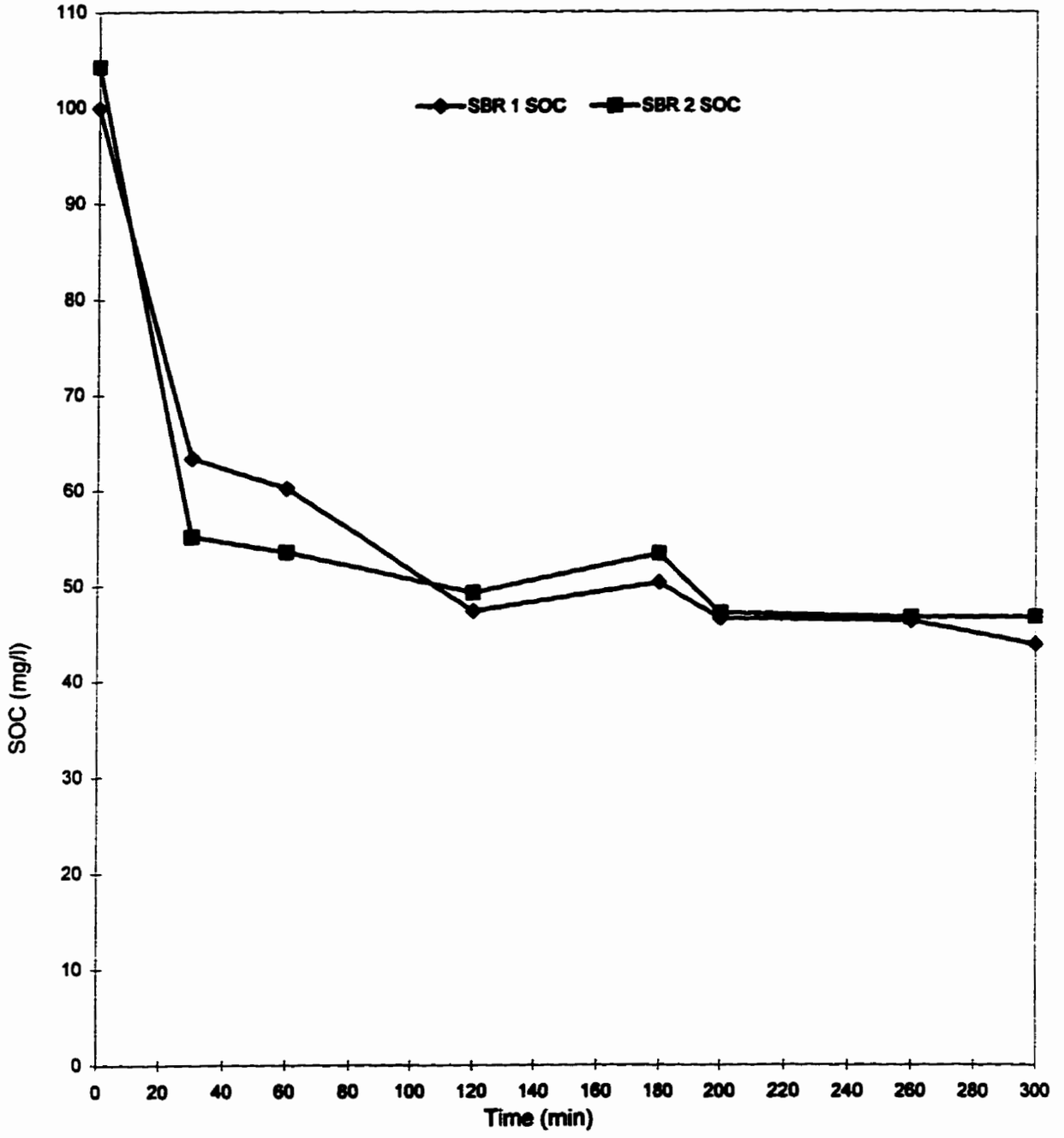


Figure 5. 5 SOC Depletion Pattern on Day 22 After the 2,4-D Introduction

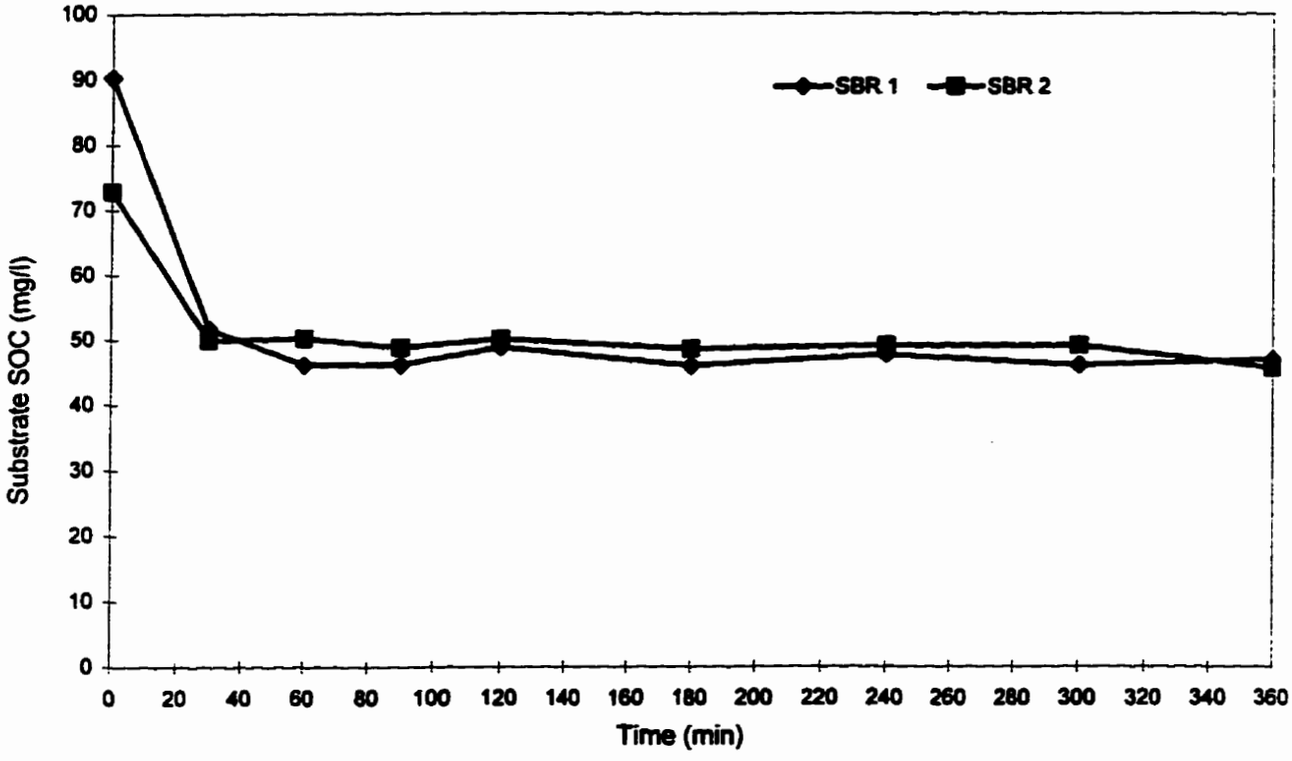


Figure 5. 6 SOC Depletion Pattern on Day 36 after the 2,4-D Introduction

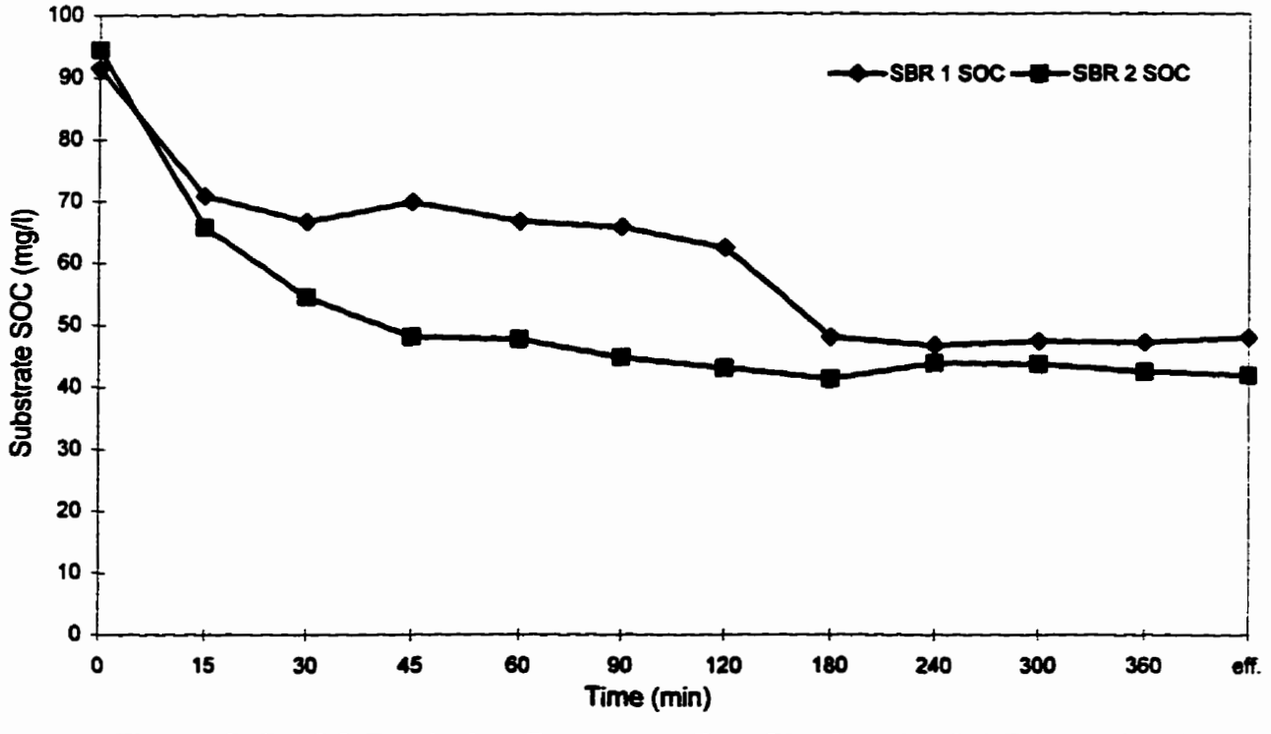


Figure 5. 7 SOC Depletion Pattern on Day 50 after the 2,4-D Introduction

Once again, the SBRs were able to degrade the supplemental substrates completely after the initial upset conditions (Tables A.1 and A. 2, Appendix A). The 2,4-D concentration was raised eventually to 100 mg/L, 17 days after the initial 2,4-D introduction (acclimation 2). The selection of this concentration was based on previous studies to provide the basis for comparison (Orhon et al., 1989, McTernan and Pereira, 1991; Yadav and Reddy, 1993). Further results indicated that both systems were able to retain their potential for complete degradation of phenol and dextrose in the presence of 100 mg/L 2,4-D concentration (Figure 5.5 to 5.7). The behavior observed in the two SBRs clearly dictates that prior to adopting a particular feed concentration of a toxic compound, it is very crucial that acute and subacute toxicity to the microflora be considered. The importance of such a step is also highlighted in previous reports (Sullivan, 1991; Tucker et al., 1975).

Also, there is ample evidence that microorganisms are able to degrade many synthetic chemicals provided that favorable conditions prevail. The degradation potential is often achieved as a result of the interaction of biological selection and three processes: mutation, recombination, and selection (Kilbane, 1986). In environmental engineering terminology such a period of evolutionary changes is termed as "acclimation stage". It is also defined as the change in the microbial community that increases the rate of transformation of the target compound as a result of prior exposure to this compound (Spain and Vanveld, 1983).

5. 2. 1 Substrate Degradation Behavior

During the first 80 days of operation after 2,4-D addition no signs of 2,4-D degradation appeared in either reactor (Tables A.3 and A.4). However, the supplemental substrates were readily consumed within the 3 hr of the react period. Dextrose was utilized at a faster rate than phenol. Specifically, dextrose was completely removed in about half the time required for phenol removal. A typical biodegradation pattern has been demonstrated in Figure 5.8. A sharp decrease in supplemental substrate concentrations in the beginning of react period represents the dilution factor after the addition of feed in reactors.

It was evident from both SOC and 2,4-D analysis that no detectable 2,4-D degradation had occurred during the first 80 days of the operation. It often involves a lengthy acclimation period before a particular complex xenobiotic substance is broken down by microbes. This period is associated with a series of mutational changes and multiplication of organisms responsible for catabolism of compounds (Kilbane, 1986). Also, a study by Greer et al (1990) has pointed out that the starting population density (of 2,4-D degraders) and initial 2,4-D concentration during acclimation period are directly related to the lag time observed prior to the start of 2,4-D degradation. The effect of initial 2,4-D concentration on lag period has been also emphasized by other researchers (Davidson et al., 1980; Fogarty and Tuovinen, 1991; Ou et al, 1978; Parker and Doxtader, 1982). Parker and Doxtader (1982) reported a direct correlation of initial 2,4-D concentrations (in the 1.3 mg/L to 135 mg/L range) and length of lag period preceding active degradation in soil systems. Another 2,4-D degradation

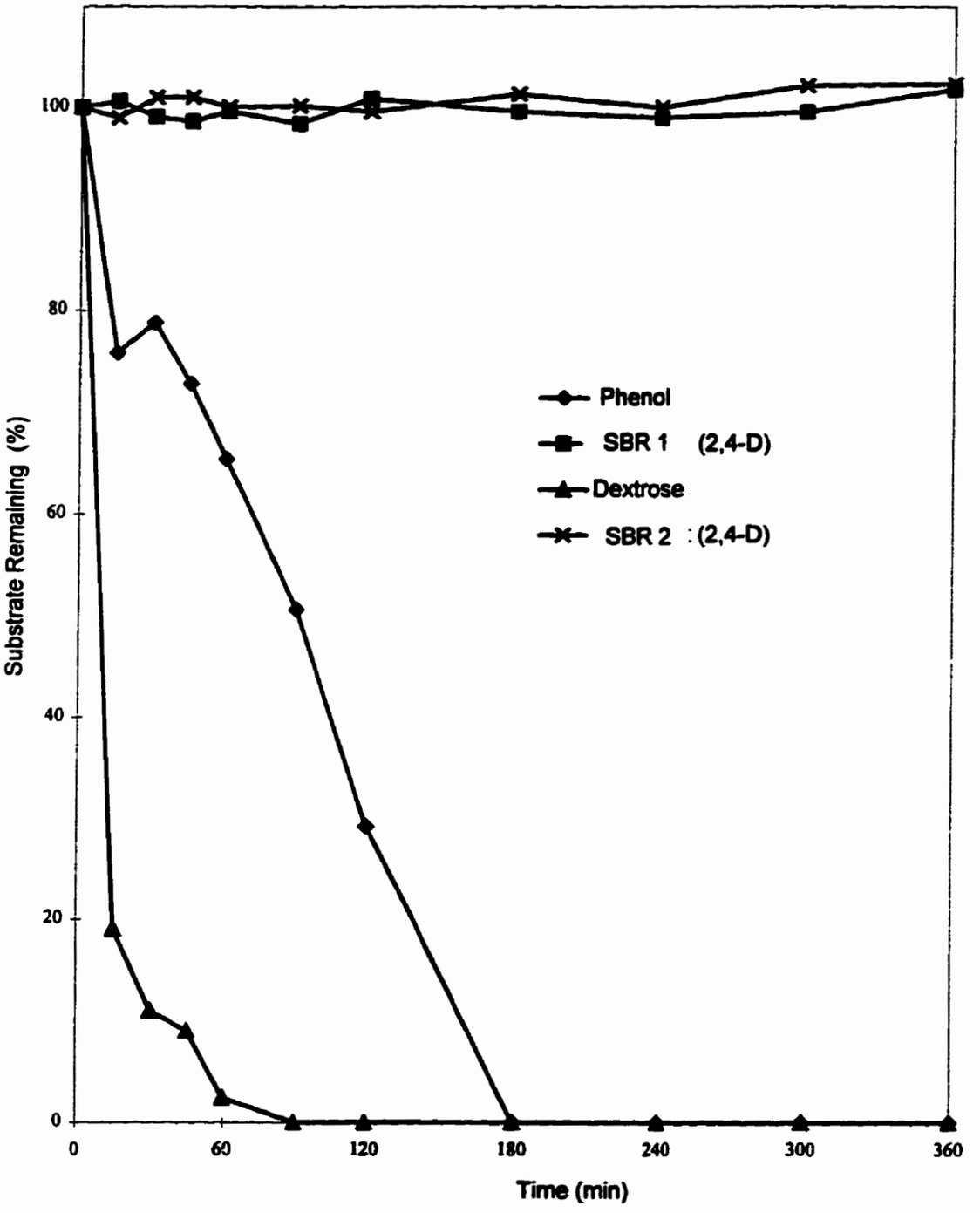


Figure 5. 8 Typical Substrate Utilization Pattern During Acclimation Period at 16 hrs HRT

study in soils indicated that extremely high concentrations (5,000 to 20,000 mg/L) inhibited the breakdown of this compound (Ou et al. 1978). A previous attempt to degrade 2,4-D (100 to 400 mg/L) similar conditions, resulted in 35 to 45 days lag period (Orhon et al., 1989). It was also indicated that in the presence of phenol, the 2,4-D degradation lag period dropped to 20 days instead of the 35 days observed when 2,4-D acted as a sole substrate. However, the authors do not specify the type or source of biomass used for reactor seeding, so it can be inferred that there might be a different type of initial flora used.

The first evidence of 2,4-D degradation appeared after 80 days of continuous monitoring. This was a partial removal stage. A 5.5% removal of 2,4-D was observed in SBR 1, and a 10.9% removal was analyzed in SBR 2 (Table A. 3 and A.4). Further results indicated that over a five days period, 56.4 % 2,4-D was removed in SBR 2. At the same time, SBR 1 had only achieved a maximum of 7.25 % decrease in 2,4-D concentration. In addition, SBR 1 the 2,4-D removal process did not follow any particular trend. Instead, there were only sporadic appearances of 2,4-D degradation. Over the next 2 weeks, degradation of 2,4-D ceased to exist. However, the reactors were kept monitored for two more weeks (until day 110), but no signs of 2,4-D degradation were noticed. In summary, for both system's, 2,4-D degradation performance was rather limited and non-sustainable, lasting only for a few days.

5. 2. 2 Actions to Achieve 2,4-D Degradation

To improve the situation, remedial action was taken. This involved increasing the reactors' HRT and feed supplemental substrate concentration values on day 110. The HRT was increased from 16 to 48 hrs, to provide the longer contact between the biomass and substrates. The supplemental substrate concentrations were doubled to 150 mg/L supplemental substrate SOC. In addition, the average MLVSS concentrations of 575 and 1055 mg/L in SBR 1 and SBR 2, respectively, were also enhanced to approximate 2000 mg/L by adding fresh activated sludge from the same source as outlined in Chapter 4. Continuous high levels of suspended solid concentrations in the effluent of SBR 1 reduced the MLVSS level in the reactor to 985 mg/L within a week (Table A.6). This was mainly the result of poor settling. The use of non-specialized biomass (i.e., non phenol degraders) for enhancement purpose may have contributed to this phenomenon. Using phenol-degrading cultures, another attempt was made to restore the diminishing MLVSS concentration to about 2000 mg/L.

Regarding the prolonged lag period, apart from the initial population of specialized bacteria and 2,4-D concentration, the possibility of the effect of supplemental substrate should not be ignored. Kilpi (1980) showed that repeated exposure of MCPA-degrading cultures to glucose further increased the lag phase for MCPA degradation from few days to few weeks.

A study by Yadav and Reddy (1993) has recorded that the enzymes responsible for 2,4-D degradation are produced constitutively (enzymes that are

permanently present in the cells) and prior acclimation of the organism for 2,4-D degradation is not required. On the contrary, this study and other studies previously mentioned have implied that proper acclimation is necessary in order to develop a viable 2,4-D-degrading population.

During the acclimation period, the physical appearance and consistency of the biomass underwent certain changes. Initially, the physical appearance of the biomass was more or less the same in both reactors, having a blackish color. During the first month of operation, the biomass in the phenol-fed system (SBR 1) turned yellowish in color, whereas the one in the dextrose-fed system (SBR 2) maintained its original color. In addition, the biomass in SBR 2 had a granular consistency and was easy to filter in contrast to the "fluffy" biomass in SBR 1. The above observations indicate that different microbial consortia developed in each reactor during acclimation. No experiments were conducted to identify the type of biomass, as taxonomic microbial determination was beyond the scope of this study.

5.3 Steady-State Operation

5. 3. 1 Degradation of 2,4-D

The successful outcome of remedial actions was noticed as greater than 99% 2,4-D degradation was achieved within one week period in SBR 2. However, the response was slower in the case of SBR 1. This reactor achieved greater than 99% 2,4-D removal 5 weeks after the remedial action was taken. Before the

successful 2,4-D degradation in SBR 1, a sporadic depletion pattern was observed, which ranged from 7% to 80 % 2,4-D removal (Table A.1). Such behavior led to the conclusion that phenol, despite its structural similarities with 2,4-D, had prolonged the acclimation period in this reactor in comparison to dextrose. The delayed response could be the result of involvement of various secondary-level enzymes (associated with intermediate compound breakdown), which may have the potential of being utilized during both phenol and 2,4-D degradation. A direct competition for enzymes between two different substrates has been shown to affect the biodegradation of chlorinated organics (Chang and Cohen, 1995). The amount of biomass and enzymes produced are directly related. Therefore the low MLVSS concentration can also explain the delayed response of SBR 1. The lower MLVSS levels in SBR 1 compared to those in SBR 2) were improved twice during this study. This validates the decision of increasing the MLVSS concentration as part of the remedial action described previously.

It should be also noted that in this study, once 2,4-D degradation was established, it reached the maximum values rather quickly, particularly in the presence of dextrose. In contrast to this observation, in activated sludge systems after 5 to 10 days of lag period (no degradation), a small amount of 2,4-D was degraded (about 10%). This was followed by a trend of gradual increase in 2,4-D degradation till it reached 25% in 45 days (Lackman et al., 1980; Orhon et al., 1989). In summary, it could be inferred that for certain species of bacteria, the induction of specific genes capable of degrading 2,4-D may be a gradual phenomenon, as was the case of phenol-degrading cultures in this study. On the

other hand, in other species (such as the dextrose-degrading biomass) these genetic changes may have occurred rather fast.

Summarizing the above thoughts, it can be inferred that two factors played a major role in achieving consistent 2,4-D degradation in both SBR systems: longer contact time between the biomass and the target compound and higher concentration of MLVSS in the reactors. In addition, increase in supplemental substrate SOC can also improve MLVSS concentration in bioreactors.

5. 3. 2 Addition of SBR 3

Followed the initial inconsistent period of 2,4-D degradation, a stable 2,4-D degradation (>99% in every cycle) was observed. At this stage the third reactor (SBR 3) was commissioned, which received 2,4-D as the sole source of carbon and energy. The reactor was seeded using a mixture of fresh activated sludge and biomass from SBR 2. Since at the time of seeding the SBR 3, there was not enough 2,4-D degrading biomass available, it was decided to employ a mixture of fresh activated sludge and 2,4-D degrading biomass (from SBR 2). Eventually a viable population of 2,4-D degraders could be obtained through the mechanisms of conjugation and transformation (Pemberton et al., 1974; Steiert and Crawford, 1985).

The initial MLVSS concentration in the SBR 3 was kept about the 2000 mg/L level. As the system started operating, the MLVSS concentration followed a

gradually decreasing trend. This was the result of high concentration of MLVSS in the effluent at the end of every cycle, due to poor settling. However, it stabilized around the 150 mg/L concentration level (Table A5, Appendix A). A possible explanation for this kind of behavior could be that the unacclimated biomass used as a seed was washed out gradually with the effluent. The rate of loss of MLVSS in the effluent may have been higher than the rate of conjugational or mutational mechanisms.

5. 3. 3 Substrate Depletion in SBRs

As far as the reactors performance in terms of SOC depletion is concerned, SBR 1 and SBR 2 were able to deplete 200 mg/L SOC in every cycle, whereas SBR 3 degraded 45 mg/L SOC levels (Table A5). In all results, a residual 10 to 20 mg/L effluent SOC was observed. This may have been due to two different reasons. First, a part of this SOC levels may have been contributed by the soluble biomass products, which may have passed through the microfilter during sample preparation for SOC analysis. Second, background SOC contributed by tap water can be also held responsible for these residual levels. Another study in this laboratory has indicated similar levels of residual SOC (Basu, 1995).

All three reactors were able to degrade an average 100 mg/L 2,4-D concentration in every cycle. Theoretically, this corresponds to 43.4 mg/L SOC. In SBR 1 and SBR 2 approximately 155 mg/L supplemental substrate SOC was being depleted at the same time. It should be noted that a previous study on the

behavior of chlorophenoxy herbicides during activated sludge treatment of municipal wastewater achieved only a partial breakdown of 2,4-D. This breakdown resulted in short-chain ester formation. However, the nature of the breakdown products was not specified (Saleh et al.,1980). There are indications that certain intermediate products during 2,4-D breakdown may be toxic to the biomass involved (Haugland et al., 1990). It was found that "dead end" (of which further degradation does not occur) intermediate products such as 2,4,5-trichlorophenol and 2,4-dichlorophenol were accumulating during 2,4-D breakdown. However, during this study regular soluble carbon analysis clearly demonstrated that no intermediate product has accumulated in the SBRs. In all three systems, a complete removal of 2,4-D could be associated with assimilation into the biomass.

5. 4 HRT Optimization Study

HRT, is defined as the average time period the liquid remains in the reactor in contact with the biomass. It can be calculated as the volume of the reactor divided by the average flow rate. During this study, HRT significantly effected the reactor behavior, at least the appearance of the first complete 2,4-D degradation activity was mainly attributed to the change in HRT of the system and biomass enhancement.

At the stage when all three reactors were able to attain a steady 2,4-D and supplemental substrate (where applicable) removal, HRT optimization studies were initiated. A set of four HRTs (i.e. 48 hrs, 24 hrs, 16 hrs, and 12 hrs) was tested. At all HRTs, aeration in the react period was extended for a minimum of 2

hrs after complete disappearance of 2,4-D was observed. This step was taken to enhance SOC removal. In the past, such an extended period of aeration has shown to improve sludge settling characteristics (Ng and Tan, 1990). Also, the good settling ability of biomass observed in this study may have been related to extended aeration. As a general observation, good floc formation was observed in both SBR 1 and SBR 2 systems. This was the reason that settling time was decreased by 15 min during the later part of the experiment. All reactors were operated at 20 days nominal SRT at any HRT. At any particular HRT study, the reactors were allowed to operate for minimum of a 1.5 SRT period (about 30 days). In addition, between the change in HRTs, no lag period was observed throughout this study.

Several representative percent depletion vs. time profiles have been plotted at various HRTs (Figures 5.9 to 5.12). Each of these profiles is the average of at least two comprehensive track studies. These degradation studies indicated that at all HRTs, the supplemental substrate and 2,4-D were being depleted sequentially. In SBR 1 and SBR 2, phenol or dextrose were the first to be removed, and 2,4-D removal always followed. It is also evident that as the system progressed towards longer operation, an accelerated degradation of both the supplemental substrate and target compound was observed. Initially, degradation of supplemental substrate at 48 hrs HRT has been occurring within 45 min. Particularly, dextrose was being utilized within 30 min of the react period, while phenol required 45 min for its complete removal. The trend at 24 hrs HRT, was such that dextrose removal accelerated to 15 min and phenol was being depleted within 30 min time period. Similarly, a 16 hrs HRT further improved the

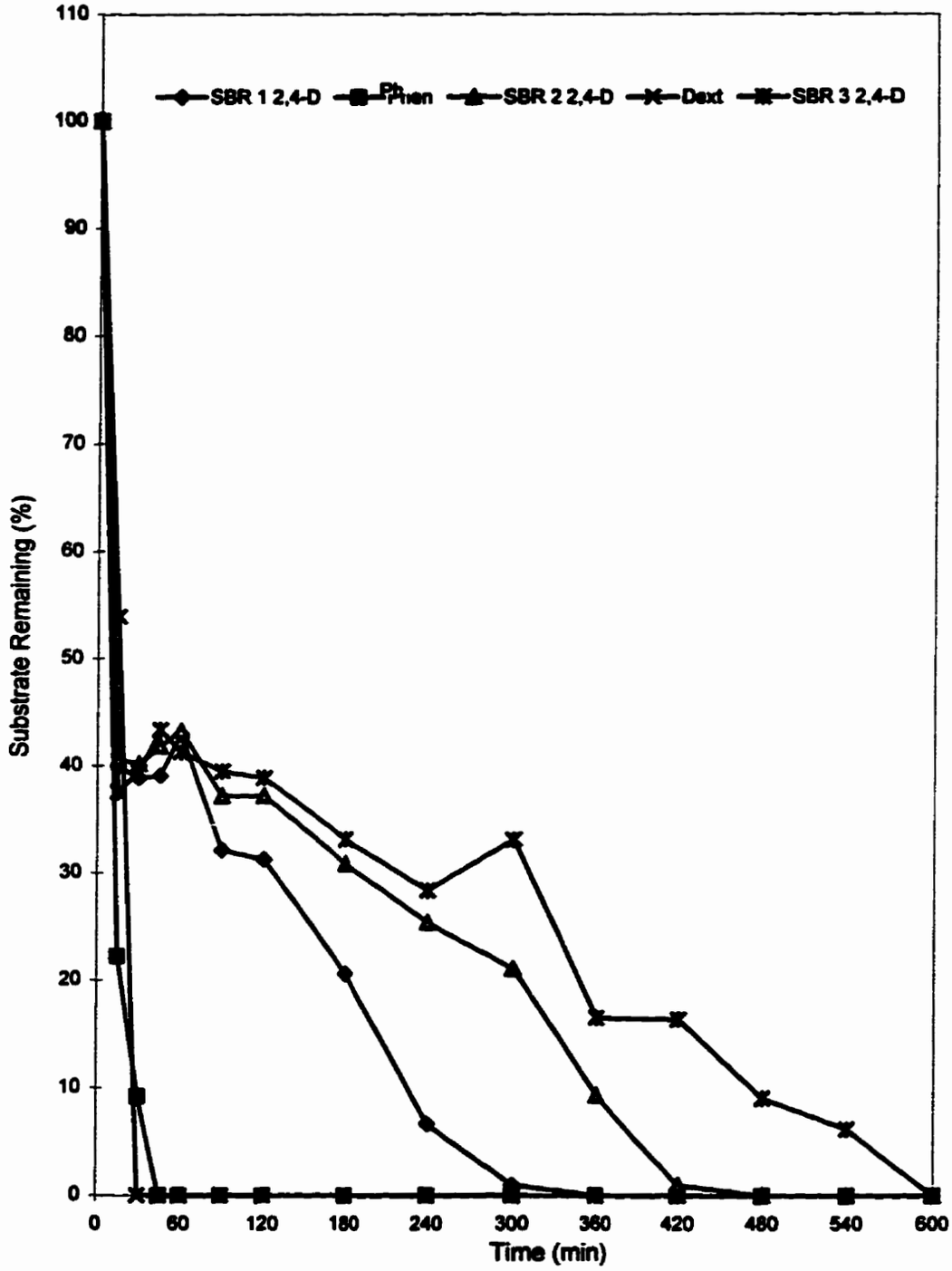


Figure 5. 9 Substrate Depletion Pattern at 48 hrs HRT

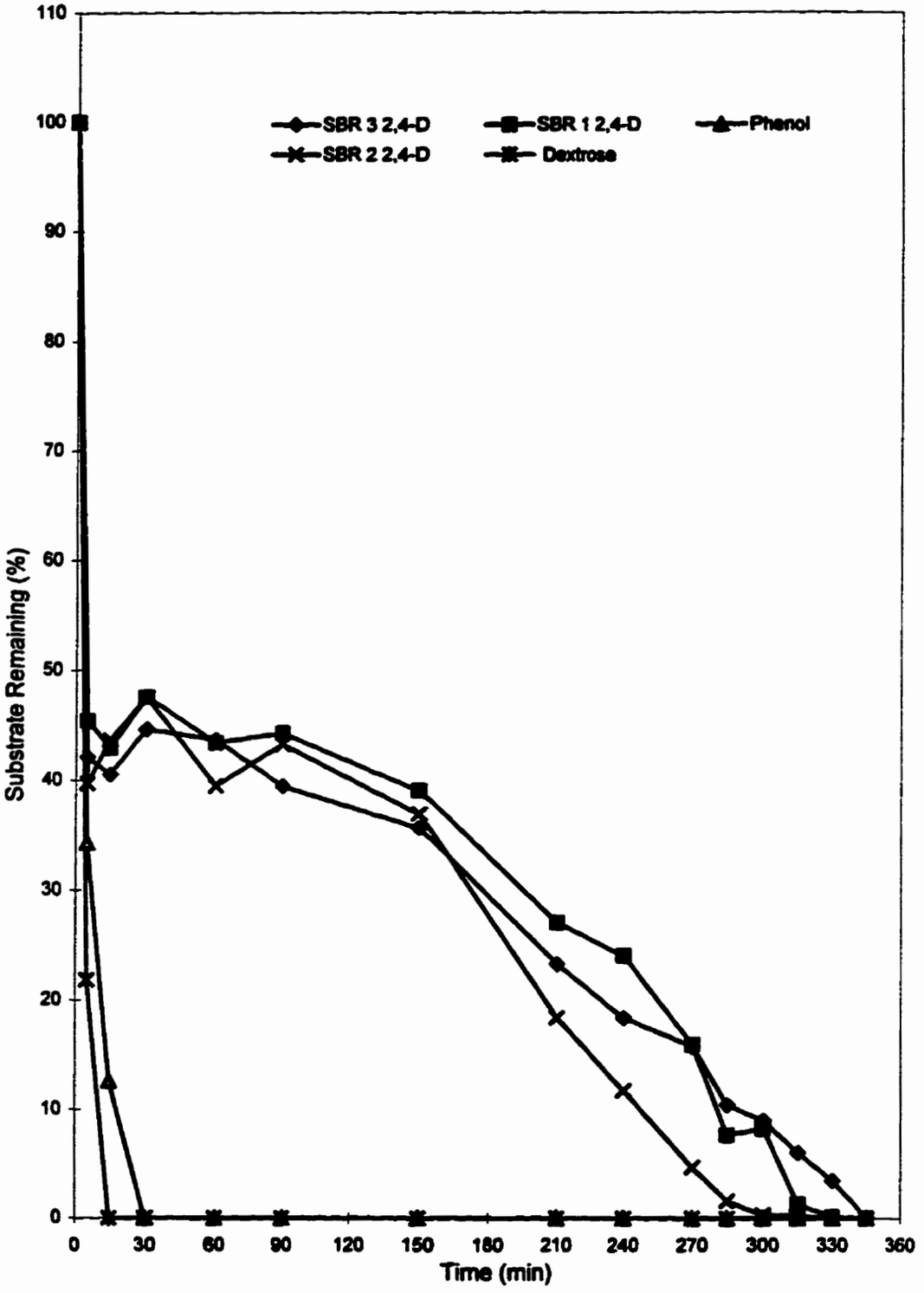


Figure 5.10 Substrate Depletion Pattern at 24 hrs HRT

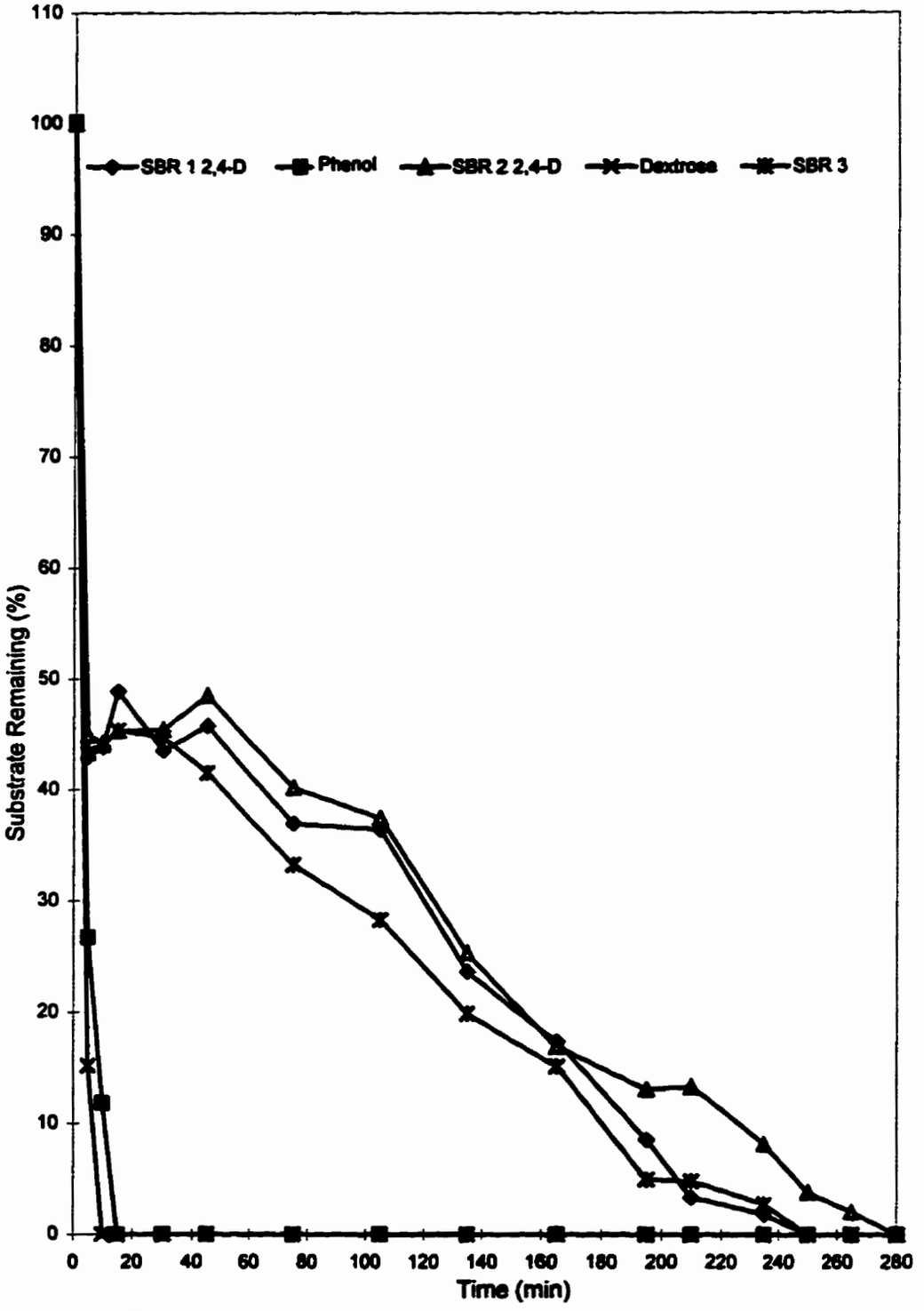


Figure 5. 11 Substrate Depletion Pattern at 16 hrs HRT

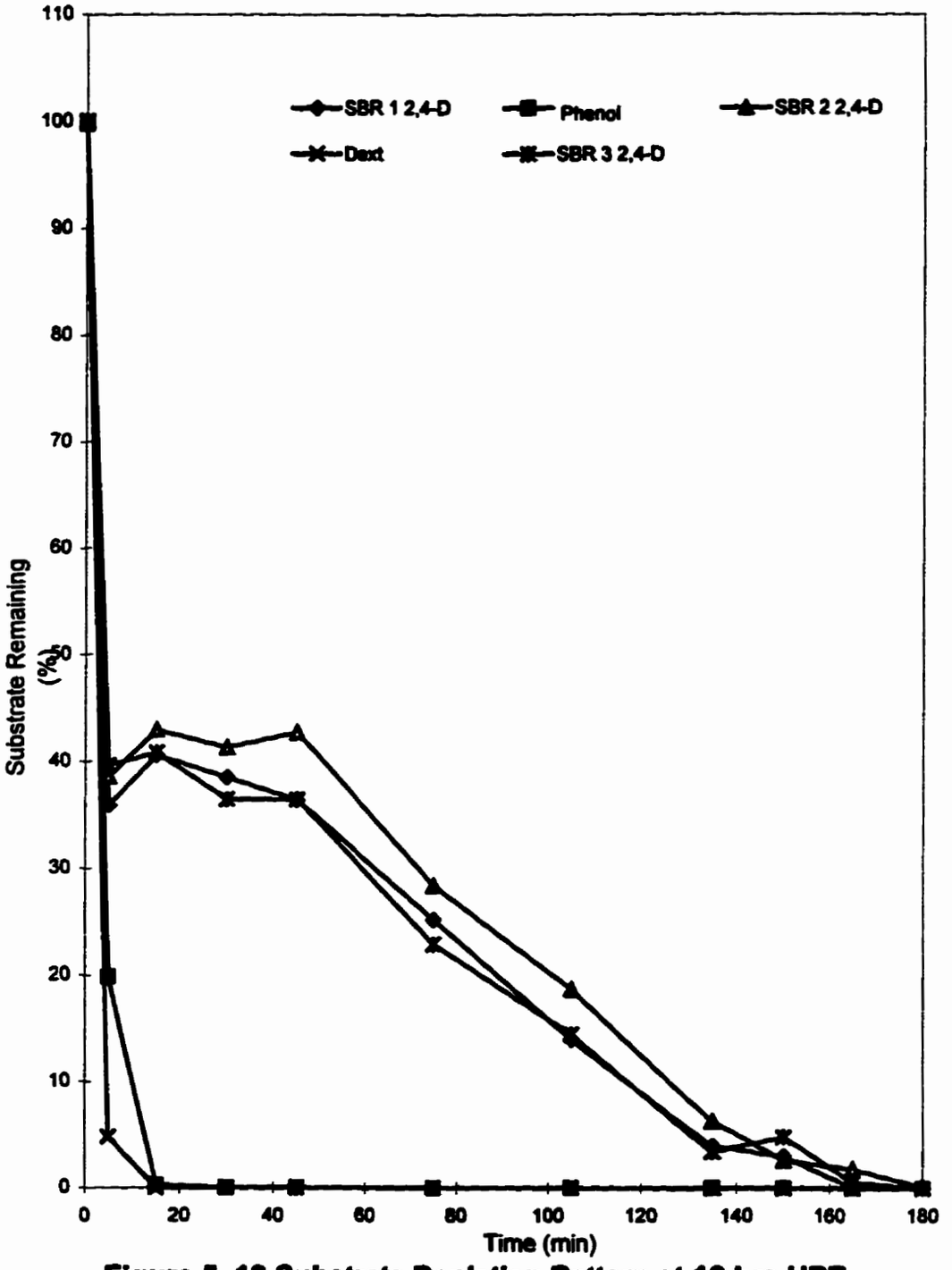


Figure 5. 12 Substrate Depletion Pattern at 12 hrs HRT

degradation time to 15 and 10 minutes for dextrose and phenol respectively. However, at 12 hrs HRT the response was similar to the one observed at 16 hrs HRT.

In addition, faster degradation of 2,4-D occurred with the progression of this study. A complete breakdown of 2,4-D was being achieved on average in 6 hrs, 5 hrs, 4 hrs, and 2.75 hrs at the HRTs of 48 hrs, 24 hrs, 16 hrs, and 12 hrs, respectively (Figure 5.9 to 5.12). Even though from the above observation, it seems evident that both phenol and dextrose have lowered the 2,4-D specific removal rate in comparison to SBR 3 (which received 2,4-D alone), yet the depletion of both of these supplemental substrates has been accelerated in the presence of 2,4-D. A comparison of the pre-acclimation and post acclimation period clearly demonstrates the effect of 2,4-D degradation on supplemental substrate depletion (Figure 5. 13). A comparison of the specific removal rates (expressed as mg of substrate per g of MLVSS per hr) in both reactors at 16 hrs HRT before and after 2,4-D degradation occurred is shown in Table 5. 1. The dextrose system has been more affected in terms of increased specific removal rates after the start of 2,4-D degradation. Such an increase was up to 14 times higher than that during the pre 2,4-D degradation period. Even though, the phenol system has also indicated enhanced removal, yet it only accelerated up to 5 times the pre 2,4-D degradation period value. This trend can only be justified on the basis of enzyme interaction, which implies that the enzymes produced for 2,4-D degradation must have been involved in supplemental substrate depletion as well. A related study which explored 2,4-D and glucose depletion in a dual substrate mode, has pointed out a similar trend of accelerated glucose depletion

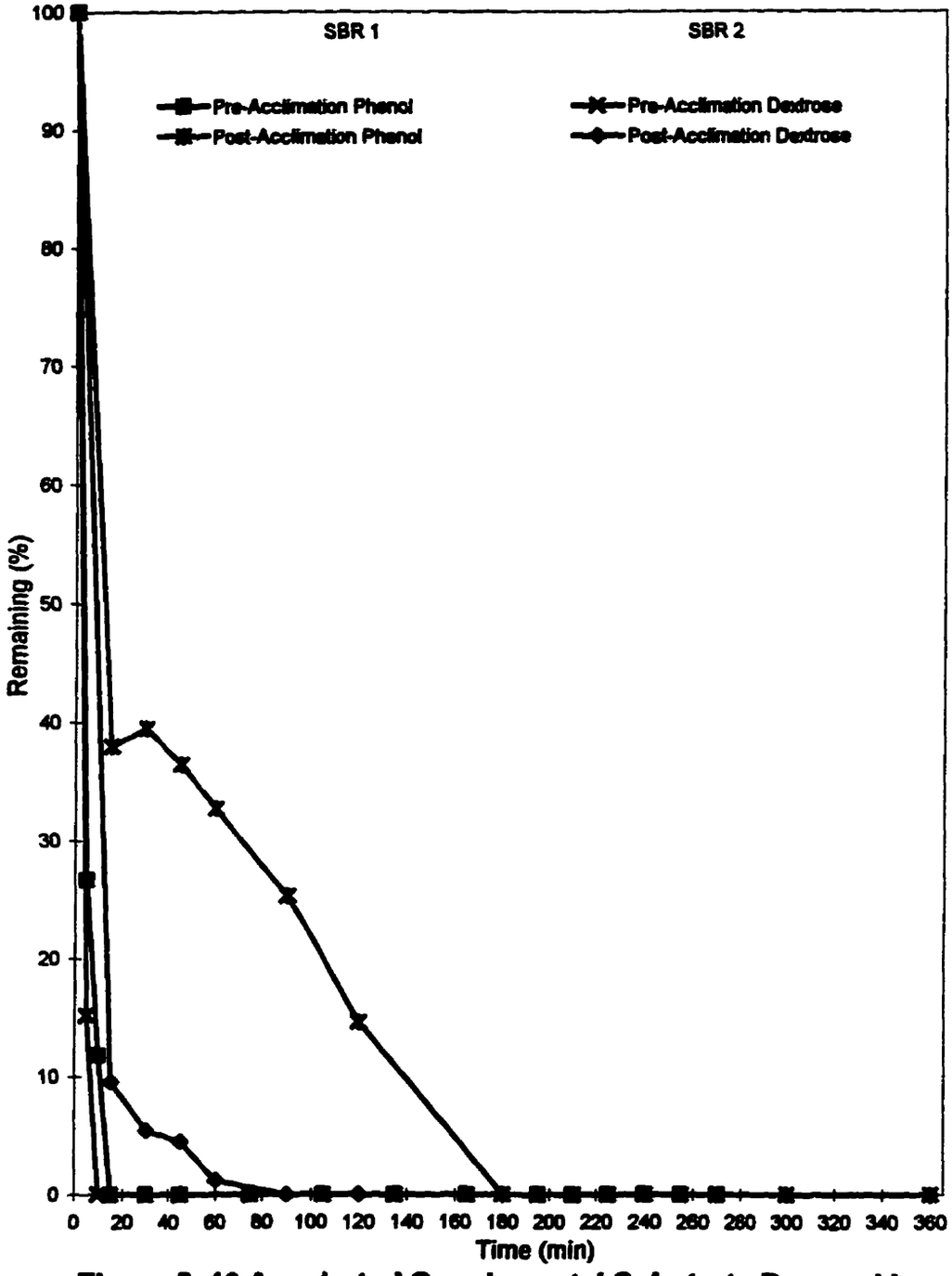


Figure 5. 13 Acceirated Supplemental Substrate Removal in SBR 1 and 2 at 16 hrs HRT

(Papanastasiou, 1982).

For the entire HRT optimization study, the 2,4-D specific removal rates were calculated. It was observed that in the presence of phenol as supplemental substrate the specific removal rates were better than that observed in the presence of dextrose. This trend of relatively lower 2,4-D specific removal rates in the presence of dextrose was consistent (Table 5. 2).

Operational Stages	Specific Removal Rate (mg Supplemental Substrate/g MLVSS. hr)	
	SBR 1 (Phenol)	SBR 2 (Dextrose)
Pre 2,4-D Degradation	18	15
Post 2,4-D Degradation	92	208

Table 5. 1 Effect of 2,4-D on Supplemental Substrate Depletion

On the other hand, in SBR 3 where 2,4-D was the sole substrate, a very sharp increasing trend in specific removal rate was noticed. At 48 hr HRT, SBR 3 had an average specific removal rate of approximately 1.3 and 2.4 times higher than SBR 1 and 2, respectively. With decreasing HRT, a very sharp rise in the specific removal rate was observed. The rate observed at 24 and 16 hrs HRT was approximately 9.5 times higher than that at 48 hrs HRT. These rates further increased about 16 times at 12 hrs HRT as compare to the initial value (Table 5.

2). A direct comparison of the specific removal rates in the three SBR systems indicated that the highest specific removal rates were obtained in SBR 3. Since the specific removal rate is a function of MLVSS concentration in the reactor, high specific removal rates for SBR 3 are the direct result of the low MLVSS concentration in this reactor (Table 5. 3).

HRT	Specific Removal Rate (mg 2,4-D/gMLVSS.hr)			
(hrs)	SBR 1	SBR 2	SBR 3	% Difference (SBR 1 & 2)
48	3.25	1.75	4.17	45.5
24	2.59	1.46	37.5	44.8
16	2.56	1.82	38.0	30.0
12	3.68	1.86	67.5	49.5

Table 5. 2 Average Specific Removal Rates (mg 2,4-D / g MLVSS. hr) at Different HRTs

5. 4. 1 Overall SBR Performance

Throughout the HRT optimization period, a decreasing trend in HRT was associated with higher MLVSS concentration in SBR 1 and 2. This could be attributed to the more frequent feed cycles as HRT decreased. Specifically, at 48

hrs, 24 hrs, 16 hrs, and 12 hrs HRT, SBR 1 and 2 systems were utilizing more than 90 % of the total of 200 mg/d, 400 mg/d, 600 mg/d, and 800 mg/d SOC respectively. On the other hand, the MLVSS concentration in SBR 3 after the initial decrease, was eventually stabilized within the 150 and 170 mg/L range. Again this can be associated with the relatively lower feed SOC value.

HRT	MLVSS Concentration (mg/L)		
(hrs)	SBR 1	SBR 2	SBR 3
48	1680	1949	1038
24	1650	2798	117
16	2115	3063	174
12	2463	3925	148

Table 5. 3 Average MLVSS Concentrations at Various Stages of HRT study

A direct comparison of all HRT studies revealed a maximum treatability at 12 hrs HRT. The total amount of waste treated per unit time indicated a four fold increase in treatability, from a 98.4 mg 2,4-D / d at 48 hrs HRT to 393.6 mg 2,4-D / d at 12 hrs HRT.

5. 4. 2 SBR Monitoring for Abiotic Losses

A control reactor was installed to explore the potential contribution of abiotic losses to the degradation phenomenon observed. Often such losses are attributed

to the processes of volatilization, photodegradation, and chemical degradation. This control reactor was operated at 48 hrs HRT, which has been the maximum applied HRT during this study, and was kept devoid of biomass. However, other parameters such as reactor volume, mixing, temperature, and aeration were same as in the other SBRs. Reactor sampling indicated no detectable decrease in 2,4-D concentrations in the effluent after the completion of the react cycle in SBR operation. This suggest that no abiotic losses were occurring in SBR systems and all the removal was due to biological processes.

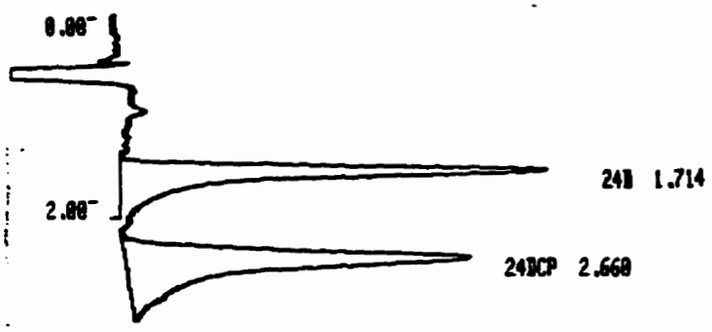
No attempt was made to quantify the potential of 2,4-D adsorption on the biomass. Previous biodegradation studies (one particularly performed using activated sludge) have clearly demonstrated that there was no such adsorption mechanism was involved during 2,4-D removal. (Hill et al., 1986; McTernan and Pereira, 1991). In addition the lack of 2,4-D removal in SBRs 1 and 2 during the long acclimation period (over 80 days) observed in the present study provides a strong evidence that 2.4-D absorption onto the biomass was negligible.

5. 4. 3 Observations Regarding the 2,4-D Degradation Pathway

The exploration of the 2,4-D degradation pathways was beyond the scope of this study. However, the HPLC equipment was calibrated to detect at least one of the main intermediate products (i.e. 2,4-DCP). This pathway involves the side chain hydroxylation of 2,4-D with the help of the enzyme alpha-keto-glutarate dioxygenase resulting in the formation of CO_2, 2,4-DCP, succinate, and glyoxylate

(Figure 2.6). There were sporadic appearances of the metabolic intermediate product 2,4-DCP over the entire study period, at concentrations below 5 mg/L. A typical HPLC chromatogram is shown in Figure 5.14. This suggests that degradation may have proceeded via the removal of side chain. Such pathways are typically followed by several bacterial species including Pseudomonas cepacia, Alcaligenes eutrophus, and Arthobacter (Clarkson et al., 1993; Daugherty and Karel, 1994; Steiert and Crawford, 1985). Details of this pathway have been already mentioned earlier (Section 2. 2. 3. C). Over the entire length of this study, 2,4-DCP was not observed to accumulate in any SBR system. This non accumulating behavior of 2,4-DCP has been also reported in another study, which indicated that less than 1 μg/l 2,4-DCP was observed in the system (Smith et al., 1994).. However, the HPLC equipment used in this study had a detection limit of 1 mg/L.

Summary: Overall, SBR technology has proved to be very effective in degrading 2,4-D. SBRs 1 and 2 were able to degrade approximately 200 mg /l SOC at each HRT tested. The sequential supplemental substrates and 2,4-D removal was also evident from this component of the study. Despite the higher concentration of supplemental substrates applied (after 2,4-D degradation) in both SBRs, their depletion was accelerated as compared to the depletion observed during the acclimation period. The higher 2,4-D removal rates observed at the final stages of the study may have been the result of biological selection which increased numbers of specialized microbial degraders. It should be noted that the main mechanism of 2,4-D disappearance is biodegradation, since adsorption on biomass and volatilization were negligible.



	SF	PA	PB
	.100000E+01	.100000E+01	.100000E+01

NO.	NAME	RT	A OR H	MK	CONC
1	24D	1.714	130490	M	4.9280
2	24DCP	2.660	159972		5.1332
	TOTAL		290463		10.0613

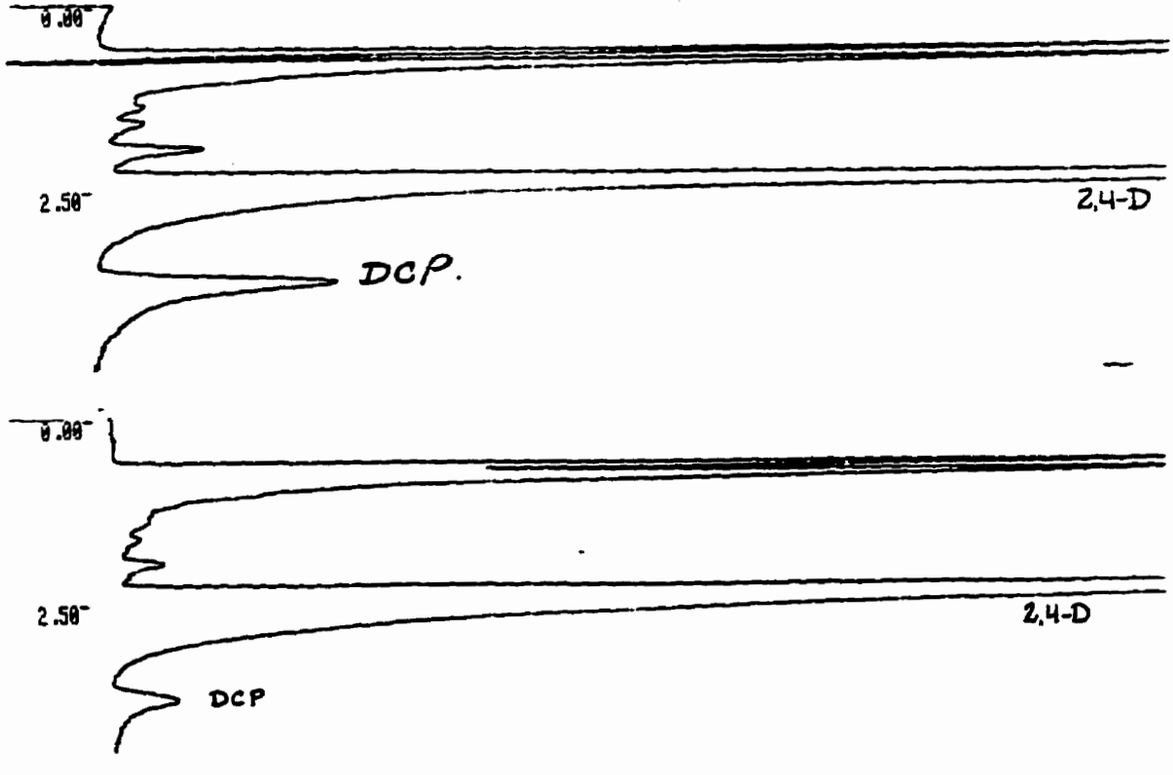


Figure 5.14 Typical HPLC Chromatogram Showing Intermediate Products after 2,4-D Breakdown

5. 5 Effect of Variation in Feed Concentration

The last part of the SBR studies was to investigate whether the increase or decrease in supplemental substrate concentration (SBR 1 and 2) or increase in 2,4-D concentration (SBR 3) would affect the 2,4-D degradation. To observe such effects, a study was organized, where, by changing the feed constituents in a particular cycle, the 2,4-D degradation patterns were studied. Observations were also focused to quantify the system's endurance towards shock loads, which are very common in real field conditions. These effects were studied by keeping the HRT constant at 12 hrs during all feed variation experiments. Depending on the increase or decrease in phenol and dextrose concentration, nitrogen and phosphorus sources were also adjusted accordingly. The potential for nutritional complication was alleviated by keeping the C:N:P ratio at 100:20:5 levels. Care was also taken to minimize accumulation of any potential residual material. Such accumulation often results in system failure (Haughland et al., 1990). To overcome this problem, between any two spike periods (the time period during which concentration changes were made in the SBR systems) the reactors were allowed to run for several normal feed cycles. In all cases the next experiment was initiated after ensuring complete removal of all the substrates by performing mass balance calculations. However, no such buildup was observed during any experiment. In addition, spike studies were duplicated to improve the accuracy of the results.

5. 5. 1 Effect of Supplemental Substrate Concentration

Three different feed variations in supplemental substrate concentration were applied in SBR 1 and 2. The feed compositions during these three sets of experiments were designated as S1, S2, S3 (Table 4.2). The S1 stage refers to the absence of supplemental substrate (phenol or dextrose) in the feed. The sole source of carbon in this case was 2,4-D. The second stage S2 implies a doubling in the feed supplemental substrate concentrations, whereby keeping the same 2,4-D levels. The maximum feed concentration applied include 3.5 times the normal supplemental substrate concentrations (S 3). A detailed discussion of these experiments is provided below.

During stage S1, the SBR 3 was also monitored for comparison purposes. In this experiment, the absence of supplemental substrate (phenol or dextrose) did not effect the 2,4-D degradation (Figure 5.15). In addition, higher removal rates were observed in SBR 1 and 2 in comparison to SBR 3. This may have been the result of longer 2,4-D stress period on biomass and consequently faster enzyme production for 2,4-D degradation. Another possibility may have been that all the enzyme system produced at these conditions is dedicated to 2,4-D and it's byproduct breakdown alone. On the other hand, a comparison of normal feed and zero supplemental substrate conditions in Figure 5. 16, shows minimal variations in 2,4-D degradation pattern. It appears that degradation was slightly faster (by about 10 min) in the absence of supplemental substrate. However, due to the nature of biological systems in general, such low level of variations may not be considered as a different response. This indicate that there was no short term

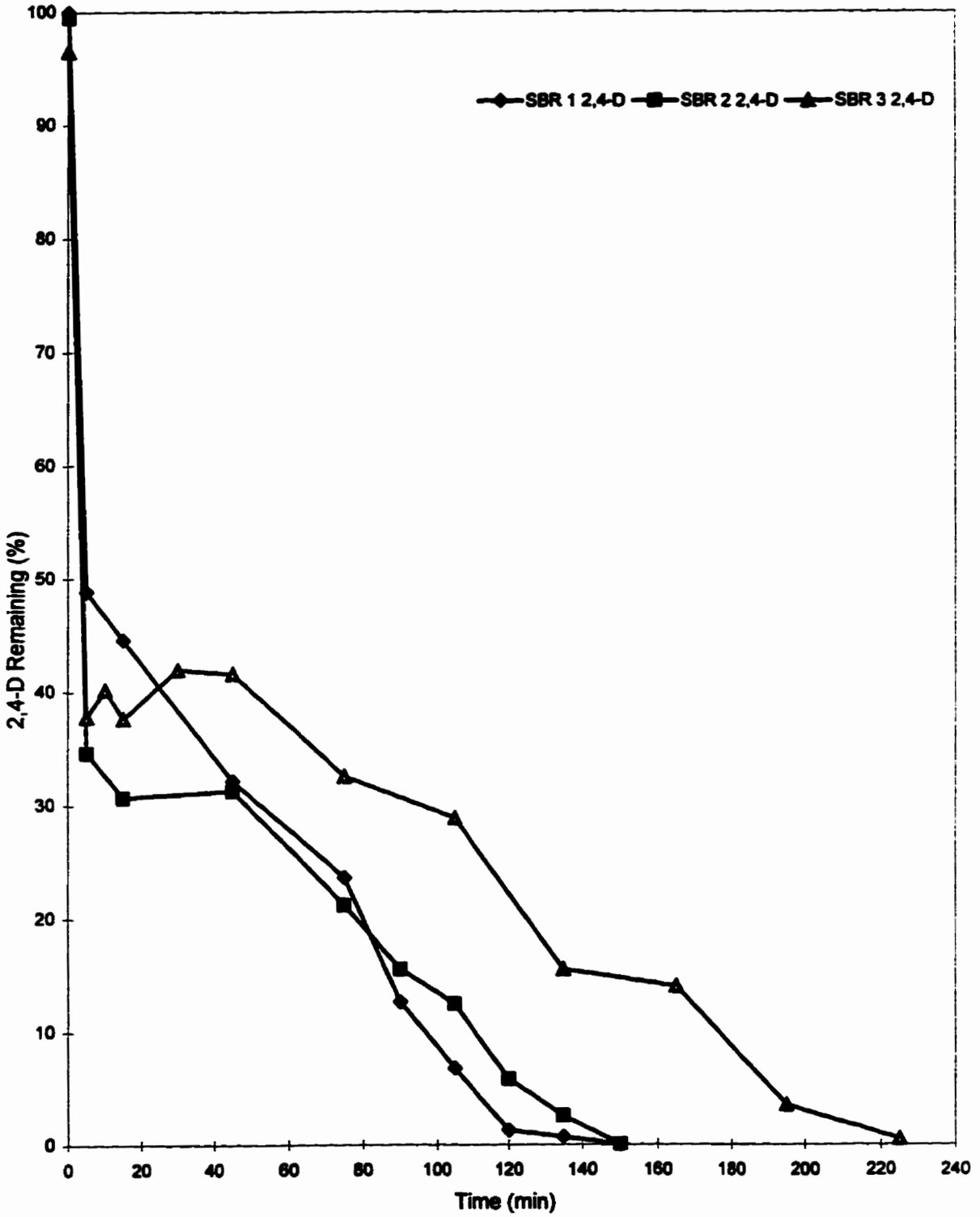


Figure 5.15 Substrate Depletion Pattern at Zero Supplemental Conc. in SBR 1 and 2 in Comparison to SBR 3

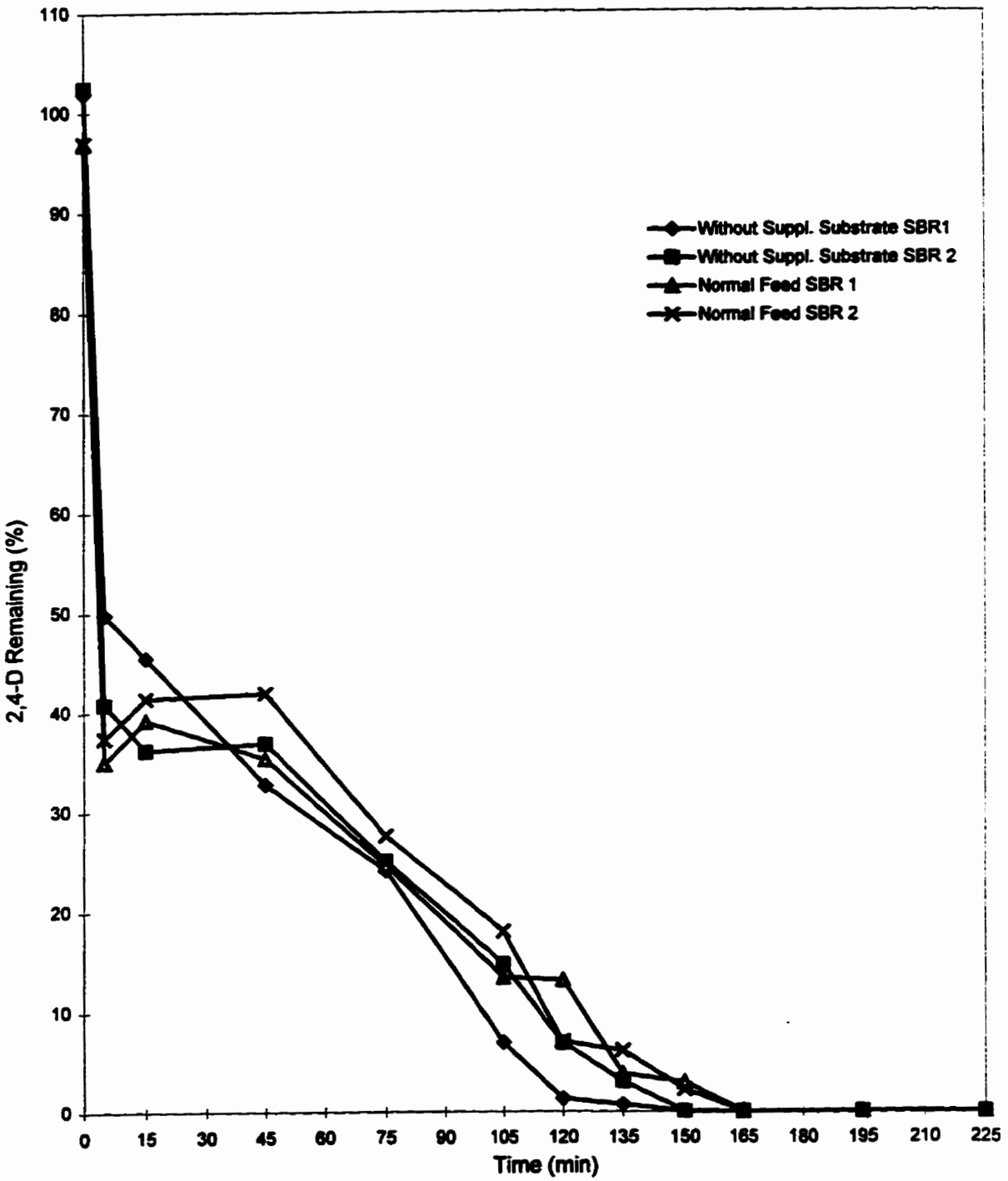


Figure 5. 16 Accelrated Degradation Rates Observed in the Abscence of Supplemental Substrate in SBR 1 and 2

effect due to the absence of either supplemental substrate. During stage S2, doubling the normal concentration of supplemental substrate, approximately to 400 mg/L phenol and 775 mg/L dextrose concentration, yielded once again complete removal of 2,4-D as well as the respective supplemental substrates. Details on reactor's performance at this level of supplemental substrate concentration are shown in Figure 5.17. It is evident from this profile that there was not significant difference in the 2,4-D degradation pattern. However, the total time for supplemental substrate depletion increased to 30 min in comparison to less than 20 minutes at the normal feed condition.

During stage S3, 3.5 times the normal amount of supplemental substrate concentrations were applied in the feed. These concentration changes resulted in significantly different degradation profiles than that observed under normal feed, and stages S1, and S2 of the experiment. The system's response is summarized in Figure 5.18. The total time required for removal of supplemental substrate has been 60 minutes for phenol and 30 minutes for dextrose. In addition, previously in both SBRs, 2,4-D and phenol were being sequentially utilized. The supplemental substrate was being utilized first and 2,4-D degradation always followed. However, at such high concentrations this trend was not repeated at least in the case of SBR 1. There is evidence of concurrent utilization of both phenol and 2,4-D in this reactor at high phenol concentration. On the other hand, the dextrose-fed system did not show any noticeable change in the preference order for the removal of substrates. Dextrose has been depleting first, always preceding 2,4-D degradation (Table 5. 4). This phenomenon of shift of the microorganisms from one source to

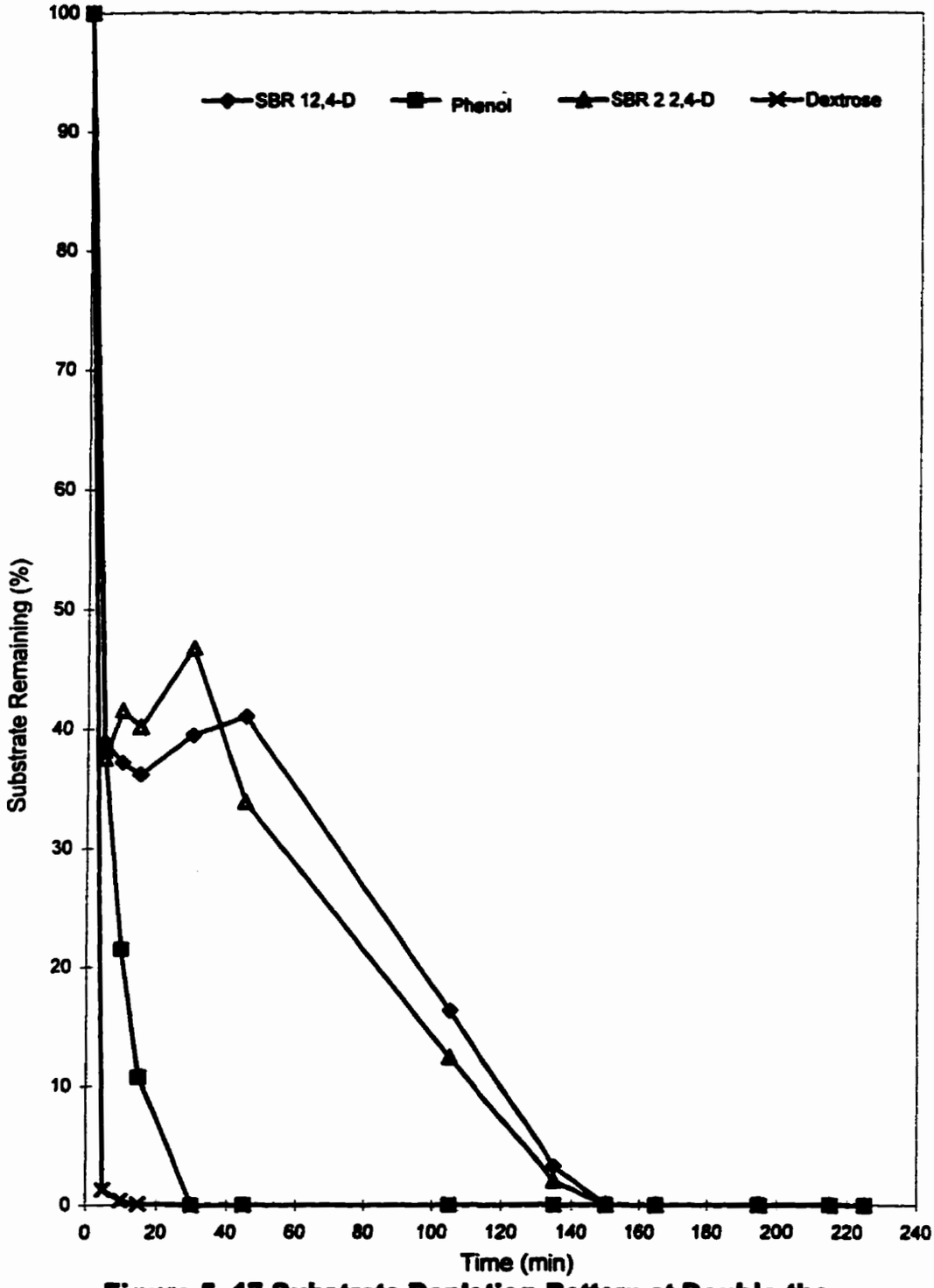


Figure 5. 17 Substrate Depletion Pattern at Double the Supplemental Substrate Concentrations

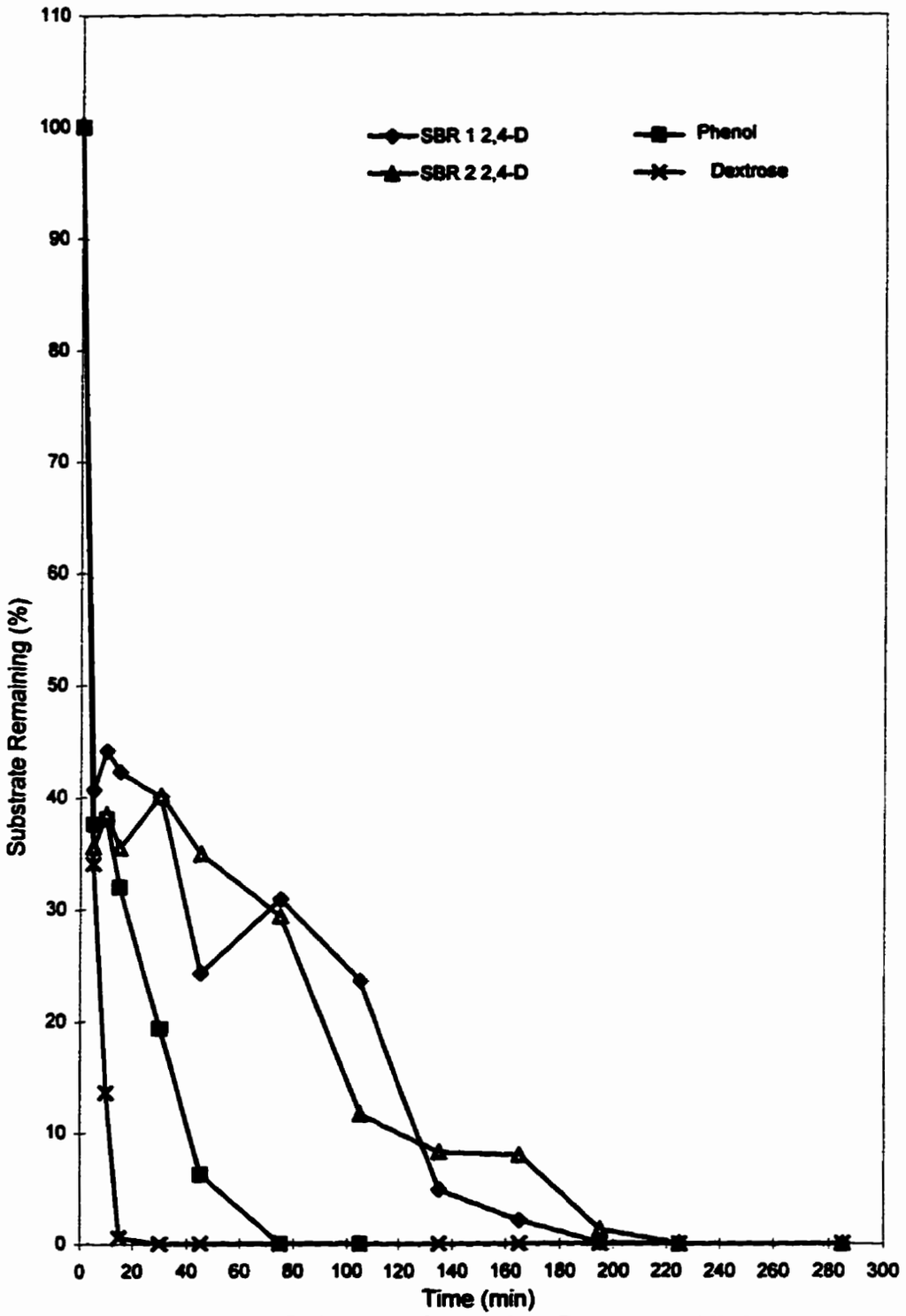


Figure 5. 18 Substrate Depletion Pattern at Highest Supplementary Substrate Concentrations (3.5xnormal)

another at high concentrations has been also reported in another study (Fournier, 1981).

Time min	SBR 1 Substrate Remaining (%)		SBR 2 Substrate Remaining (%)	
	Phenol	2,4-D	Dextrose	2,4-D
0	100	100	100	100
5	38	41	34	35.6
10	38	44	14	38.5
15	32	42	1	36
30	19	40	0	40
45	6	24	0	35
75	0	31	0	29
105	0	24	0	12
135	0	49	0	8
165	0	2	0	8
195	0	0	0	1
225	0	0	0	0
285	0	0	0	0

Table 5. 4 Substrate Degradation Pattern at the Highest Supplemental Substrate Applied

Overall, the maximum applied phenol and dextrose concentrations of 680 mg/L and 1350 mg/L respectively did not induce any toxic effects. In oppose to this

observation, a previous study by Oh and Tuovinen (1990) reported that 500-1000 mg/L of glucose had shown severe effects on 2,4-D degradation. On the other hand, no information was found in the literature regarding the direct effect of phenol on 2,4-D degradation. However, a system with two supplemental substrates (phenol and bacto nutrient) showed an inhibitory effect on 2,4-D degradation at 490 mg/L phenol levels (Orhon et al., 1989). Similarly, present study found minimal effect on 2,4-D degradation up to 400 mg/L phenol concentration. However, a further increase (3.5 times the normal level) did show a delay in total 2,4-D degradation. Details of this response are depicted in Figure 5.19.

The specific removal rates in SBR 1 and 2 are given in Table 5. 5. It was evident from these observations that as the supplemental substrate concentrations increased in both reactor systems, a concurrent specific removal rate increase was also observed. However, this trend only continued up to 310 mg/L SOC levels. A sharp decrease in the specific removal rate was noticed, when the SOC value further increased to 535 mg/L. Such decrease was as lower as 50 percent of the highest specific removal rate observed. The above observations clearly demonstrate that there is a threshold value above which the 2,4-D degradation would experience detrimental effect. High supplemental substrate concentrations have been shown to induce detrimental effect on the 2,4-D degradation in a previous study (Daugherty and Karel, 1994). In this study increasing amount of succinate had resulted in very low 2,4-D removal rates. A comparison of consistent trend of specific removal rates between SBR 1 and SBR 2 is highlighted in Figure 5.20. In addition, an average of 42 percent lower specific removal rate in dextrose-

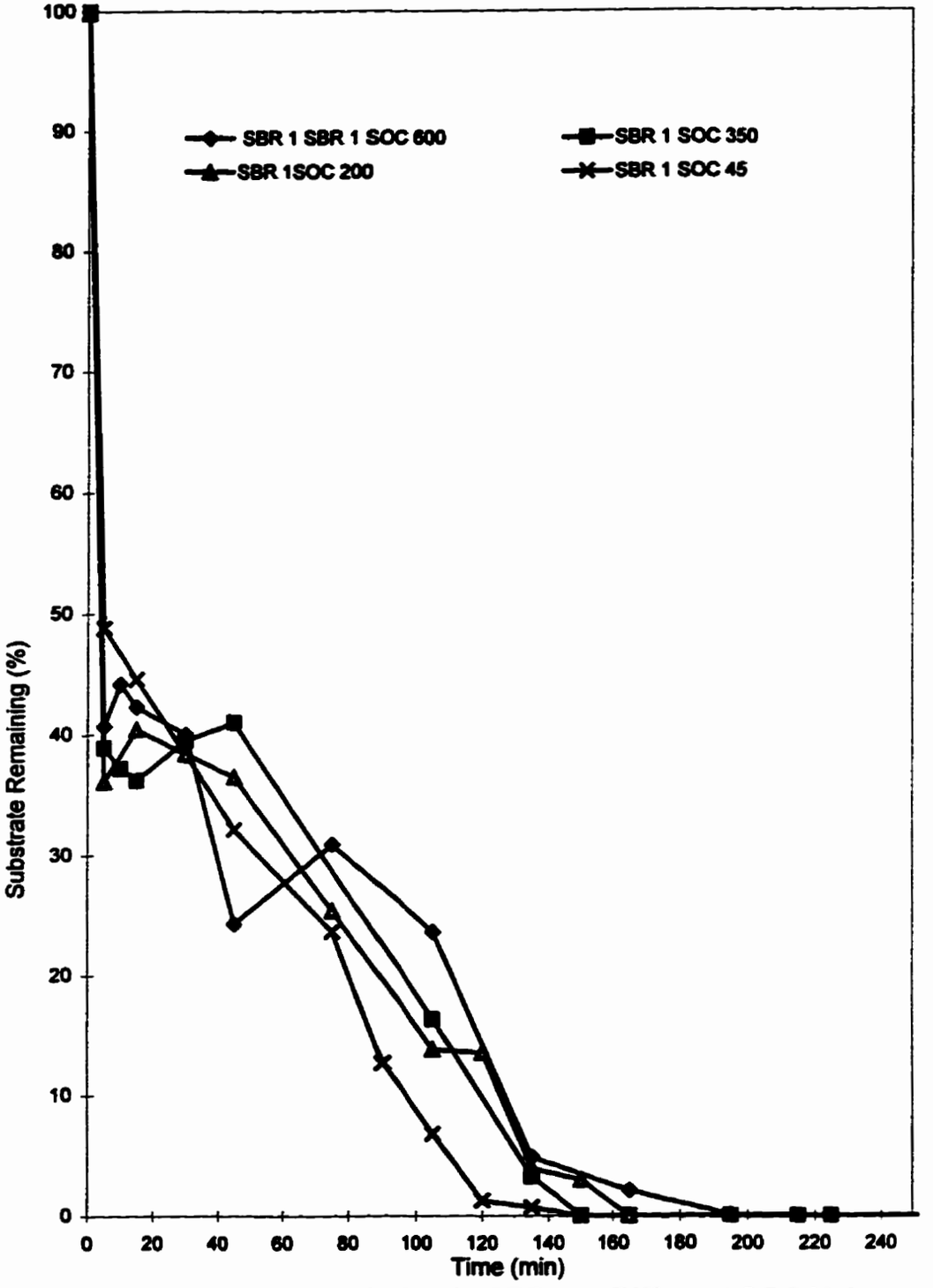


Figure 5. 19 2,4-D Degradation in SBR 1 at Different SOC Values

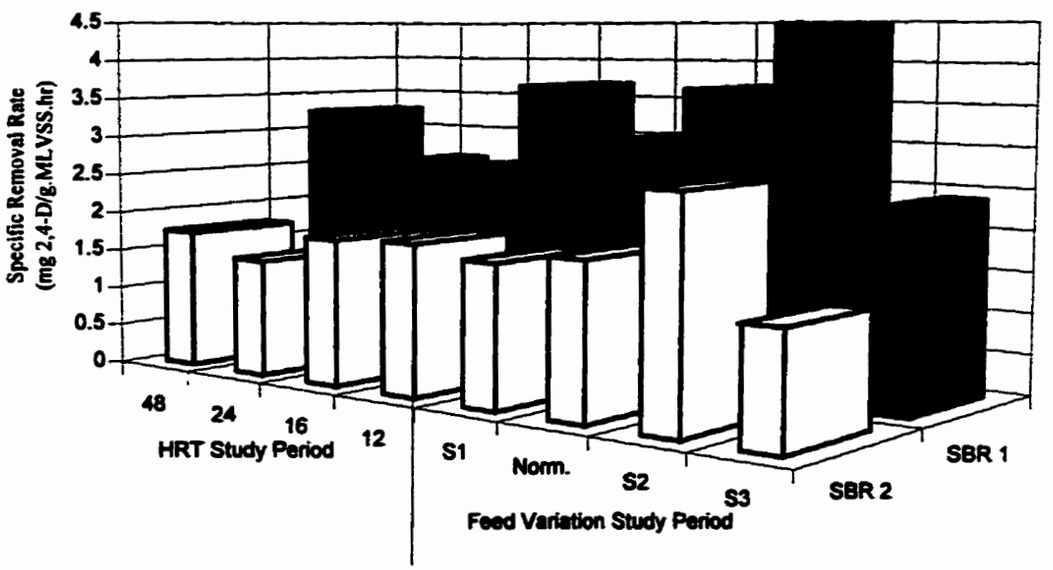


Figure 5.20 Specific Removal Rates at Various Stages of the Experimental Period

fed system (SBR 1) relative to the phenol-fed system (SBR 2) at all S 1, S 2, S 3, and normal feed conditions was also evident. As outlined earlier, degradation of both supplemental substrates increased with the increase in reactors operation time. The particular significance of enhanced degradation time is in terms of phenol removal, since phenol is a potential contaminant of natural

SOC (Suppl.)	Specific Removal Rates (mg 2,4-D/g.MLVSS. hr)		
mg/L	SBR 1	SBR 2	%Difference
(S1) 0	3.02	1.73	42
(Normal) 155	3.64	2.16	41
(S2) 310	4.47	2.70	40
(S3) 535	2.38	1.35	44

Table 5. 5 Specific Removal Rates at Various Supplemental SOC in SBR 1 and SBR 2 at Similar HRT

environments. This method has also shown promising results as far as phenol removal is concerned. This study has been able to achieve a maximum of 160 mg phenol/g MLVSS.hr (Table 5. 6). Another trend noticed during phenol removal has been that as the phenol concentration in feed was increased decrease in specific removal rate was noticed.

5. 5. 2 Effect of 2,4-D Concentration Variations in SBR 3

In SBR 3, 2,4-D alone was employed to quantify the maximum treatability potential at 12 hrs HRT. Providing adequate nutrients, 2,4-D concentration was increased to 2 and 3 times the normal concentration applied (Table 4.3 T1 and T2 Stages). As far as the first application is concerned (i.e. 200 mg / L), 2,4-D was being depleted within 225 min time, which was well within the react period (4.75 hrs) at 12 hrs HRT. SOC removal also followed a similar trend (Figure 5.21).

Phenol Conc. (mg/L)	MLVSS (mg/L)	Specific Removal Rate (mg phenol/g MLVSS.hr)
100	510	100
200	2440	160
407	2635	90
680	2635	40

Table 5. 6 Specific Removal Rates at Various Levels of Phenol Concentrations in SBR1

The 2,4-D concentration was further increased in SBR 3 to about 300 mg/L. This corresponds to nearly 3 times the normal concentration in SBR 3. The profile of 2,4-D degradation is given in Figure 5. 22. As indicated, the system could not completely degrade the applied 2,4-D amount, but a significant removal was noticed. A total of 95% of 2,4-D was degraded during this period. In addition, because of partial removal of the applied concentrations, this can be considered

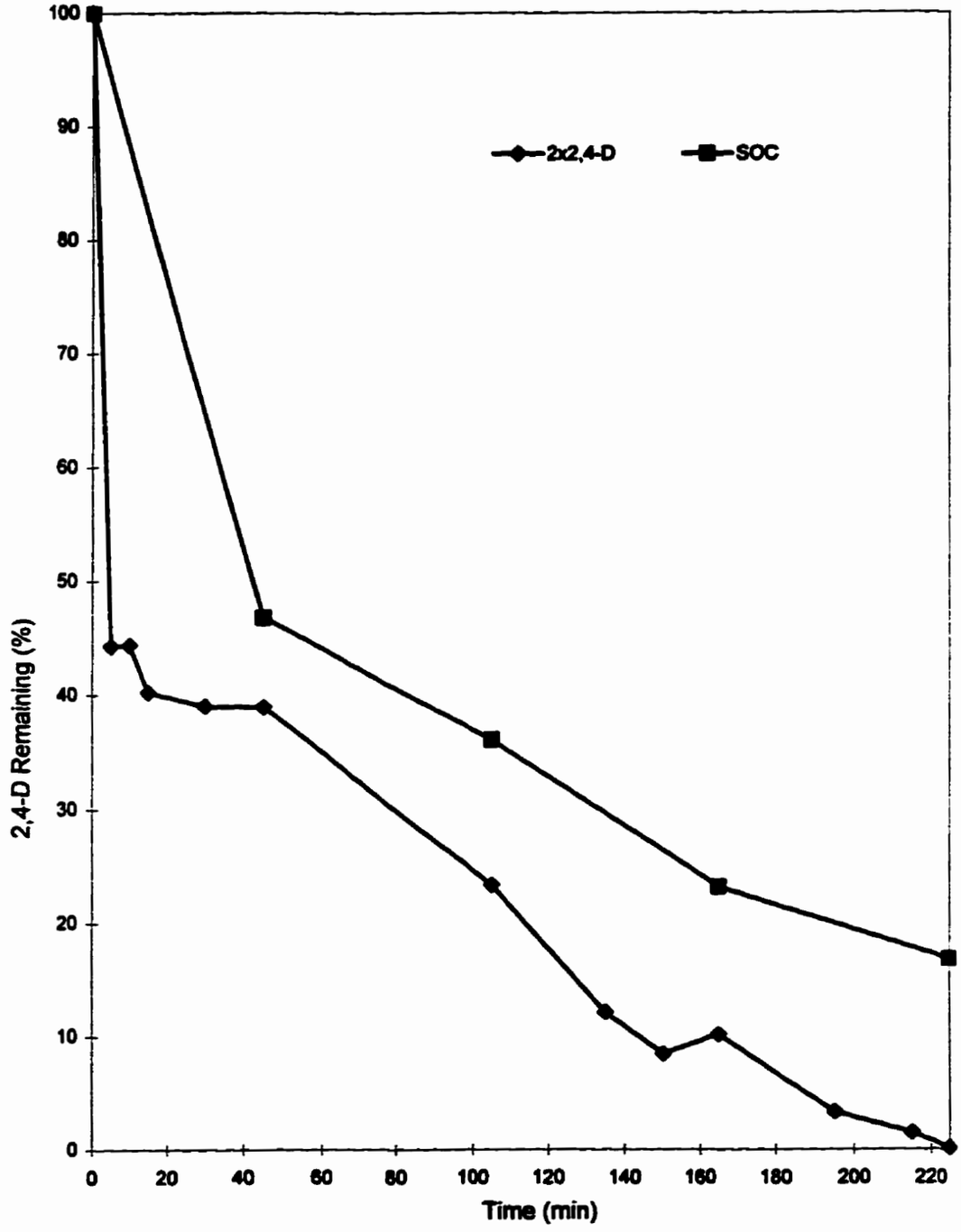


Figure 5. 21 2,4-D Depletion Pattern at Two Times the Normal Concentrations in SBR 3

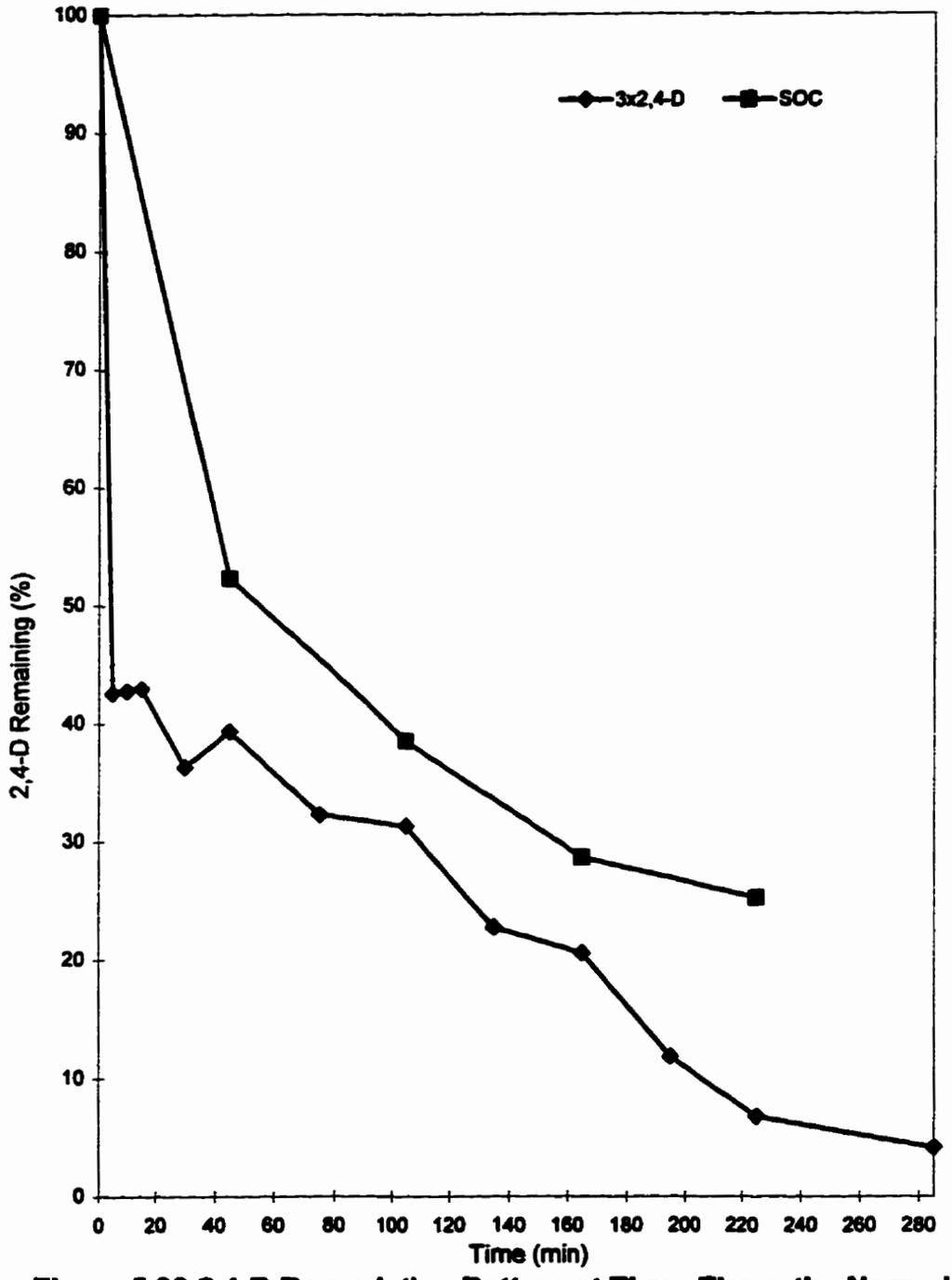


Figure 5.22 2,4-D Degradation Pattern at Three Times the Normal Concentration

as a breakpoint for system performance at 12 hrs HRT. Fournier (1981) recorded that at high 2,4-D concentrations, there is quicker degradation.

The 2,4-D specific removal rates are given in Table 5. 7. The system's performance improved with the increase in the feed 2,4-D as the specific removal rate reached maximum value of (95 mg/g.MLVSS.hr) highest concentration applied. It is apparent that no adverse effects were noticed as 2,4-D concentration increased.

A comparison of degradation profiles at the various concentrations applied in SBR 3 is provided in Figure 5.23. A sharp decrease in percent removal rate in the beginning is the result of dilution of the feed in all cases. However, after the dilution factor, there was a variation in the degradation patterns between normal and increasing feed conditions. During normal feed conditions, the reactor did not indicate immediate breakdown of 2,4-D. There has been always a delay of more than 45 min (after the start of the react

Feed Pattern	2,4-D Conc. (mg/l)	Specific Removal Rate (mg 2,4-D/g.MLVSS.hr)
Normal	100	67.5
T 1	200	93
T 2	300	95

Table 5. 7 2,4-D Specific Removal Rates in SBR 3 at Various Feed Patterns

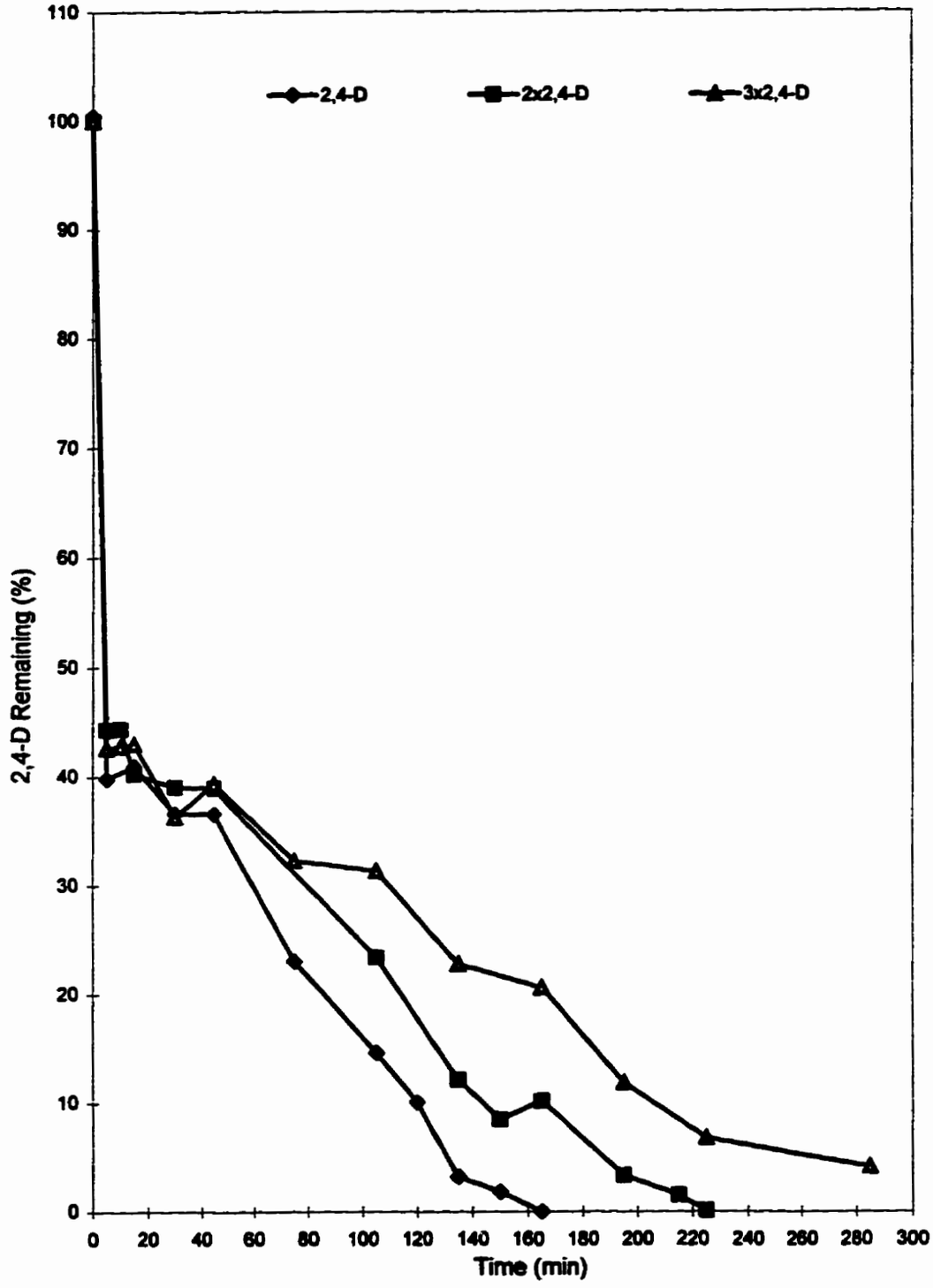


Figure 5.23 Degradation Pattern at Various Concentrations of 2,4-D in SBR 3

period) before significant removal could occur (>10%) (Figure 5.24). On the other hand, when high concentration (3 times the normal) was applied more than 15% 2,4-D was removed within 30 min.

5. 6 Salient Features of the SBR Study

Over the entire study period, a very important observation has been that at all stages (i.e. HRT optimization and supplemental substrate effect studies), consistent high specific removal rates were observed in SBR 3 reactor as compared to SBR 1 and SBR 2. This can be attributed to very low MLVSS concentration present in SBR 3. Since by deleting supplemental substrate from the influent, no 2,4-D degradation hindrance was noticed, it is believed that the absence of phenol or dextrose does not show any short-term effects on the reactor's performance in terms of 2,4-D degradation. However the need for supplemental substrate can not be ignored all together, specially considering the situation during the starting phase of the experiment. However, if the system's stability is concerned, undoubtedly, the reactors with supplemental substrate addition appeared to exhibit a better biomass settling behavior in comparison to SBR 3. Through visual examination, the settling ability in SBR 1 and SBR 2 has been much better and particularly floc sizes appeared to be larger than in SBR 3. So it can be concluded that there is a requirement of the presence of supplemental substrate to harbor target compound degraders. Results on the role of supplemental substrate suggest that it may increase flocculation and hence improve settling of the target compound degraders. Few studies have indicated such role of dextrose in enhancing flocculation, and not to increase the growth rate

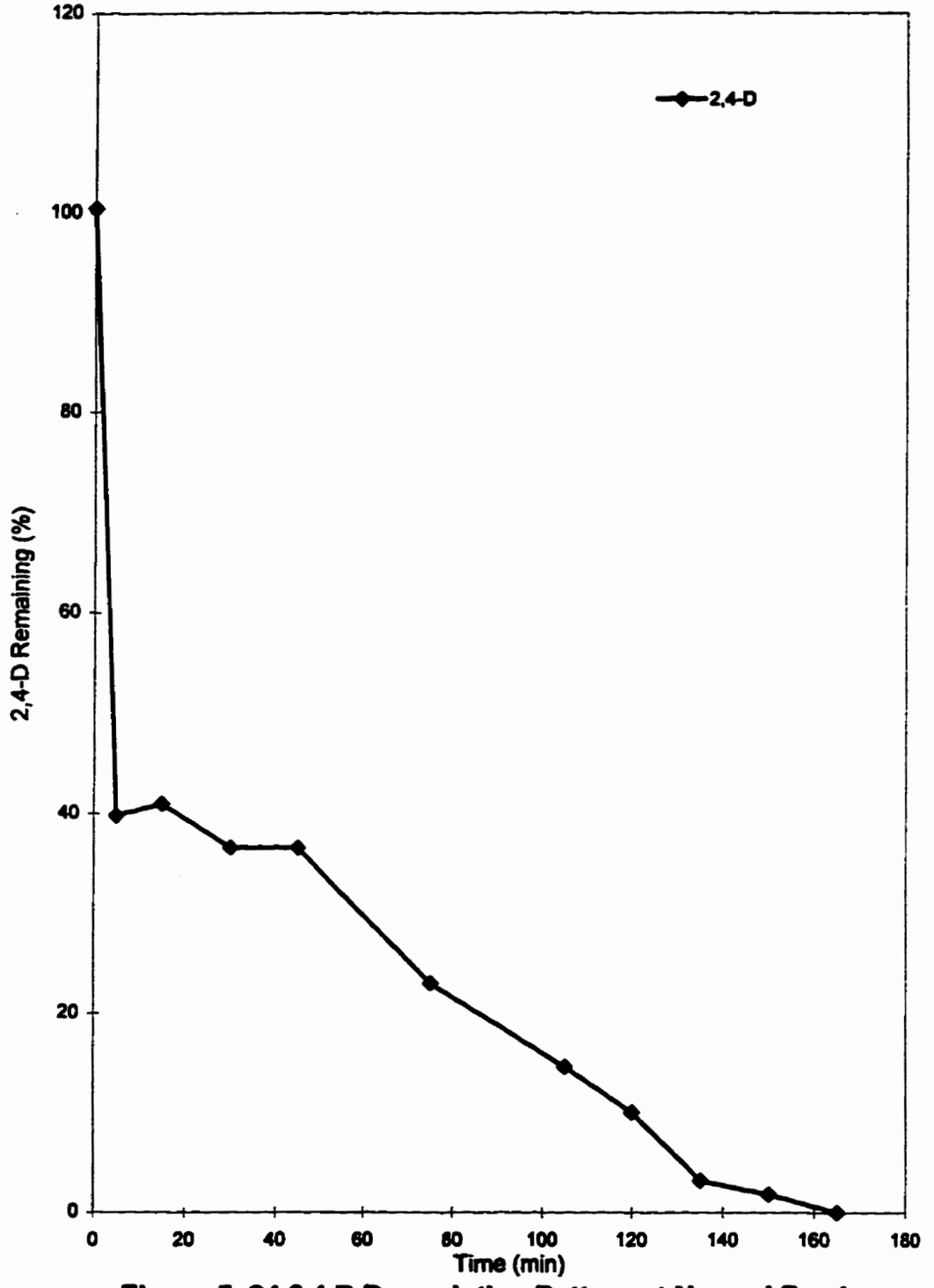


Figure 5. 24 2,4-D Degradation Pattern at Normal Feed Conditions in SBR 3

of 2,4-Dinitrophenol (DNP) degraders (Schmidt et al., 1992; Silverstein, 1990). During the above experiments the settling biomass was able to harbor an increased number of active 2,4-DNP degrading population.

No attempt was made during this project to quantify the amount of biomass associated with 2,4-D degradation vs. the biomass which obtain it's carbon and energy by utilizing supplemental substrate or through cometabolism. Hence the MLVSS concentration value may in some cases represent biomass that is not actually associated with the 2,4-D degradation population. Fournier et al (1980) reported that such a bacterial population could be 1000 to 10,000 fold higher (in soil systems) than the 2,4-D as a sole source of carbon and energy. It could be argued that since the same concentration of 2,4-D was applied in the feed, similar numbers of 2,4-D specialized population may have developed in all systems.

As far as sequential utilization of supplemental substrate is concerned, a supporting observation by Papanastasiou (1982) has been confirmed during this study. Such preferential utilization of a mixture in dual substrate conditions has been frequently reported in the past. Kleck and Maier (1988) indicated that pentachlorophenol (PCP) and phenol, when fed as a mixture, resulted in PCP utilization first, which followed by phenol degradation. Other studies have indicated such a sequential or concurrent utilization trend as well (Saez and Rittmann, 1991; Shimp and Pfaender, 1987; Tabak et al., 1964). Sequential utilization can be explained on the basis of enzyme requirements and/or complexity of the chemical structures. It has been reported that a competition may exist for enzyme system requirements. This competition is among the various carbon sources for specific

enzymes (i.e., oxygenase). The slightly different picture was observed in the present study at high phenol concentrations may be the result of effect of direct or indirect toxicity (i.e., intermediate product inhibition). Often biomass shifts its feeding pattern in such adverse conditions (Chang and Cohen, 1995). Lastly, bacteria preferentially tend to disintegrate and utilize simple-structured compounds first.

Intermediate product inhibition appeared to be insignificant in this case, since all reactors did not show any signs of toxic inhibition throughout the period of operation of over 200 days.

5. 7 Summary of SBR Performance

Overall, SBRs have been very successful in treating high concentrations of 2,4-D waste. In comparison to other biological systems, the SBR system has been the most effective biological treatment of 2,4-D contaminated waste. As high as 300 mg/L applied 2,4-D concentration was removed to a significant amount (95%). Although it is a significant removal, yet an 11.9 mg/L 2,4-D effluent concentration may not satisfy the disposal requirements. According to Canadian standards the maximum 2,4-D limit regarding freshwater aquatic life is 4 μg/l (CWG, 1995). The system is robust against changing feed conditions, which is realistic in field conditions.

The HRT has been a critical parameter during this study, first in the beginning to achieve degradation and later to treat high concentrations. The longer the compound was in contact with the biomass the higher were the depletion

percentages. The only exception at the highest concentrations tested may be attributed to other factors such as inhibition toxicity etc.

Specific removal rates for 2,4-D in SBRs were influenced by the presence and nature of supplemental substrate like dextrose or phenol. In terms of percent removal rates the presence of supplemental substrate has an insignificant effect on total time required for 2,4-D removal. Irrespective of the presence of very low concentration of MLVSS in SBR 3, degradation occurred within approximately the same time at all stages. On the other hand, the presence of 2,4-D certainly has enhanced the supplemental substrate depletion as well.

Wherever supplemental substrates were present, their utilization for microbial growth was observed regardless of the presence of 2,4-D. It was Observed that organisms either utilized the supplemental substrate preferentially and completely before acting on the target substrate, or utilize concurrently both substrates. Unlike other chloroorganics such as 4-chlorophenol, prior exposure of biomass to phenol is not the only way of degradation of 2,4-D. The particular biomass used in this study was able to use 2,4-D as a sole source of energy and carbon (SBR 3).

5. 8 Effect of pH on 2,4-D Degradation

The pH study was performed using the reactor set-up described in Table 4.4. Two experiments were performed at 30°C temperature in a temperature-controlled chamber. All conditions were kept similar, except in the first case no pH adjustment was practiced whereas in the second case the pH of the reactor system was constantly adjusted using NaOH or HCl.

5. 8. 1 Uncontrolled pH Study

Results on 2,4-D concentration vs. time are plotted in Figure 5.25. For this experiment, the initial pH of the reactors was in the range of 7.0t o 7.7. First sample analysis after 24 hrs indicated the complete removal of both supplemental substrates in all reactors, except reactor P200, which was able to deplete only 10% of the applied phenol. The particular reactors, which depleted maximum supplemental substrate had experienced a pH drop from their respective initial values. For instance, reactor D200 had the highest drop in pH to 5.5. All other reactors were at the 6.0 to 6.8 pH range. Hence both dextrose and phenol mineralization was also associated with a pH drop in the systems. After 48 hrs of operation a slight depletion of 2,4-D was noticed in the reactor having 2,4-D as sole source of carbon. This was associated with pH drop down to 6.7. By this time P200 had shown complete removal of phenol, which also resulted in pH drop down to 5.7. Further sampling on a daily basis did not show any significant variations from above described levels until after 144 hrs of operation (day 6). At this stage, a

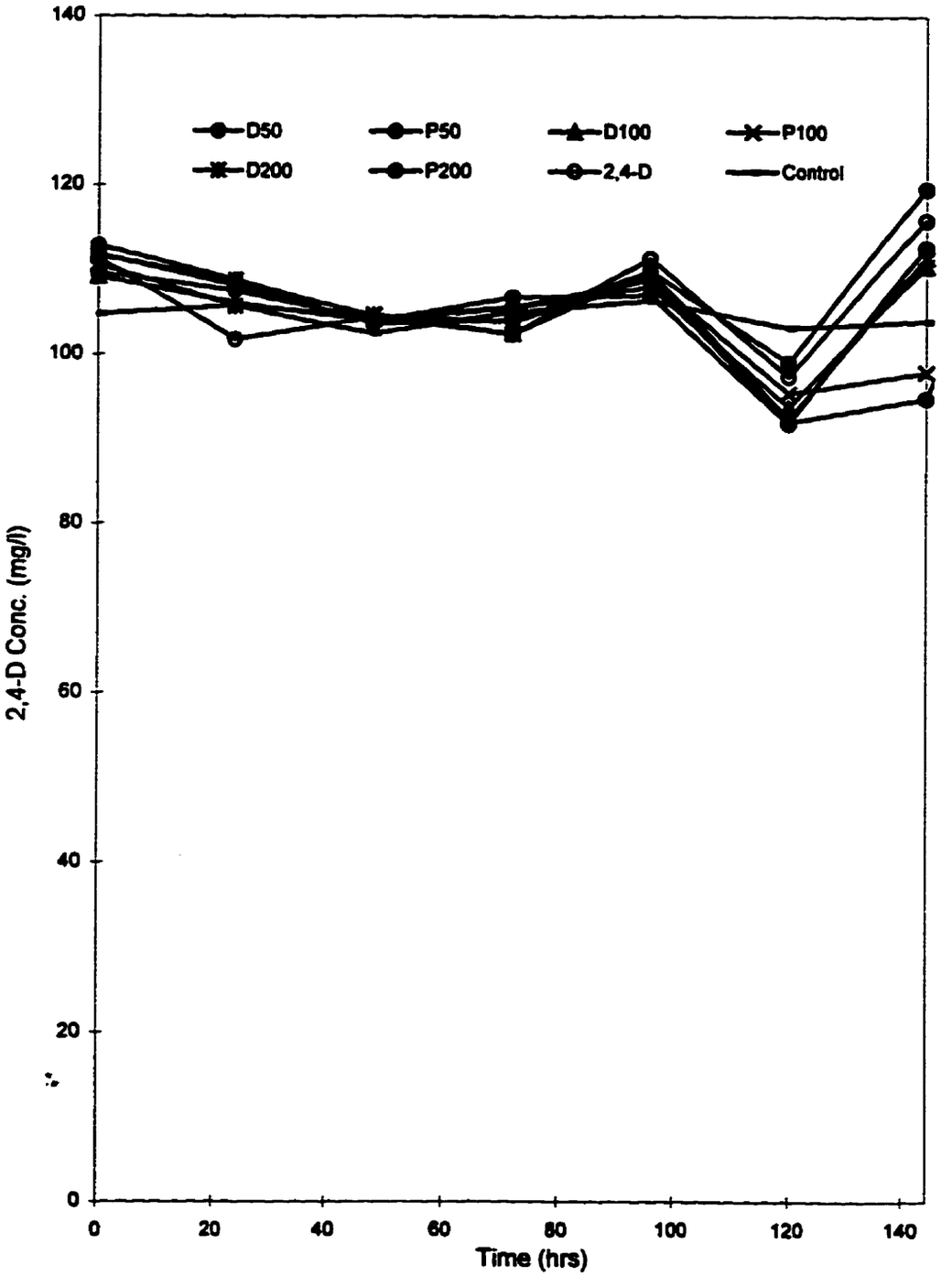


Figure 5. 25 2,4-D Degradation at 30^{0}C Temperature Without pH Control

further decrease in pH was noticed along with a slight decrease in 2,4-D concentration. In most of the reactors 8-10% decrease was in 2,4-D was evident. A pH drop in the 5.2 to 5.4 range was also observed. After this sampling period, no further significant decrease in 2,4-D was noticed, up to a total of 192 hrs (8 days) of operation. This observation led to believe that practically no degradation was possible in these systems. Similarly, Oh and Tuovinen (1990) revealed that degradation of 2,4-D is an acid-yielding step and degradation below pH 5.3 is not possible. A follow up study by the same authors indicated that only 40% reduction in 2,4-D in non-pH adjusting cultures occurred (Oh and Tuovinen, 1991). There is direct association of the enzyme system involved in 2,4-D breakdown and pH of the system (Fogarty and Tuovinen, 1991).

5. 8. 2 Controlled pH Study

In order to further investigate the reasons for the non-degradation behavior in the previous experiment, a second experiment was performed under similar operating conditions. Temperature, biomass concentration, and feed conditions were standardized according to the previous experiment. The only variation was to adjust the pH to 7.0-8.0 range in the event a pH drop occurred in the reactors below pH 6.0. A detailed degradation pattern is summarized in Figure 5.26.

Degradation patterns are shown in Tables 5.9 and 5.10. There has been a 24 hrs lag period before any noticeable (~5%) 2,4-D degradation occurred.

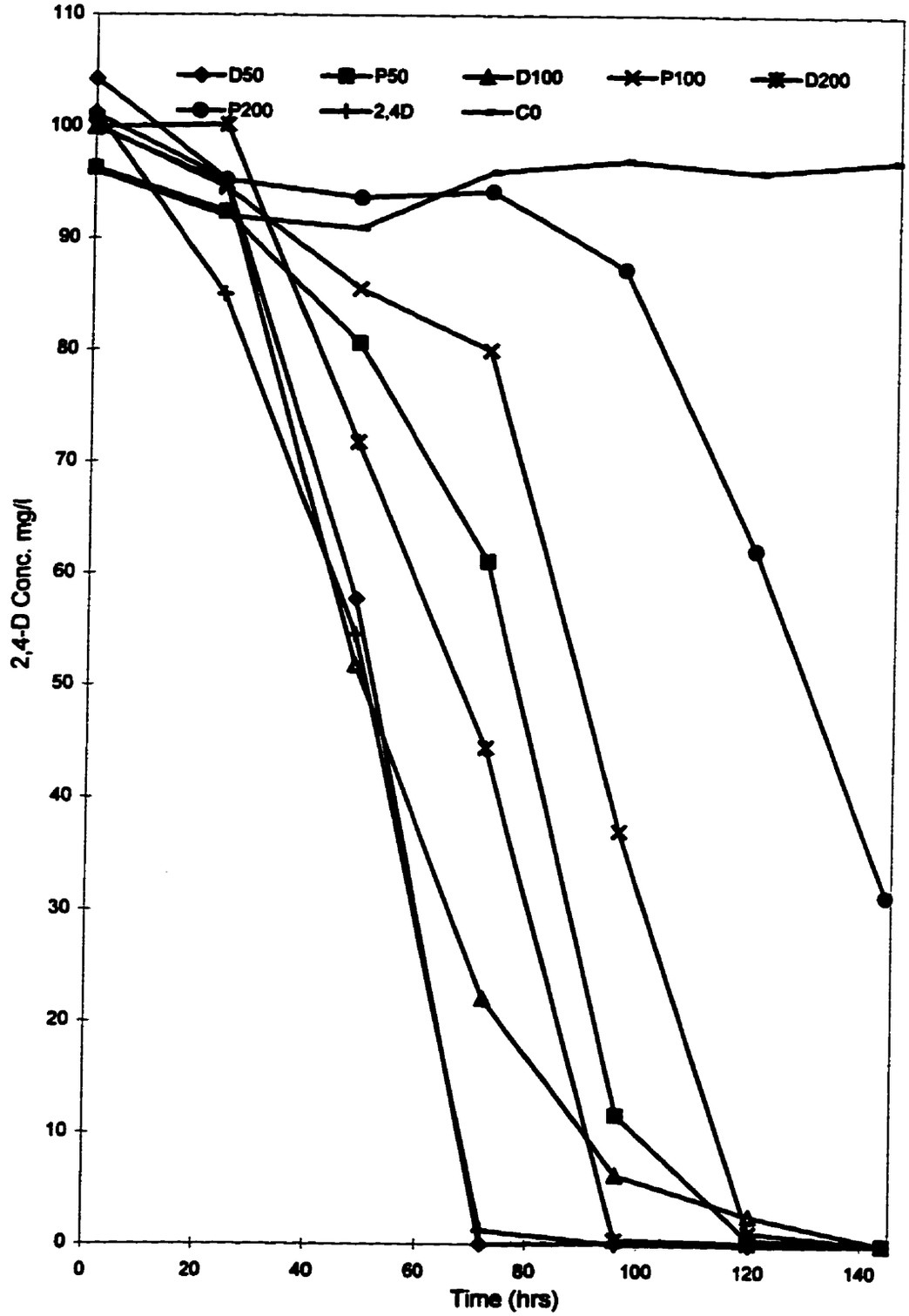


Figure 5. 26 2,4-D Degradation Pattern at 30^{0}C pH Control

Time	2,4-D Removal (%)						
hrs	P50	P100	P200	D50	D100	D200	2,4-D
0	0	0	0	0	0	0	0
24	4	5	6	9	5	0	16
48	16	15	7	45	48	28	46
72	36	20	7	100	78	56	99
96	88	63	14	100	94	100	100
120	100	99	39	100	97	100	100
144	100	100	70	100	100	100	100

Table 5. 8 Percent 2,4-D Removal at 30 °C with pH Control

Time	Supplemental Substrate Removal (%)					
(hrs)	P50	P100	P200	D50	D100	D200
0	0 (8.1)	0 (7.7)	0 (7.2)	0 (8.1)	0 (7.5)	0 (7.4)
24	70 (6.7)	20 (7.4)	14 (7.3)	100 (6.6)	100 (6.1)	62(5.9)
48	100 (6.7)	100 (6.2)	100 (5.7)	100 (5.8)	100 (5.9)	100 (5.9)
72	100 (6.6)	100 (6.4)	100 (6.7)	100 (5.7)	100 (6.2)	100 (6.7)
96	100 (6.3)	100 (6.5)	100 (6.8)	ND	100 (5.1)	100 (6.3)
120	100 (6.7)	100 (6.8)	100 (6.8)	ND	100 (5.2)	100 (5.4)
144	ND	100 (6.5)	100 (6.7)	ND	ND	ND

Table 5. 9 Supplemental Substrate Depletion Pattern with pH Control
(Values in Parentheses Represent pH Values)

Phenol-fed reactors achieved lower removal rates in comparison to those in the dextrose-fed reactors.

Among the phenol-fed reactors, the increasing phenol concentration resulted in decreasing 2,4-D removal rates. Similar response was recorded for the dextrose-fed reactors. In spite of similar environmental conditions, P200 reactor degraded only 70% 2,4-D after 6 days of operation (experiment termination stage).

Detailed pH changes in all reactors are shown in Figure 5.27. Overall a direct comparison of the above two 30°C experiments shows that without pH adjustment 2,4-D removal can not be achieved during batch studies. In contrast, during the 10°C temperature studies, there was not any pH adjustment required. This implies that the effect of pH is severe at high temperature during 2,4-D degradation. A possible explanation for this behavior may be that when 2,4-D disintegrates, it releases chloride ion. If the released Cl^- ion remains in the reactor system, it results in lower pH values. The fate of chloride ion is temperature and aeration dependent (Steenson and Walker, 1957). Tyler and Finn (1974) indirectly indicated the optimum growth of 2,4-D grown cultures at 25°C and at 6.2 to 6.9 pH range.

It can be inferred that due to the time period elapsed between the halt of degradation after pH drop and readjusting the pH, the observed degradation rates may not describe accurately the performance of the system as it is a non continuous operation. During this experiment pH studies (30°C control pH) the sampling frequency was lower than the previously conducted temperature study

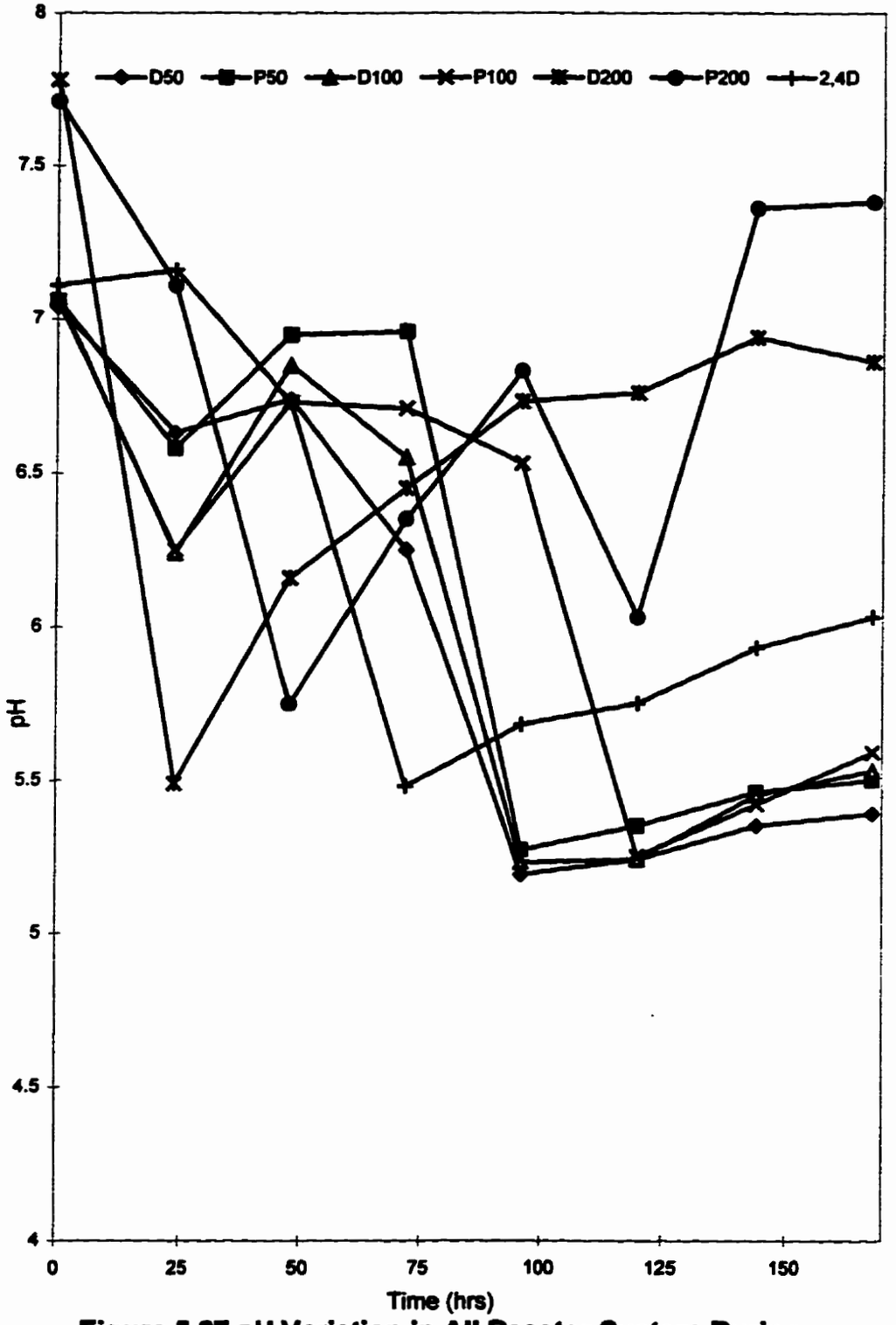


Figure 5.27 pH Variation in All Reactor System During Batch Operation

(also 30°C uncontrolled pH). Also, in one of the 2,4-D degradation studies reported in the literature, adjustment of pH every fourth day would not be a true indication of degradation rates (Oh and Tuovinen, 1991). In fact, biomass exposed to such decreased pH values may require substantial recovery time before further degradation to occur.

5. 9 Effect of Temperature on 2,4-D Degradation

A series of batch studies was conducted to investigate the effect of temperature on 2,4-D degradation. The effect of temperature was further explored in the presence of two different supplemental substrates. Phenol and dextrose were the two substrates used in this part of the study as well.

In Canada, wastewater temperature can vary significantly over seasons. Liquid temperatures below 10°C and above 25°C are usually encountered during climatic changes (Basu, 1995). These variations in temperature could greatly effect the biodegradation rates. Smith et al. (1994) have indicated a temperature range of 25 to 30 °C as optimum for 2,4-D degradation. A large number of studies have tried to investigate the temperature effect on chlorinated pesticides as well (Baker et al., 1980; Tyler and Finn, 1974; Viranaghavan et al, 1993).

5. 9. 1 10°C Temperature Study

The reactors were seeded with equal amount of biomass from SBR 1, SBR 2, and SBR 3 units as described in Table 4. 2. The degradation pattern exhibited at 10° C has been shown in Table 5.10 and 5.11. Even though an acclimated biomass was used in this experiment, yet a 15 hrs lag phase was experienced before any disappearance of 2,4-D noticed. The reactors with either no or minimum amount of supplemental substrate (i.e., 50 mg/L SOC of supplemental substrate) exhibited a similar 2,4-D degrading trend. In the case of

reactors with higher supplemental substrate concentrations (100 and 200 mg/L SOCs), the dextrose-fed reactors were experiencing faster degradation rates than phenol-fed reactors. Such faster 2,4-D removal rates were more pronounced in the case of D200 reactors. Removal rates up to 70% faster than P200 reactors were observed. Among the different phenol reactors, increasing phenol concentrations indicated decreasing removal rates. However, such trend was reversed in the case of dextrose reactors. Another observation has been that the supplemental substrate and 2,4-D removal occurred concurrently.

Time	Supplemental Substrate Removal (%)					
(hrs)	P50	P100	P200	D50	D100	D200
15	43	12	8	0	0	0
40	71	45	38	17	13	14
90	68	27	43	27	24	38
115	68	27	43	42	54	73
185	100	78	44	77	98	100
280	100	87	28	100	100	100
330	100	100	100	100	100	100

Table 5. 10 Supplemental Substrate Removal at 10 °C Temperature

In addition, except in the case of presence of high concentration of supplemental substrate (200 mg/L SOC), supplemental substrate has not affected the 2,4-D degradation. This is also supported by the same rate of 2,4-D degradation in the reactor 2,4-D.

Time (hrs)	2,4-D Substrate Removal (%)						
	P50	P100	P200	D50	D100	D200	2,4-D
15	0	0	0	0	0	0	0
40	21	11	7	17	13	14	18
90	16	11	11	27	24	38	15
115	40	30	20	42	54	73	35
185	87	77	36	77	98	100	77
280	100	100	89	100	100	100	100
330	100	100	100	100	100	100	100

Table 5. 11 2,4-D Removal at 10 °C Temperature

A follow-up analysis indicated that irrespective of the presence of phenol still in P100 and P200 systems, 2,4-D was the last to be depleted. A detailed reactor performance at 10°C has been depicted in Figure 5.28. It can be seen that at the end of 330 hrs (≈13 days) period, complete removal of 2,4-D was observed. In comparison, a study by smith et al (1994) has found that at 10°C temperature, 2,4-D degrading cultures were able to degrade 5% of the applied 2,4-D over 7 days period.

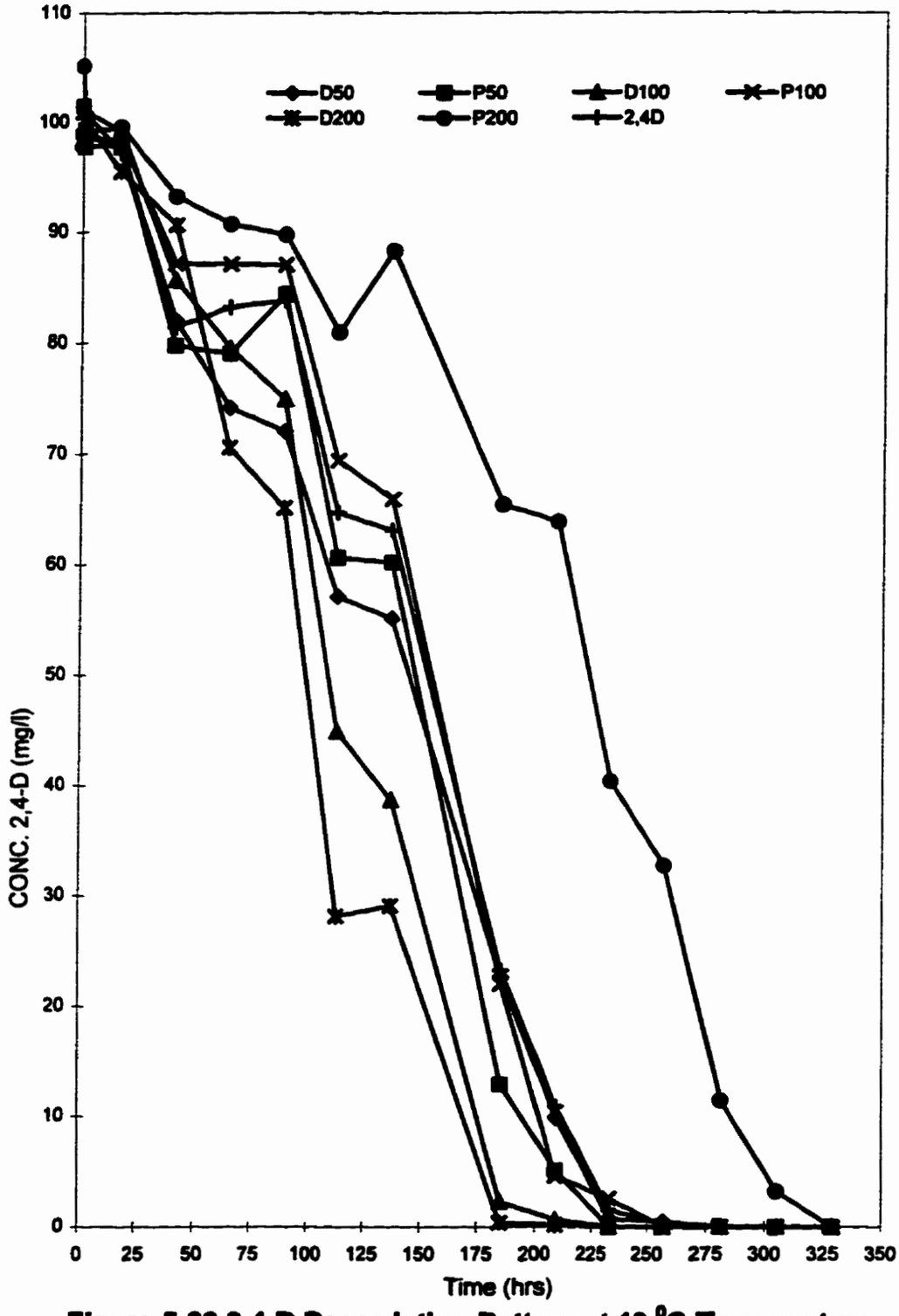


Figure 5.28 2,4-D Degradation Pattern at 10 ^{0}C Temperature

5. 9. 2 30°C Temperature Study

A study at 30°C temperature was performed under similar experimental conditions as the 10°C, except that pH was adjusted at regular intervals. More frequent sampling was performed to alleviate any potential adverse effects due to pH decrease. Figure 5.29 summarizes the 2,4-D degradation exhibited by all reactor system at this temperature.

Initial sample analysis indicated that the supplemental substrate removal has been particularly faster in the phenol and the dextrose reactors containing 50 and 100 mg/L SOC. This was also associated with a decrease in pH from its initial values 7.4-7.9 to 7.0-7.1 range (Table 5. 12). No 2,4-D removal was noticed at this stage. As the 2,4-D removal started after the complete removal of supplemental substrate, the pH value further decreased to 6.2-6.9 range for all reactors. So It can be inferred that drop in pH has been associated with both supplemental substrate and target compound.

The 2,4-D degradation pattern is given in Table 5.13. All reactors except reactor D50, experienced an initial lag period of 12 hrs before any measurable 2,4-D degradation. Reactor D50 showed faster removal as compared to all other reactors. In the case of both phenol and dextrose-fed reactors the presence of increasing supplemental substrate resulted in decreasing removal rates. However, at a lower temperature condition (i.e. 10°C) the dextrose-fed system experienced a reverse trend.

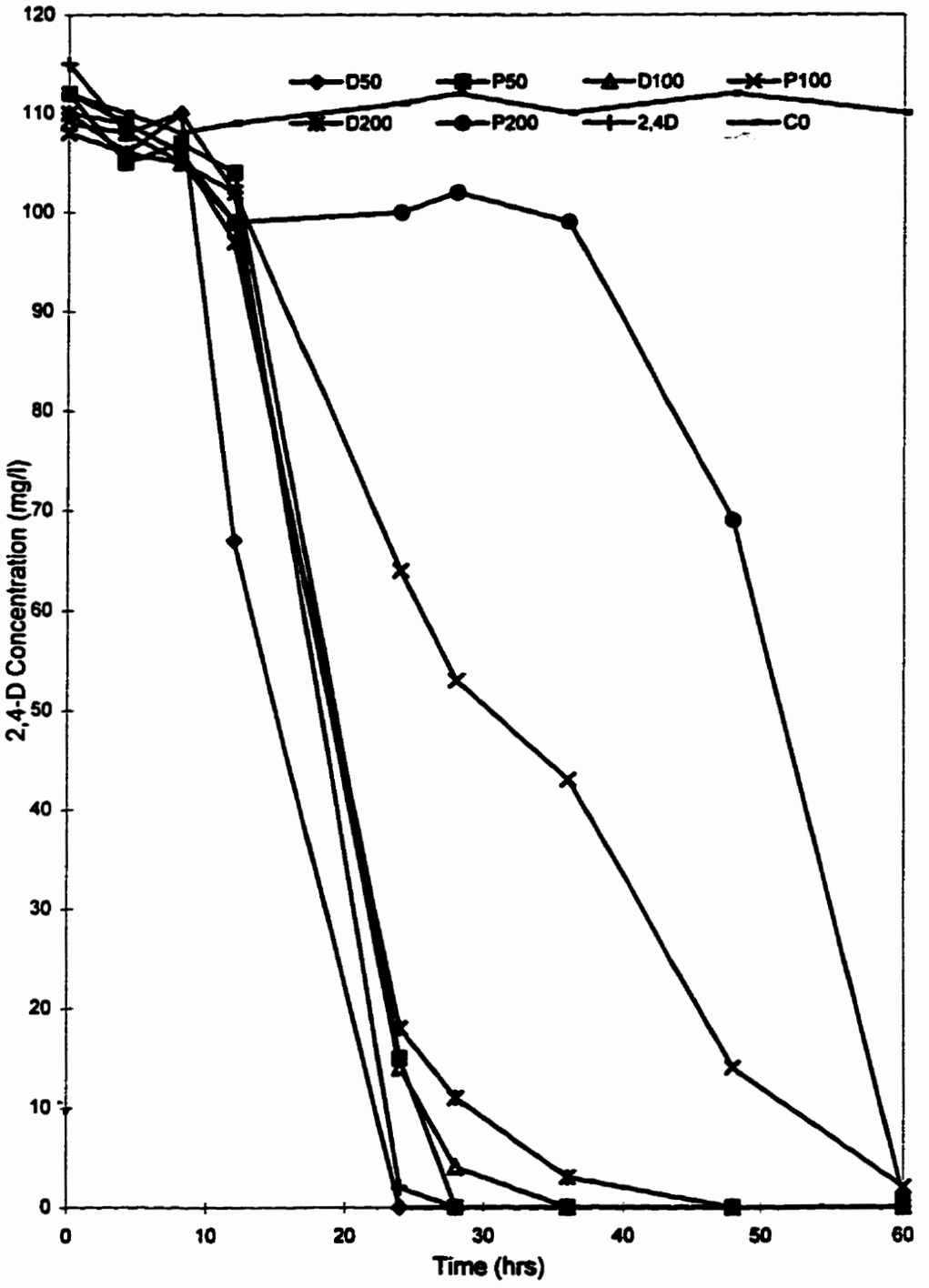


Figure 5. 29 2,4-D Degradation Profile at 30 ^{0}C Temperature

Time (hrs)	Supplemental Substrate Removed (%)					
	P50	P100	P200	D50	D100	D200
0	0(7.9)	0 (7.8)	0 (7.5)	0 (7.9)	0 (7.7)	0 (7.4)
4	39 (7.3)	19 (7.2)	5 (7.3)	28 (7.3)	24 (7.3)	0 (7.2)
8	58 (7.0)	30 (7.1)	8 (7.1)	50 (7.1)	23 (7.1)	9 (7.1)
12	100 (6.7)	100 (6.3)	49 (6.3)	98 (6.4)	98 (6.2)	63 (6.9)
24	100 (6.0)	100 (6.3)	100(5.8)	100 (5.0)	100 (5.6)	100 (6.20)

Table 5. 12 Percent Supplemental Substrate Depletion at 30° C

(Values in Parentheses Represent pH Values)

2,4-D specific removal rates at both temperature conditions are given in Table 5. 14. At 10°C, the specific removal rates were relatively lower than at 30° C. In addition, as the supplemental substrate concentration increased the specific removal rate decreased in all reactors except in the case of the dextrose-fed systems at 10° C temperature. Minimum specific removal rate was observed in the presence of highest phenol concentration at 10°C temperature. Overall, phenol-fed system indicated decreasing specific removal rates in comparison to dextrose-fed systems. This shows that phenol has a detrimental effect on 2,4-D degradation at high concentrations.

Time	2,4-D Concentration (mg/L)						
(hrs)	P50	P100	P200	D50	D100	D200	2,4-D
0	112	108	112	110	109	110	115
4	105	106	109	106	108	109	108
8	107	105	106	110	105	106	110
12	104	102	99	67	99	97	102
24	15	64	100	0	14	18	2
28	0	53	102	0	4	11	0
36	0	43	99	0	0	3	0
48	0	14	69	0	0	0	0
60	0	2	1	0	0	0	0

Table 5. 13 Remaining 2,4-D Concentration at 30^0 C Temperature

The much lower specific removal rates at low temperature may be the result of several factors. According to the literature, temperature can exert an effect in two ways : by influencing the rate of enzymatically catalyzed reactions; and by affecting the rate of diffusion of the substrate to the cell (Grady and Lim, 1980). Even though, there was a delay in 2,4-D breakdown at both 10^{0}C and 30^{0}C, after the start of the reactor operation, this period was prolonged at low temperature. The much low rates observed at 10^0 C can also be explained on the

basis that 2,4-D is taken into the microbial cell and degraded intracelluarly (Stott et al., 1983). As stated above, since temperature has an effect on cellular diffusion, it is possible that at low temperatures 2,4-D molecule diffusion into the bacterial cells may be reduced.

REACTOR TYPE	Specific Removal Rates (mg2,4-D/gMLVSS.hr)	
	10°C	30°C
P50	4.7	53
P100	4.2	24
P200	3.7	25
D50	4.6	61
D100	5.0	40
D200	5.7	31
2,4-D	4.3	55

Table 5. 14 Summary of Specific Removal Rates at Different Temperatures

The variation in the degradation pattern observed at 10°C temperature studies indirectly indicate that different enzyme systems are responsible for breakdown of the supplemental substrate and target compound. Hence there must be a variation in population type, at least two or more different species may be responsible for different responses observed at the same temperature. This observation is based on the fact that at 10^0 C, the effect on the breakdown of both phenol and degradation was severe in comparison to 2,4-D breakdown.

Overall, at high temperature, the degradation has been at much higher rate than at low temperature. Phenol has detrimental effect on 2,4-D degradation at high concentrations. The disappearance of sequential mode of degradation (as observed at high temperature) may be related to the adverse effect of low temperatures on the mixed cultures. Degradation at 10^0 C did not need any pH adjustment, whereas, the pH was continuously adjusted during the 30^0C experiment.

5. 10 Effect of Seed on 2,4-D Degradation

During the SBR 3 operation, 2,4-D degraders were never exposed to supplemental substrates (i.e. phenol and dextrose). Further exploration of the 2,4-D-degrading culture's potential to degrade different substrates was considered important. Such a study would indicate whether or not a separate biomass proliferated in SBR 3, where 2,4-D was the sole source of carbon. To investigate this degradation pattern two different experiments were conducted, using the same reactor setup as in previous batch experiments (Table 4. 4). The only variation was the source of seed (from SBR 3). In the first set the biomass was a mixture of phenol-, dextrose-, and 2,4-D-degraders, whereas, in the second set, only pure 2,4-D-degraders were used. The temperature was controlled at $22^{o}C \pm 1$.

5. 10. 1 Degradation Profile Using Mixed Cultures

Sampling at 24 hrs indicated a decrease in the supplemental substrate concentrations both in phenol and dextrose reactors as outlined in Figure 5.30. As shown in this figure, no degradation was observed in the control reactors. The depletion of supplemental substrate was faster in the dextrose reactors than in the phenol reactors. This trend of faster dextrose depletion was continuously observed in subsequent analysis as well. Further analysis after 96 hrs of operation revealed complete supplemental substrate removal in all reactors except in D200 reactor.

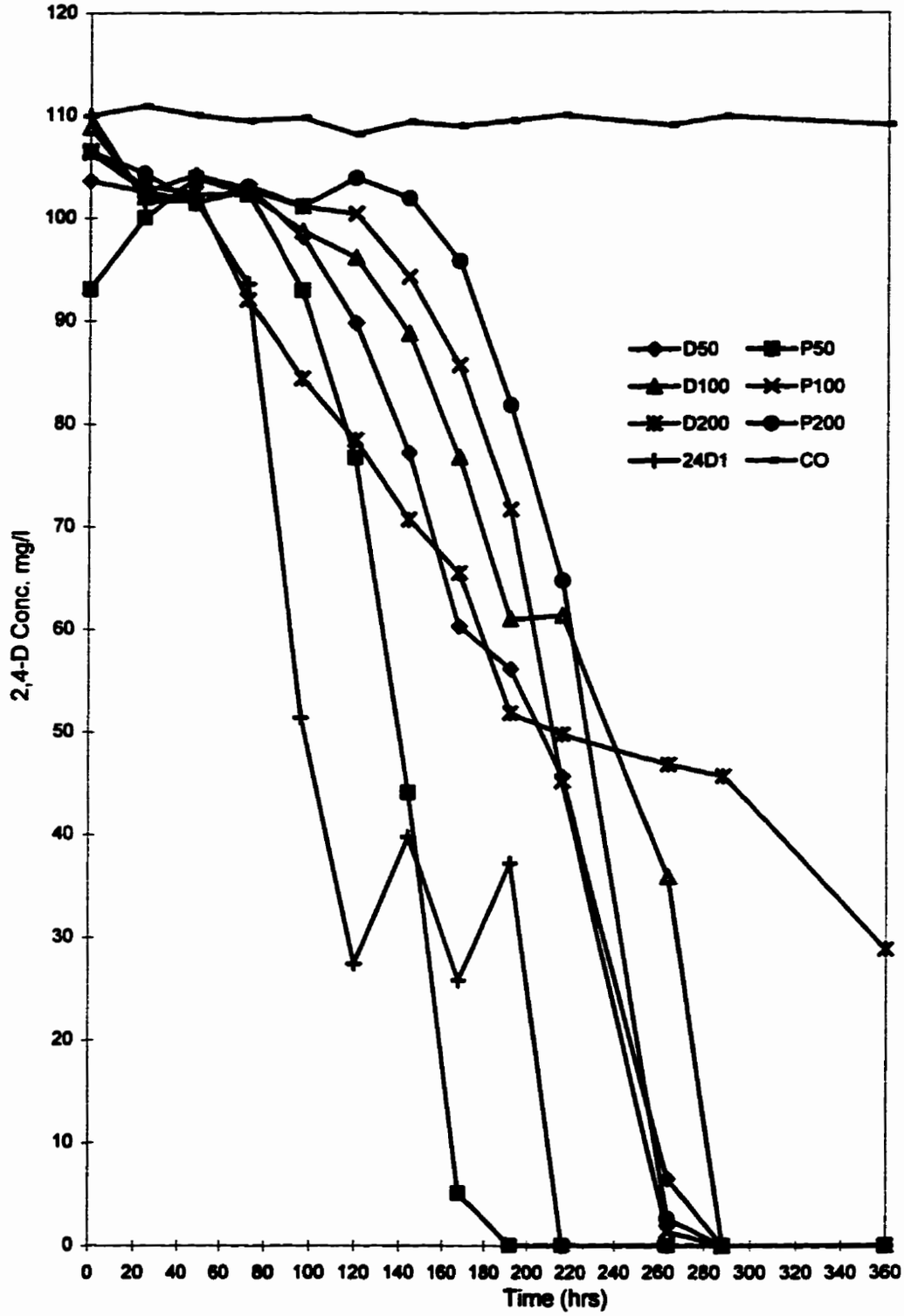


Figure 5.30 2,4-D Degradation Pattern by Mixed Inocula

Time	2,4-D removal (%)						
hrs	P50	P100	P200	D50	D100	D200	2,4-D
24	0	0	0	0	6	0	8
48	0	0	0	0	6	0	7
72	0	0	0	0	6	14	15
96	0	0	0	0	9	21	53
120	18	6	0	13	12	26	75
144	53	11	0	26	18	34	64
168	95	19	10	42	29	39	77
192	100	33	23	46	44	51	66
216	100	58	39	56	44	53	100
264	100	99	98	94	67	56	100
288	100	100	100	100	100	57	100
360	100	100	100	100	100	73	100

Table 5. 15 Percent 2,4-D Removed in the Mixed Culture Reactors at 22 °C

As outlined in Tables 5. 15 and 5.16, during the first 48 hrs of reactor operation 2,4-D removal was negligible or minimal in all reactors. However, after the disappearance of supplemental substrate, increased 2,4-D removal was noticed. This indicated that almost all reactors had an initial preference for supplemental substrate.

Although the phenol reactors were the last to start degrading 2,4-D, yet at the end all dextrose systems had very slow degradation. For instance, after 264 hrs of reaction period, all phenol systems showed more then 97% depletion. On the other hand, the dextrose reactors were still left with 5%-45% levels of 2,4-D. So initially, the dextrose reactor did start degrading before the phenol ones, but later staggering degradation was evident in dextrose systems. Such behavior can be explained on the basis that the initial breakdown had resulted in the decrease of the pH in all dextrose systems. This may be the result of some intermediate products producing acid. As the pH adjustment was conducted on daily basis, the period between decrease in pH and its adjustment to normal levels (pH around 7) may have been long enough to effect the degradation rates at the later stages of this experiment.

Time	Percent Supplemental Substrate Removed					
(hrs)	P50	P100	P200	D50	D100	D200
24	24	18	20	90	65	37
48	100	72	48	100	95	74
72	100	98	69	100	100	100
96	100	100	83	100	100	100
120	100	100	94	100	100	100
144	100	100	100	100	100	100

Table 5.16 Percent Supplemental Substrate Removed in the Mixed Culture Reactors at 22 °C

5. 10. 2 Degradation Profile Using 2,4-D Degraders

Under similar environmental and operational conditions, the 2,4-D degraders showed a different pattern of degradation for supplemental substrate and target compound (Figure 5.31). No degradation was observed in the control reactor. After 24 hrs of operation, a concurrent utilization of both supplemental substrate and 2,4-D in all reactors was observed (Tables 5. 18 and 5.19). Reactors with increasing phenol concentrations showed decreasing 2,4-D removal trend. However, in the case of dextrose-fed reactor significantly higher 2,4-D removal was observed in comparison to phenol-fed reactor. Except in highest phenol concentration reactor (P200), all other reactors had complete 2,4-D removal. Apart from lowest supplemental substrate concentration reactors (50 mg/L), all other reactors had remaining supplemental substrate concentration. The P200 reactor was further monitored up to 120 hrs, when there was a complete removal of both phenol and 2,4-D.

The above observations indicate that between the two supplemental substrates depletion patterns, phenol depletion was faster in comparison to dextrose. This is a very different response in relation to the majority of the observations throughout this in this study, where dextrose was the first substrate to be depleted. This experiment also indicates that 2,4-D degraders have a tendency for faster degradation of structurally related compounds, such as phenol. During this study, experiments with phenol-acclimated biomass (during the SBR phase) showed a slower 2,4-D degradation pattern, which may be

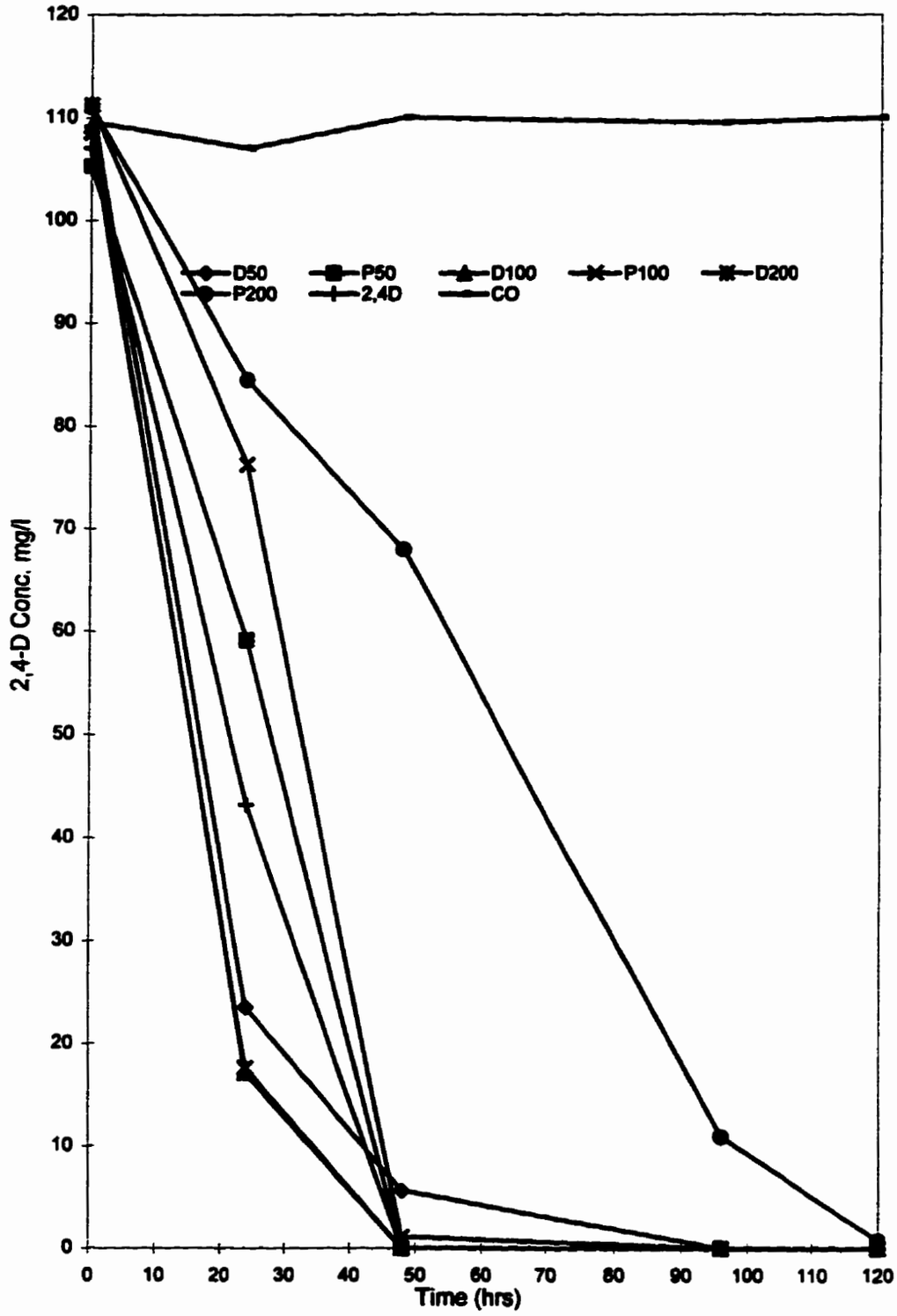


Figure 5. 31 2,4-D Degradation Pattern by Pure 2,4-D Degrading Inocula

strongly related to the enzyme system involved (hence bacterial species). It has been often observed that a particular enzyme system capable of degrading a "x" compound may degrade another "y" compound with same enzyme mechanism. However, the reverse process may not be feasible. For instance, Steenson and Walker (1956) indicated similar response with MCPA and 2,4-D mixture, where MCPA-acclimated biomass degraded both 2,4-D and MCPA, but 2,4-D-acclimated biomass could not degrade MCPA.

Time	2,4-D Removal (%)						
(hrs)	P50	P100	P200	D50	D100	D200	2,4-D
24	44	32	24	79	84	84	60
48	100	99	40	95	100	100	100
96	100	100	90	100	100	100	100

Table 5. 17 2,4-D Removal Using 2,4-D Degraders

It should be mentioned that 2,4-D depletion occurred irrespective of the presence of supplemental substrates. A representative example of such concurrent degradation is shown in Table 5.17 and 5.18. Papanastasiou (1982) also indicated that 2,4-D acclimated cultures when exposed to glucose (55 mg/L) and 2,4-D (50 mg/L) simultaneously in a batch reactor, showed an initial preferences for 2,4-D at 20 ^{0}C. The MLVSS concentration used was 0.5 mg/L which is much lower than the concentration used in this study (75 mg/L). Despite the difference in MLVSS and 2,4-D concentrations, the present study found that

depletion occurred in approximately 50 hrs of reaction period in comparison to approximately 55 hrs in the study by Papanastasiou (1982).

Time	Supplemental Substrate Removal (%)					
hrs	P50	P100	P200	D50	D100	D200
24	44	42	37	37	31	24
48	100	84	17	100	28	61
96	100	100	100	100	100	100

Table 5.18 Supplemental Substrate Removal Using Pure 2,4-D Degraders

Overall, it can be inferred that both systems behaved differently because of different biomass involved in each study. Pure 2,4-D degrading biomass from SBR 3 exhibited no preferential behavior for supplemental substrates.

Chapter 6

RESEARCH OVERVIEW

6. 1 Conclusions

Several conclusions can be drawn from both SBR and batch reactor studies during this project.

A summary of important observations from the SBR study is presented below:

- There is a need for acclimation of the biomass to 2,4-D

- The prolonged acclimation period (of about 110 days) observed indicated the requirement for adequate initial biomass concentration and contact time between the biomass and the target compound.

- All reactors were able to achieve 99% removal of both 2,4-D and the corresponding supplemental substrates, at all the HRT applied ranging from 12 to 48 hrs.

- The use of SBR technology was effective in treating a simulated wastewater containing up to 300 mg/L 2,4-D at the minimum applied HRT of 12 hrs.

- Even though neither of the supplemental substrates appear to enhance 2,4-D degradation, yet they both contributed to better reactor stability.

- Dextrose-fed reactors indicated a consistently lower (by about 42%) 2,4-D specific removal rate in comparison to phenol-fed reactors.

- Even though reactors with supplemental substrates exhibited lower 2,4-D specific removal rates than the reactor without supplemental substrate, yet no inhibitory effect was observed at any concentrations applied.

- The frequently observed pattern of sequential degradation of supplemental substrate and 2,4-D appeared to change/shift to concurrent degradation mode at higher phenol concentrations.

- The removal of supplemental substrates has been observed to accelerate after the onset of 2,4-D degradation.

- 2,4-D can be used as a sole source of carbon and energy by the biomass for its growth and proliferation.

- There is no need to adjust the reactor pH during SBR operation for treating 2,4-D liquid waste. Well aerated and continuously mixed reactors will have minimal or no pH effect.

Similarly, a number of observations were made during batch studies on 2,4-D degradation and are summarized below:

- The 2,4-D degradation rate was greatly affected by the variation in temperature and it was much higher at 30 ^{0}C than at 10^{0}C.

- Due to the effect of temperature (lower degradation rate) on supplemental substrate depletion, a concurrent utilization of 2,4-D and supplemental substrate was observed, which was more pronounced in the case dextrose.

- A concurrent utilization of both substrates (2,4-D and supplemental) was also observed during 2,4-D degradation studies using a 2,4-D degrading culture.

- Phenol appeared to have a detrimental effect on 2,4-D degradation at concentrations higher than 125 mg/L.

- There is a need for pH control during batch degradation experiments conducted at 22 ^{0}C or higher temperatures.

- At pH below 6.0, 2,4-D degradation was severely affected, whereas complete lack of degradation was observed at pH below 5.5.

6. 2 Recommendations For Future Work

Even though this study has provided a number of plausible explanations for the long lag period observed before 2,4-D degradation, yet it would be beneficial to further explore the relationship between the application of two specific substrates used vs. the lag period observed. This could help to optimize the start-up an operation time in various practical applications.

Some very important operational parameters such as temperature was explored using only batch reactors. It is believed that exploration of the effect of temperature on 2,4-D degradation in continuous-running systems could provide a great insight into the operation of full-scale treatment systems.

Previous studies have reported that in batch reactors, pesticides with similar functional groups are amenable to degradation by a specific biomass. However, this study focused on the treatment of a single toxic compound (2,4-D). thus it would be useful to explore the biodegradation potential of SBRs using a variety of other herbicides from the phenoxy group. This will address the problem of waste segregation for biotreatment. Instead of applying a particular pesticide for biodegradation in SBRs, a mixture of several herbicides could be degraded at the same time.

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APPENDIX A

Table A1 Substrate Depletion in SBR 1

DATE	DAY	2,4-D			Phenol		
		Inf	Eff	%Rem	Inf	Eff	%Rem
Oct-23	1	37.8	16.6	56	98.5	0.1	99.9
Oct-26	4	39.0	39.0	0	92.5	0	100
Nov-01	10	39.6	37.9	4	86.4	23	
Nov-03	12	37.8	36.8	3	97.3	0	100
Nov-06	15	36.0	39.0	0	92.5	0	100
24-D CONC. INCREASED to 100 mg/l							
Nov-08	17	109	108	1	113.4	0	100
Nov-10	19	106	89.3	16	104.9	0	100
Nov-13	22	91.4	91.2	0	108.6	0	100
Nov-15	24	101	100	1	92.2	0	100
Nov-17	26	100	100	0	75.7	0	100
Nov-20	29	104	100	4	87.4	0	100
Nov-22	31	98.0	98.0	0	85.0	0	100
Nov-24	33	101	101	0	97.5	0	100
Nov-27	36	97.0	96.0	0	81.4	0	100
Nov-29	38	98.0	99.0	0	90.3	0	100
Dec-01	40	103	101	2	99.7	0	100
Dec-06	45	101	103	0	88.3	0	100
Dec-08	47	105	102	3	99.4	0	100
Dec-11	50	102	104	0	100.6	0	100
Dec-13	52	98.6	98.2	0	87.2	0	100
Dec-18	57	101	101	0	95.8	0	100
Dec-20	59	101	105	0	97.6	0	100
Dec-22	61	106	104.3	0	101.7	0	100
Dec-25	64	88.2	89.7	0	82.7	0	100
Dec-27	66	102	102	0	92.8	0	100
Dec-29	68	102	102	0	98.1	0	100
Jan-01	71	103	104	0	98.1	0	100
Jan-03	73	100	104	0	106.8	0	100
Jan-08	78	96.9	96.7	0	100.9	0	100
Jan-10	80	102	96.5	5	97.7	0	100
Jan-12	82	103	104	0	112.3	0	100
Jan-15	85	101	101	0	96.3	0	100
Jan-17	87	101	94.0	7	97.4	0	100
Jan-19	89	103	103	1	92.0	0	100
Jan-20	90	103	96.7	6	112.7	0	100
Jan-22	92	102	101	0	97.6	0	100
Jan-24	94	101	103	0	96.0	0	100
Jan-26	96	103	103	0			

Table A1 Substrate Depletion in SBR 1

DATE	Time (days)	2,4-D Inf	2,4-D Eff	2,4-D %Rem	Phenol Inf	Phenol Eff	Phenol %Rem
Jan-29	99	102	104	0	ND	ND	ND
Jan-31	101	102	104	0	"	"	"
Feb-02	103	102	102	0	"	"	"
Feb-05	106	92.2	94.2	0	82.9	0	100
Feb-12	113	92.5	87.6	5	229.9	0	100
Feb-14	115	107	96.0	11	236.2	0	100
Feb-16	117	104	96.4	7	228.6	0	100
Feb-22	123	102	102	0	225.0	0	100
Feb-26	127	97.0	78.0	20	215.6	0	100
Feb-28	129	101	90.0	11	224.6	0	100
Mar-01	131	99.2	91.8	7	201.5	0	100
Mar-07	137	86.1	16.9	80	216.0	0	100
Mar-11	141	99.6	0.4	100	186.0	0	100
Mar-14	144	96.0	36.5	63	103.3	0	100
Mar-18	148	99.2	0.3	100	196.1	0	100
Mar-20	150	94.6	0.4	100	146.7	0	100
Mar-22	152	101	0.0	100	226.4	0	100
Mar-25	155	95.8	0.1	100	209.6	0	100
Mar-27	157	102	0.2	100	209.9	0	100
Mar-30	160	105	0.0	100	218.8	0	100
Apr-01	162	108	0.0	100	247.1	0	100
Apr-06	169	91.7	1.4	99	161.4	0	100
Apr-10	171	84.9	0.3	100	167.5	0	100
Apr-12	173	97.2	0.0	100	199.7	0	100
Apr-15	176	96.7	0.3	100	215.8	0	100
Apr-18	179	96.4	0.0	100	219.9	0	100
Apr-24	185	102	0.0	100	230.5	0	100
Apr-26	192	103	0.0	100	230.7	0	100
May-3	194	104	0.2	100	234.7	0.5	100
May-6	197	101	0.2	100	225.6	0	100
May-8	199	97.0	0.0	100	205.6	0	100
May-10	201	96.6	0.0	100	215.6	0	100
May-14	205	102	0.4	100	224.6	0	100
May-16	207	89.7	0.0	100	192.2	0	100
May-19	210	99.1	0.0	100	213.8	0	100
May-27	218	100	0.0	100	175.7	0	100
Jun-4	226	96.7	0.0	100	211.2	0	100
Jun-10	232	94.8	0.0	100	205.9	0	100

Table A 2 Substrate Depletion in SBR 2

Date	Day	2,4-D			Dext.		
		Infl.	Effl.	%DP	inf.	effl.	%DP
Oct-23	1	40.9	17.2	58			
Oct-26	4	40	40	0			
Nov-01	10	39.2	36.5	7			
Nov-03	12	37.5	37.1	1			
Nov-06	15	37.5	37.1	1	236	0	100
100 mg/liter 24-D CONC INCREASED							
Nov-08	17	111	109	2			
Nov-10	19	105	105	0	282	0	100
Nov-13	22	93.6	93.2	0			
Nov-15	24	99.9	99.9	0	272	0	100
Nov-17	26	100	100	0			
Nov-20	29	99.3	98.2	1	174	0	100
Nov-22	31	99.8	97.2	3	179	0	100
Nov-24	33	101	101	0	261	0	100
Nov-27	36	101	99	2	86.4	0	100
Nov-29	38	98.7	98	1			
Dec-01	40	98	98	0	254	0	100
Dec-06	45	97	97	0			
Dec-08	47	97.3	95.8	1			
Dec-11	50	98	98	0			
Dec-13	52	91.7	97.3	-6	339	0	100
Dec-15	54	96.6	95.8	1			
Dec-18	57	99	95.3	4	260	0	100
Dec-20	59	99	99	0	356	0	100
Dec-22	61	103	102	2			
Dec-25	64	86.2	88	-2			
Dec-27	66	88	86.1	2			
Dec-29	68	101	100	0			
Jan-01	71	101	101	0			
Jan-03	73	102	98.4	4			
Jan-08	78	98.2	102	-4			
Jan-10	80	102	91.3	11			
Jan-12	82	103	77.5	25			
Jan-15	85	105	45.7	56			
Jan-17	87	100	66.2	34			
Jan-19	89	101	92.4	8			
Jan-20	90	103	91.3	11			
Jan-22	92	95.8	86.4	10			
Jan-24	94	96	94.1	2			
Jan-26	96	96	94.6	2			

Date	Day	2,4-D			Dext.		
		Infl.	Effl.	%DP	inf.	effl.	%DP
Jan-29	99	102	96.3	6			
Jan-31	101	101	94.6	6			
Feb-02	103	102	95	7			
Feb-05	106	99.5	96.7	3			
Feb-12	113	99.9	4.85	95	390	0	100
Feb-14	115	104	2.35	98	382	0	100
Feb-16	117	105	0.6	99	380	0	100
Feb-22	123	104	4.63	96	380	0	100
Feb-26	127	101	2.56	97	446	0	100
Feb-28	129	103	0.11	100	415	0	100
Mar-01	131	102	0.04	100			
Mar-07	137	92.7	0.34	100	407	0	100
Mar-11	141	103	0.42	100			
Mar-14	144	102	0.55	99			
Mar-18	148	101	0.28	100	431	0	100
Mar-20	150	102	0.15	100	394	0	100
Mar-22	152	98.3	0	100	401	0	100
Mar-25	155	101	0.11	100	344	0	100
Mar-27	157	103	0.06	100			
Mar-30	160	106	0	100	425	0	100
Apr-01	162	107	0.09	100			
Apr-08	169	91.4	0.03	100	431	0	100
Apr-10	171	84.3	0	100	406	0	100
Apr-12	173	92.2	0.2	100	175	0	100
Apr-15	176	99.9	0.07	100	469	0	100
Apr-18	179	93.1	0	100	588	0	100
Apr-24	185	96.3	0	100	377	0	100
May-01	192	103	0	100			
May-03	194	103	0.5	100	396	0	100
May-06	197	103	0.31	100	409	0	100
May-08	199	97.5	0.09	100	396	0	100
May-10	201	96.8	0	100	443	0	100
May-14	205	103	0	100	395	0	100
May-16	207	90.2	0	100	388	0	100
May-19	210	100	0.23	100	406	0	100
May-27	218	96.3	0	100	448	0	100
Jun-04	226	99.9	0	100	443	0	100
Jun-10	232	94.1	0	100	410	0	100

Table A3 SOC Data For SBR1 and SBR 2

Date	Day	SBR1			SBR 2		
		Inf.	Eff.	%Rem	Inf.	Eff.	%Rem
10/23/95	1	94.3	16.9	82	86.6	26.2	70
10/26/95	4	94.3	37.5	60	87.9	70.7	20
11/1/95	10	86.0	32.5	63	80.8	77.1	5
11/3/95	12	93.8	32.0	66	82.4	71.0	14
11/6/95	15	85.1	55.9	34	82.8	47.9	42
11/8/95	17	100.5	47.5	53	114.6	44.7	61
11/10/95	19	92.6	42.4	54	109.1	45.8	58
11/13/95	22	99.9	43.6	56	104.3	46.7	55
11/15/95	24	79.9	39.4	51	90.3	39.0	57
11/17/95	26	78.1	42.2	46	73.1	40.5	45
11/20/95	29	114.5	53.6	53	116.9	55.5	53
11/22/95	31	114.0	58.4	49	113.9	56.2	51
11/24/95	33	108.0	52.7	51	109.3	52.6	52
11/27/95	36	90.3	46.6	49	72.8	45.1	38
11/29/95	38	100.1	46.7	54	105.6	49.3	53
12/1/95	40	93.1	43.7	53	106.4	42.4	60
12/4/95	43	99.0	47.1	52	109.0	45.3	58
12/6/95	45	101.2	49.8	51	106.0	46.4	56
12/8/95	47	102.1	46.9	54	109.5	45.2	59
12/11/95	50	91.5	47.8	48	94.4	41.8	56
12/13/95	52	95.0	47.1	50	108.1	49.6	54
12/15/95	54	92.0	45.2	51	101.7	46.0	55
12/18/95	57	91.9	42.9	53	101.0	43.1	57
12/20/95	59	90.4	42.9	53	104.8	43.5	58
12/22/95	61	87.9	42.8	51	78.3	42.8	45
12/25/95	64	106.5	43.2	59	92.3	42.6	54
12/27/95	66	94.8	44.0	54	101.6	42.8	58
12/29/95	68	84.9	48.4	43	100.5	47.6	53
1/1/96	71	88.3	49.9	44	84.6	48.2	43
1/3/96	73	88.9	42.9	52	78.5	41.0	48
1/5/96	75	93.3	52.9	43	72.1	46.7	35
1/8/96	78	85.0	47.8	44	76.0	48.0	37
1/10/96	80	85.0	40.5	52	92.3	40.9	56
1/12/96	82	84.3	42.1	50	93.2	34.2	63
1/15/96	85	85.2	40.7	52	90.4	25.3	72
1/22/96	92	101.2	49.8	51	106.0	38.5	64
1/24/96	94	85.5	41.3	52	92.3	38.1	59
1/31/96	101		47.9		108.0	46.9	57
2/2/96	103		39.1		90.6	38.3	58
2/5/96	106	90.8	41.3	54	92.4	37.8	59
2/12/96	113	173.0	38.7	78	192.0	11.2	94
2/14/96	115	207.0	72.0	65	208.0	51.9	75
2/16/96	117	236.0	64.3	73	235.3	35.3	85
2/19/96	120	230.0	47.7	79	232.0	21.5	91

Table A4 SOC Data for SBR 1, SBR 2, and SBR 3

Date	Day	SBR 1			SBR 2			SBR 3		
		Inf.	Eff.	%Rem	Inf.	Eff.	%Rem	Inf	Eff	%Rem
2/26/96	127	206.7	45.0	78	239.6	19.1	92	36.3	22.7	41
2/28/96	129	206.9	42.3	80	197.0	15.8	92	38.6	19.1	50
3/1/96	131	225.0	54.5	76	185.6	15.7	91	37.6	18.5	51
3/6/96	136	224.8	48.3	78	239.2	50.4	79	74.2	54.4	27
3/8/96	138	225.0	18.0	92	283.0	51.7	82	74.2	13.0	82
3/11/96	141	239.3	19.8	92	225.0	13.9	94	40.2	13.4	67
3/14/96	144	37.1	25.7	31	213.6	17.7	92	37.9	18.0	52
3/18/96	148	229.1	21.6	90	222.5	12.3	94	37.0	24.0	35
3/20/96	150	230.0	10.6	95	223.5	13.4	94	38.0	10.6	72
3/25/96	155	244.3	15.4	94	226.9	13.5	94	38.9	16.1	59
3/27/96	157	196.7	13.6	93	193.9	15.1	92	45.7	15.0	67
3/30/96	160	202.0	17.9	91	202.1	20.9	90	61.0	14.2	77
4/1/96	162	201.6	15.6	92	199.7	15.4	92	59.0	15.2	74
4/8/96	169	184.8	15.6	92	190.3	15.7	92	46.2	15.5	66
4/10/96	171	189.0	18.3	90	187.4	18.0	90	46.9	19.7	58
4/12/96	173	188.3	17.2	91	151.2	74.1	51	47.0	12.2	74
4/16/96	177	195.7	15.9	92	216.6	15.0	93	43.9	14.1	68
4/18/96	179	209.5	16.0	92	248.1	15.0	94	65.9	15.0	77
4/24/96	185	263.0	15.0	95	265.0	13.0	95	48.0	12.0	75
4/26/96	187	261.0	13.0	95	252.0	14.0	94	50.0	14.0	72
5/3/96	194	190.2	11.8	94	183.7	12.6	93	46.2	11.3	76
5/8/96	199	227.7	14.0	94	221.5	14.0	94	47.0	15.0	68
5/10/96	201	249.5	15.0	94	250.0	13.0	95	50.0	16.0	68
5/14/96	205	213.3	8.9	96	211.3	8.2	96	46.4	8.2	82
5/16/96	207	210.1	11.4	95	202.6	9.7	95	47.4	10.0	79
5/19/96	210	204.6	13.7	93	202.9	15.8	92	45.0	13.4	70
5/22/96	213		15.7			16.8			16.8	
5/24/96	215	202.3	8.8	96	179.8	7.9	96	44.4	6.9	84
5/31/96	222	206.1	15.6	92	179.8	18.7	90	45.7	12.9	72
6/3/96	225	171.1	9.1	95	165.1	8.9	95	47.1	7.8	83
6/5/96	227	194.5	12.2	94	212.0	13.2	94	44.0	14.3	68
6/10/96	232	190.3	17.4	91	202.9	16.7	92	42.5	15.0	65

Table A5. MLVSS Values During Periodic Sampling

Time	SBR 1		SBR 2	
Day	inf.	eff.	inf.	eff.
1	843		413	
8	710	68	273	70
12	497	71	373	40
15	593	75	393	39
17	457	65	533	72
22	367	14	657	21
26	823	73	1053	105
29	747	26	797	52
31	820	24	737	63
36	997	31	1023	46
40	810	36	1188	31
45	503	27	1320	27
47	497	24	1433	33
50	460	24	1343	54
52	433	10	1115	45
54	570	13	1163	46
57	633	26	1253	32
59	457	12	997	40
61	513	50	1023	87
64	473	35	1147	60
66	510	52	1040	67
68	413	27	1163	20
71	623	53	1363	66
73	503	60	1287	75
75	503	21	1313	52
78	430	25	1337	
80	510	36	1233	78
82	430	29	1247	76
85	447	20	1293	71
87	597	35	1013	90
89	573	13	910	18
92	680	6	1140	32
96	630	102	970	0
99	590	25	1230	36
101	500	22	1250	35
103	455	23	1370	34
106	595	55	1655	75
113	1480	100	2340	186
115	1075	110	2080	193
117	1030	115	2055	130
120	985	56	2085	67

Time	SBR 1		SBR 2		SBR 3	
Day	inf.	eff.	inf.	eff.	inf.	eff.
124	2050	60	1710	70	2270	
129	1885	97	1860	92	1615	56
131	1905	53	1885	60	1400	59
135	1940	94	2045	42	1210	58
137	1905	72	2045	28	1070	59
141	1985	56	2160	36	985	40
144	1595	85	2040	96	985	36
146	1595	62	1900	40	795	27
150	1600	83	2035	42	910	60
152	1430	73	1785	17	885	73
155	1460	66	1785	48	915	122
157	1390	70	1815	36	660	136
159	1385	76	1910	36	660	114
169	1415	34	2314	46	220	41
171	1290	40	2205	45	140	0
175	1545	45	2785	42	185	48
179	1750	56	3045	38	100	26
181	1930	34	2995	38	50	2
185	1735	60	2960	78	110	16
188	1970		3060		140	
194	2115	20	3260	49	300	22
197	1835	46	3180	52	130	22
199	2230	54	3240	70	175	30
204	2285	74	2945	70	140	24
206	2275	74	2885	70	160	16
213	2380	75	3400		190	
218	2430	12	3535	20	160	16
220	2430	80	3570		105	
225	2440	34	4475	18	130	16
229	2635		4645		155	

APPENDIX B

Table B1 Experiment Performed at 30 C Temperature with pH control.

DATE: March 14,1996 DAY:0

REACTOR	2,4-D		PHENOL		DEXTROSE		SOC		pH
SD501	105.46	104.24	na	na	131.03	na	66.22	120.92987	8.16
SD502	103.02		na	na	117.86	na	62.15	111.82534	8
SP501	93.58	96.305	67.31	na	na	na	60.71	108.60406	7.96
SP502	99.05		66.13	na	na	na	62.22	111.98193	8.15
SD1001	100.28	99.915	na	na	233.1	na	82.57	157.5046	7.63
SD1002	99.55		na	na	227.85	na	85.86	164.66426	7.49
SP1001	99.22	100.055	134.03	na	na	na	84.56	161.9562	7.66
SP1002	100.89		134.4	na	na	na	86.55	166.4078	7.68
SD2001	100.14	99.975	na	na	495.55	na	120.65	242.68904	7.38
SD2002	99.81		na	na	491.33	na	118.5	237.87952	
SP2001	100.63	101.04	250		na	na	127.85	258.79534	7.29
SP2002	101.45		250		na	na	123.1	248.16985	7.19
S24D1	101.96	101.27	na	na	na	na	39.53	61.224705	7.96
S24D2	100.58		na	na	na	na	36.16	53.686061	
SC01	96.02	96.02	na	na	na	na	35.26	51.672774	
March,15,1996									DAY:1
SD501	97.71	95.215	n.a	na			44.11	71.470103	6.6
SD502	92.72		n.a	na			43.52	70.150281	6.5
SP501	89.49	92.415	26.14	na			56.11	98.313939	6.77
SP502	95.34		13.39	na			54.89	95.584815	6.84
SD1001	93.74	94.9	na	na			50.11	84.892021	6.12
SD1002	96.06		na	na			42.94	68.852829	6.1
SP1001	94.52	94.565	110.81	na			82.17	156.6096	7.37
SP1002	94.61		111.38	na			81.99	156.20714	7.39
SD2001	101.09	100.195	na	191.02			80.22	152.24768	5.92
SD2002	99.3		na	195.96			77.97	147.21446	5.82
SP2001	95.69	95.275	217.34	na			120.65	242.68904	7.31
SP2002	94.86		214.01	na			116.95	234.41219	7.28
S24D1	76.01	85.02	na	na			47.04	78.024473	7.21
S24D2	94.03		na	na			45.02	73.50576	7.1
SCO1	92	92	na	na					7.2
March,16,1996									DAY:3
SD501	60.83	57.72					35.67	52.589936	5.8
SD502	54.61						31.95	44.288349	5.8
SP501	78.19	80.64					40.63	63.68539	6.66
SP502	83.09						41.39	65.3855	6.66
SD1001	41.77	51.775					38.87	59.748294	5.9
SD1002	61.78						35.1	51.314856	5.91
SP1001	84.53	85.545					41.05	64.624925	6.2
SP1002	86.56						41.63	65.922377	6.2
SD2001	75.32	71.76					43.59	70.30687	5.94
SD2002	68.2						38.53	58.987719	5.94
SP2001	93.62	93.7					49.4	83.30376	5.61
SP2002	93.78						58.42	103.48138	5.83
S24D1	67.38	54.54					38.39	58.674541	6.4
S24D2	41.7						31.46	43.172226	6.12
SCO1	91	91							

Table B1 Experiment Performed at 30 C temperature with pH control.

March,17,1996 DAY:4

REACTOR	2,4-D		PHENOL		DEXTROSE		SOC		pH
SD101	0.19	0.255					13.58	3.17491	
SD102	0.32						13.37	2.7051428	
SP501	52.24	61.06					29.17	36.049527	
SP502	69.92						34.37	49.681856	
7D1001	14.31	22.02					20.84	19.415431	
7D1002	29.73						21.97	21.943225	
7P1001	79.13	79.995					36.62	54.715075	
7P1002	80.86						36.13	53.618652	
7D2001	47.24	44.405					27.17	33.575554	
7D2002	41.57						27.66	34.671678	
7P2001	95.23	94.275					42.94	68.852829	
7P2002	93.32						43.17	69.367336	
724D1	1.9	1.255					15.37	7.1791155	
724D2	0.61						13.27	2.4614442	
7CO1		#DIV/0!							

March,18,1996 DAY:5

SD101	0.2	0.1							
SD102	0								
SP501	6.2	11.63					13.71	3.4657182	
SP502	17.06						18.96	15.209896	
7D1001	0.15	6.22					14.06	4.2486634	
7D1002	12.29						16.49	9.6845402	
7P1001	33.44	36.99					22.85	23.911773	
7P1002	40.54						25.08	28.900253	
7D2001	0.53	0.385					13.95	4.0025949	
7D2002	0.24						13.86	3.8012961	
7P2001	96.18	87.29					41.62	66.347404	
7P2002	78.4						39.22	60.53124	
724D1	0	0							
724D2	0								
7CO1									

March,19,96 DAY:6

7D501									
7D502									
7P501	0.19	0.51					14.8	5.9040333	6.47
SP502	0.83						14.19	4.5394716	5.89
7D1001	0.06	2.59					16.85	10.466655	5.12
7D1002	5.1						15.53	7.5370333	5.41
7P1001	0.76	1.13					13.45	2.8841017	6.39
7P1002	1.5						13.68	3.3966086	7.27
7D2001	0.21	0.18					12.35	0.4234166	4.97
7D2002	0.15						13.6	3.2196497	5.8
7P2001	93.03	62.11					41.67	66.011856	6.97
7P2002	31.19						28.61	32.322842	6.73
724D1									
724D2									
7CO1									

TABLE B1 CONT..

Table B1 Experiment Performed at 30 C temperature with pH control.

Mar20,1996 DAY:7

REACTOR	2,4-D		PHENOL		DEXTROSE		SOC		pH
SD202									
SD102									
SP501									
SP502									
7D1001									
7D1002									
7P1001	0.29								
7P1002	0.25								
7D2001									
7D2002									
7P2001	61.6	31.205					52.2	89.567322	
7P2002	0.81						33.5	47.735678	
724D1									
724D2									
7CO1									

March21,1996 Day:8

7D601									
7D602									
SP501									
SP502									
7D1001									
7D1002									
7P1001									
7P1002									
7D2001									
7D2002									
7P2001	35								
7P2002	0.81								
724D1									
724D2									
7CO1									

Table B 2. Batch Study Performed with no pH Control

Feb 23, 96 — DAY:0

REACT	2,4 -D		PHENOL		DEXTROSE		SOC		pH
D501	108.8						65.34	64.51	7.08
D502	110.37						67.59	66.2	7.01
P501	110.9		68.62				68.22	68.06	7.1
P502	112.66		70.2				68.14	67.43	7.03
D101	110						90.6	90.21	7.01
D102	108.35						90.18	90.05	7.05
P101	110.6		149.51				90.94	89.37	7.04
P102	112.63		151.73				89.76	91.11	7.09
D201	110.63						124	123.1	7.01
D202	110.45						123.2	123.2	8.55
P201	114.89		305.78				132.2	132.4	7.69
P202	110.81		296.61				134.1	133.8	7.73
24D1	113.42						37.71	38.69	7.09
24D2	109.13						40.56	39.77	7.13

Feb, 24,96 — Day:1

REACT	2,4 -D		PHENOL		DEXTROSE		SOC		pH
D501	107.42						37.74	38.54	6.49
D502	107.59						40.66	41.1	6.77
P501	108.74		0.05				38.2	38.04	6.63
P502	107.45		0.06				42.74	38.76	6.53
D101	104.99						37.5	38.16	6.17
D102	107.07						42.7	42.02	6.3
P101	107.91		0.5				40.34	40.89	5.99
P102	109.58		0.15				42.3	42.75	6.11
D201	105.9						39.61	39.89	5.38
D202	105.44						37.06	38	5.6
P201	109.5		266.04				124.5	122.5	7.05
P202	108.45		264.79				125.1	122.8	7.16
24D1	105.66						37.21	38.2	7.12
24D2	97.89						37.3	37.95	7.2

Feb, 25,96 — Day:2

REACT	2,4 -D		PHENOL		DEXTROSE		SOC		pH
D501	105.52						39.25	39.24	6.7
D502	102.97						39.9	39.4	6.77
P501	103.57						38.43	38.6	6.9
P502	102.42						38.79	39.7	6.99
D101	104.24						39.18	38.39	6.93
D102	103.93						40.26	41.66	6.77
P101	103.06						38.17	39.08	6.75
P102	105.27						38.51	38.31	6.7
D201	107.04						39.51	40.31	6.15
D202	102.36						39	38.33	6.17
P201	102.87						41.92	44.67	5.82
P202	104.37						41.43		5.68
24D1	105.14						39.08	38.21	6.82
24D2	103.71						38.68	39.41	6.63

TABLE B2 CONT...

Feb, 26,96 Day:3

REACT	2,4 -D		PHENOL		DEXTROSE		SOC		pH
D501	106.84						63.51	63.14	6.17
D502	104.33						45.91	45.43	6.33
P501	107.51						63.51	63.04	6.97
P502	106.03						51.78		6.94
D101	106.4						68.56		6.61
D102	104.51						57.73		6.48
P101	103.86						43.34	43.29	6.69
P102	103.82						52.63		6.72
D201	102.1						43.58	43.19	6.5
D202	102.71						42.54	43.34	6.4
P201	103.99						45.28	45.6	6.4
P202	104.09						42.63	43.91	6.3
24D1	101.98						44.48	44.14	5.5
24D2	102.82						44.31	45.05	5.45

Feb, 28,96 Day:5

REACT	2,4 -D		PHENOL		DEXTROSE		SOC		pH
D501	111.89								5.19
D502	105.82								5.2
P501	107.23						55.3	45.44	5.24
P502	106.8						52.58	51.36	5.29
D101	107.79						47.37	44.49	5.21
D102	108.28						55	43.78	5.24
P101	109.69						45.91	45.04	6.3
P102	108.53								6.75
D201	109.43								6.82
D202	110.16						44.86	46.13	6.64
P201	109.88						49.99	49.67	6.86
P202	109.87						55.3	45.49	6.8
24D1	110.01						43.89	43.66	5.77
24D2	112.78						44.44	43.15	5.59

TABLE B2 CONT....

Feb, 29,96 Day:6

REACT	2,4 -D		PHENOL		DEXTROSE		SOC		pH
D501	94.64								5.29
D502	89.01								5.18
P501	98.24								5.28
P502	89.36								5.42
D101	102.97								5.35
D102	87.81								5.32
P101	92.42								5.18
P102	92.35								5.32
D201	94.05								6.84
D202	104.09								6.69
P201	107.59								7.06
P202	114.91								6.97
24D1	95.63								5.83
24D2	99.18								5.67

Mar 1,96 Day:7

REACT	2,4 -D		PHENOL		DEXTROSE		SOC		pH
D501	118.37								5.38
D502	71.34								5.33
P501	112.72								5.43
P502									5.49
D101	111.54								5.47
D102	109.39								5.42
P101	86.49								5.43
P102	109.76								5.4
D201	112.33								7.1
D202	110.64								6.77
P201	119.62								7.38
P202									7.33
24D1									6
24D2	115.95								5.85

TABLE B2 CONT....

Mar 2,96 Day:8

REACT	2,4 -D		PHENOL		DEXTROSE		SOC		pH
D501	134.53								5.43
D502									5.35
P501	102								5.49
P502	111.92								5.51
D101	128.33								5.5
D102									5.56
P101	101.48								5.58
P102	125.73								5.8
D201									7.06
D202	125.79								6.68
P201	123.4								7.4
P202	104.63								7.37
24D1	110.76								6.1
24D2	127.92								5.95

Table B 3 Batch Study Performed at 10 C with Mixed Biomass

May 22, 1996 DAY:0

REACTOR	2,4-D		PHENOL		DEXTROSE		SOC		pH
9D501	97.48	99.29			139.1	155.52	93.55	92.115	7.4
9D502	101.1				133.5	136.65	90.68		7.6
9P501	101.17	101.49	73.32	74.47			92.28	92.155	7.4
9P502	101.81		75.62				92.03		
9D1001	100.06	98.45			277.38	284.65	133.5	134.65	
7D1002	96.84				298.55	285.87	135.8		7.2
7P1001	98.91	98.115	133.92	134.57			126.6	127.55	
7P1002	97.32		135.22				128.5		7.2
7D2001	100.43	100.975			580.05	596.36	197.3	198.75	7.2
7D2002	101.52				597.19	611.14	200.2		7.2
7P2001	110.78	105.175	264.7	257.12			199.7	196.25	
7P2002	99.57		249.54				192.8		7.2
724D1	98.35	99					47.93	47.205	7.3
724D2	99.65						46.48		

May 23,1996 DAY:1

7D501	97.42	98.845			120.97	139.26	94.72	95.26	7.4
7D502	100.27				116.94	136.79	95.8		8.27
7P501	99.6	97.72	46.26	49.23			84.73	84.305	7.39
9P502	95.84		50.18				83.88		7.31
7D1001	100.96	100.985			282.26	279.94	135.9	135.8	7.62
7D1002	100.97				283.3	285.21	135.7		7.44
7P1001	99.48	98.58	115.6	116.605			119.8	119.9	7.22
7P1002	97.68		117.61				120		7.43
7D2001	100.46	100.275			527.36	559.4	202.3	202.65	7.46
7D2002	100.09				579.07	607.97	203		7.49
7P2001	97.64	99.085	225.32	229.34			178.4	177.85	7.4
7P2002	100.53		233.36				177.3		7.34
724D1	98.49	100.88					67.84	62.23	7.86
724D2	103.27						56.62		7.87

May, 24,1996 DAY:2

7D501	96.1	97.525	NA		133.1	113.77	93.63		7.77
7D502	98.95		NA		115.88	106.4	95.85		7.72
9P501	98.86	98.05	44.35				73.95		7.29
9P502	97.24		35.88				72.92		7.01
7D1001	96.37	99.285	NA		247.51	236.26	144.8		7.37
7D1002	102.2		NA		239.39	261.06	139.4		7.34
7P1001	99.16	98.42	112.06				123.8		7.24
7P1002	97.68		110.46				112.2		7.27
7D2001	99.62	95.51	NA		587.64		243.7		7.13
7D2002	91.4		NA		567.78		236.6		7.24
7P2001	100.39	99.59	221.76				207.5		7.27
7P2002	98.79		228.54				205.5		7.31
724D1	94.43	97.195	NA				54.82		7.64
724D2	99.96		NA				49.56		7.66

TABLE B3 CONT.-

Table B 3 Batch Study Performed at 10 C with Mixed Biomass

DATE: MAY25,96 DAY:3

REACTOR	2,4-D		PHENOL		DEXTROSE		SOC		pH
7D501	73.67	82.04			111.74				7.32
7D502	90.41				82.69	89.95			7.25
7P501	79.91	79.765	20.26						7.15
7P502	79.66		20.43						7.16
7D1001	88.72	85.705			221.85				7.18
7D1002	82.69				211.94				7.06
7P1001	87.19	87.19	88.67						7.21
7P1002									7.11
7D2001	90.3	90.665			500.33				6.91
7D2002	91.03				552.64				7.01
7P2001	90.99	93.255	142.01						7.17
7P2002	95.52		159.84						7.14
724D1	80.55	81.44							7.53
724D2	82.33								7.45

May,26,96 DAY:4

7D501	78.35	74.175							7.1
7D502	70								7.04
7P501	79.35	79.07	22.4						7
7P502	78.79		24.5						6.99
7D1001	80.7	79.56							6.95
7D1002	78.42								6.92
7P1001	85.6	87.15	78.31						7
7P1002	88.7		79.56						6.99
7D2001	70.56	70.56							6.22
7D2002	72..41								6.23
7P2001	92.32	90.76	143.7						7.1
7P2002	89.2		140.3						7.05
724D1	82.81	83.265							7.2
724D2	83.72								7.3

May 27,96 Day 5

7D501	82.1	72.035			18.84	15.48	58.86	54.75	6.74
7D502	61.97				17.89	19.49	65.64		6.84
7P501	89.61	84.435	24.15				82.86		6.93
7P502	79.26		21.07				73.48		6.85
7D1001	78.55	74.93			97.24	109.89	97.8		6.62
7D1002	71.31				40.64	19.82			6.43
7P1001	88.93	87.11	93.66				104.8		6.99
7P1002	85.29		91.44				99.51		6.87
7D2001	64.83	65.085			250.99		180.7		6.08
7D2002	65.34				270.69		174.3		6.13
7P2001	93.15	89.775	211.55				196		7
7P2002	86.4		137.74	130.37			200		7.26
724D1	85.95	83.87					50.97		7.1
724D2	81.79						49		7.14

TABLE B3 CONT.

Table B 3 Batch Study Performed at 10 C with Mixed Biomass

DATE:MAY 28, 96 DAY:6

REACTOR	2,4-D		PHENOL		DEXTROSE		SOC		pH
7D601	61.31	57.075							6.97
7D602	52.84								6.81
7P601	58.89	60.595	9.32						6.86
P602	62.3		10.04						6.74
7D1001	45.13	44.855							6.23
7D1002	44.58								6.29
7P1001	69.24	69.36	56.52						6.8
7P1002	69.52		74.51						6.8
7D2001	28.76	28.08							5.36
7D2002	27.4								5.28
7P2001	79.38	80.94	146.43						6.93
7P2002	82.5		142.27						7.1
724D1	66.13	64.66							7.1
724D2	63.19								6.03

MAY,29,96 DAY:7

7D601	55.18	55.105					42.57		6.71
7D602	55.03						46.64		6.73
P601	64.5	60.165	7.14				50.06		6.76
P602	55.83		0.3				41.77		6.6
7D1001	42.17	38.64					36.64		6.25
7D1002	35.11						37.6		6.26
7P1001	71.43	65.895	73.04				87.4		6.79
7P1002	60.36		66.57				78.89		6.73
7D2001	30.49	29.025					41.04		6.63
7D2002	27.56						36.84		6.59
7P2001	87.49	88.355	205.91	195.27			156.1		6.89
7P2002	89.22		207.71				158.7		7.03
724D1	67.34	63.07					42.65		7.01
724D2	58.8						47.25		6.92

MAY 31,96 DAY:9

7D601	14.69	22.59							6.36
7D602	30.49								6.56
7P601	22.75	12.86	0.7						6.51
P602	3.01		0.18						6.45
7D1001	3.74	2.27							6.04
7D1002	0.8								6.48
7P1001	31.33	21.945	38.92						6.5
7P1002	12.56		16.86						6.31
7D2001	0.17	0.385							6.57
7D2002	0.6								6.5
7P2001	61.21	65.405	127.97						6.7
7P2002	69.6		142.01						6.8
724D1	34.02	23.21							6.76
724D2	12.4								6.6

TABLE B3 CONT..

Table B 3 Batch Study Performed at 10 C with Mixed Biomass

JUNE 1, 1996 DAY:10

REACTOR	2,4-D		PHENOL		DEXTROSE		SOC		pH
7D601	0.14	9.86					20.64	23.955	6.32
7D602	19.58						27.27		6.4
7P601	9.98	5.06					22.62	21.725	6.5
P602	0.14						20.83		6.54
7D1001	1.16	0.61					16.63	17.485	6.12
7D1002	0.06						18.34		6.61
7P1001	9.11	4.555	16.08	16.08			40.73	40.73	6.3
7P1002	0								6.07
7D2001	0.29	0.145					19.06	22.985	6.69
7D2002	0.43						26.87		6.62
7P2001	57.06	63.635	164.81	175.28			133.2	135.65	6.55
7P2002	70.61		185.75				138.1		6.7
724D1	21.68	10.85					29.06	24.35	6.65
724D2	0.02						19.64		6.49

JUNE 2, 96 DAY:11

7D601									
7D602	0.86	0.735							6.38
7P601	0.61								6.47
P602	0								
7D1001									
7D1002									
7P1001	3.16	2.545	0.08						6.25
7P1002	1.93		0.13						6.13
7D2001									
7D2002									
7P2001	32.59	40.335	94.74						6.4
7P2002	48.08		121.76						6.6
724D1	1.53	1.53							6.6

Jun-03 DAY:12

7D602	0.02	0.47	0.08				19.93		
7P601	0.92		0.06				18.56		
P602									
7D1001									
7D1002									
7P1001	0.03	0.08	0.12				18.33		
7P1002	0.13		0.62				19.99		
7D2001									
7D2002									
7P2001	25.32	32.685	94.96				79.6		
7P2002	40.01		143.47				107.4		
724D1	0.32	0.32					18.36		
724D2									
7CO1									

TABLE B3 CONT..

Table B3 Batch Study Performed at 10 C with Mixed Biomass

June 4,96 DAY:13

REACTOR	2,4-D		PHENOL		DEXTROSE		SOC		pH
7P2001	5.09	11.39	0.27	37.44					
7P2002	17.69		74.61						

June 5,96 DAY:14

7P2001	3.43	3.21							
7P2002	2.99								

June 6,96 DAY:15

7P2001	0								
7P2002	0								

Table B 4 Batch Study Performed at 30 Temperature

START DAY:1

REACT	2,4-D	PHEN	DEXT	SOC	pH
D50	110		141	228	7.23
P50	112	78.2		217	7.57
D100	109		279	306	7.67
P100	108	154		293	7.43
D200	110		520	457	7.35
P200	112	271		438	7.49
2,4D	115			157	7.62
C0	112				7.8

Time 2.30 PM Day: 1

REACT	2,4-D	PHEN	DEXT	SOC	pH
D50	106		102	120	7.2
P50	105	47.5		109	7.2
D100	108		213	183	7.3
P100	106	125		166	7.2
D200	109		533	320	7.2
P200	109	257		286	7.3
2,4D	108			76.8	7.65
C0	110			70.3	7.8

TIME 6:30 PM DAY:1

REACT	2,4-D	PHEN	DEXT	SOC	pH
D50	110		71.5	109	6.94
P50	107	32.8		92.8	7
D100	105		216	168	7.1
P100	105	108		161	7.1
D200	106		472	302	7.1
P200	106	249		272	7.1
2,4D	110			71.5	7.5
C0	108			70	7.7

TIME 10:30 AM DAY:2

REACT	2,4-D	PHEN	DEXT	SOC	pH
D50	66.7		2.4	61.4	6.35
P50	104	0.1		61.7	6.7
D100	99.4		3.55	59.5	6.21
P100	102	0.2		59.5	6.32
D200	96.7		194	117	5.89
P200	99.2	139		137	6.34
2,4D	102			61.4	6.81
C0	109			72	7.8

TIME 10:30 AM DAY:3

REACT	2,4-D	PHEN	DEXT	SOC	pH
D50	0.02		0	17.5	5
P50	15	0		24.7	6.1
D100	13.9		0	24.1	5.55
P100	64	0		44.3	6.27
D200	18.1		0	32.4	6.18
P200	100	0		59.3	5.82
2,4D	1.63			17.4	5.96
C0	111			71.3	7.75

TIME 2:30 DAY:3

REACT	2,4-D	PHEN	DEXT	SOC	pH
D50	Discontinued				
P50	0.28	0		19	6.7
D100	4.2		0	21	6.72
P100	52.6	0		40.8	6.49
D200	10.8		0	30.3	6.32
P200	102	0		62.6	6.87
2,4D	Discontinued				
C0	112				6.7

TIME 6:30 DAY:3 TIME 10:30 DAY:4 TIME 10:30 DAY:5

REACT	2,4-D	PHEN	DEXT	SOC	pH	REACT	2,4-D	PHEN	DEXT	SOC	pH	REACT	2,4-D	PHEN	DEXT	SOC	pH
D50	0		0			D50	0		0			D50					
P50	0	0				P50	0	0				P50					
D100	0.17		0	14.9	6.71	D100	0		0			D100					
P100	42.7	0		32.3	6.46	P100	14.145	0		52.4	6.04	P100	1.6			33	5.79
D200	3.06		0	18.5	6.31	D200	0		0	54.7	5.97	D200					
P200	98.6	0		64	6.92	P200	69.26	0		88.7	6.89	P200	0.5			52.6	6.78
2,4D	0					2,4D	0					2,4D					
C0	110			70.5		C0	112					C0	110				

Day 6 Time 10:30

P101	0	0	na	12.2	

Calibration curve for SOC standards

10	10.04
20	20.89
40	38.12
80	70.55
100	82.21
120	97.4
140	110.1
180	132.8
240	164.4

Regression Output:

Constant		11.0239
Std Err of Y Est		5.87257
R Squared		0.98869
No. of Observations		9
Degrees of Freedom		7
X Coefficient(s)	0.67451	
Std Err of Coef.	0.02726	

Table B 5 BATCH STUDIES AT 22 C USING MIXED CULTURE

DATE: FEBURARY 4, 1996 DAY:0

REACT.	2,4-D		PHENOL		DEXTROSE		SOC		pH
D601	106	104			141		66.1	66.5	7.5
D602	102						71.73	71	7.5
P601	93	93.1	60.7	61.8			76.69	73.9	7.7
P602	93.2		60.4				64.8	65.9	7.5
D1601	107	109					96.27	94.9	7.8
D1602	110				317.5		95.16	96.6	7.7
P1601	106	106	142				94.78	93.1	7.6
P1602	107		146				88.72	90.1	7.6
D2601	106	107					121.7	123	7.9
D2602	105				500.1	518	128.3	126	7.9
P2601	107	106	286				139.7	142	7.6
P2602	106		290				133.6	132	7.6
24D1	107	110					41.17	47.7	7.4
24D2	110						38.38	39.3	7.4
CO1	110	110					37.38	37.7	7.76

FEBURARY 5, 1996 DAY:1

D601	104	103			13.91		61.83	61.9	
D602	101	101			13.86	15.9	95.58	93.7	
P601	96.3	102	46.5				68.29	72.4	
D1601	101	101					108.8	108	
D1602	103	103					82.56	81.3	
P1601	99.4	101	117				91.92	91.4	
P1602	107	103	118				94.61	94.6	
D2601	100	101					117.2	116	
D2602	107	105					130.3	131	
P2601	104	105	235				133.1	134	
P2602	106	103	232				127.2	126	
24D1	104	103							
24D2	101	99					49	48.6	
CO1	110	112					44.16	43.2	

DATE: FEBURARY 6, 1996 DAY:2

REACT.	2,4-D		PHENOL		DEXTROSE		SOC		pH
D601	105	105			2.42		52.58	50.7	7.6
D602	105	102			0		78.31	77.6	7.6
P601	103	105					41.96	41.3	7.4
D1601	102	100			6.43		82.66	83	7.5
D1602	105	103			27.29		54.52	55.9	7.5
P1601	104	98.7	38.4	36.5	-		57.34	55	7.3
P1602	102	101	42.5	43			64.18	64.3	7.2
D2601	101	100			22.2		46.66	47.4	6.7
D2602	106	102			247.1		106.5	107	7.4
P2601	102	103	165	166			106.5	106	7.1
P2602	101	99.4	142	140			100.13	102	7.46
24D1	103	103					37.83	37.8	7.8
24D2	101	100					45.95	-	7.1
CO1	111	109					44.5	43.8	7.8

TABLE B5 CONT.

Table B. BATCH STUDIES AT 22 C USING MIXED CULTURE

FEBURARY 7, 1996 **DAY:3**

REACT.	2,4-D		PHENOL		DEXTROSE		SOC		pH
D601	105	102			0	0	52.9	50.1	7.04
D602	103	101			0	0	73.2	72	7.41
P601	103	102	0	0			41.3	42	7.06
D1001	102	101			0	0	77.9	78.8	7.02
D1002	106	101			0	0	53.8	53.6	7.03
P1001	102	104	0	0			41.5	41	7.3
P1002	103	102	2.42	2.39			50.1	52.2	6.66
D2001	80.2	78			0	0	43.6	43.6	6.2
D2002	109	101			0	0	94	94.8	6.86
P2001	105	102	109	105			89.6	89.7	6.66
P2002	103	102	75.8	75			81.6	82.2	6.52
24D1	101	99.7					38.5	38.2	6.96
24D2	87.4	86.2					38.4	38.2	6.44
CO1	110	109					47.7	50.9	7.5

FEBURRAY 8, 1996 **DAY:4**

D601	101	101			0	0	53.6	53.5	7.04
D602	96	95			0	0	71.3	69.1	7.41
P601	93.5	92.4	0	0			41.6	42	7.06
D1001	97.1	96.3			0	0	78.1	77.4	7.02
D1002	104	97.9			0	0	54.4	56	7.03
P1001	102	99.5	0	0	–		44.6	44.7	7.3
P1002	102	101	0	0			58	51.7	6.66
D2001	60.7	60.6			0	0	46.7	43.7	6.2
D2002	109	107			0	0	84.4	85	6.86
P2001	104	99.2	70.8	67.2			80.5	80.7	6.66
P2002	102	99.2	33.5	32.2			70.4	72.8	6.52
24D1	87.7	86.4					36.9	36.5	6.96
24D2	15.6	15.5					23.6	20.1	6.44
CO1	109	111					45.3	46.7	7.6

FEBURRAY 9, 1996 **DAY:6**

D601	89.9	87.2			0	0	45.3	47.3	47
D602	89.9	92.2			0	0	62.2	61.8	7.83
P601	75.4	78	0	0			35		6.96
D1001	92	94.1			0	0	66.8		7.72
D1002	100	98.4			0	0	50.2	52.2	7.3
P1001	101	100	0	0			40.3	40.4	7.7
P1002	103	98.4	0	0			47.1	46.6	6.92
D2001	51.1	50.4			0	0	35.3	35.6	6.96
D2002	106	104			0	0	70.6	71.1	7.16
P2001	105		36.2	35.6			68.2	67.6	6.92
P2002	105	103	0	0			54.6	54	6.95
24D1	80						33.5	33.6	5.74
24D2	1	1.1					17.9	14.6	6.66
CO1	109	107					45.4	44.6	7.7

TABLE B5 CONT.

Table B5 BATCH STUDIES AT 22 C USING MIXED CULTURE

DATE: FEBURARY 10, 1996 DAY:6

REACT.	2,4-D		PHENOL		DEXTROSE		SOC		pH
D501	72.1	73.1			0	0	43.49	43.4	7.03
D502	81.2	82.3			0	0	59.72	59.8	7.81
P501	43.6	44.5	0	0			27.4	27.3	6.86
D1001	88.3	89.1			0	0	63.94	64	7.82
D1002	87.2	90.5			0	0	48.69	48.6	7.17
P1001	94	95.7	0	0			40.67	40.5	7.82
P1002	92.6	94.9	0	0			45.26	45.5	7.13
D2001	34.8	36			0	0	33.58	35	6.83
D2002	107	105			0	0	66.79	67.2	7.5
P2001	103	104	0	0			54.54	52.6	7.01
P2002	100	99.9	0	0			53.52	53.2	7.39
24D1	78.2	79					31.81	32.6	5.7
24D2	0.67	0.83					16.26	17.2	7.12
CO1	111	108					-	-	-

DATE: FEBURARY 11, 1996 DAY:7

REACT.	2,4-D		PHENOL		DEXTROSE		SOC		pH
D501	65.2	64.3					37.03		7.26
D502	51.4						55.66		7.9
P501	5.12	5.1					15.55		6.77
D1001	82.6						59.86		7.8
D1002	73.1	74.5					44.13		7.23
P1001	85.4	85.9					37.22		7.82
P1002	86	85.5					41.51		7.52
D2001	1.37						26.67		6.82
D2002	86.5	107					71.77		7.71
P2001	102	93.1					46.97		7.13
P2002	92.4	96.2					49.54		7.56
24D1	74						34.06		5.86
24D2	3.36	0					22.35		7.13
CO1	109	109					-	-	-

FEBURARY 12, 1996 DAY:8

D501	58.6						38.67		7.77
D502	53.6						58.19		7.88
P501	0	0					21.7		7.84
D1001	56.1						61.61		7.82
D1002	65.8						44.2		7.38
P1001	76						39.29		7.84
P1002	67.3						42.31		7.25
D2001	1.51						26.77		6.82
D2002	102						66.67		7.81
P2001	84.7						53.98		7.12
P2002	76.9						42.46		7.62
24D1	74.3						37.28		7.45
24D2	0						21.28		7.25
CO1	108	111					48.07		-

TABLE B5 CONT

Table B. 5 BATCH STUDIES AT 22 C USING MIXED CULTURE

FEBURARY 13, 1996 DAY:9

REACT.	2,4-D		PHENOL		DEXTROSE		SOC		pH
D501	49.1	53.2					67.4		7.68
D502	40.2	39.9					61.5	67.4	7.93
P501	0	0							
D1001	66.3								7.85
D1002	56.3						74.6	73.5	7.7
P1001	57.7	57.4					42.2		7.77
P1002	20.3						69.1	71.2	6.89
P2001	0	0							
D2002	99.1	99.6					80.4	77.9	7.84
P2001	63.8						79.2		7.15
P2002	65.6						51.3	50.6	7.6
24D1	0	0					45.3	4659	7.62
24D2	0	0					-	-	-
CO1	110	111					-	-	-

FEBURRAY 14, 1996 DAY:10

D501	30.5	31.7							7.7
D502	38.9	38.6							7.83
P501	0	0							
D1001	54.5	55.3							7.6
D1002	40.3	41.8							7.2
P1001	30.8	31.9							7.2
P1002	10.2	10.8							7.7
D2001	0	0							7.96
D2002	95.6	96.8							-
P2001	30.8	31.9							-
P2002	32.8	33.1							-
24D1	60.8	60.3							-
24D2	0	0							-
CO1	110	110							-

FEBURRAY 15, 1996 DAY:11

D501	1.86	1.31							7.84
D502	11.2	11.5							
P501	0	0							
D1001	49.6								
D1002	22						43.4	46.4	7.8
P1001	1.86	1.13					51.5	54.8	7.93
P1002	0.99						58.1	59.2	7.92
D2001	0	0							
D2002	90.5	96.5							7.72
P2001	2.3	2.3							7.37
P2002	3.2								7.5
24D1	0	0							
24D2	0	0							
CO1	108	110							

Table B5BATCH STUDIES AT 22 C USING MIXED CULTURE

DATE: FEBURARY 16, 1996 DAY:12

REACT.	2,4-D		PHENOL		DEXTROSE		SOC		pH
D661	0	0					66.9	49.1	7.95
D662	0	0					77.8	70.5	
7P661	0	0					66.86	61.3	
D1661	0	0							
D1662	0	0							7.37
P1661	0	0							7.92
P1662	0	0					53.81	51.5	7.76
D2661	0	0					61.35	56.6	7.74
D2662	91.5	90.6							
P2661	0	0							
P2662	0	0							
24D1	0	0							
24D2	0	0							
CO1	110	110							

FEB. 17,1996 DAY 13

D2662	67.3	68.2							

FEB. 19,96 DAY16

D2662	57.3	57.6					34.64		

Table B 6 Batch Study Performed to Study the Degradation Pattern by Pure 2,4-D Degraders at 22 C

DATE: April. 3, 1996 DAY:0

REACTOR	2,4-D		PHENOL		DEXTROSE		SOC		pH
9D501	115.22	111.305	na		138.59	142.75	89.23	115.94506	7.82
9D502	107.39		na		146.91		92.4	120.64476	7.71
9P501	101.89	105.32	76.06	75.15	na		84.31	108.65067	7.82
9P502	108.75		74.24		na		89.63	116.53808	7.86
9D1001	109.17	109.17	na		290.06	302.935	121.9	164.38022	7.73
7D1002			na		315.81		122.5	165.28975	7.75
7P1001	112.82	111.245	145	142.3	na		119.7	161.11859	7.7
7P1002	109.67		139.6		na		127.1	172.08952	7.72
7D2001	110.06	108.545	na		624.69	599.73	167.7	232.28136	7.76
7D2002	107.03		na		574.77		170.9	237.02554	7.8
7P2001	112.61	111.05	246.02	252.455	na		178.6	248.44124	7.72
7P2002	109.49		258.89		na		180.8	251.70287	7.84
724D1	104.48	107.025	na		na		49.68	57.309869	7.64
724D2	109.57		na		na		52.3	61.1942	7.76
7CO1	na		na		na		50	57.784318	7.7
									DAY:1
7D501	25.15	23.52			82.99	89.075	49.88	49.235	6.45
7D502	21.89				95.16		48.59		6.42
7P501	57.54	59.11	46.34	42.85			69.91	68.175	6.94
9P502	60.68		39.36				66.44		6.87
7D1001	17.69	17.12			208.36	208.78	74.7	74.86	6.38
7D1002	16.55				209.2		75.02		6.29
7P1001	77.74	76.225	81.77	83.165			103.8	102.1	7.14
7P1002	74.71		84.56				100.4		7.1
7D2001	12.73	17.625			441.96	453.76	122.5	126.05	6.31
7D2002	22.52				465.56		129.6		6.37
7P2001	80.95	84.485	127.6	157.995			156.3	157.3	7.44
7P2002	88.02		188.39				158.3		7.39
724D1	42.35	43.155					32.4	33.255	6.61
724D2	43.96						34.11		6.62
7CO1							50.2		7.4
									DAY:2
7D501	11.23	5.615	NA		3.5	3.33	65.83	65.83	6.18
7D502	0		NA		3.16		65.83		6.12
9P501	0	0	0	0			64.19	66.065	6.16
9P502	0		0				71.94		7.17
7D1001	0	0	NA				79.74	81.09	5.56
7D1002	0		NA				82.44		5.74
7P1001	1.23	1.095	19.19	23.15			74.28	74.28	5.85
7P1002	0.96		27.11						5.95
7D2001	0	0	NA		217.39	228.5	86.49	86.49	5.66
7D2002	0		NA		239.61				6.88
7P2001	66.91	67.93	205.15	208			189.8	134.77	7.05
7P2002	68.95		210.85				79.74		7.07
724D1	0	0	NA				80.92	66.615	6.42
724D2	0		NA				52.31		6.46
7CO1			NA						7.3

Table B6 Batch Study Performed to Study the Degradation Pattern by Pure 2,4-D Degraders at 22 C

DAY:4

REACTOR	2,4-D		PHENOL		DEXTROSE		SOC		pH
7P2001	11.01	10.92							6.4
7P2002	10.83								6.3

DAY:5

REACTOR	2,4-D		PHENOL		DEXTROSE		SOC		pH
7P2001	0.76								6.4
7P2002	0.83								6.5

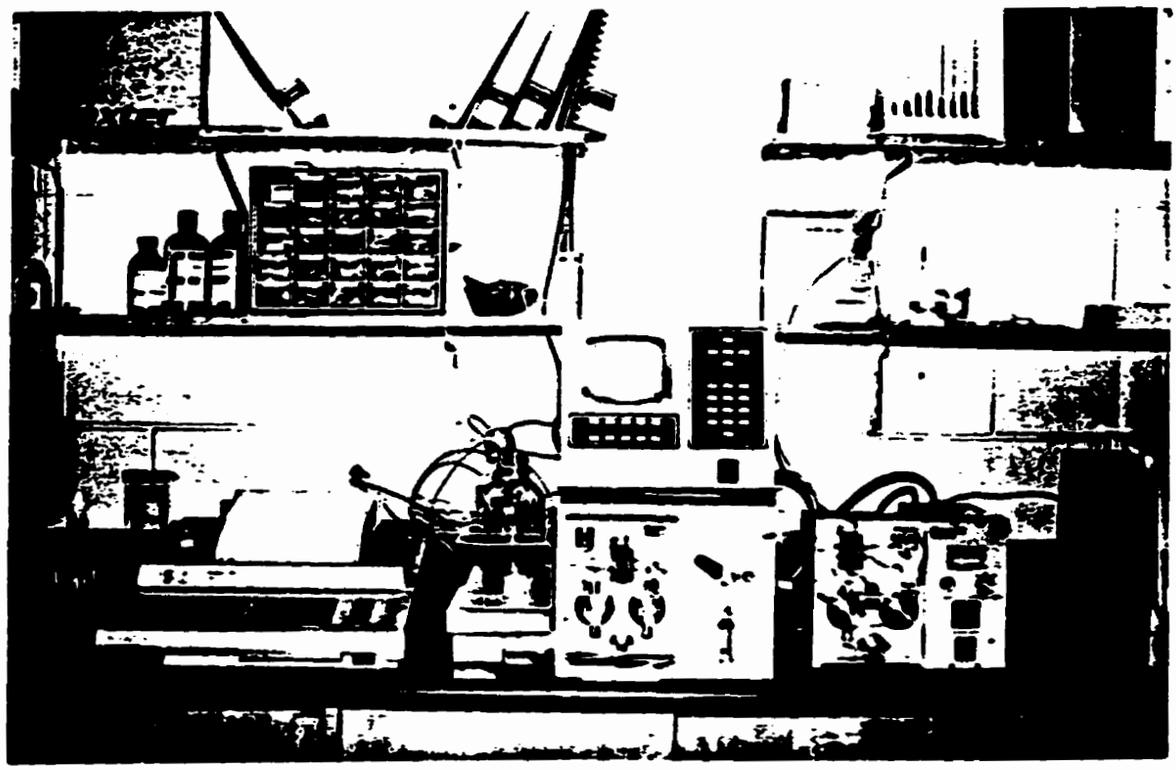

Figure A View of Typical HPLC Set-up