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**Environmental Engineers and Scientists**

REVIEW AND CRITICAL EVALUATION OF THE SCIENTIFIC
LITERATURE TO DETERMINE IMPORTANT
ENVIRONMENTAL VARIABLES CAPABLE
OF INFLUENCING BIODEGRADATION
RATES OF CHEMICALS

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ABSTRACT

This report reviews and evaluates the scientific literature that describes environmental variables that influence the biodegradation rates of chemicals. Information sources for this review and evaluation include relevant papers, books, review articles, abstracting services, computer searches, and the current investigators files of the Smithsonian Science Information Exchange.

There are numerous variables that can affect biodegradation rates of chemicals in the environment. These may include physical variables (such as temperature, dilution, mixing, diffusion, sorption, hydrostatic pressure, and light), chemical variables (such as pH, redox potential, nutrients, toxins, and water availability), and biological variables (such as microbial interactions and adaptation).

An extensive body of literature is available that identifies relationships between a single environmental variable and the biodegradation rate of a particular chemical or class of chemicals for a specific set of environmental conditions. In some cases, mathematical expressions have been developed which characterize these relationships. However, such mathematical expressions generally cannot be used to accurately predict biodegradation rates behavior in natural settings. Clearly, much remains to be learned concerning the influence of environmental variables on biodegradation.

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# SECTION 1

## INTRODUCTION

Chemicals that are considered to be persistent in most environments are of two types. First, there are natural organic compounds including paleobiochemicals such as lignin, tannin, melanin, and complexes such as lignified wood, melanized fungal walls, and tanned proteins. Secondly, there is the enormous variety of synthetic organic chemicals (xenobiotics) that enter the environment either deliberately, via agricultural practice for example, or inadvertantly as a consequence of industrialization. While not all xenobiotics are of a persistent nature, those that have caused most concern due to their recalcitrance include pesticides, detergents, coolants, polymers, resins and solvents. The number of such chemicals is ever expanding as are also the purposes for which they are being used (Bull, 1980). The latter group of chemicals will be the major concern of this investigation.

Biodegradation of chemicals, as discussed in this report, refers to the microbiological transformation of chemicals. This process can result in complete mineralization of the chemical or it can lead to the loss of some or all of the chemical's characteristic properties. Biodegradation may be accompanied by utilization of the chemical as a source of energy or nutrients; likewise it may result in detoxification with reference to either the transforming population, a specific target organism or organisms in general.

There are numerous variables that can affect biodegradation rates of chemicals in the environment. These may include physical variables (such as temperature, dilution, mixing, diffusion, sorption, hydrostatic pressure, and light), chemical variables (such as pH, redox potential, nutrients, toxins, and water availability), and biological variables (such as microbial interactions and adaptation). The objective of this study was to identify significant environmental variables that influence biodegradation rates of chemicals, and to evaluate algorithms for correlation biodegradation rates and environmental variables.

An extensive body of literature is available that identifies relationships between a single environmental variable and the biodegradation rates of a particular chemical or class of chemicals for a specific set of environmental conditions. In some

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cases, mathematical expressions have been developed which characterize these relationships. However, such mathematical expressions generally cannot be used to accurately predict biodegradation rate behavior in natural settings. Unlike the controlled laboratory, the real world is highly dynamic, and biodegradation rates are influenced by interactions between a number of everchanging environmental factors, microbial systems, and organic substrates. Moreover, the interpretation of data concerning the effects of even a single environmental variable is complicated by differences in experimental approaches and techniques, such as pure versus mixed culture, single versus multiple substrates, batch versus continuous systems, in vitro (laboratory) versus in situ (field), and so on. Clearly, much remains to be learned concerning the influence of environmental variables on biodegradation.

In this report, the results of the literature review and evaluation are organized into four topics:

- Physical environmental variables;

- Chemical environmental variables;

- Biological environmental variables; and

- Biodegradation algorithms.

# SECTION 2

## CONCLUSIONS AND RECOMMENDATIONS

### CONCLUSIONS

The preparation of this report involved a scientific review of pertinent literature for identification and evaluation of the effects of environmental variables on biodegradation rates. Specific environmental variables are classified as physical, chemical, or biological, and are summarized in the following discussion.

### Physical Environmental Variables

Temperature--

- Within the physiologically tolerated range of temperature, biodegradation rates increase as the temperature increases. The "Van't Hoff Rule" and "Arrhenius Equation" are frequently used to express such relationships.

Concentration--

- In general, there is a positive correlation between the concentration of a chemical and its rate of biodegradation. However, little or no biodegradation may occur for certain substrates at very low concentrations, and a threshold may exist below which no significant biodegradation occurs. Biodegradation rates can also be greatly reduced when substrate concentrations are higher than certain inhibitory levels.

- Concentrations of chemicals may be affected greatly in natural environments by a variety of physical mechanisms, such as dilution, mixing, diffusion, etc. Such effects may either enhance or reduce biodegradation rates depending upon whether concentrations increase or decrease.

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Sorption--

- Sorption of organic compounds to organic and mineral components of the soil can act to enhance or inhibit biodegradation. Most studies have found that clay minerals with high cation exchange capacities can enhance biodegradation rates.

Hydrostatic Pressure--

- In general, biodegradation rates are inversely proportional to hydrostatic pressure. However, biodegradation rates can be enhanced or reduced by high pressure depending on the co-effects of temperature and pressure. High pressure can also result in an extended lag period.

Light--

- Light can enhance or inhibit the biodegradation of chemicals depending on the specific conditions and microorganisms involved. Ultraviolet light acts to inhibit biodegradation because of its lethal and mutagenic effects. Visible light can also be lethal or mutagenic to those species which are without protective carotenoid pigment.

- Light can be used by photosynthetic bacteria to degrade low molecular weight organic compounds. Evidence suggests that certain light intensities may inhibit substrate utilization by bacteria; though few data are available on this subject.

## Chemical Environmental Variables

pH--

- Biodegradation generally occurs within a pH range of 5 to 8, pHs which are commonly encountered in natural systems. For some chemicals, variation within this range will influence biodegradation rates.. For others, variations within this range have a negligible effect on biodegradation rates.

Redox Potential--

- The rate and extent of biodegradation is profoundly affeted by redox potential. Though biodegradation rates are generally found to increase with increasing redox potentials, certain chemicals are degraded, and some reactions (e.g., dechlorination) are enhanced, at lower redox potentials.

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Nutrients--

- Nutrients may or may not be limiting depending
  on the availability of readily available carbon-
  aceous substrates. Nitrogen and/or phosphorous
  are often implicated in inhibiting biodegradation
  by being in limited amounts. When either nitrogen
  or phosphorous is provided in excess, biodegradation
  may be limited by the amount of the other nutrient.

- Increased microbial activities associated with
  nutrient rich soils may lead to misleading inter-
  pretations of the effects of nitrogen and phos-
  phorous on biodegradation.

- Organic cosubstrate enrichment may inhibit or stimu-
  late the biodegradation of xenobiotics.

Toxins/Inhibitors--

- Very little systematic work has been conducted on
  the effects of inorganic and organic toxins on bio-
  degradation rates. Contradictory evidence indicates
  that certain concentrations of a given substance
  may be inhibitory to biodegradative processes,
  though higher concentrations are reported to be
  harmless to such activities.

- Considerable literature is available which describes
  the inhibitory, synergistic, and antagonistic effects
  of heavy metals, inorganic ions, and organics on
  microbial activities in biological treatment processes.
  Such data may be of limited value in the prediction
  of general trends in biodegradation rates.

Water Availability--

- Water availability in the aqueous environment is
  usually measured in terms of water activity, osmotic
  pressure, ionic strength, or salinity. Water activity
  has been used in most studies to evaluate the effects
  of water availability on microbial growth. Quantitative
  relationships indicating the effects of water avail-
  ability on biodegradation rates have not been found
  in the literature.

- In non-aqueous environments, water availability
  is usually measured in terms of percent moisture
  content or field capacity. Most studies have shown
  that when the water content was within a certain
  range, biodegradation rates increased with increasing

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water content. However, when the water content was too high, biodegradation rates either decreased or remained unchanged.

## Biological Environmental Variables

Microbial Interactions--

- Interactions among microbiological populations can have significant impact on chemical biodegradation. Basic types of interactions include mutualism, commensalism, amensalism, predator-prey relationships, and competition. Mutual and commensal interactions may provide complete degradation of a subject chemical and its metabolic products.

Adaptation--

- The phenomenon of adaptation, whereby organisms acquire new physiological or morphological traits which enable them to operate under a new set of environmental conditions, has considerable ecological significance. Recent studies show that the ability of organisms to adapt to a new substrate or environmental condition depends on the presence of specific microorganisms.

## Biodegradation Algorithms

- A complete generic algorithm(s) which includes the relationships between biodegradation rates and various environmental variables is still lacking.

- Basic biodegradation algorithms (i.e., algorithms only addressing the rate of disappearance of a growth substrate as a function of substrate concentration) are usually based on one of two basic approaches: decay or enzymatic reactions. The latter is believed to be superior to the former for the quantitative expression of biodegradation rates.

- Very few algorithms have been derived for expressing the effects of environmental variables on biodegradation rates. Variables that have been most extensively studied for algorithm development include temperature, substrate inhibition and moisture content. Algorithms for other variables are either impractical or do not exist at the present time. Due to the complexity of environmental factors affecting biodegradation rates, it is unlikely that useful mathematical expressions relating dynamic

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interactions in these factors to biodegradation rates
will become available in the near future.

RECOMMENDATIONS

Review of the literature revealed that little systematic
work has been performed in many areas relevant to the effects of
environmental variables on biodegradation rates.  Subject areas
which warrant further study are summarized in the following
discussion.

- The literature describing attempts to establish
  relationships between certain environmental factors
  and biodegradation rates often neglect to report
  the state of other significant environmental factors
  during experimentation.  Future efforts to develop
  relationships and algorithms in this area would be
  aided greatly by adequate description of all the
  significant variables in the experiments.

- Most 'studies on the relationships between environ-
  mental variables and biodegradation rates emphasized
  the descriptions of results rather than causes.
  In order to have fruitful understanding in this
  area, studies attempting to determine the causes
  of the effects of variables should also be conducted.

- The co-effects of environmental variables were
  usually neglected in the biodegradation studies
  reviewed.  For example, the effects of pH on bio-
  degradation may be significantly influenced by Eh.
  Soils of different organic matter contents may
  affect the biodegradation rate differently at
  similar pH levels.  The mobility of chemicals that
  may be toxic to microorganisms is affected by pH
  and Eh.  Pressure effects may be greatly influenced
  by temperature, D.O., and metabolic products.  The
  effects of sorption may be affected by pH, Eh, the
  type and distribution of adsorbent (medium), etc.
  Such co-effects should be considered in any bio-
  degradation studies.

- The literature search revealed that quantitative
  data on the effects of the following variables
  on biodegradation rates are greatly lacking and
  require future research:  diffusion of xenobiotics
  in the natural environment; light intensity; aerobic/
  anaerobic conditions in terms of different redox
  (or D.O./dissolved sulfide) levels; inhibitors/
  toxins; water availability in the aqueous environ-
  ment; and biological variables.

- If generic algorithms describing effects of environmental variables on biodegradation rates are to be derived, controlled environmental conditions and more experiments to cover wide varieties of chemicals and environmental variables are urgently needed.

# SECTION 3

## PHYSICAL ENVIRONMENTAL VARIABLES

### OVERVIEW

The biodegradation of chemicals by microorganisms in terrestrial and aquatic environments may be affected by a multitude of physical environmental variables. The purpose of this section is to identify and characterize those physcial environmental variables that significantly affect biodegradation rates and processes. The significant factors to be discussed include: (1) temperature; (2) concentration; (3) sorption; (4) hydrostatic pressure; and (5) light.

Biodegradation of chemicals involves complicated enzyme-catalyzed reactions which need heat or thermal energy to proceed (Farrell et al., 1967; Rose, 1976; and Welker, 1976). Within the physiologically tolerated range of temperature, biodegradation rates increase as the temperature increases. The "Van't Hoff Rule" or "Arrhenius Equation" are frequently used to express such relationships. However, when temperatures are higher or lower than the optimum temperatures for growth, biodegradation rates are reduced. The literature review also revealed that temperature can indirectly affect biodegradation rates through changes in the lag period and in biodegradation pathways.

Several physical variables, such as dilution, mixing, and diffusion, are found to affect the concentration or opportunity for contact between organisms and substrates, and, therefore, influence biodegradation rates of chemicals. In general, a positive correlation between the concentration of substrates and their biodegradation rates is found. However, little or no biodegradation may occur for certain substrates at very low concentrations, and a threshold may exist below which no significant biodegradation occurs. On the other hand, when substrate concentrations are higher than certain inhibitory levels, biodegradation rates can be greatly reduced.

Sorption also plays an important role in controlling rates of biodegradation. Sorption of organic compounds to organic and mineral components of the soil can act to enhance or inhibit biodegradation, depending on site-specific factors and the compound of interest. Most studies, as shown in the following pages, have found that clay minerals with high cation exchange

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capacities can enhance biodegradation rates.  Chemicals may be
protected from biodegradation once bound to the inner lattice
of clay minerals (e.g., montmorillonite), or when strongly bound
to humic substances.  In aqueous environments, enhanced bio-
degradation through adsorption effects have been reported.

Large numbers of microorganisms live below the surface of
water masses, where hydrostatic pressure may be much higher than
one atmosphere.  Evidence shows that pressure affects biodegrada-
tion in many ways.  Biodegradation rates can be increased or
reduced by high pressure, depending on the co-effects of tempera-
ture and pressure.  High pressure can also result in an extended
lag period, enzyme denaturation, biosynthetic pathway changes,
reduction in the pH range for growth, and loss of potassium from
the cells.

Light can enhance or inhibit the biodegradation of chemicals
depending on the specific conditions and microorganisms involved.
Ultraviolet light acts to inhibit biodegradation because of its
lethal and mutagenic effects.  Visible light can also be lethal
or mutagenic to those species which are without protective carot-
enoid pigments. ' Light can be used by photosynthetic bacteria to
degrade low molecular weight organic compounds.  Evidence suggests
that certain light intensities may inhibit substrate utilization
by bacteria; though few data are available on this subject.

TEMPERATURE

Biodegradation of chemicals involves complicated enzyme-
catalyzed biochemical reactions, and in order for these reactions
to proceed at a satisfactory rate, the organism needs to be
supplied with heat or thermal energy (Farrell et al., 1967; Rose,
1976; and Welker, 1976).  A small fraction of the heat require-
ment of living organisms comes from the organism itself.  However,
the main source of heat is the environment (Farrell et al., 1967).
Because of this environmental heat dependency, temperature is
one of the most important environmental variables affecting
the biodegradation of chemicals.

Review of the scientific literature on biodegradation studies
revealed that temperature can affect biodegradation in the follow-
ing ways:

- Within the physiologically tolerated range of
  temperature, biodegradation·rates increase as
  the temperature increases;

- When temperatures are higher than or lower than
  the optimum temperatures for growth, biodegradation
  rates are reduced as the temperature is raised or
  lowered.

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- Temperature can affect the lag time prior to biodegradative activity, and therefore, change the overall time requirement for biodegradation; and

- Temperature can affect biodegradation pathways, and therefore, change the rates and products of parent chemical degradation.

## Effect of Temperature Within The Physiologically Tolerated Range

Forward (1960) stated that as early as 1864 an empirical quantification had been attempted by De Fauconpret to correlate biochemical reactions with temperature within the temperature limits of growth. The correlation revealed that biochemical reactions were similar to chemical reactions; that is, reaction rate increases as temperature increases. Later, a quantitative relationship was established by Van't Hoff as the well-known "Van't Hoff Rule". Van't Hoff suggested that rates of bioactivity increase by a factor of 2 to 3 when temperature increases by 10°C. This ratio is called the "temperature coefficient", or "$Q_{10}$ value", as expressed by the following equation:

$$Q_{10} = \frac{k_{t+10}}{k_t} = 2 \text{ to } 3 \quad \text{------------------------- (1)}$$

where k is the reaction rate constant and t is the temperature in °C. Based on this relationship, the $Q_{10}$ value can be calculated for any temperature interval ($t_2$-$t_1$):

$$\log Q_{10} = \frac{10}{t_2 - t_1} \log \frac{k_2}{k_1} \quad \text{------------------------- (2)}$$

A generalized, and more complicated, expression quantifying the effects of temperature on biological systems within the temperature limits of growth was suggested by Arrhenius:

$$k = Ae^{-E/RT} \quad \text{------------------------------------- (3)}$$

where k is the reaction rate, E is activation energy, R is the gas constant, and T is absolute temperature. Biologists frequently substitute the symbol $\mu$ for E, and call $\mu$ the "temperature characteristic" (Forward, 1960).

Although the concepts of Van't Hoff's Rule and the Arrhenius' equation have been used extensively to express the temperature dependence of microbiological activities, especially the growth of microorganisms, only a few studies have applied such concepts to biodegradation of chemicals. In the following, examples of such studies are presented.

In a study concerning the biodegradation of glucose, acetate, and formate in agar, Ingraham et al. (1959) found that the $Q_{10}$ values range from 2.4 to 3.6 for mesophiles[*], and from 1.5 to

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[*] Mesophiles: Microorganisms that grow at temperatures between 20 and 50°C, with optimal growth between 35 and 42°C (Welker, 1976).

2.2 for psychrophiles.[*] Tiedje (1977) reported that the $Q_{10}$ value for the biodegradation of EDTA was approximately 2. Howard et al. (1979), in their study of the biodegradation of tree and shrub litter in soil, found that the mean $Q_{10}$ values ranged from 2.3 to 2.6. Helweg (1981) showed that for the biodegradation of maleic hydrazide in soils at from 1 to 30°C, the average $Q_{10}$ value was about 3.1. Toerien et al. (1982) found that for glucose in lake sediments at 10 to 36°C, the $Q_{10}$ values ranged from 1.5 to 1.6 and 2.4 to 2.5 for two types of sediment.

Most biodegradation studies have not derived $Q_{10}$ values. Table 1 was prepared from various reports for which $Q_{10}$ values could be calculated. In some cases $Q_{10}$ values had to be estimated because the essential experimental conditions were unknown or incompletely described. The calculated results (Table 1) show that $Q_{10}$ values for biodegradation of chemicals mostly fell in the range of 1.0 to 5.0. By examination of the $Q_{10}$ values, several trends in the temperature effects can be identified:

- Higher $Q_{10}$ values were found for mesophiles than psychrophiles, which suggests that the former microorganisms may be more strongly affected by temperature changes (data of Ingraham et al., 1959);

- Biodegradation of chemicals in relatively dry soils may be more drastically affected by temperature changes in comparison to that of wet soils (results of Yaron et al. (1974); and

- Higher temperatures (especially higher than room temperature) usually showed lower $Q_{10}$ values when compared to that of lower temperatures (Rose, 1976; Ward et al., 1976; Cserhati et al., 1977; and Helweg, 1979).

Attempts were made in some biodegradation studies to identify the applicability of the Arrhenius equation for quantitating temperature effects. A plot of log k against 1/T was generally used to study the suitability of the equation or the applicable temperature range of the equation. A straight line relationship of the log k against 1/T indicates an applicable situation, and the slope is equal to -E/2.303R (calculated from Equation (3)). Walker (1974), in his study of napropamide biodegradation in soil, found that in both laboratory and field conditions, biodegradation followed the Arrhenius equation, with activation energies (or temperature characteristics) of 7.80 and 7.85 kcal/mole for 7.5 and 10% soil moisture content, respectively. A linear plot was also observed for the biodegradation of linuron in soil in the temperature range of 10 to 26°C, with an activation energy of 13.3 kcal/mole (Cserhati et al., 1977). Helweg (1979) found

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[*] Psychrophiles: Microorganisms that grow optimally at temperatures below 20°C (Welker, 1976; Morita, 1976).

TABLE 1. TEMPERATURE COEFFICIENTS OF BIODEGRADATION[a]

| Chemicals | Medium | Temperature Range Studied (°C) | Other Environmental Conditions | Temperature Coefficient ($Q_{10}$) | Reference |
|-----------|--------|-------------------------------|-------------------------------|----------------------------------|-----------|
| Glucose | Nutrient agar and trypticase soy agar | 10 to 30 | (i) Mesophiles: Escherichia coli / Pseudomonas aeruginosa / (ii) Psychrophiles: Pseudomonas perolens / Pseudomonas sp. | ←3.0 to 3.4→[+] / ←2.4 to 3.6→ / ←1.5 to 1.9→ / ←1.7 to 2.2→ | Ingraham et al. (1959) |
| Acetate | Nutrient agar and trypticase soy agar | 10 to 30 | (i) Mesophile: Escherichia coli / (ii) Psychrophile: Pseudomonas sp. | ←2.8→ / ←1.9→ | Ingraham et al. (1959) |
| Formate | Nutrient agar and trypticase soy agar | 10 to 30 | (i) Mesophile: Pseudomonas aeruginosa / (ii) Psychrophile: Pseudomonas perolens | ←3.2→ / ←2.0→ | Ingraham et al. (1959) |
| Petroleum | Seawater | 5 to 20 | cultured for 60 days (i) fresh Sweden crude oil; 1% concentration; summer seawater. (ii) fresh Sweden crude oil; 1% concentration; winter seawater. (iii) weathered Sweden crude oil; 0.7% concentration winter seawater. | ←2.2→[+] / ←1.7→ / ←1.3→ | Atlas et al. (1972a) |

(Continued)

TABLE 1. (Continued)

| Chemicals | Medium | Temperature Range Studied (°C) | Other Environmental Conditions | Temperature Coefficient ($Q_{10}$) | Reference |
|---|---|---|---|---|---|
| Bunker C oil | Inorganic liquid medium | 5 to 28 | Mixed microbial cultures; 0.125% concentration | ◄——— 3.5 ———► (Range: 1.6 to 8.0 Medium: 3.5) | Mulkins-Philips et al. (1974) |
| Azinphosmethyl (pesticide) | Soil | 6 to 40 | (i) Dry soil (3% moisture content)  (ii) Wet soil (50% moisture content) | ◄— 2.8 —►  ◄—1.2——► | Yaron et al. (1974) |
| Napropamide (herbicide) | Soil | 14 to 28 | (i) 10% moisture content  (ii) 7.5% moisture content | ◄—1.5—►  ◄—1.4—► | Walker (1974) |
| Glucose | Glucose-basal salts broth | 5 to 30 | Organism· Pseudomonas fluorescens | ◄—1.7 —►◄—2.1—► | Lynch et al. (1975) |
| ·—— | —— | 15 to 45 | Organism: E. coli | ◄—4.2—►   ◄—1.04—► | Rose (1976) |
| Mineral oil | Lake water | 4 to 37 | (i) Summer water sample  (ii) Fall water sample  (iii) Winter water sample | ◄—2 1—►◄— 1.1—►  ◄— 2.9—►◄— 1.5—►  ◄—2.8—►◄— 1.6—► | Ward et al. (1976) |

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(Continued)

TABLE 1.   (Continued)

| Chemicals | Medium | Temperature Range Studied (°C) | Other Environmental Conditions | Temperature Coefficient ($Q_{10}$) 0  10  20  30  40  50°C | Reference |
|---|---|---|---|---|---|
| Hexadecane | Lake water | 4 to 37 | (i) Summer water sample<br>(ii) Fall water sample<br>(iii) Winter water sample | ←1.9→ ←1.6→<br>←1.5→ ←3.7→<br>←2.8→ ←1.1→ | Ward et al (1976) |
| Linuron | Soil | 10 to 37 | ---- | ←—2.1—→ ←0.9→ | Cserhati et al. (1977) |
| Tree and Shrub litters | Soil | --- | Field testing | Oak= 2.50<br>Ash= 2.56<br>Hazel= 2.29<br>Hawthorn= 2.31 | Howard et al. (1979) |
| 2-amino-benzimidazole | Soil | 1 to 40 | Initial concentration = 4 ppm. | ←—5.0—→ ←—1.0—0.2→ | Helweg (1979) |
| 1,2,3-tri-chlorobenzene | Soil | 12 to 28 | Initial concentration = 50 ppm. | ←—1.0→←—2.0→ | Marinucci et al. (1979) |
| N-chloro-alanine | Aqueous | 19 to 35 | Initial concentration = 7.1 x 10$^{-5}$M to 7.1 x 10$^{-2}$M   alanine | ←—4.6—→ | Stanbro et al. (1979) |
| O-chloro-phenol | Sediment | 0 to 20 | pH = 6.9<br>Moisture content = 95.5%<br>Organic matter = 24% (dry wt.)<br>Initial concentration = 100 ug/ml (slurry) | ←1.75—→ | Baker et al. (1980a) |
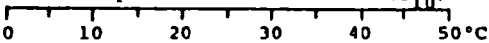
(Continued)

TABLE 1. (Continued)

| Chemicals | Medium | Temperature Range Studied (°C) | Other Environmental Conditions | Temperature Coefficient ($Q_{10}$) 0  10  20  30  40  50°C | Reference |
|---|---|---|---|---|---|
| m-chloro-phenol | Sediment | 0 to 20 | pH = 6.9 Moisture content = 95.5% Organic matter = 24% (dry wt.) Initial concentration = 100 μg/ml (slurry) | ←—2.0—→ | Baker et al. (1980a) |
| p-chlorophenol | Sediment | 0 to 20 | Same as above | ←—1.5—→ | Baker et al. (1980a) |
| 2,4-dichloro-phenol | Sediment | 0 to 20 | Same as above | ←—1.5—→ | Baker et al. (1980a) |
| Pentachloro-phenol | Sediment | 0 to 20 | Same as above | ←—1.0—→ | Baker et al (1980a) |
| P-chlorophenol | Stream water | 0 to 20 | pH = 7.1 Initial concentration = 100 μg/ml | ←—1.0—→ | Baker et al. (1980a) |
| 2,4-dichloro-phenol | Stream water | 0 to 20 | Same as above | ←—2.4—→ | Baker et al. (1980a) |

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(Continued)

TABLE 1. (Concluded)

| Chemicals | Medium | Temperature Range Studied (°C) | Other Environmental Conditions | Temperature Coefficient ($Q_{10}$) 0 10 20 30 40 50°C | Reference |
|---|---|---|---|---|---|
| Maleic hydrazide | Soils (3 types) | 1 to 30 | Moisture content = air dry to 2x field capacity. | ←——— 3.1 ———→ | Helweg (1981) |
| Glucose | Lake sediment | 10 to 36 | (i) Sediment #1 | ←1.5←—1.6→ | Toerien et al. (1982) |
| | | | (ii) Sediment #2 | ←2.5←—2.4→ | |

• Values estimated from the results of cited references

+ Applicable temperature range for the indicated $Q_{10}$ value

that the degradation of 2-aminobenzimidazole (2-AB) in soil was
in accordance with the Arrhenius equation within the temperature
interval of 1 to 20°C (Figure 1). Maximum biodegradation of 2-AB
was at 22°C, while between 25 and 35°C, the biodegradation re-
mained almost constant, and at 40°C it was almost nil (Figure 1).
Larson (1979) showed that the biodegradation of NTA and alkyl-
benzene sulfonate (LAS) could also be described by the Arrhenius
equation (Figure 2), with calculated activation energies of 14.5
and 9.1 kcal/mole for NTA and LAS, respectively. Maleic hydra-
zide biodegradation in soil also displayed a linear curve within
the range of 1 to 30°C, with an activation energy of about 18.6
kcal/mole (Helweg, 1981). A recent study conducted by Toerien
et al. (1982) showed glucose biodegradation in sediments with
activation energies in the range of 7.6 to 15.3 kcal/mole. The
calculated results of activation energies from other biodegrad-
ation studies are shown in Table 2.

As illustrated in Table 2, the activation energies for bio-
degradation of chemicals are in the range of 3 to 40 kcal/mole,
with the majority in the range of 10 to 20 kcal/mole. It is
indicated that temperature effects on biodegradation are more
pronounced for chemicals having higher activation energies.

Effects of Temperature Outside the Physiologically Tolerated
Range

Reduction in biodegradation rates can be found when the
growth temperatures are higher than, or lower than, the optimum
temperature range for growth (Forward, 1960; Farrell et al., 1967;
Welker, 1976; Rose, 1976). In petroleum biodegradation tests,
Ludzack et al. (1956) found that biodegradation of medium grade
motor oil was not measurable at 4°C, but that 20 to 30%, 30
to 50%, and 50 to 80% per week was biodegraded at 10, 20, and
25°C, respectively. ZoBell (1969) reported that the maximum
rates of crude oil biodegradation occurred between 25 and 37°C,
and below 10°C the biodegradation rate was markedly reduced.
Atlas et al. (1972a) also found that at low temperatures bio-
degradation rates were significantly lower. Figure 3 shows
that when the temperature was reduced to 5°C, the biodegradation
rate of fresh crude oil in seawater collected in late summer was
negligible. Mulkins-Phillips et al. (1974) also reported that
low temperature resulted in a reduced biodegradation rate for
Bunker C fuel oil. At 15°C, 41 to 85% of Bunker C disappeared
after 7 days, but at 5°C, only about 21 to 52% disappeared after
14 days of incubation. Similar trends of low temperature effects
were also observed by Ward et al. (1976) for hexadecane and min-
eral oil biodegradation. Figure 4 shows that when temperatures
were lower than the optimum range (20 to 25°C) biodegradation
rates were greatly reduced.

At high temperatures (higher than the optimum temperature),
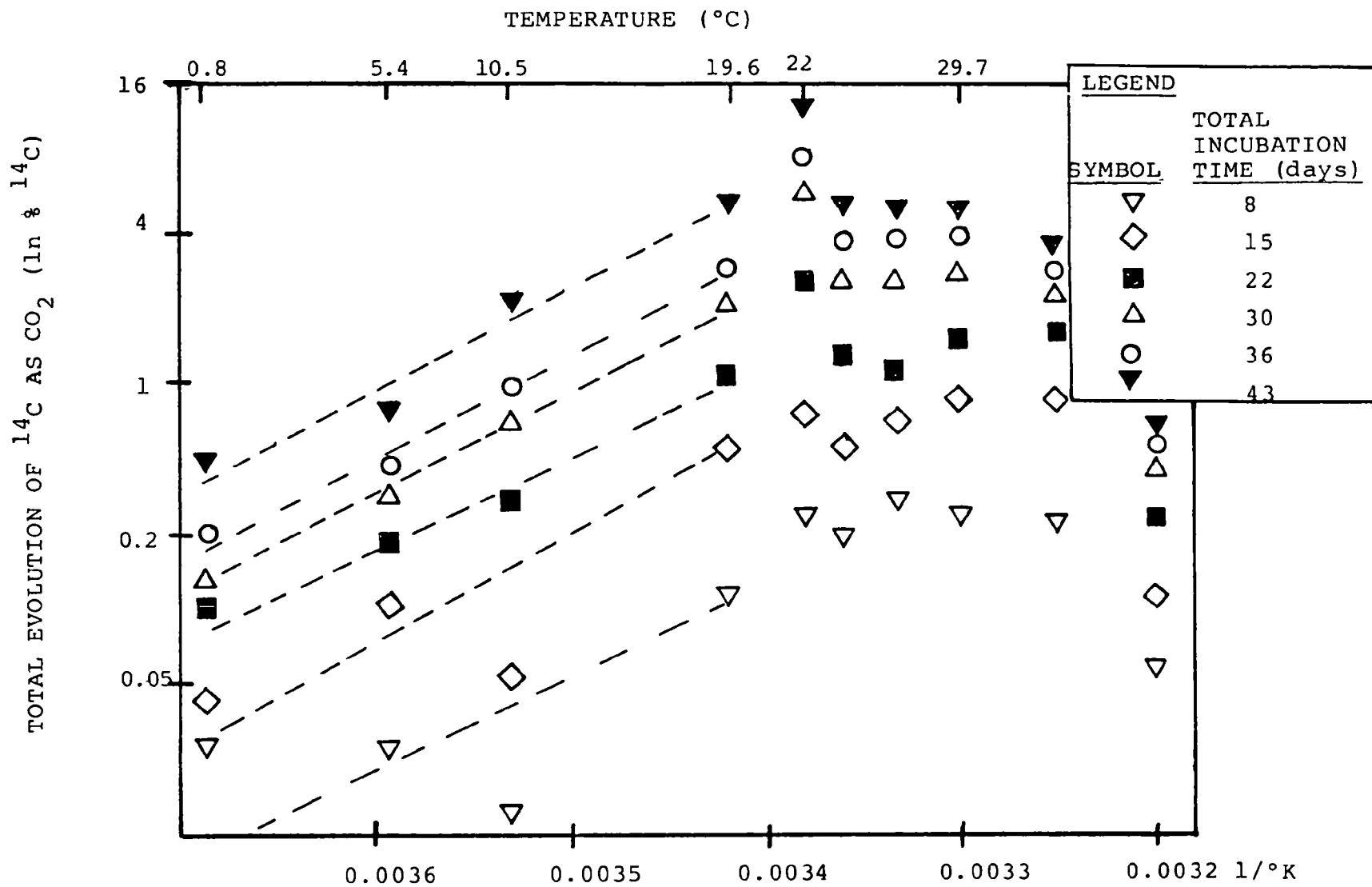
Figure 1. Arrhenius plots for biodegradation of 2-AB (Helweg, 1979).
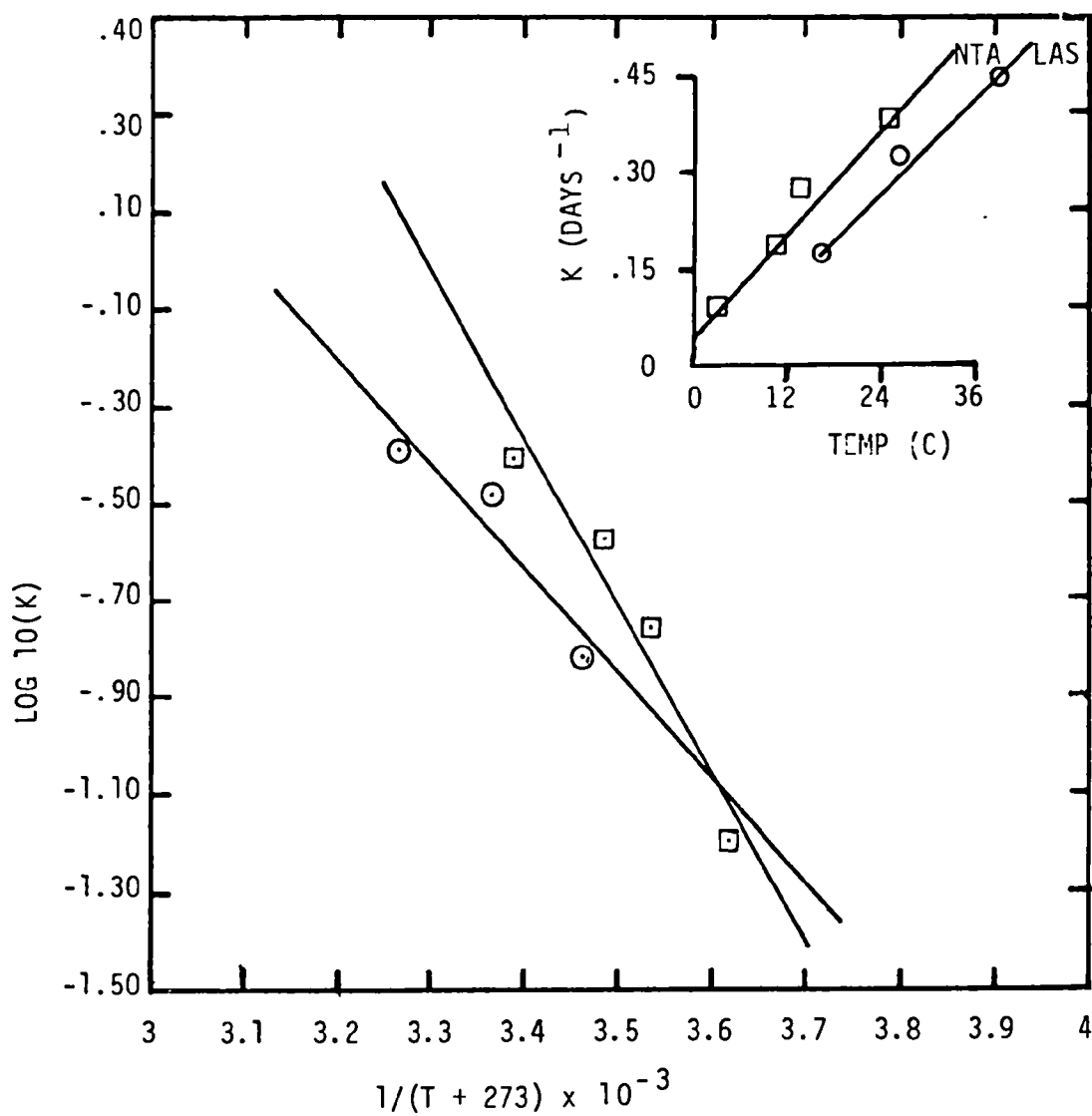
Figure 2. Arrhenius plots for biodegradation of NTA and LAS (Larson, 1979).

TABLE 2. ACTIVATION ENERGIES FOR BIODEGRADATION*

| Chemicals | Medium | Environmental Conditions/ Microorganisms | Linear Tempera- ture Range (°C) | Activation Energy (Kcal/mole) | Reference |
|---|---|---|---|---|---|
| Malate | Nutrient agar and trypticase soy agar | (i) Psychrophile: Pseudomonas sp. (Temperature tested: 5 to 35°C) | < 5[†] to 25 | 11.2 | Ingraham et al. (1959) |
| | | (ii) Mesophile: Escherichia coli (Temperature tested 5 to 35°C) | < 5 to 25 | 11.4 | |
| Isocitric Acid | Nutrient agar and trypticase soy agar | (i) Psychrophile: Pseudomonas sp. (Temperature tested: 3 to 35°C) | < 3 to > 35 | 11.4 | Ingraham et al. (1959) |
| | | (ii) Mesophile: Escherichia coli (Temperature tested: 3 to 35°C) | < 3 to > 35 | 10.5 | |

(Continued)

TABLE 2.    (Continued)

| Chemicals | Medium | Environmental Conditions/ Microorganisms | | Linear Tempera- ture Range (°C) | Activation Energy (Kcal/mole) | Reference |
|---|---|---|---|---|---|---|
| Glucose- 6-phos- phate | Nutrient agar and trypticase soy agar | (i) | Psychrophile: Pseudomonas sp. (Temperature tested: 5 to 40°C) | < 5 to > 40 | 11.4 | Ingraham et al. (1959) |
| | | (ii) | Mesophile: Escherichia Coli (Temperature tested: 5 to 40°C) | 15 to > 40 | 10.3 | |
| Glucose | Glucose- basal salts broth | | Pseudomonas fluorescens (Temperature tested: 3 to 33°C) | < 3 to 20 | 4.5 | Palumbo et al. (1969) |
| Azinphos- methyl | Soil | | 3% moisture content (Temperature tested: 6 to 40°C) | --- | 14.4 | Yaron et al. (1974) |

(Continued)

TABLE 2 (Continued)

| Chemicals | Medium | Environmental Conditions/ Microorganisms | Linear Temperature Range (°C) | Activation Energy (Kcal/mole) | Reference |
|---|---|---|---|---|---|
| Napropamide | Soil | (i) 10% moisture content (Temperature tested: 14 to 28°C) | --- | 7.85 | Walker (1974) |
| | | (ii) 7.5% moisture content (Temperature tested: 14 to 28°C) | --- | 7.80 | |
| Glucose | Glucose-basal salts broth | Pseudomonas fluorescens (Temperature tested: 5 to 30°C) | 5 - 20 | 2.9 | Lynch et al. (1975) |
| Mineral Oil | Winter lake water | Temperature tested: 4 to 37°C | 10 - > 37 | 14.0 | Ward et al. (1976) |
| Nitrite | --- | (i) pH = 6.5 (ii) pH = 7.3 (optimum) (iii) pH = 8.5 | --- | (i) 14.0 (ii) 6.6 (iii) 39.6 | Alexander (1977) |
| Ammonium | --- | (i) pH = 6 (ii) pH = 7.5 (optimum) (iii) pH = 8.5 | --- | (i) 19.8 (ii) 16.0 (iii) 20.0 | Alexander (1977) |
| Linuron | Soil | Temperature tested: 10 to 37°C | 10 - 26 | 13.3 | Cserhati et al. (1977) |
| EDTA | Soil | EDTA = 2.8 ppm (Temperature tested: 10 - 50°C ) | 10 - 30 | 15.5 | Tiedje (1977) |

(Continued)

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TABLE 2 (Continued)

| Chemicals | Medium | Environmental Conditions/ Microorganisms | Linear Tempera- ture Range (°C) | Activation Energy (Kcal/mole) | Reference |
|---|---|---|---|---|---|
| Benefin | (i) Ascalon soil (ii) Weld soil | Temperature tested: 15 - 30°C | --- | (i) 11.0 (ii) 13.1 | Zimdahl et al. (1977) |
| Trifluralin | (i) Ascalon soil (ii) Weld soil | Temperature tested: 15 - 30°C | --- | (i) 16.5 (ii) 14.9 | Zimdahl et al. (1977) |
| 2,6-dinitro- N-(3-pentyl) -$\alpha,\alpha,\alpha$- trifluoro-p- toluidine | (i) Ascalon soil (ii) Weld soil | Temperature tested: 15 - 30°C | --- | (i) 11.9 (ii) 10.9 | Zimdahl et al. (1977) |
| 2-amino- benzimidazole | Soil | Initial concentration = 4 ppm Temperature tested: 1 to 40°C | 1 - 20 | 19.7 | Helweg (1979) |
| NTA | Surface water | Temperature tested: 3 to 36°C | 0 - > 36 | 14.5 | Larson (1979) |
| LAS | Surface water | Temperature tested: 3 to 36°C | 0 - > 36 | 9.1 | Larson (1979) |

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TABLE 2 (Concluded)

| Chemicals | Medium | Environmental Conditions/ Microorganisms | Linear Tempera- ture Range (°C) | Activation Energy (Kcal/mole) | Reference |
|---|---|---|---|---|---|
| 2,4-D | River water | Temperature tested: 6 to 25°C | --- | 40.4 | Nesbitt et al. (1980) |
| Maleic hydrazide | Soils | Temperature tested: 1 to 30°C | --- | 18.6 | Helweg (1981) |
| Glucose | Lake sediments | (i) Sediment #1 (Temperature tested: 10 to 36°C) (ii) Sediment #2 (Temperature tested: 10 to 36°C) | --- | (i) 7.6 <br> (ii) 15.3 | Toerien et al. (1982) |

* Estimated from the results of cited references.

⊦ "<" or ">" indicates the linear temperature range may exceed the range as shown.

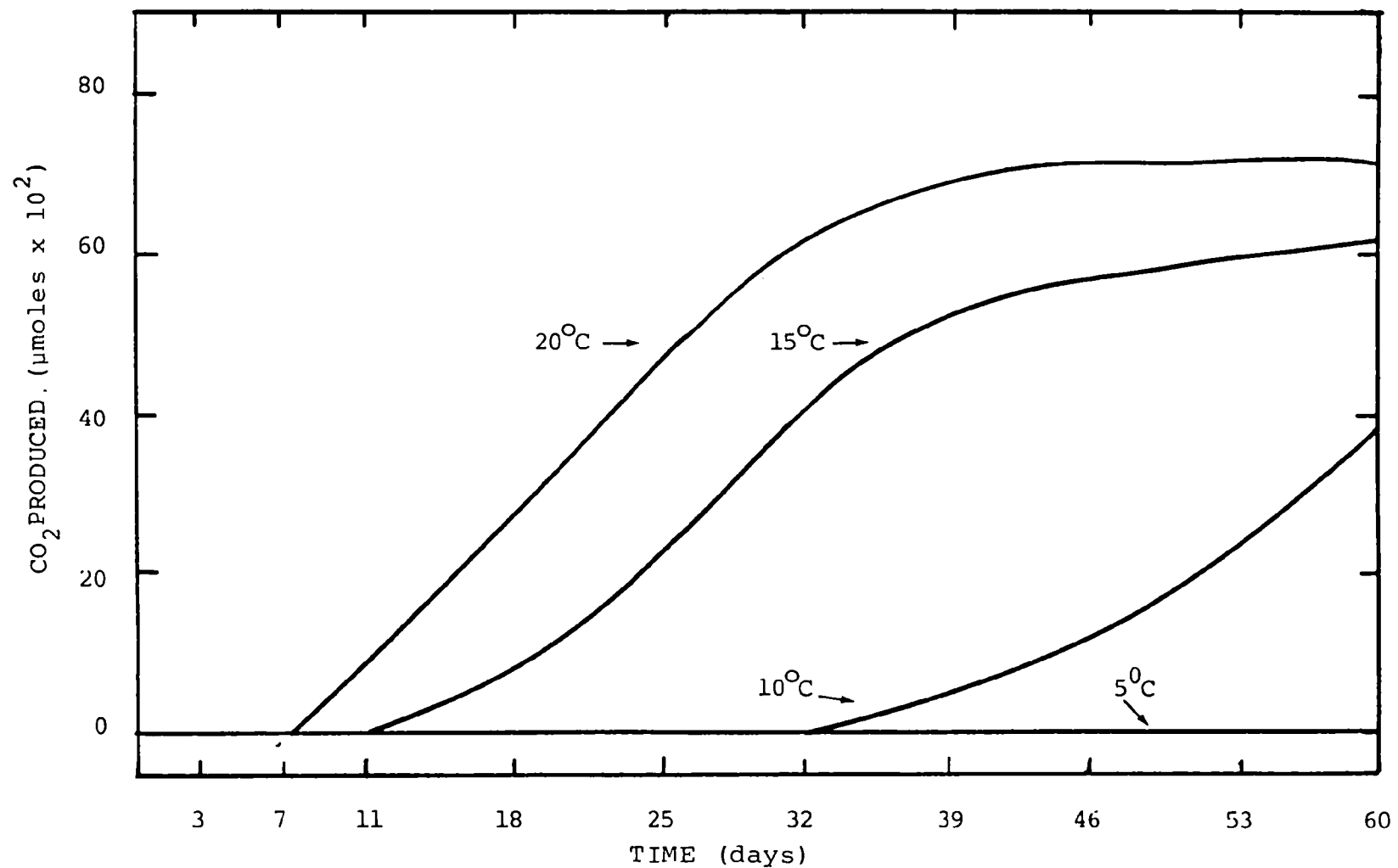# Data not provided or not tested by the cited references.

Figure 3.   Mineralization at various temperatures of 1% (v/v) fresh Sweden crude oil in seawater collected in late summer (Atlas _et al._, 1972a).
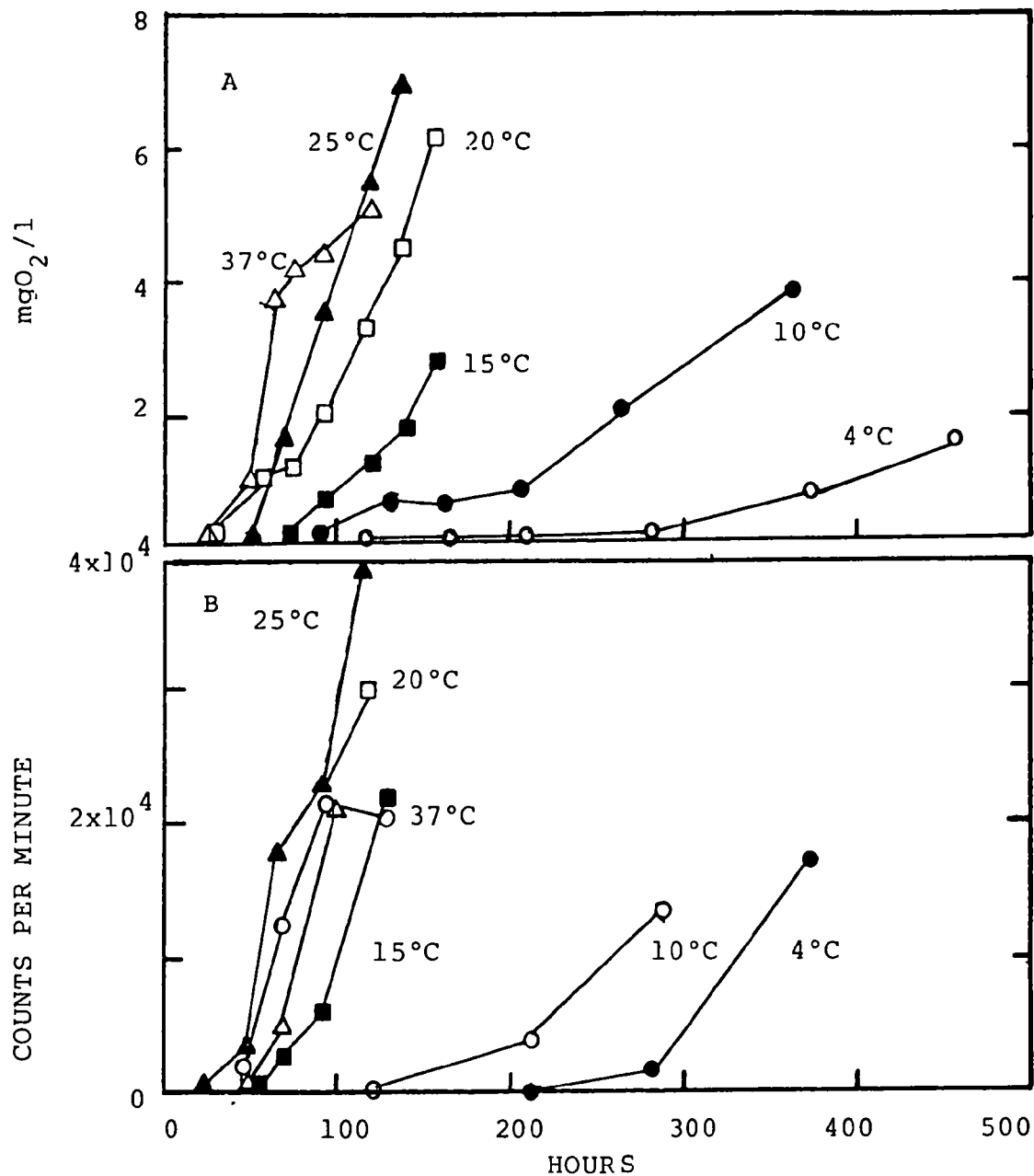
Figure 4 . Effect of temperature on (A) oxygen uptake during mineral oil oxidation and (B) $^{14}CO_2$ production during hexadecane oxidation by Lake Mendota surface water samples (Ward et al., 1976).

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reduction in biodegradation rates was also reported by many re-
searchers. Data as shown in Figure 4 indicate that at 37°C
rates of mineral oil and hexadecane biodegradation were greatly
depressed as compared to the optimum temperature range (20 to 25°C)
(Ward et al., 1976). Cserhati et al. (1977) in their linuron bio-
degradation tests also observed a decline in biodegradation rates
when temperature increased from 26 to 37°C. In another typical
example, shown in Figure 1, biodegradation rates for 2-AB were
reduced when temperatures were higher than about 20°C (Helweg, 1979).

The reduction of biodegradation rates as observed in the low
and high temperature regions can also be seen from the Arrhenius
plots. Figure 5 shows a typical Arrhenius plot for growth of P.
flourescens on glucose. In the high (higher than 30°C) and low
(lower than 5°C) temperature regions, the reduction of biodegrada-
tion rates can easily be observed. This effect is usually explained
by the denaturation of cell proteins in the high temperature region
and inhibition of enzyme activity in the low temperature region
(Forward, 1960; Rose, 1967; Farrell, 1967; Rose, 1976; Welker, 1976;
and Stanier et al., 1976).

## Other Temperature Effects

Temperature also may indirectly affect biodegradation rates
through its effect on lag time and pathways of biodegradation.
Lag time is usually considered to be a reflection of the need for
acclimation of a degrading population, including enzymatic adapta-
tion to degrade the chemical. In recent studies, lag time was
found to be correlated with temperature. For example, Atlas et al.
(1972a) reported that low temperature could cause lag periods to
increase (see Figure 3 as shown previously). The lower the tempera-
ture, the longer the lag period. Such phenomena have also been
observed by other researchers, such as Mulkins-Phillips et al. (1974),
Yaron et al. (1974), and Ward et al. (1976). Atlas et al. (1972a)
and Yaron et al. (1974) further reported that such lag periods were
found to be directly proportional to temperature, as can be seen in
Figure 6.

Temperature effects on biodegradation pathways were reported
by Palumbo et al. (1969) and Lynch et al. (1975). Such effects
were believed to indirectly affect the biodegradation rates of the
parent chemical. Lynch et al. (1975) reported that at low growth
temperatures (0 to 5°C), 2-ketogluconate (2-KG) was the major
biodegradation product from glucose (up to 70%). When the growth
temperature raised to 20°C, only 25% of glucose was recovered as
2-KG. At the optimum temperature (30°C) or above, no 2-KG was
detected at any time during the tests. Lynch et al. (1975)
suggested that at low temperature, the major route for biodegrada-
tion of glucose was the direct oxidative non-phosphorylated pathway.

Other indirect temperature effects on biodegradation rates,
such as effects on limiting biodegradation concentration, effects
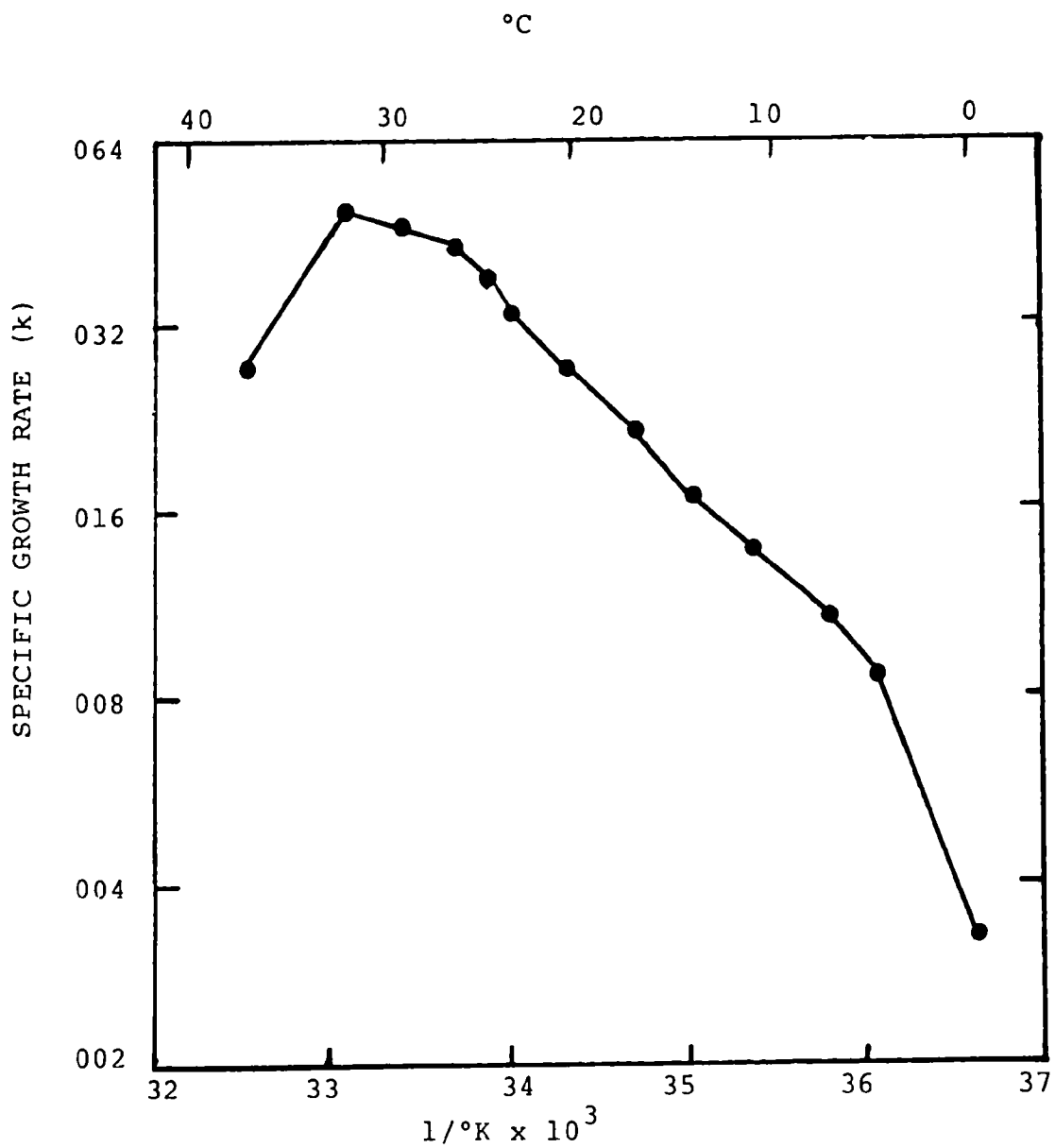
Figure 5. Arrhenius plot of the specific growth rate of P. fluorescens on glucose (Lynch et al., 1975).
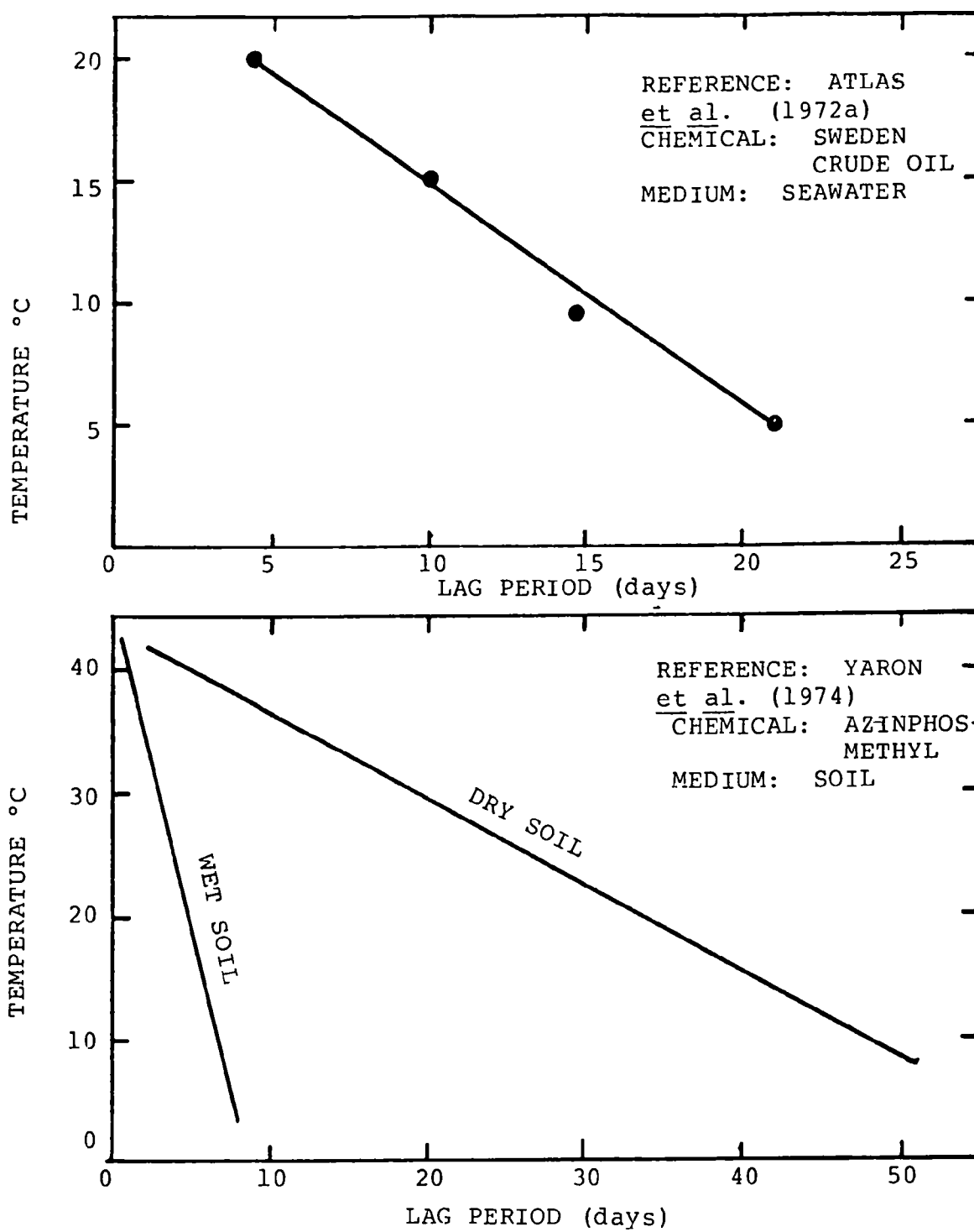
Figure 6.   The effects of temperature on the "lag period" for biodegradation.

on acclimation of organisms in different media, effects on other essential environmental factors such as pH, chemical speciation, etc. were also suggested in the literature. But quantitative evaluations are still lacking. Temperature is one of the difficult factors to evaluate as a determinant of biodegradation rates of chemicals because of its association with the kinetics of all physical and chemical reactions. There are possible a myriad of indirect effects of temperature on biodegradation. It does not seem to be fruitful with the present state of knowledge to attempt precise correlation of temperature with biodegradation under a multitude of environmental conditions.

CONCENTRATION

Effects of chemical concentration on biodegradation rates have long been known to microbiologists. The first work describing such relationships in a mathematic model was performed by Michaelis and Menten, as early as 1913, in their enzyme reaction study (Michaelis et al., 1913). The concentration factor can be affected by physical mechanisms such as mixing, diffusion, dilution, and other physical-chemical factors affecting the mobility of the chemical (e.g., sorption, complexation, solubilization, etc.). In this subsection, only those factors deemed physical in nature will be discussed. Since concentration is the direct factor affecting biodegradation, the concentration effects will be discussed before the description of the physical variables influencing concentration.

## Effects of Concentration

It is generally true that the biodegradation rate (K) increases as substrate concentration (S) increases. Such a relationship holds until a certain maximum biodegradation rate ($K_o$) is reached, as shown in Figure 7 (Sawyer et al., 1978; Sundstrom et al., 1979). As illustrated in Figure 7, when $S \gg K_m$ (where $K_m$ = the substrate concentration at which the reaction rate is one-half of maximum), the reaction rate is a maximum and independent of the substrate concentration--the reaction is zero-order. When $S \ll K_m$, the reaction rate becomes first-order with regard to concentration.

In recent years, results of research have emphasized the importance of chemical concentration, especially trace concentrations of xenobiotic chemicals, on biodegradation. DiGeronimo et al. (1979), in studying the effect of concentration of p-chlorobenzoate, dimethylamine and 2,4-D on their biodegradation, found that 2,4-D may be more persistent at very low than at higher concentrations. They further pointed out that many organic pollutants could persist in aquatic ecosystems owing in part to their low prevailing concentration. In a study of the biodegradation of phenol in river water, Borighem et al. (1978) were able to distinguish three different phases in the degradation curve (Figure 8): an induction period, a linear decrease in the phenol concentration as a function of time, followed by a slow decrease to zero

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The figure shows a graph with axis labels:
- Vertical axis: SUBSTRATE UTILIZATION RATE (K), with marks $K_0$ and $\dfrac{K_0}{2}$
- Horizontal axis: SUBSTRATE CONCENTRATION (S), with mark $K_m$

MAXIMUM RATE

WHERE $K_m$ = SUBSTRATE CONCENTRATION AT WHICH THE REACTION RATE IS ONE-HALF OF MAXIMUM.
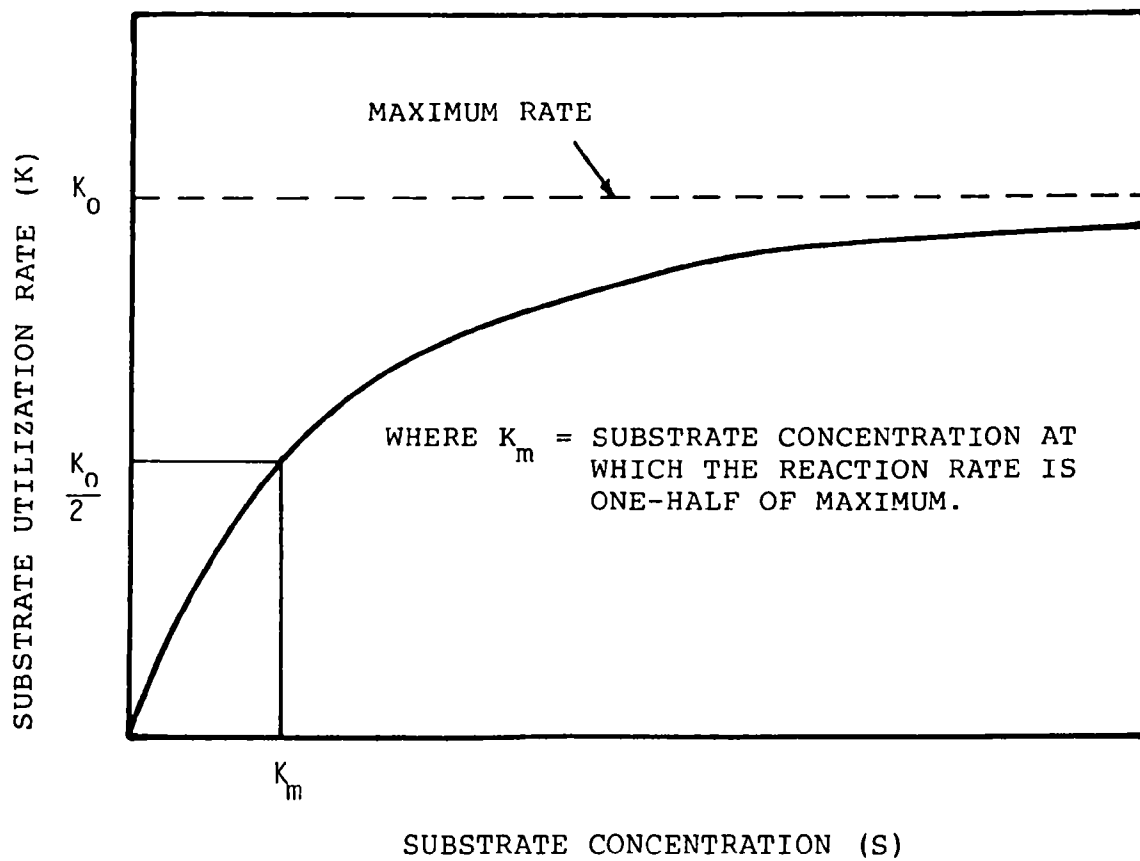
Figure 7. The relationship between substrate concentration and substrate utilization rate.

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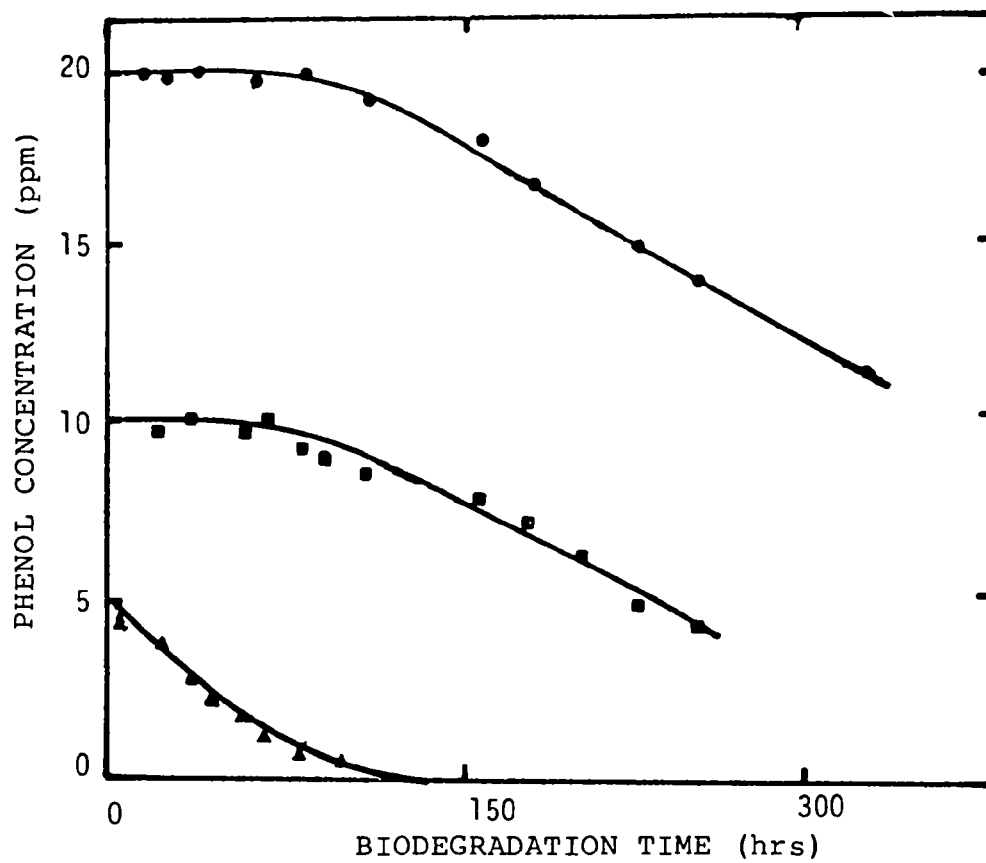
FIGURE 8.  Biodegradation of phenol as a function of time and
concentration (Borighem et al., 1978).

concentration. With increasing phenol concentration, the induction period and the time for complete degradation also increased.

Mason et al. (1979) found a positive correlation between the concentrations of chemicals and their biodegradation rates. They showed that the initial rate of demethylation of methylmercury followed a first order relationship at each pH to a methylmercury concentration of at least 2 mg/l.

The positive correlation between a chemical's concentration and its biodegradation rate is further verified by Boethling and Alexander (1979a and 1979b). In their studies on biodegradation rates of glucose, dimethylamine, diethylamine, diethanolamine, p-chlorobenzoate, chloroacetate, 2,4-D, and 1-naphthyl N-methyl-carbamate at various concentrations, two important findings were obtained for some of the chemicals studied:

(1) The biodegradation rate may be directly proportional to chemical concentration at the trace level. A decrease of approximately one order of magnitude in biodegradation rate was observed for each successive decrease in one order of magnitude in the initial chemical concentration.

(2) Little or no biodegradation may occur at low chemical concentrations, and a threshold would exist below which no significant biodegradation occurs. The existence of a threshold is not predicted by Michaelis-Menten kinetics.

When the concentration of chemicals increases, according to the Michaelis-Menten model (illustrated in Figure 7), the biological reaction rate approaches a maximum and thereafter exhibits no correlation with concentration. However, many biological reactions are susceptible to inhibitors which can affect biochemical activity within the cell (Sundstrom et al., 1979). Sundstrom et al., (1979) showed that whenever inhibition is present, the biodegradation rate decreases. More discussion on substrate inhibition is provided in Section 6, "Biodegradation Algorithms".

From the above discussion, it can be concluded that:

● Biodegradation rates are positively correlated with the substrate concentration when the concentration is within a certain range;

● Little or no biodegradation may occur for certain substrates at very low concentrations, and a threshold may exist below which no significant biodegradation occurs.

● When substrate concentrations are higher than the range mentioned above, substrate inhibition effects may cause a lowering of biodegradation rates.

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## Physical Effects On Concentration

Physical effects can influence concentrations of chemicals in many ways. Dilution (e.g., discharge of xenobiotics into a stream) could result in a reduction of chemical concentration and, therefore, influence the biodegradation rate of that chemical. Diffusion and dispersion may increase or decrease chemical concentration and, as a result, accelerate or hinder the biodegradation process (Kozak et al., 1979; Gerstl et al., 1979; and Parker, 1979). Mixing may increase the opportunity for contact between microorganisms and chemicals (Lo et al., 1978), especially for the biodegradation of chemicals having very low concentrations.

Dilution is especially important in the biodegradation of chemicals in surface waters. Diffusion and dispersion effects on biodegradation may occur in both soil and aqueous environments. In marine environments, for example, diffusion of oil will greatly enhance its biodegradation rate (Gerstle et al., 1979). Diffusion of a substance in soil may also accelerate or hinder the biodegradation process. Kozak et al. (1979) reported that a material may diffuse from one soil setting to another (such as clay), providing a more susceptible setting for degradation. Movement of the substance into an air-water, air-soil, or water-soil interface, where the material is usually concentrated, desorbed or adsorbed can also affect transformation rates (Parker, 1979).

Mixing usually improves the rate of biodegradation. Swilley et al. (1964) investigated the influence of mixing on substrate biodegradation and obtained a higher reaction rate under stirred conditions. Theoretical studies on the effects of micromixing and macromixing on microbial growth have been conducted by several workers (e.g., Tsai et al., 1969; Fan et al., 1970 and 1971; Dohan et al., 1973; and Lo et al., 1978). Their results can be summarized as follows:

> In the case of pure cultures of non-aggregatable microorganisms, good mixing can prevent individual cells from settling and increase the frequency of contact of limiting substrate with active sites of cells. In the case of a pure culture consisting of dispersed and flocculated microorganisms, the overall limiting substrate removal rate would depend upon the distribution of dispersed and flocculated microorganisms in the culture; thus, it should be a function of degree of mixing because the flocculation-deflocculation process was found experimentally to vary with agitation rate when other environmental factors were kept constant. The specific surface of a particle has been defined as its surface area divided by its mass. Thus, specific surface is larger for dispersed microorganisms, giving more active sites per unit of biomass. As a rule, the larger the number of active sites per unit of biomass, the greater will be the amount of

limiting substrate removed. In the case of mixed culture systems, where aggregatable and non-aggregatable micro-organisms of different species exist, good or strong mixing prevents both kinds of microorganisms from settling and provides more contact between limiting substrate and cells and flocs per unit time per unit of biomass. Each species has its own particular shape and average size and weight. Species of aggregatable microorganisms flocculate or deflocculate under the influence of mixing and other environmental factors. Consequently, the limiting substrate removal rate for a given biomass concentration in a mixed culture system should depend not only on the degree of mixing but also on the aggregation state and particle (cell and floc) size of different species. When other controlling or limiting factors are assumed to be constant, it can be reasoned from the above discussions that the common factor to control removal rate in the above mentioned three cases is the frequency of contact of microorganisms with limiting substrate per unit of biomass.

Literature search also revealed that in certain cases mixing showed no effects on biodegradation rates. For example, Ward et al. (1976) found that incubation of samples on a rotary shaker to promote mixing did not influence the kinetics of hydrocarbon biodegradation.

SORPTION

Considerable research has been conducted regarding the effects of bacterial and substrate sorption in influencing rates of biodegradation in various soils. Beyond terrestrial ecosystems, however, biodegradation studies have generally not considered the effects of sorption on biodegradation.

Sorption can be defined as both the taking up of one substance at the surface of another (adsorption) and the penetration of one substance into the body of another (absorption). Because it is often difficult to distinguish between adsorption and absorption processes, they are commonly grouped under a single heading—sorption.

Sorption can be viewed as both a physical and chemical phenomenon, involving both weak attractive forces (van der Waal's forces) and stronger ionic or molecular attraction. The availability of organic substrates to soil microorganisms may be enhanced or reduced through sorption by particulate matter (Marshall, 1976). For substrates that are sorbed, the availability will depend on the substrate location relative to that of the degrading microorganisms, or whether extracellular enzymes are involved and whether these are sorbed, and on the configuration and arrangement of the substrates and enzymes in the sorbed state. Some contradictory results have

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been reported on the metabolism of specific organic substrates in different soils, but these probably reflect the complexity of soils as microbial systems. Several significant studies are mentioned in the following discussion.

The sorption of maleic hydrazide by clay and organic material and its influence on substrate biodegradation was studied by Helweg (1981). It was found that the rate of decomposition of maleic hydrazide, at varying temperatures and moisture content, was lowest in soil with the highest content of organic matter and the lowest clay content. Helweg suggested that adsorption of maleic hydrazide by organic matter reduced the biodegradation rate, but that the clay content of soil may play an important role (i.e., the degradation rate may be higher in soil with a higher clay content). Evidence suggests that the metabolic products of the biodegradation of certain pesticides can bind to soil·humic substances and increase the chances of their persistence (Katan et al., 1976; Hsu et al., 1976). An assessment of the influence of sorption by soil organic matter on herbicide stability was made by Hance (1974). In contrast to the results of the above-mentioned studies, it was found that the breakdown rates of atrazine and linuron were not related to their extent of organic adsorption, rather that herbicide degradation occurred incidentally to the general metabolism of organic material in the soil. Gibson and Burns (1977) also demonstrated that colloidal organic matter itself, or the fraction associated with it, is the most important single factor concerned with the rapid breakdown of malathion in the soil studied. The persistence of malathion in soil and soil fractions is illustrated in Figure 9. The breakdown of malathion in nonsterile soil components is contrasted with autoclaved fractions in which no breakdown occurred.

Soil clays appear to play an important role in influencing biodegradation. Researchers including Stotzky (1966a and b) and Stotzky and Rem (1966, 1967) reported that some clays (e.g., kaolinite) exerted little influence on bacterial respiration with glucose as substrate, whereas other clays (e.g., montmorillonite) stimulated respiration. Montmorillonite also stimulated microbial degradation of aldehydes, but kaolinite did not (Kunc and Stotzky, 1970). Conversely, $^{14}$C-diquat sorbed by kaolinite was readily degraded by the soil microflora, while decomposition of the same herbicide sorbed by montmorillonite was almost completely inhibited (Weber and Coble, 1968). Specific sorption mechanisms influencing bacterial respiration and substrate biodegradation, and mechanisms controlling the degradation of sorbed chemicals, are described below.

Evidence suggests that one mechanism by which clay minerals stimulate bacterial respiration is by maintaining a pH suitable for growth (Stotzky and Rem, 1966; Stotzky, 1966a). This mechanism is dependent on the initial pH of the system and the buffering capacity of the particles. When the initial pH was sufficiently high the bacterium was able to initiate and maintain growth until the accumulation of acidic metabolites reduced the pH to an
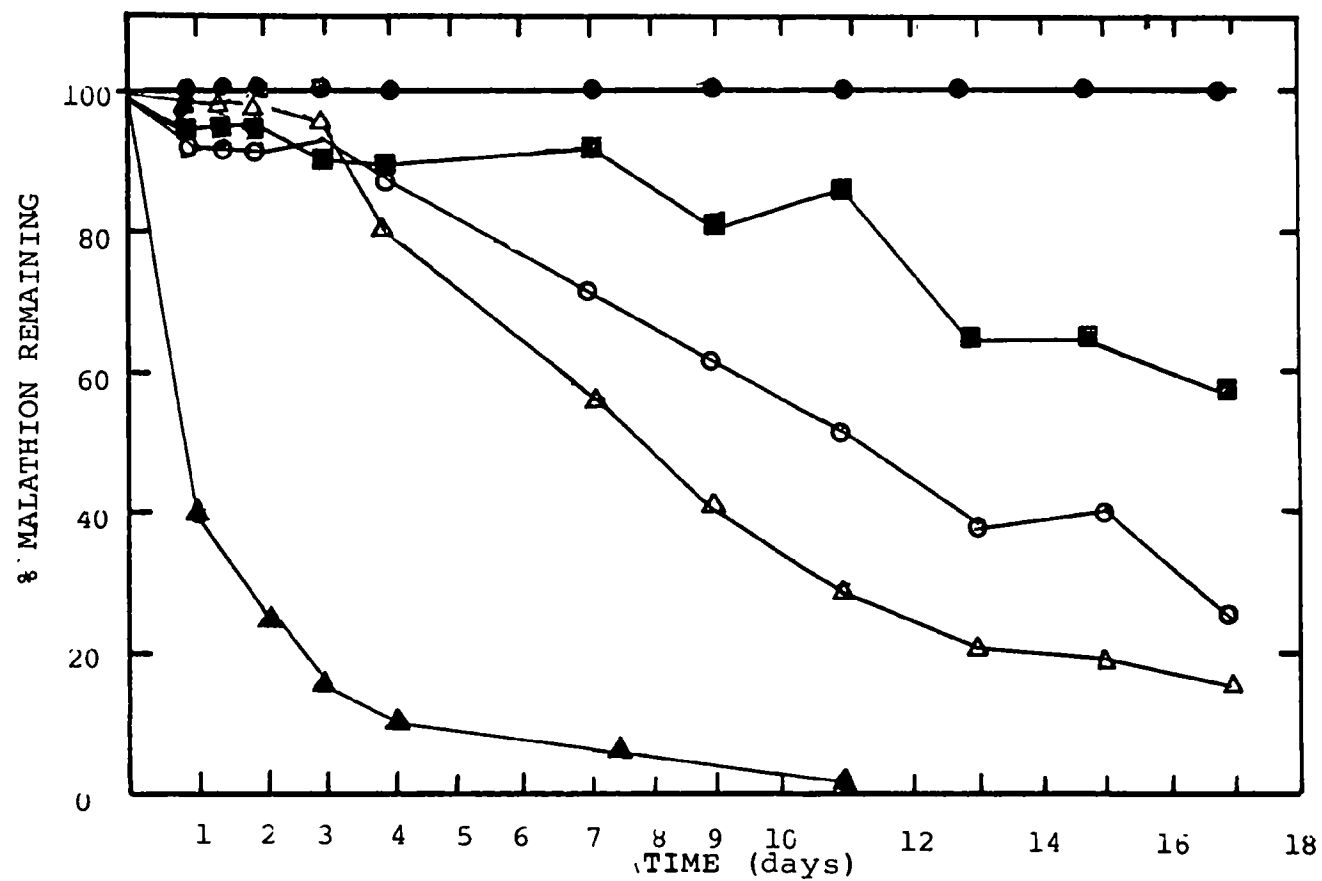
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Figure 9. Malathion breakdown in sedimented and centrifuged soil fractions. ● , all four
autoclaved; ■ , clay; △ , silt; O , sand; ▲ , organo-mineral complex (Gibson and Burns, 1977).

inhibitory level (Stotzky, 1966a). When the particle was also cap-
able of neutralizing these metabolites, thereby maintaining a pH
adequate for growth, the bacterium continued to metabolize until
the buffering capacity was exhausted.

The cation exchange capacity (CEC) of soil is also found to
be correlated with substrate decomposition rates. Novakova (1972a
and b) reported that montmorillonitic clays with saturating cations
Na and Ca stimulated glucose decomposition by a mixed soil micro-
organism culture, but that these forms of kaolinite were somewhat
inhibitory. Using samples of montmorillonite made homoionic to
a range of cations, Stotzky (1966a) reported rates of bacterial
respiration with saturating cations to be in the order Na>Ca>Mg>K>H.
This order reflects the relative basicity of the cations (except
for potassium), rather than their ease of replacement from the
exchange complex, or the ability to displace other cations from
the complex. The correlation of bacterial respiration and cation
exchange is illustrated in Figure 10. The establishment of cation
exchange capacity as a dominant factor in bacterial respiration is
consistent with aforementioned pH effects, presumably because clay
minerals with high cation exchange capacity are capable of exchang-
ing more $H^+$ ions produced during metabolism than those with a lower
cation exchange capacity, thus maintaining the requisite pH of the
ambient solution for a longer time (Stotzky, 1966b). Although no
biodegradation rates were mentioned in the above discussion, due
to the close relationship between bacterial respiration and bio-
degradation, the similar effects of CEC on biodegradation also can
be expected.

Protection of a substrate from decomposition is often associa-
ted with sorption within the inner lattice of expanding lattice
clays. Weber and Coble (1968) demonstrated that the decomposition
rate of the cationic herbicide diquat was significantly reduced
when associated with montmorillonite clay, presumably because
sorption of the diquat in interlayer spaces rendered it inaccessible
to microorganisms. Kaolinite clay, however, did not inhibit de-
gradation. Burns and Audus (1970) observed similar degradation
rate inhibition from montmorillonite for the herbicide paraquat.
Wszolek and Alexander (1979) found that an increased resistance
to microbial attack was provided to n-alkylamines sorbed to
montmorillonite, and that higher resistance was found for amines
with higher molecular weights (Figure 11). The researchers also
concluded that the rate of substrate desorption does not limit the
rate of biodegradation, and that microrganisms may facilitate re-
moval of substrate from clay surfaces by production of extracellular
enzymes.

The biodegradation of herbicides in soil has been described
using first order kinetics (Hamaker, 1972). Zimdahl and Gwynn
(1977), in their degradation studies of herbicides, found that all
herbicides studied degraded more rapidly in loamy soil than in sandy
soil. They speculated that it may have been due to greater microbial
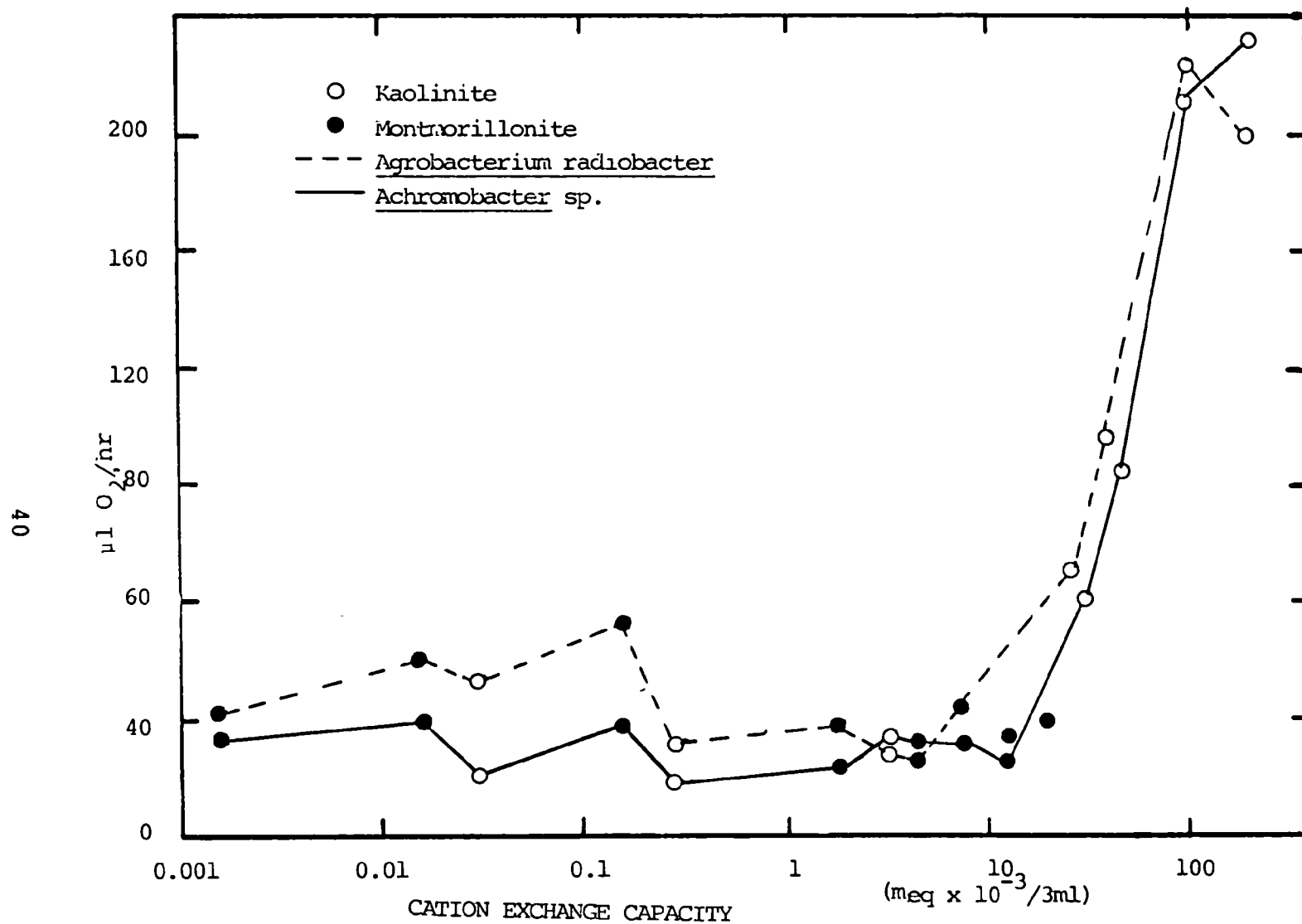activity in the loamy soil. Loamy soil contains higher clay and

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Figure 10. Effect of cation exchange capacity on respiration of Agrobacterium radiobacter and Achromobacter sp. (Stozky, 1966b)
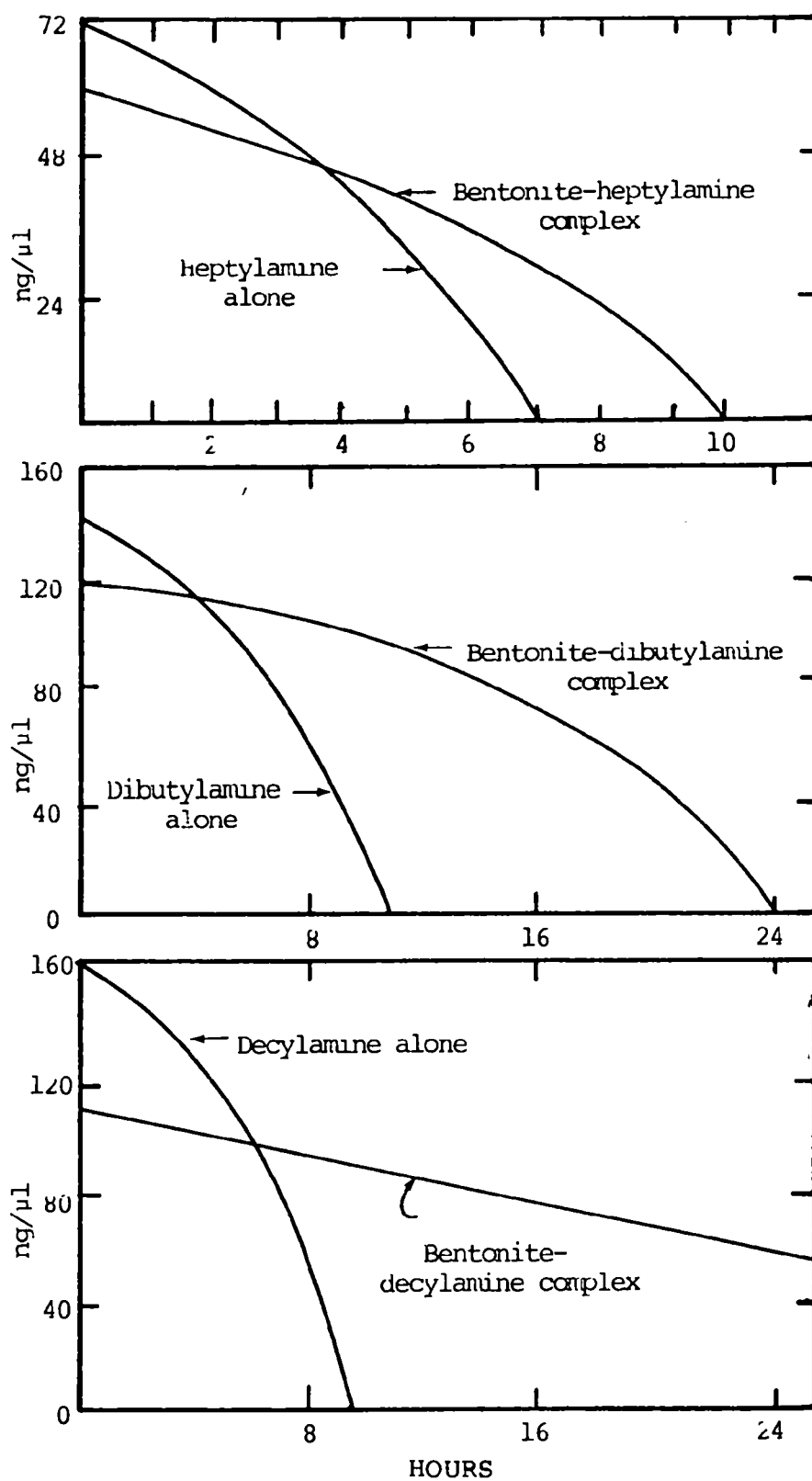
Figure 11. Degradation of bentonite-n-alkylamines and n-alkylamines free in an inorganic salts solution (Wszolek and Alexander, 1979)

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organic contents than sandy soil. Degradation that is catalyzed by adsorption on clay or organic matter may be expressed by (Zimdahl and Gwynn, 1977):

$$- \frac{d[H]}{dt} = k \; [H] \; [clay] \; [OM] \text{--------------------(4)}$$

where [H] and [OM] represent the concentration of herbicide and organic matter, respectively. Such a reaction would be third order, but for a given medium the concentration of clay and organic matter remain constant and only the herbicide concentration is time-dependent, the reaction appears to be first order.

Enzymes may play an important role in the biodegradation of sorbed organic chemicals. Early work by McLaren (1963) showed that proteolytic enzymes may be adsorbed on clay and hydrolyze adsorbed proteins. Studies have shown that not only protein adsorbed on the outside surfaces of clay particles but also protein present in the interlayer space is utilized by micro-organisms, suggesting that extracellular proteolytic enzymes have access to the interlayer space (Estermann et al., 1959). Studies have also shown that the types of clay involved in sorption can have a drastic influence on enzyme activity: kaolinite clays inhibited protease activity to a much greater extent than mont-morillonite clays (Aomine et al., 1964). Interestingly, the adsorption of enzymes has been reported to be as much as 100 times greater (per unit weight) on montmorillonite than on kaolinite (Haska, 1975), suggesting that the activity of sorbed enzymes may be enhanced or inhibited as a result of selective adsorption. Immobilized enzymes have, however, been observed to obey Michaelis-Menten kinetics (Makboul et al., 1979). Attachment of such enzymes to solid interfaces may affect their affinity towards the substrate. Makboul et al. (1979) observed that at each substrate (p-nitrophenyl phosphate, PNP) level tested, the addition of increasing amounts of clay decreased the activity and increased the $k_m$ values (the higher the $k_m$, the lower the affinity for a given substrate, according to Michaelis-Menten kinetics). The authors found that the affinity of sorbed acid phosphatases for PNP was significantly lower with montmorillonite than with kaolinite or illite.

It should be noted that a major limitation in evaluating the results of the above-mentioned studies is that only one chemical was applied to a soil at a time. Combinations or mixtures of chemicals might be a common situation in nature. The behavior of a chemical mixture may or may not be independent or additive, but rather based upon the influence of one chemical and/or the formulation associated with a given chemical.

In review, the availability of organic substrates to soil microorganisms may be either enhanced or reduced when sorbed to soil organics and minerals. Clay minerals with high cation ex-change capacities can enhance biodegradation by maintaining the suitable pH for growth. Evidence has indicated that chemicals

may be protected from biodegradation once bound to the inner lattice of clay soils (e.g., montmorillonite) or when strongly bound to humic substances. Enzymes also play a significant role in governing the biodegradation rates of sorbed chemicals. Because of conflicting evidence regarding effects of sorption on biodegradation of organic substrates, and because of the heterogeneous and complex nature of soil systems, much more data are needed to develop empirical relationships between the effects of sorption and biodegradation.

## HYDROSTATIC PRESSURE

Many microorganisms thrive and multiply in environments, such as soils, that are at atmospheric pressure. But there are large numbers of microorganisms living below the surface of a water mass that are subjected to hydrostatic pressures greater than one atmosphere. In the aqueous environments, pressure increases at a rate of about one atm for every 10 m depth. Over half of the earth's surface is covered with water at depths of 3800 m or more, which create 380 atm or more of hydrostatic pressure. The greatest hydrostatic pressures known for the ocean floor and freshwater lakes are about 1160 and 164 atm, respectively.

There is abundant evidence that biodegradation of chemicals can occur on the ocean floor, in deep oil well brines, and in other habitats characterized by high pressure (ZoBell et al., 1949; Rose, 1976; and Morita, 1976). No clear understanding, however, has been reached concerning the effects of pressure on biodegradation rates of chemicals.

In studies of the effects of pressure on terrestrial and marine bacteria, ZoBell et al. (1949) concluded that at temperatures below the normal optimum, a pressure of 500 atm greatly retarded the biochemical reactions. This is because biodegradation reactions proceed with a volume increase, but high pressure tends to reduce the overall volume of the reactants and products, and, therefore, results in a retardation of the reactions. At higher temperatures, and most noticeably above the optimum, the critical enzyme undergoes a reversible denaturation that proceeds with an even larger increase in volume of reaction. At these temperatures, the net effect of pressure is to increase the rate of the reaction by reversing the denaturation of the enzyme to a greater extent than the opposing catalytic reaction. Therefore, ZoBell et al. (1949) suggested that the effects of pressure are likely, in all cases, to depend upon temperature, and a complete picture is obtainable only after exhaustive studies of the reciprocal relationships of both factors. The above-mentioned temperature-pressure relationship was also reported by Brown et al. (1942), Strehler et al. (1954), and ZoBell (1970).

These authors suggested that increased hydrostatic pressure may exert an effect on the cells, raising the minimal growth temperature. They hypothesized that in an environment of low temperature, an increasing pressure will eliminate growth

and biochemical activity of bacteria, as their minimal growth temperature are shifted toward, and ultimately surpass, the environmental temperature.

A more complete description of the co-effects of temperature and pressure on biodegradation was reported by Morita (1976). When the temperature was kept constant for psychrophiles, the pressure increase could result in a decrease in the biodegradation rate. However, when the pressure was kept constant, a temperature increase could enhance the biodegradation rate. These relationships are shown in Figure 12. As shown by this figure, in order to maintain the same rate of substrate uptake, an increase in hydrostatic pressure should be accompanied by an increase in temperature.

Morita (1957), in a study of the inactivation of various dehydrogenases of the tricarboxylic acid (TCA) cycle, reported that increased hydrostatic pressures had a marked effect on the biodegradation of formate, malate, and succinate as compared to one atmosphere as follows:

| Pressure (atm) | Percent Degraded (%) | | |
|---|---|---|---|
| | Formate | Malate | Succinate |
| 1 | 100 | 100 | 100 |
| 200 | 97 | 98 | 88 |
| 600 | 81 | 74 | 52 |
| 1,000 | 21 | 13 | 9 |

However, he indicated that, although the dehydrogenase systems were inactivated by hydrostatic pressure, such pressure effects appear to have little or no effect on the absolute biochemical reaction rates.

Jannasch et al. (1971) reported that the rates of organic matter (e.g., acetate, mannitol, sodium glutamate, casamino acids, starch, galactose, peptone, and albumin) degradation were 10 to 100 times slower in the deep sea (1540 m) when compared to controls at comparable temperatures but atmospheric pressure. In another study, Jannasch et al. (1973) reported that the in situ (in the deep sea at depth 1830 m) biodegradation rates of various organic substrates (e.g., starch, agar, gelatine, bond paper, paper towels, balsa wood, beech wood) were between one to three orders of magnitude lower than in the controls (4°C, performed in the laboratory). These authors reported that the response of deep-sea microbial populations was similar to that of surface-water populations incubated in the deep sea or in the laboratory. They further indicated that successive compression and decompression of marine bacteria during testing had little or no effect on the viability of the bacteria. The low rate of microbial activity also can not be explained by too small an inoculum or possible effects of oxygen tension. They suggested that the slow biodegradation rates of organics in the deep-sea
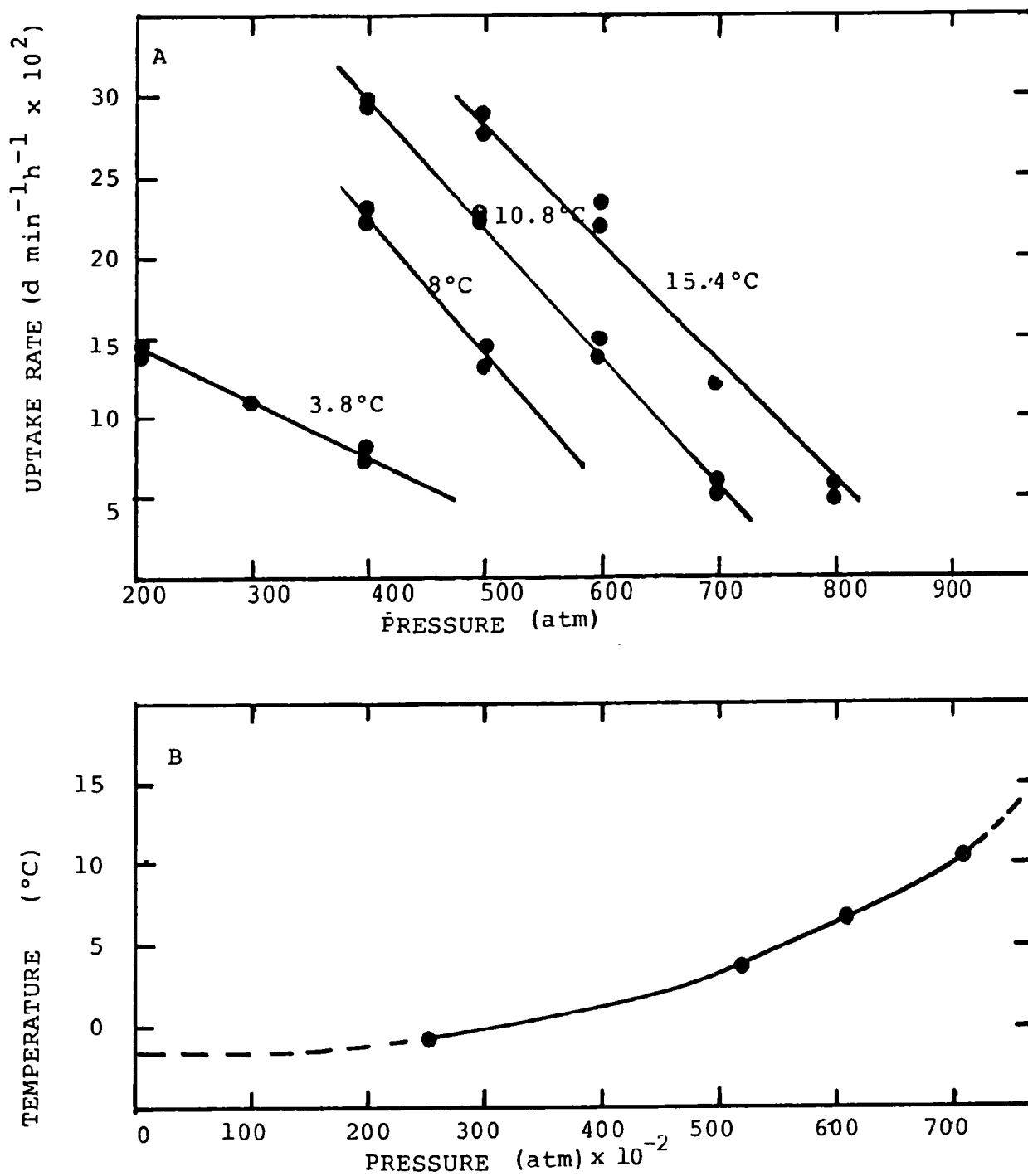
Figure 12. (A)  Uptake rate of $[U^{-14}C]$ glutamic acid by the antarctic psychrophile Ant-300 at various temperatures and pressures.

(B)  Constant uptake isopleth for $[U^{-14}C]$ glutamic acid by Ant-300 prepared from the above Figure (A) (Morita, 1976).

environment could be the result of a relative retardation of
certain critical metabolic processes.

It has been suggested in the literature that some of the
organisms may increase their activities at elevated pressure
compared to that of one atm pressure (ZoBell et al., 1949; Morita,
1976). Wirsen et al. (1976), in their studies of the degradation
of various solid organic materials (e.g., seaweed, wood, paper,
chitin, fish meta, foodstuffs) in the deep sea at depths of 1830-
5300 m for up to 15 months, did not observe a "barophilic" response.
In a comprehensive literature review, ZoBell (1970) also concluded
that the growth of virtually all microbial species examined has
been found to be retarded or completely inhibited by pressures
50 to 500 atm higher than those normally encountered. This
generalization applies to barophilic as well as to barophobic
species, both marine and terrestrial organisms (ZoBell, 1970).

Besides the pressure inhibition effects discussed above,
hydrostatic pressure was also reported to affect biodegradation
lag periods (Rose, 1976). These lag periods may indirectly
affect biodegradation rates because of the overall time require-
ment for biodegradation. Schwarz et al. (1975) demonstrated in
laboratory experiments that Pseudomonas bathycetes, an organism
isolated from the Challenger Deep, had an extremely long lag
period for growth of approximately four months at 1000 atm and 3°C.

Hydrostatic pressure was also found to cause a marked
narrowing of pH ranges for growth and a reduction in growth
yield for a variety of bacteria. Although the direct relation-
ships between pressure and biodegradation were not measured, as
discussed in Section 7 "Biodegradation Algorithms", a positive
relationship between biodegradation rate and growth has been
found. Examples of the pressure effect on pH ranges for growth
are shown in Figure 13 (Matsumura et al., 1974). As can be seen
from the figure, for each bacterium, pH ranges for growth were
progressively narrowed with increasing pressure, and growth yields
were also progressively reduced. The authors suggested that the
reduced yields under pressure could be directly related to in-
creased sensitivities to metabolic acids that accumulated in the
system. The increased sensitivity to low pH of bacterial growth
under pressure could be partially reversed with magnesium and
calcium ions.

Other pressure effects that could indirectly influence bio-
degradation include changes in the biodegradation pathways (Rose,
1976), an increase in the loss of potassium from the cells under
high hydrostatic pressure (Matsumura et al., 1974), and denatura-
tion of enzymes by moderate (100 to 500 atm) to high hydrostatic
pressures (Rose, 1976; and Morita, 1976). However, as mentioned
by Rose (1976), there is little likelihood of a clearer under-
standing of the effects of hydrostatic pressure on microbes emerg-
ing in the near future, mainly because of a wide variety of factors
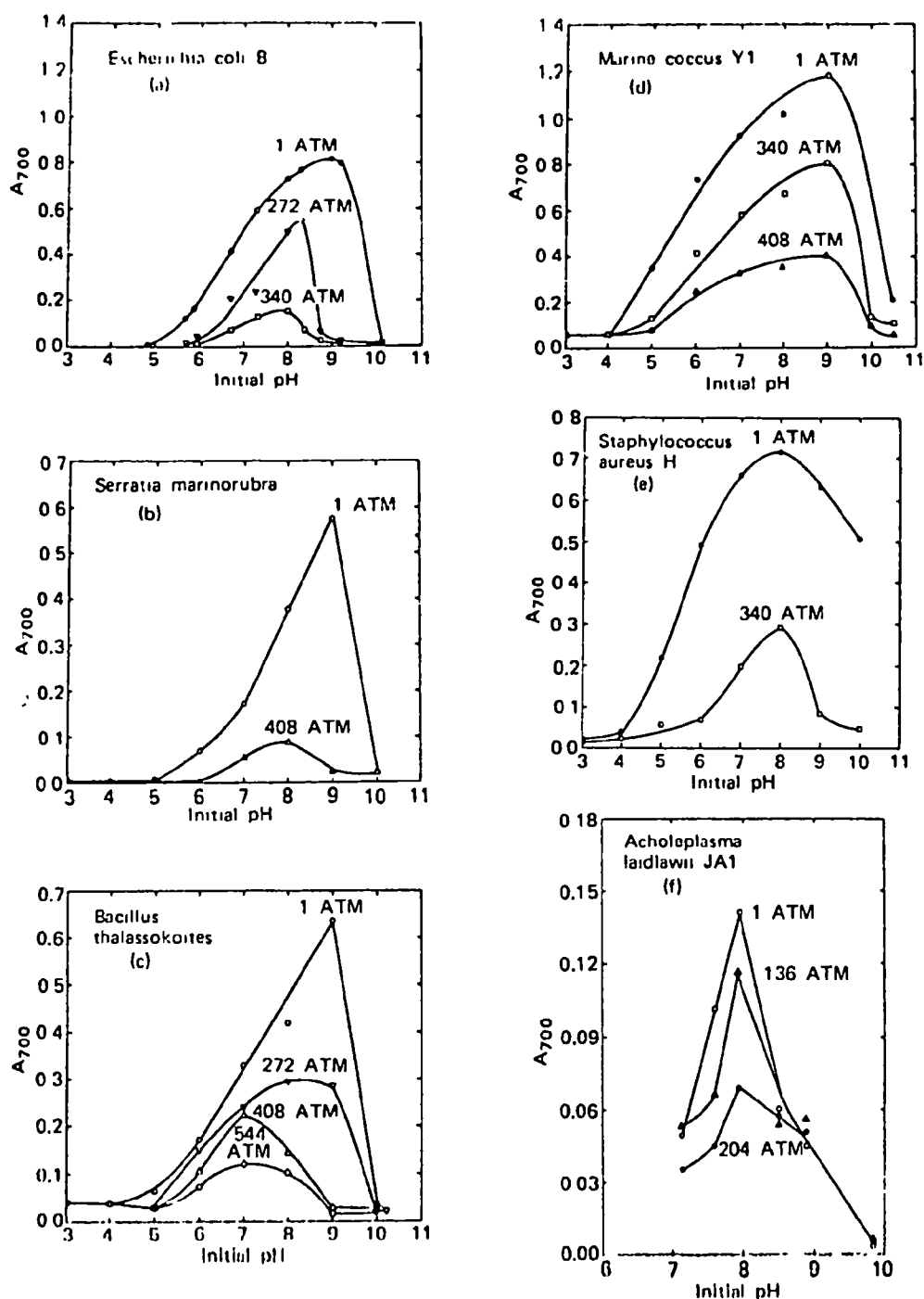
Figure 13. pH ranges for growth of (a) E. coli, (b) S. marinorubra, (c) B. thalassokoites, (d) marine coccus Yl, (e) S. aureus, and (f) A. laidlawii. Incubation times and temperatures were: 24 hr at 24°C for E. coli and S. marinorubra, 24 hr at 30°C for B. thalassokoites, 48 hr at 24°C for marine coccus Yl, 49 hr at 30°C for S.aureus, and 48 hr at 30°C for A. laidawii. $A_{700}$ is absorbance at 700 nm (Matsumura et al., 1974).

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associated with pressure (e.g., temperature, biochemcial reaction rates, solubilities of substrate and gases, etc.) and the diffi-culties encountered in carrying out experiments on these effects.

LIGHT

Unlike many environmental factors, light can directly affect organic substrates: certain recalcitrant xenobiotics can be chemically transformed by light into forms more usable by micro-organisms. Some organisms are receptive to light and its effects; e.g., by using light as an energy source, through damage to cellu-lar structures caused by light, by movements in response to light, etc. Before exploring light and its potential effects on biode-grading microorganisms, a short review of the forms of light, or radiation, is in order.

Radiation is generally classified according to its wavelength. Most radiation reaching the earth is in the near ultraviolet (UV), visible, or infrared (IR) regions of the spectrum illustrated below.

Wavelength (nm)

$10^{-5}$   $10^{-3}$   $10^{-1}$   $10$   $10^{3}$   $10^{5}$

Cosmic rays   Gamma rays   X-Rays   Ultra-violet radiation   Infrared radiation

Visible Radiation
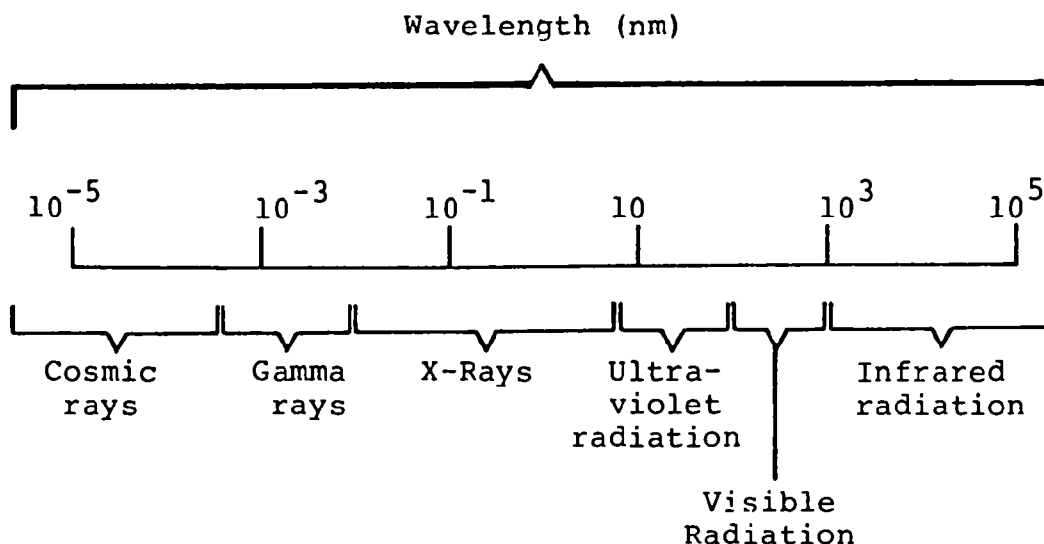
Ultraviolet radiation (10-300 nm) is important in biodegrada-tive processes largely because of its potential lethal or mutagenic effects upon organisms. Vegetative microbes are varied in their response to ultraviolet radiation. Rose (1976) reports that the dose of UV radiation needed to inactivate 90% of a population of E. coli is less than $10^{-7}$ $Jmm^{-2}$ (i.e., 1 erg $mm^{-2}$), whereas a dose of 7 x $10^{-4}$ $Jmm^{-2}$, is needed to achieve the same effect with M. radiodurans. Ultraviolet radiation below 280 nm can damage cellular DNA and RNA. Visible radiation (300-1000 nm) can also be lethal or mutagenic to some microbes, but the most important effects of visible light are in photosynthesis and light-induced changes in growth rates. Little is known about the effects of infrared radiation on microorganisms since the energy of this radiation is immediately converted into heat or thermal energy on contact with absorbing materials.

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A particularly important aspect of light is its effect on photosynthetic organisms, and in our case, photosynthetic bacteria capable of biodegrading organic chemicals. Certain bacterial genera, e.g. Rhodospirillum, are capable of using reduced organic compounds as electron donors. The photosynthetic bacteria are found in oxygen-deficient environments where light is not a limiting factor. These bacteria are found in sediments of ponds, in estuarine sediments, and in a narrow band at a depth of approximately 80 to 150 feet in deep lakes and in the open ocean where oxygen has been depleted and there is still a low light intensity (Clayton and Sistrom, 1978). Photosynthetic bacteria are able to photoassimilate a wide variety of low molecular weight organic compounds, including acetate, pyruvate, fatty acids, methanol and ethanol, and some are able to degrade simple sugars and sugar alcohol. The bacteria generally lack the capacity to break down organic macromolecules such as starch, cellulose, pectin, lipids and proteins. In natural habitats, they therefore depend on the activity of chemoorganotrophic bacteria capable of degrading such macromolecules.

The effects of light intensity on bacterial growth rates and substrate utilization have been the subject of limited investigation. Nakamura (1937) observed that the rate of oxygen uptake by Rhodopseudomonas palustris was decreased by illumination. Partial suppression of oxygen uptake has been observed in Rp. sphaeroides and Rp. capsulata (Clayton, 1955). The diminished utilization of oxygen in light is not, however accompanied by an inhibition of growth, and growth continues essentially at the same rate in air as under anaerobiosis (Cohen-Bazire et al., 1957). The utilization of substrates can even remain unchanged (van Niel, 1941; Morita, 1955), for van Niel demonstrated that acetate utilization was the same whether suspensions were illuminated in air or nitrogen, while oxygen uptake was completely suppressed in the latter instance. This is not necessarily so, however, for Clayton (1955) found a different rate of succinate utilization in light and dark. Furthermore, Clayton demonstrated that the relative amount of substrate utilized via either respiration or photosynthesis depended on both light intensity and oxygen tension. The preferential mode of substrate utilization was through photosynthesis. Johnson and Brown (1954) demonstrated that light, rather than oxygen tension, was inhibiting respiration, by demonstrating that the $^{18}O/^{16}O$ ratios did not change during illumination of Rhodospirillum rubrum. Later studies by Thore et al. (1969) determined that light inhibited NADH oxidation in Rs rubrum at an intensity of $1.2 \times 10^{-5}$ ergs cm$^{-2}$ sec$^{-1}$.

In review, light can enhance or inhibit the biodegradation of chemicals depending on the specific conditions and microorganisms involved. Ultraviolet light can inactivate microbial population and, therefore, inhibit biodegradation. Visible light

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can also be lethal or mutagenic to those species which are without protective carotenoid pigment. Photosynthetic bacteria are able to photoassimilate a wide variety of mostly non-xenobiotic low molecular weight organics. However, the effects of light intensity on biodegradation rates are largely undocumented. The capability of photosynthetic bacteria and/or algae to photoassimilate xenobiotic organics are also greatly unknown.

# SECTION 4

## CHEMICAL ENVIRONMENTAL VARIABLES

### OVERVIEW

The chemical environment can influence the biodegradation rate of chemicals in two ways:  (1) by direct transformation of a chemical to a form that is more or less biodegradable or available than the parent chemical, or (2) promoting or inhibiting the activity of biodegrading microorganisms.  Conversely, the chemical environment may have no effect in influencing chemical biodegradation.  Chemical environmental variables discussed in this section are:  (1) pH; (2) redox potential; (3) nutrients; (4) toxins/inhibitors; and (5) water availability.

The optimum pH required for growth of microorganisms varies considerably, though the physiological limits of pH range from about pH 4.0 to 9.0.  For most organisms, there is a fairly narrow range within these limits that is most favorable for growth.  A review of the literature revealed many investigations on the effect of pH on growth rates in pure culture; however, very little is known about these effects and mechanisms in natural habitats.

The pH of the environment affects microbial activity and thus biodegradation as a result of the interaction between hydrogen ions and enzymes (and presumably transport proteins) in the plasma membrane.  Certain pH values will be optimal for activities of specific enzymes.  Optimal pH values may depend on other factors such as salt concentration (Cockrane, 1958; Doetsch and Cook, 1973; and Dickinson and Pugh, 1974).

In addition to affecting microorganisms and microbial enzymes directly, pH influences the dissociation of many molecules which directly influence microorganisms.  Of particular importance are the effects of pH on the availability of required nutrients, e.g., ammonium and phosphate, which limit microbial growth rates in many ecosystems; and on the mobility of heavy metals which may be toxic to microorganisms.

Many enzymatic reactions are oxidation-reduction, or redox, reactions.  The ability to carry out redox reactions by an organism depends on the oxidation reduction potential (Eh) of the environment.  It is found that many compounds are resistant to biodegradation under anaerobic conditions but can be readily

biodegraded in oxidizing conditions. Alternatively, some chemical transformations, such as dechlorination, can proceed at a faster rate under anaerobic conditions. It should be noted, however, that the Eh of an environment may be quite different than that of a cell, and thus intracellular enzymatic reactions can occur in spite of environmental redox conditions.

Gradients in Eh are widely evident in nature, and the location of many populations is quite obviously correlated with the potential that allows for their development. For example, populations capable of reducing inorganic or organic substances may be activated by a reduction in Eh as $O_2$ is consumed. Linked with anaerobiosis and low redox potential is the appearance of other toxicants, $H_2S$ and organic acids in particular, and hence an array of forces make anaerobiosis an effective means whereby one portion of the community does material harm to a second (Mitchell and Alexander, 1962).

Among the microbial activities directly affected by Eh in soil are mineral transformations, alterations in organic products, and changes in pH. Little is known, however, about the effects of Eh on various physiological processes of microbes in soil (e.g., enzyme activity, release of reducing metabolites by drying cells, toxin production, nutrient uptake) although some of these effects have been studied in pure culture (Dolin, 1961).

Rose (1976) defined nutrients as compounds which must be taken by microorganisms from the environment in order to satisfy their requirements for biosynthetic raw materials and for energy. Rose categorized nutrients as: (1) water, (2) energy sources, (3) biosynthetic raw materials (4) growth factors, and (5) inorganic minerals. The effects of water on microbial growth and biodegradation are discussed under water availability.

Biosynthetic raw materials include carbon, hydrogen, oxygen, nitrogen, phosphorous and sulfur. Carbon, hydrogen and oxygen account for the bulk of the dry weight of microorganisms. If growth is to take place, utilizable compounds containing these elements must be available in the environment in relatively high concentrations.

Concentrations and sources of available nitrogen and phosphorus often limit biodegradation in aquatic and soil habitats. The limiting effects of inorganic nutrients in soil are usually only apparent when substantial amounts of readily oxidizable carbonaceous substrates are introduced (Stotzky and Norman, 1961; Stotzky and Norman, 1964). The concentration of the limiting nutrients then determines the rate of substrate oxidation.

Growth factors are organic compounds such as amino acids, vitamins, purines, pyrimidines, and nucleotides, which are either essential or stimulatory to the growth of microorganisms. The requirements of a microorganism for growth factors are not fixed

but may vary with the conditions under which the organisms are grown. The requirement for growth factors by different species is dependent upon environmental conditions, such as pH, Eh, temperature, ionic composition and the concentration of nutrients. Species not requiring such factors, or able to make use of those present, probably develop more rapidly after the introduction of readily utilizable substrates than those species dependent on growth factors. These "secondary populations" may be dependent upon growth factors that are synthesized by the primary populations (Laskin and Lechevalier, 1974).

Small amounts of many inorganic cations and anions are required for growth of all microorganisms. These inorganic nutrients fall into two classes. Macronutrient elements that are required in relatively high concentrations include $Mg^{2+}$, $K^+$, $Fe^{3+}$, $Mn^{2+}$, $Zn^{2+}$, $Na^+$, $Ca^{2+}$, and $Cl^-$, and the metabolic functions of most of these ions are reasonably well understood. Micronutrient elements (e.g., Co, Ni, Se, V) are required in very much lower concentrations. It is usually very difficult to establish a requirement for a micronutrient because of contamination in the purest of medium constituents (Rose, 1976).

Mineral elements function in microbial metabolism mainly as activators of various enzymes. Little is known, however, about which mineral nutrients are absolutely required for microbial life in soil or aquatic environments. Deficiencies of some minerals can affect the synthesis of enzymes and other biopolymers, the stabilization of cell walls and tertiary structures of DNA and RNA, cell division, mobility, and a variety of other physiological and biochemical processes (Weinberg, 1962).

The presence of other chemicals can have an impact on the fate of a specific chemical in soil. For example, the presence of detergents was found to result in an increased persistence of parathion and diazinon residues in water (Lichtenstein, 1966). Studies by Kaufman et al. (1970, 1971, 1977) have shown that the presence of methylcarbamate and pesticide combinations have increased the persistence of certain pesticides and herbicides in soils. Likewise, Kecskes and Cserhati (1977) observed decreased biodegradation of linuron in the presence of other pesticides in soil. Conversely, Liu (1980) reported that the rate of biodegradation of Arochor 1221 was enhanced by enrichment with sodium ligninsulfonate.

Microorganisms possess a range of tolerance mechanisms, most featuring some kind of detoxification. Many of these detoxification mechanisms occur widely in the microbial world. A feature of heavy metal physiology is that even though many metals are essential for growth, they are also reported to have toxic effects on cells, mainly as a result of their ability to denature protein molecules. There are, however, many reports in the literature of microbial resistance to heavy metals. The phenomenon of microbial resistance is particularly relevant to biodegradation, especially

in connection with the roles of microbes in polluted ecosystems and in the reclamation of metal-contaminated natural habitats. It is also important to understand the mechanisms of microbial tolerance because of the extensive use of some metals and metal compounds as fungicides and disinfectants (Rose, 1976).

Water accounts for between 80 and 90% of the weight of a microorganisms. Chemical reactions that take place in living organisms generally require an aqueous environment, and water must therefore be in the environment if the organism is to grow and reproduce. It must be, moreover, in the liquid phase, and this confines biological activity and biodegradation to temperatures ranging from around -2°C (or lower in solutions of high osmotic pressure) to approximately 100°C (Rose, 1976).

In order to adequately describe the environment in which a microorganism exists, some measure is required which indicates the amount and form of water in that environment. This measure must give some indication of the suitability of the solvent environment for the reaction sequences necessary for normal metabolism (Reid, 1980). A measure popular with early workers was osmotic pressure. This has been discussed by Brown (1976), who commented on the difficulty of accurately measuring the osmotic pressure in many systems. Scott (1977) suggested that a suitable measure of water availability was the thermodynamic water activity, $a_w$, of the equilibrium system.

Microorganisms can grow in media with $a_w$ values between 0.63 and about 0.99. For any one organism, the important values within this range are the optimum and minimum $a_w$ values. These have been determined for a number of microorganisms, and they seem to be remarkably constant for a particular species and to be independent of the nature of the dissolved solutes (Rose, 1976). Brown (1976) and Troller and Christian (1978) have discussed in detail the advantages and disadvantages of the use of water activity as a measure of water availability.

In soil, water availability is usually measured in terms of percent moisture content and percent of field capacity. It is generally found that rates of microbial activity increase with increasing water content of the medium to certain critical values. When the water content exceeds the critical value, biodegradation rates will be constant or reduced.

pH

On a gross level, the pH of natural soil/aquatic systems has been shown to affect biodegradation, which appears to be due to the influence of pH on the predominance of one group of organisms over another. Gradients in pH are widely evident in nature. A review of the literature revealed that most studies on the effect of pH on biodegradation were concerned with a pH range of 5 to 8, which are the limits favorable for the growth of most microorganisms.

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The effects of pH on the extent and rate of biodegradation are discussed under individual headings for specific organic compounds.

## Dimethylamine

Tate and Alexander (1976) assessed the rate of dimethylamine (DMA) biodegradation in soils with different pH values. The four soils tested were Hudson Collamer silt loam (pH 4.5), Langford channery silt loam (pH 5.2), Williamson silt loam (pH 6.8), and Lima loam (pH 7.0). Their organic matter levels were 1.8, 4.1, 1.6, and 3.6%, respectively. DMA was metabolized in samples of all of the soils tested. Figure 14 shows that in Williamson silt loam adjusted to different pH values, the rate and extent of DMA disappearance decreased as the acidity increased. Figure 14 shows the disappearance of DMA in three other soils of different pH. The disappearance was slowest in Hudson Collamer silt loam (pH 4.5) and was more rapid in Lima loam (pH 7.0) and Langford channery silt loam (pH 5.2). The differences could have been due to differing percentages of organic matter in the tested soils. The biodegradation rate of dimethylamine was more rapid in Lima loam (3.6%) and Langford channery silt loam (4.1%) than in Hudson Collamer silt loam (1.8%). This apparent correlation is also supported by the rate of biodegradation in two soils of different percentage organic content having similar pH values (i.e., Lima loam, pH 7.0, 3.6% organic content; Williamson silt loam, pH 6.8, 1.6% organic content). The data show that the biodegradation rate was significantly greater at the higher organic soil content. The results suggest that the effect of pH on biodegradation rates may be difficult to interpret in soils having a wide range of organic content.

## Naphthalene

Hambrick, DeLuane, and Patrick (1980) studied the biodegradation of naphthalene in estuarine sediments at varying pH and redox potentials. Results indicated that naphthalene biodegradation was significantly greater at pH 6.5 or 8.0 than at 5.0. Figure 15 shows that naphthalene was biodegraded to a greater extent at all pH levels under oxidizing conditions than under more reducing conditions. These results are significant in that they indicate that the determination of pH effects on biodegradation in other studies under conditions of unknown redox potential may at best indicate only a qualitative trend. Strawinski and Stone (1955) also studied the effect of pH on the biodegradation of naphthalene though utilizying a pure culture of Pseudomonas sp. The yield of non-naphthalenic extract calculated on a basis of 1% naphthalene in the medium increased from 2 to 29% by adjusting the initial pH to 8.0 and aerating during aeration. This pure culture study, while not representative of conditions existing in nature, serves to substantiate the results of Hambrick et al. (1980). It is apparent from both studies that pH-Eh relationships should be developed in order to obtain a better quantitative evaluation and comparison of pH effects on biodegradation.

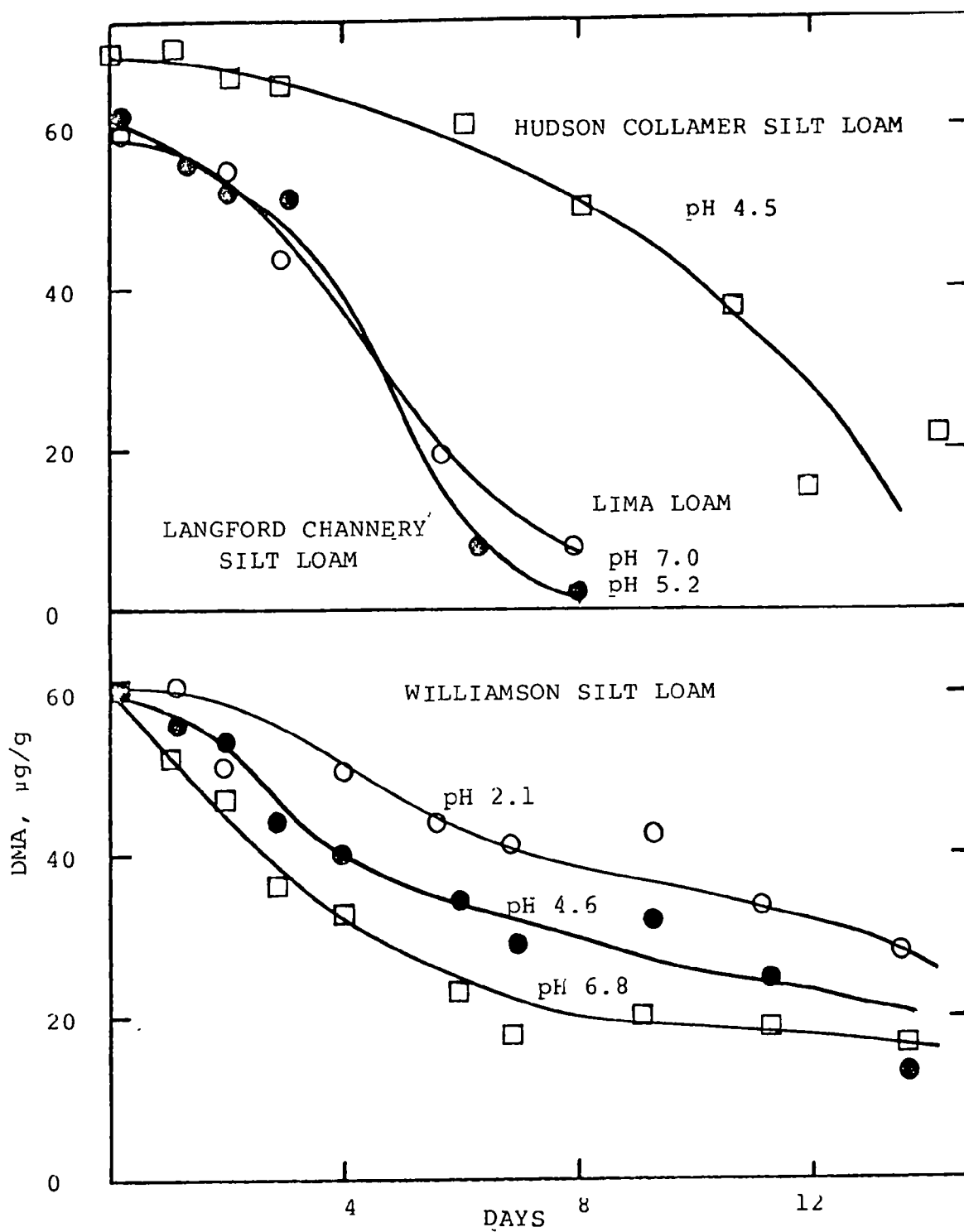Figure 14. Disapearance of dimethylamine from soils of varying
pH. The Williamson soil was adjusted to the pH
values shown (Tate and Alexander, 1976).

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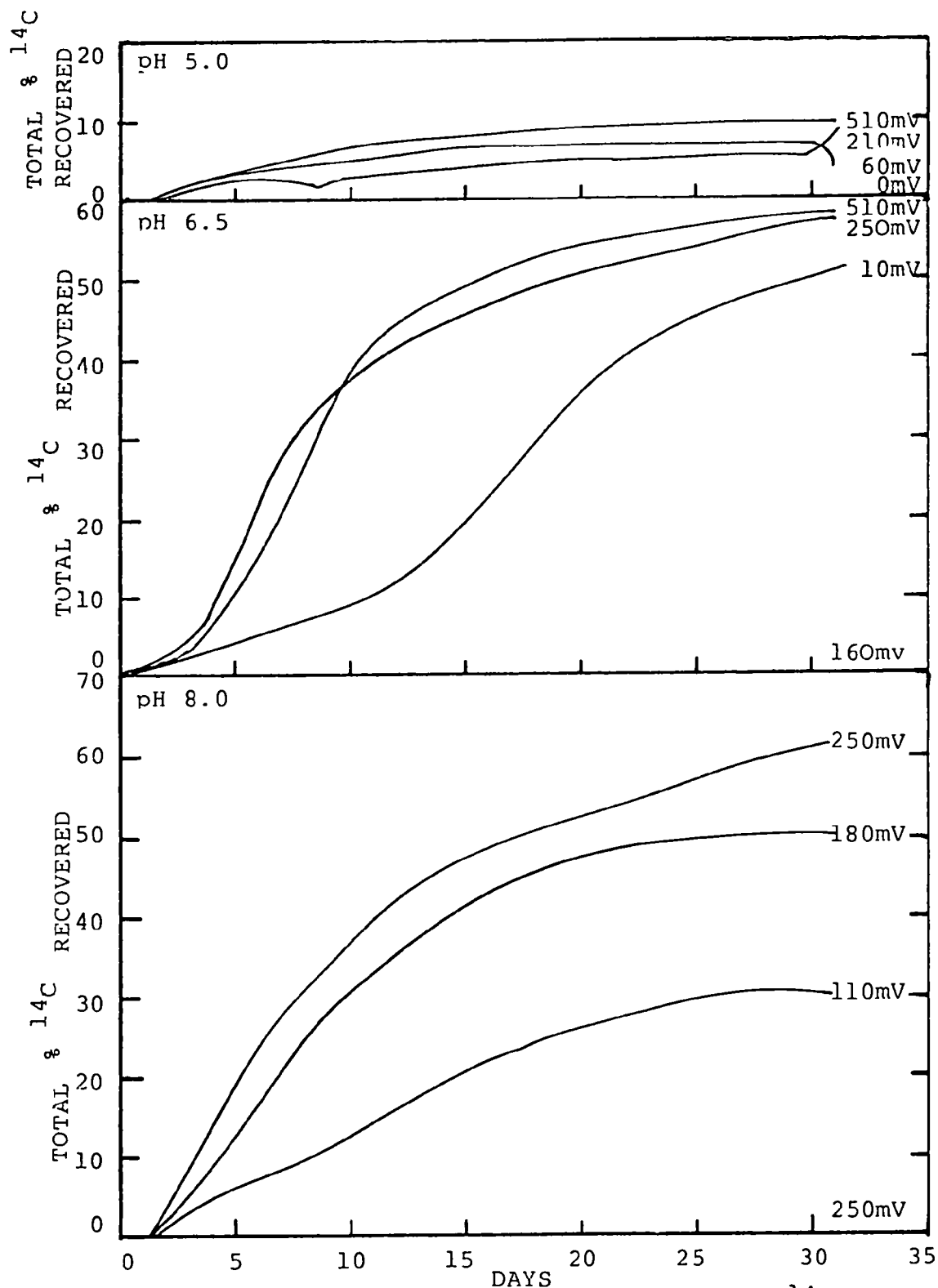Figure 15. Total percentage of $^{14}C$ recovered as $^{14}CO_2$ summed per day for the mineralization of $[1(4,5,8)-$ $^{14}C]$ naphthalene in 12 sediment-water suspensions incubated at four redox potentials and three pH levels (Hambrick et al., 1980).

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Octadecane

Hambrick et al. (1980) determined the biodegradation rate
of octadecane in estuarine sediments as affected by different pH
levels under oxidizing and reducing conditions. Highest biodeg-
radation rates occurred at pH 8.0 and the lowest at pH 5.0.
Figure 16 shows that octadecane was biodegraded to a greater
extent at all pH levels under oxidizing conditions than under
more reducing conditions. Generally, biodegradation rates for
octadecane were greater than those for naphthalene (Figure 15).
The results of this study are similar to those obtained for
naphthalene with respect to the importance of pH-Eh diagrams in
the evaluation of pH effects on biodegradation of hydrocarbons.

Chloroaniline

Studies by Hsu and Bartha (1973), who measured $^{14}CO_2$ pro-
duction from soil containing radio-labeled chloroaniline/soil
organic matter complex under various environmental conditions,
indicate that the biodegradation of chloroaniline in anaerobic,
sterilized soils is not significantly affected by variations in
pH from 5.0 to 8.0.

Summary

The extent and rate of biodegradation are generally enhanced
with increasing pH from a level of pH 5 to an approximate limit-
ing value of pH 7 to 8. Exceptions have been reported in which
variations of pH in natural systems have a negligible effect on
the biodegradation rate. Significant variation in soil organic
content may limit the interpretation of pH effects on biodegrada-
tion. Several studies have also shown that pH-Eh diagrams should
be developed in order to make more valid comparisons of pH effects
on biodegradation in different natural soil/water systems.

REDOX POTENTIAL

The effect of redox potential in influencing biodegradation
has been extensively documented in the literature. The ability
of an organism to carry-out oxidation-reduction reactions depends
on the redox potential of the environment. Many enzymatic
reactions are oxidation-reduction reactions. Variations in bio-
degradation rates of persistent compounds in natural systems may
be attributed, in part, to the effect of redox potential on the
enzymatic action of indigenous microorganisms.

Gradients in Eh are widely evident in nature. For example,
the redox potential of submerged sediments may range from +700
mv (highly oxidized) to -400 mv (highly reduced). Below the
aerobic sediment surface, facultative bacteria decrease the redox
potential resulting in development of a two-layer system con-
sisting of an oxidized surface layer and an underlying reduced
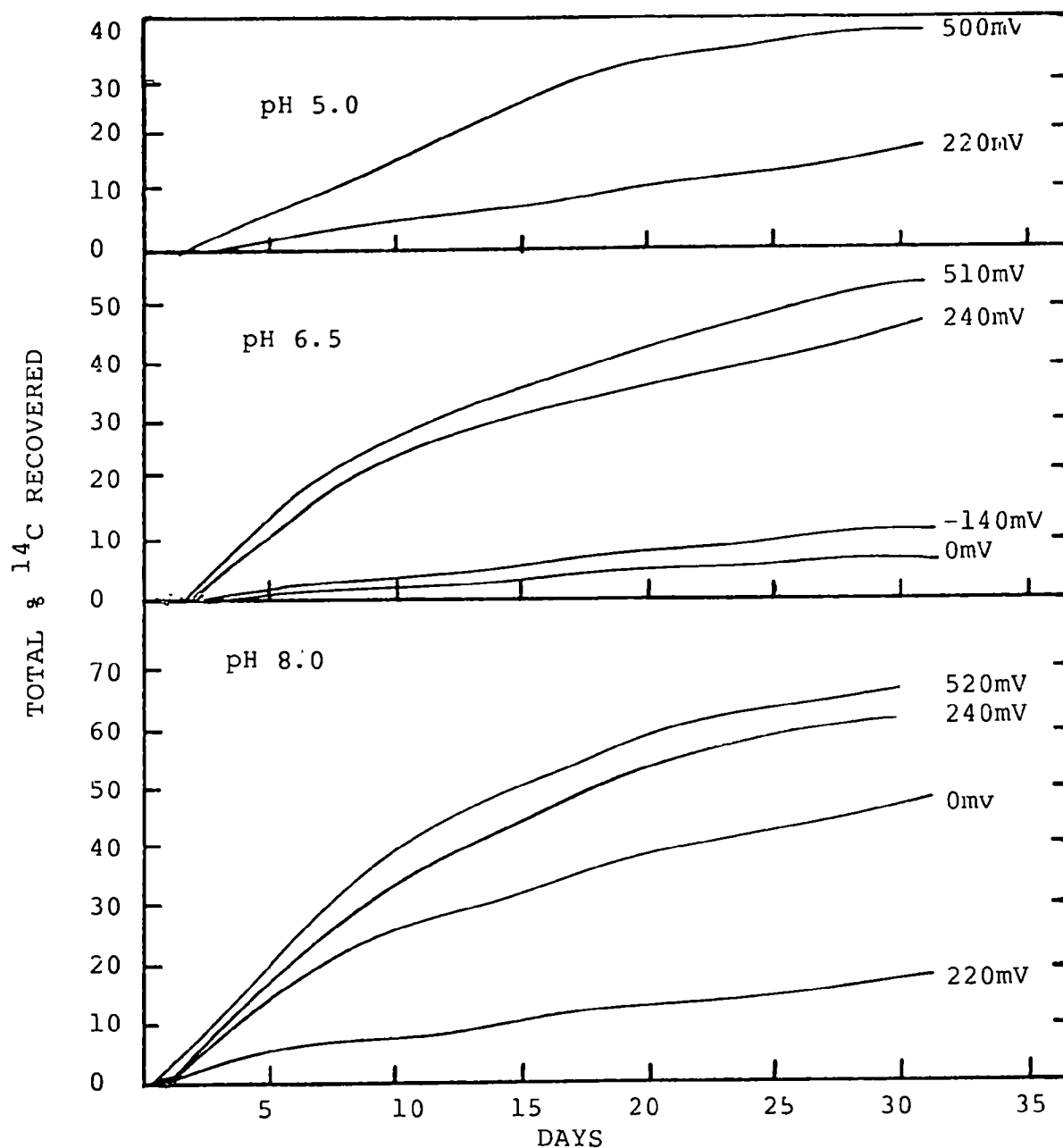layer.

Figure 16. Total percentage of $^{14}C$ recovered as $^{14}CO_2$ summed per day for the mineralization of octadecane in 12 sediment water suspensions incubated at four redox potentials and three pH levels (Hambrick et al., (1980).

Although Eh apparently has a gross effect on microbial growth, and therefore, may have some predictive value for biodegradation, the definition of its effect will depend on the ability to measure this environmental factor at levels which approximate the micro-habitat.

The influence of redox potential on the extent and rate of bio-degradation is discussed under individual headings for specific organic compounds. The effect of redox potential is presented for several studies; however, this information is greatly lacking in the literature.

## Di-2-Ethylhexyl Phthalate (DEHP) and Di-n-Butyl Phthalate (DBP)

Johnson and Lulves (1975) reported on the biodegradation of two widely used plasticizers, di-2-ethylhexyl phthalate (DEHP) and di-n-butyl phthalate (DBP) by unidentified aerobic and anaerobic microorganisms found in hydrosoil of freshwater ponds. The results of this study show that the biodegradation of DEHP and DBP is much more rapid under aerobic conditions than under anaerobic conditions as shown in Table 3. For example, under aerobiosis, 53% of the DBP was biodegraded within 25 hrs, and 98% within 5 days. Under the same conditions, nearly 14 days were required for the biodegradation of approximately 50% of the DEHP. Anaerobosis slowed biodegradation of both DBP and DEHP. Although nearly 98% of the DBP was biodegraded after 30 days in the hydrosoil, the retarding influence of anaerobiosis was evident. Approximately 30% of the DBP was biodegraded after 5 days under anaerobic conditions, whereas 98% of the DBP was bio-degraded after 5 days under aerobic conditions. After 30 days, no significant biodegradation of DEHP was observed under anaerobic conditions. The results of this study are in general agreement with

TABLE 3. BIODEGRADATION OF $^{14}$C-DI-N-BUTYL PHTHALATE AND $^{14}$C-DI-2-ETHYLHEXYL PHTHALATE IN FRESHWATER HYDROSOIL (Johnson and Lulves, 1975).

| | Percent recovery of radioactivity from hydrosoil | |
|---|---|---|
| Incubation (days) | Aerobic | Anaerobic |
| $^{14}$C-di-n-butyl phthalate | | |
| 1 | 47 | 100 |
| 5 | 2 | 69 |
| 7 | 5 | 59 |
| 14 | 8 | 39 |
| 30 | 3 | 2 |
| $^{14}$C-di-2-ethylhexyl phthalate | | |
| 7 | 100 | 100 |
| 14 | 50 | 100 |
| 30 | 41 | 100 |

other investigations which indicate that the biodegradation of organic materials is often enhanced under aerobic condtions and significantly reduced or prevented under anaerobic conditions. Unfortunately, this study like many others, did not qunatify the effects of aerobic and anaerobic environments on biodegradation rates in terms of a measured redox potential of the studied soil/water systems.

## Dimethylamine (DMA)

Studies by Tate and Alexander (1976) indicate that anaerobiosis had a significant effect on the stability of dimethylamine (DMA) in soil. Although DMA was metabolized in Williamson silt loam under anaerobic as well as aerobic conditions, the extend and rate of biodegradation was markedly retarded under anaerobiosis. Figure 17 clearly demonstrates the persistence/biodegradability of DMA under the studied conditions.

## Ethylenedimethyltriacetate (EDTA)

EDTA had been thought to be resistant to microbial degradation (Alexander, 1973), but studies by Tiedje (1975) and Belly et al. (1975) concluded that EDTA can be degraded by natural microbial populations. Tiedje (1977) studied the rate of EDTA biodegradation in three different sediments. Figure 18 shows that significant biodegradation occurred under aerobic conditions, although the rate and extent of biodegradation varied for different sediments. These variations are probably due, in part, to different soil constituents which may be significant in governing the extent and rate of biodegradation.

Figure 18 shows that EDTA biodegradation under anaerobic conditions did not result in $^{14}CO_2$ production. This finding, in itself does not confirm the absence of anaerobic biodegradation, since fermentation would not necessarily yield $^{14}CO_2$, but rather could produce organic end products. Such products, however, would be expected to be rapidly metabolized to $^{14}CO_2$ under aerobic conditions. After periods of anaerobic incubation, subsequent aerobic metabolism did not result in a rate of $^{14}CO_2$ production in excess of that found for continuous aerobic metabolism (Figure 18). Thus, EDTA does not appear to be subject to significant anaerobic biodegradation.

When the rate of degradation was determined from the linear portion of the biodegradation curves, the rate of aerobic biodegradation appeared to be first order for concentrations ranging from 0.4 to 90 ppm. These findings by Tiedje (1977) indicate that EDTA should be biodegradable in most aerobic soils and sediments, provided sufficient organic matter is available to support a general microbial population over a prolonged period of time.
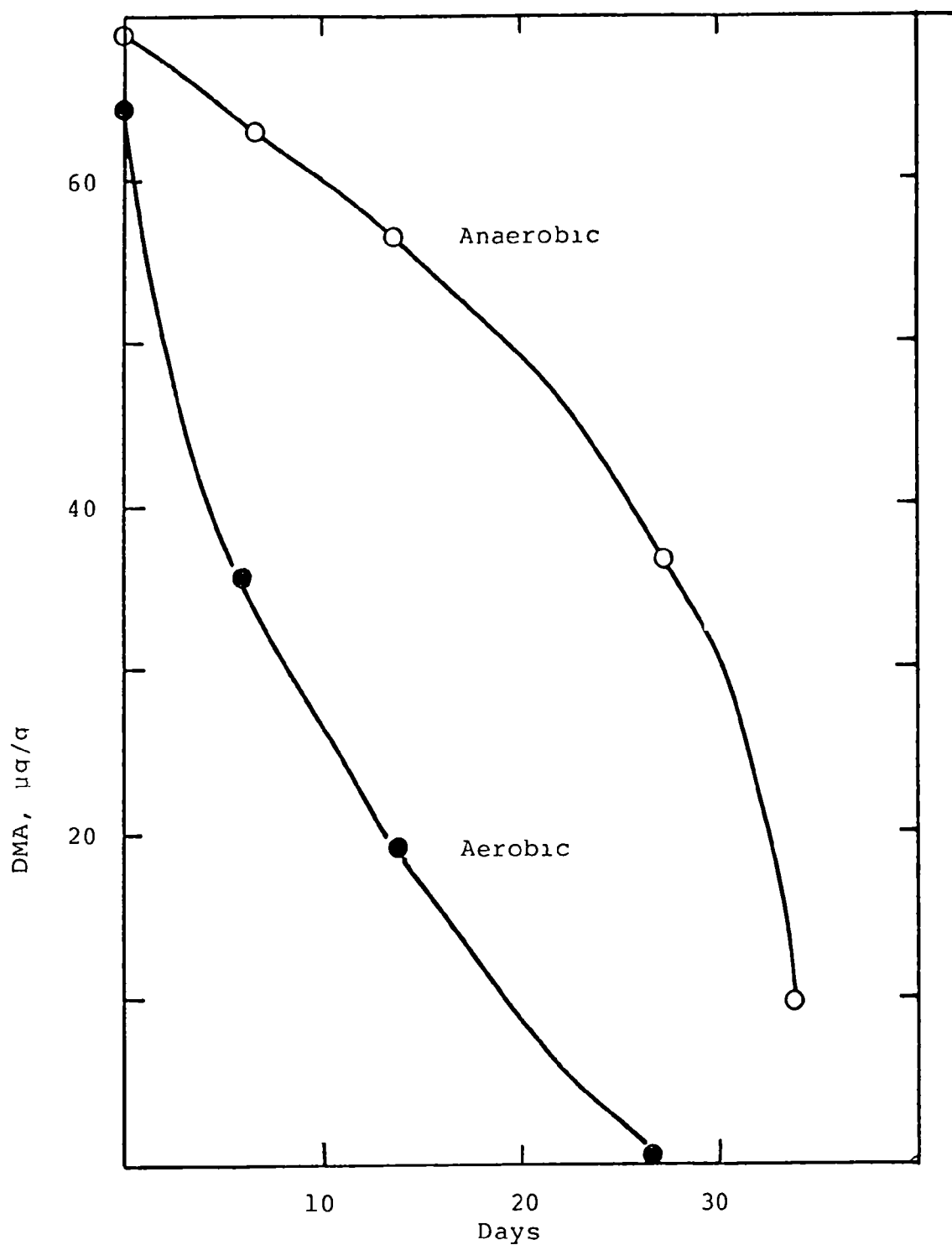
61

Figure 17.  Effect of anaerobic conditions on dimethylamine
disappearance from a Williamson silt loam
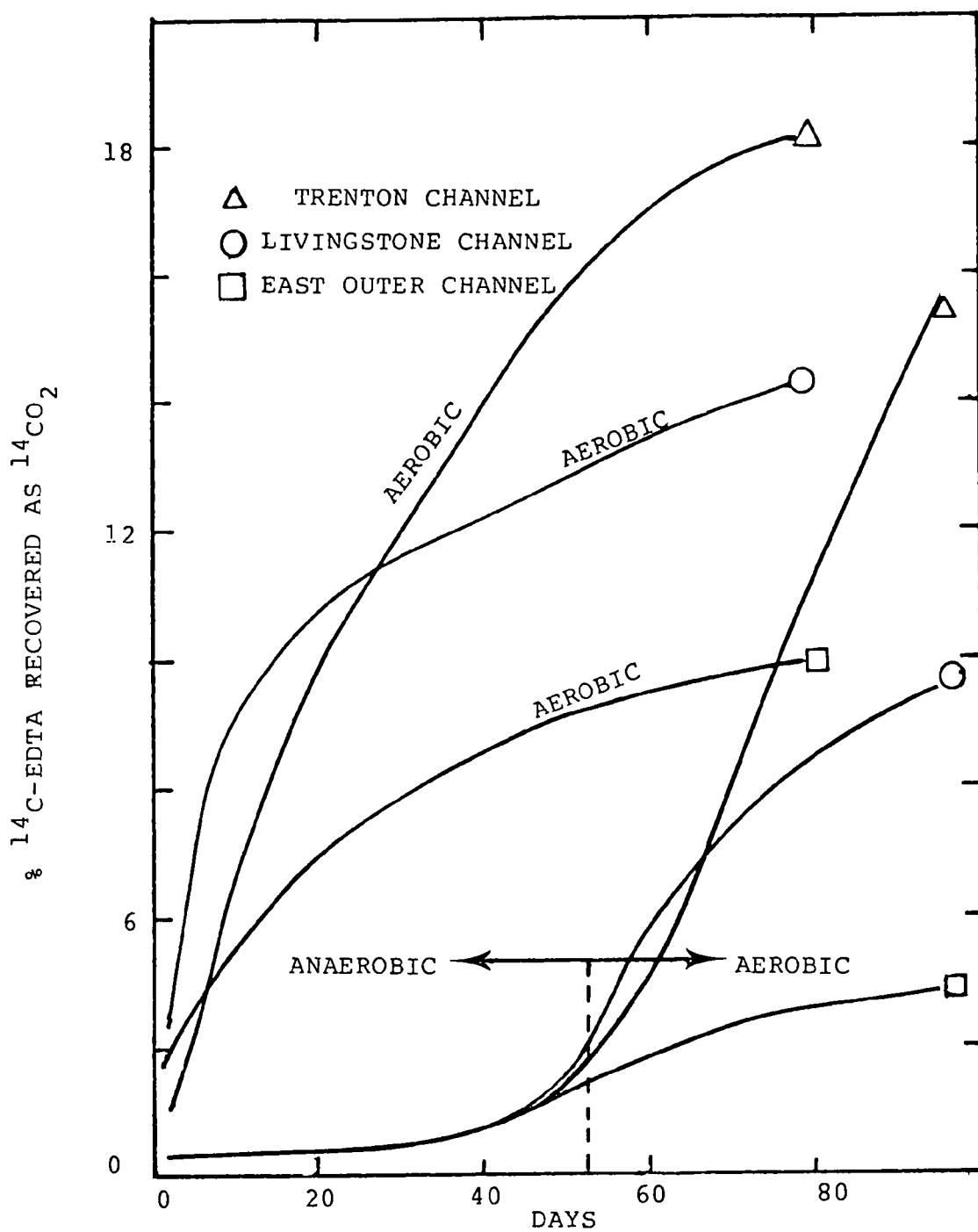(Tate and Alexander, 1976).

Figure 18. Biodegradation of $^{14}C$-carboxyl-EDTA (4.4 ppm) under aerobic and anaerobic-to-aerobic conditions in sediments (Tiedje, 1977).

## Lignin

Hackett et al. (1977) studied lignin biodegradation under aerobic and anaerobic conditions in a variety of natural materials. The observed biodegradation of lignin in aerobically incubated lake sediment implies that lignin degradation in such sediment could occur when oxygen is provided during and after seasonal turnover of lake waters. Biodegradation under anaerobic conditions was observed to be extremely slow, in agreement with other studies (Harken, 1973).

## Octadecane and Naphthalene

The ecological significance of the biodegradability of crude oil hydrocarbons is related to the persistence in the environment of those hydrocarbons that are more slowly degraded and, that are often also the more toxic components of petroleum. Several researchers have reported that the extent of hydrocarbon degradation quickly decreases with sediment depth because of the development of a two-layer system consisting of an oxidized or aerobic surface layer and an underlying reduced or anaerobic layer (Blumer and Sass, 1972; Hughes and McKenzie, 1975; Ward and Brock, 1978). The oxygen required for more rapid hydrocarbon biodegradation is not available in deeper sediments. Submerged sediments display a range of redox potential from 700 mV, which indicates highly oxidized sediments, to -400 mV, which indicates highly reduced sediments (DeLaune et al., 1976).

Hambrick et al. (1980) determined the biodegradation rates in estuarine sediments of naphthalene and octadecane as affected by different redox potentials at three pH levels. Hydrocarbon mineralization rates were inferred from the total respired $^{14}CO_2$. Figures 15 and 16 show that, in general, the biodegradation rates for both naphthalene and octadecane increased rapidly with increasing redox potential (i.e., increasing sediment aerobiosis) at all pH levels. Biodegradation rates for octadecane were generally greater than those for naphthalene. The presence of a lag phase suggests the transformation is microbial in nature. Ward and Brock (1978) reported similar results in that the biodegradation of hexadecane in freshwater lake sediments was very rapid under aerobic conditions but very slow under anaerobic conditions.

One of the major fates of released petroleum hydrocarbons in the coastal environment is their incorporation into bottom sediments. The studies of Hambrick et al. (1980) are of considerable ecological significance in that they show that hydrocarbons deposited in reduced sediments, where facultative and anerobic bacteria predominate, will persist for much greater lengths of time than hydrocarbons remaining in the sediment surface layer,

where aerobic bacteria predominate. This study also shows the
value of using redox potential as a possible indicator of bio-
degradation rates of petroleum hydrocarbon or other compounds.

## Pentachlorophenol (PCP)

Pentachlorophenol (PCP) and its salt, sodium pentachloro-
phenate, (NaPCP) are two of the most versatile pesticides now in
use in the United States. PCP has a wide distribution in the
total environment; therefore, knowledge of its environmental
behavior and fate is essential for effective management of its
use. Microorganisms including bacteria (Watanabe, 1973; Suzuki,
1971; and Reiner et al., 1978) and fungi (Cserjesi and Johnson,
1972) have demonstrated their ability to degrade PCP and other
chlorophenols.

Liu et al.(1981) determined the rate of NaPCP degradation
under aerobic/anaerobic conditions in laboratory fermentors.
Among the factors studied, oxygenation was found to have a sig-
nificant effect on the rate of PCP degradation (Figure 19). By
measuring the disappearance of PCP from the fermentor broth
(primary degradation), the researchers found that the con-
centration of PCP in the aerobic fermentor had decreased to a
negligible amount after 3 days, while 100% of the added PCP
remained unchanged in the anaerobic fermentor. Further incubation
up to 28 days resulted in only 5% biodegradation, indicating the
inherent persistence of PCP under anaerobic conditions. The half-
lives of PCP in aerobic and anaerobic fermentors were calculated
to be 0.36 and 192 days, respectively. PCP biodegradation rates
determined by Boyle et al. (1980) indicated that PCP had a shorter
half-life ($t_{\frac{1}{2}}$ = 19 days) under aerobic conditions than under
anaerobic conditions ($t_{\frac{1}{2}}$ = 80 days). In consideration of the
complexity involved in the determination of a biocide's bio-
degradability, the results obtained by Liu et al. (1981) could be
considered in agreement qualitatively with those of Boyle et al.
(1980). The difference observed in these two studies may be
due, in part, to the fact that Liu et al. (1981) used an acclima-
tized culture, while natural pond water was employed in the study
by Boyle et al. (1980). Regardless, the role of redox potential
in determining the persistence/biodegradability of PCP is clearly
demonstrated in both studies.

## Phenol and Chlorophenols

The ecological significance of phenol and chlorophenols is
in part related to the fact that they have been shown to be
intermediates in the degradation pathways of some pesticides.
They have also been found in pesticide preparations and industrial
wastes.

The aerobic and anaerobic degradation of phenol and selected
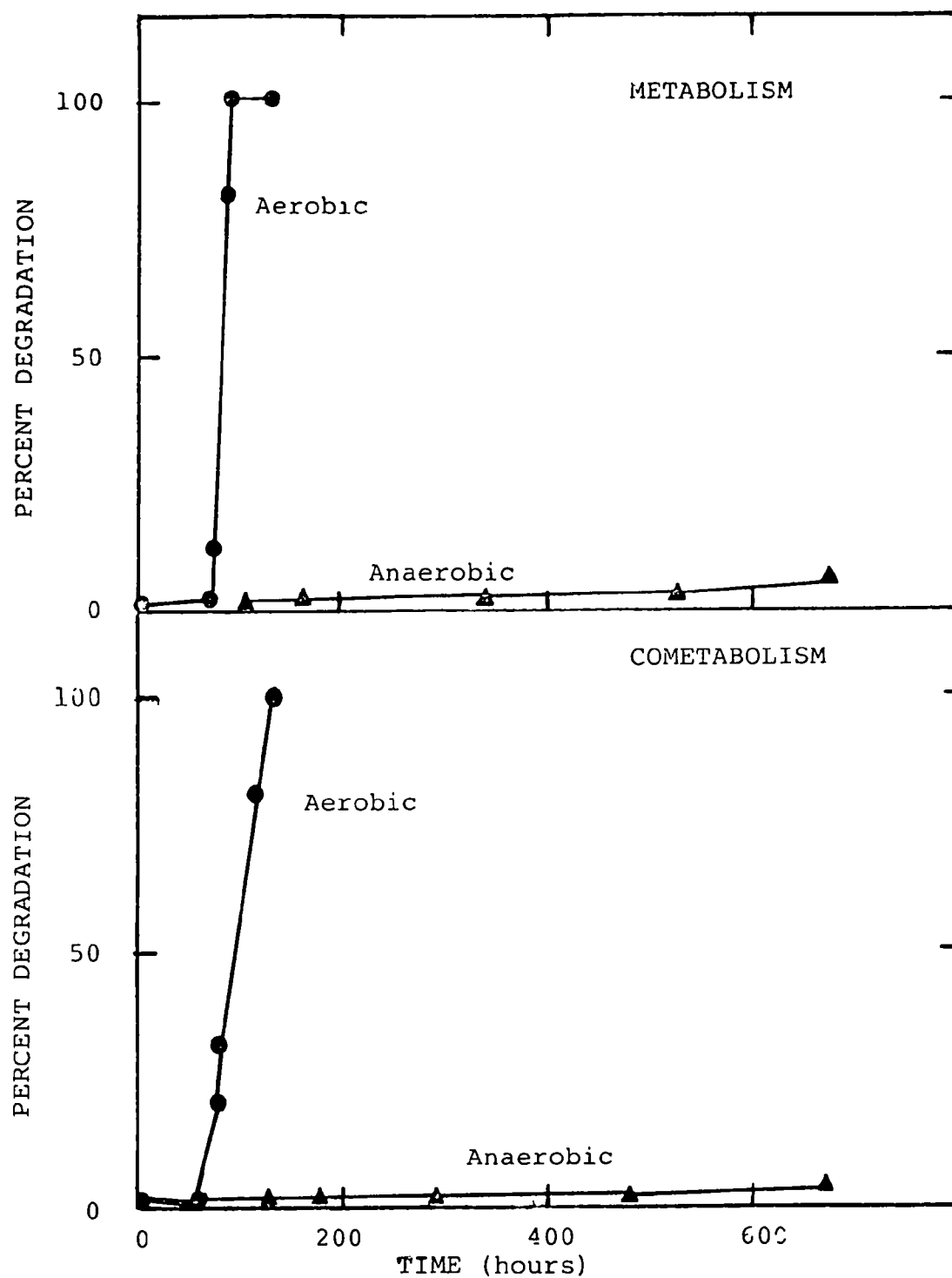chlorophenols in a clay loam soil containing no added nutrients

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Figure 19. Disapearance of PCP in cyclone fermentors (Liu et al., 1981).

was examined by Baker and Mayfield (1980). Results of this study showed that phenol, o-chlorophenol, p-chlorophenol, 2,4-dichloro-phenol, 2-6-dichlorophenol and 2,4,6-trichlorophenol were rapidly degraded by aerobic soil microorganisms. Pentachlorophenol, 2,4,5-trichlorophenol, 3,4-dichlorophenol and m-chlorophenol were degraded at much slower rates, while 3,4,5-trichlorophenol and 2,3,4,5-tetrachlorophenol persisted in aerobically incubated soils for over 160 days. None of the compounds examined were degraded by microrganisms in anaerobically-incubated soil at 23°C. Redox potentials were not reported.

The times required for the biodegradation of these compounds were, in some cases, much shorter than those determined by Alexander and Aleem (1961). It is speculated that the different biodegradation rates observed in these two studies may be due to the smaller amounts of soil used in the study by Alexander and Aleem (1961), or perhaps to differences between incubation in soil vs soil suspension systems. Nevertheless, the persistence of phenol and chlorophenols under anaerobic conditions is substantiated by both of these studies.

## Dinitroanilines

Contrary to the above-mentioned evidence linking higher biodegradation rates of selected chemicals to higher redox potential or more oxygenated environments, the biodegradation of dinitroanilines (e.g., trifluralin, benefin) appears to be more rapid in anaerobic soils than soils of higher redox potential. For example, Probst et al. (1967) reported a more rapid rate of degradation of dinitroanilines at 200% of soil field capacity (anaerobic conditions) than at moisture levels of 0, 50, and 100% of field capacity. However, the experiment lasted only 40 days.

## Other Chlorinated Hydrocarbons

Generally, organochlorine insecticides degrade slowly and are strongly adsorbed to sediments, but selected compounds have been observed to decompose readily under anaerobic conditions. For example, the rapid degradation of lindane has been observed in flooded rice soils (Raghu et al., 1966; Yashida and Castro, 1970), lake muds (Lichtenstein et al., 1966) and in simulated impoundments under anaerobic conditions (Newland et al., 1969). The degradation rate under laboratory conditions for lake mud and rice soils was rapid and normally complete within 8 days to 50 days, but was effectively impeded by soil sterilization (Lichtenstein et al., 1966). Moreover, the rate of degradation for aldrin and lindane was significantly greater in lake muds than in soils (Lichtenstein et al., 1966), and more rapid for lindane in flooded compared to nonflooded rice soils (MacRae et al., 1967; Yashida and Castro, 1970). The evidence here suggests that the rate of biologically mediated dechlorination may be more rapid under anaerobic conditions (or at lower redox potential) then under aerobic conditions.

Summary

A review of the literature showed that the extent and rate of biodegradation is profoundly affected by redox potential. Many of the chemicals studied were biodegraded much more rapidly under aerobic conditions than under anaerobic conditions. Limited data show that biodegradation rates increase rapidly with increasing redox potential (i.e., increasing aerobiosis). However, results of selected biodegradation studies involving dinitroanilines and certain pesticides suggests the opposite. These studies point out that the persistence of toxic compounds deposited in reduced sediments is compound specific. Mathematical expressions for predicting the effect of redox potential on biodegradation are generally lacking. In most cases, quantitative comparisons of biodegradation rates cannot be made because the redox potential of the studied systems was not measured.

NUTRIENTS

Biodegradation of xenobiotic chemicals in aquatic and soil habitats is often limited by the concentrations and sources of available nutrients. The limiting effects of inorganic nutrients in soil are usually only apparent when substantial amounts of biodegradable carbonaceous substrates are available. Mineral nutrition can limit the development of indigeneous microorganisms. This limitation is usually not the result of a scarcity of a specific nutrient, but rather its biological availability. Phosphorous and sulfur, though required in smaller quantities than nitrogen, may also be present in forms not readily assimilable by the microbial community. The availability of other nutrients in natural environments is usually adequate to sustain high levels of microbial growth. Most studies on the effect of nutrients on biodegradation have been concerned with the concentration levels and sources of nitrogen and phosphorous.

## Cellulose and Lignin

Biodegradation of specifically labeled $^{14}C$-cellulose and $^{14}C$-lignin-labeled lignocelluloses was examined in an organic-rich sediment in response to manipulation of various environmental factors (Federle and Vestal, 1980). Mineralization was determined by measuring the amount of radio-labeled $CO_2$ released from the labeled substrates.

Nitrogen (as $NH_4NO_3$) and phosphorus (as $PO_4^{3-}$) added alone had only a slight stimulatory effect on the biodegradation of Carex aquatilis cellulose. Especially noteworthy was the effect of nitrogen and phosphorous added together (Figure 20). At a nutrient concentration of $10\mu M$, the amount of $^{14}C$-cellulose recovered as $CO_2$ was nearly twice that of the control treatment. The data suggest also that phosphorous may be limiting. The degree to which phosphorous limitation severely affects cellulose biodegradation is
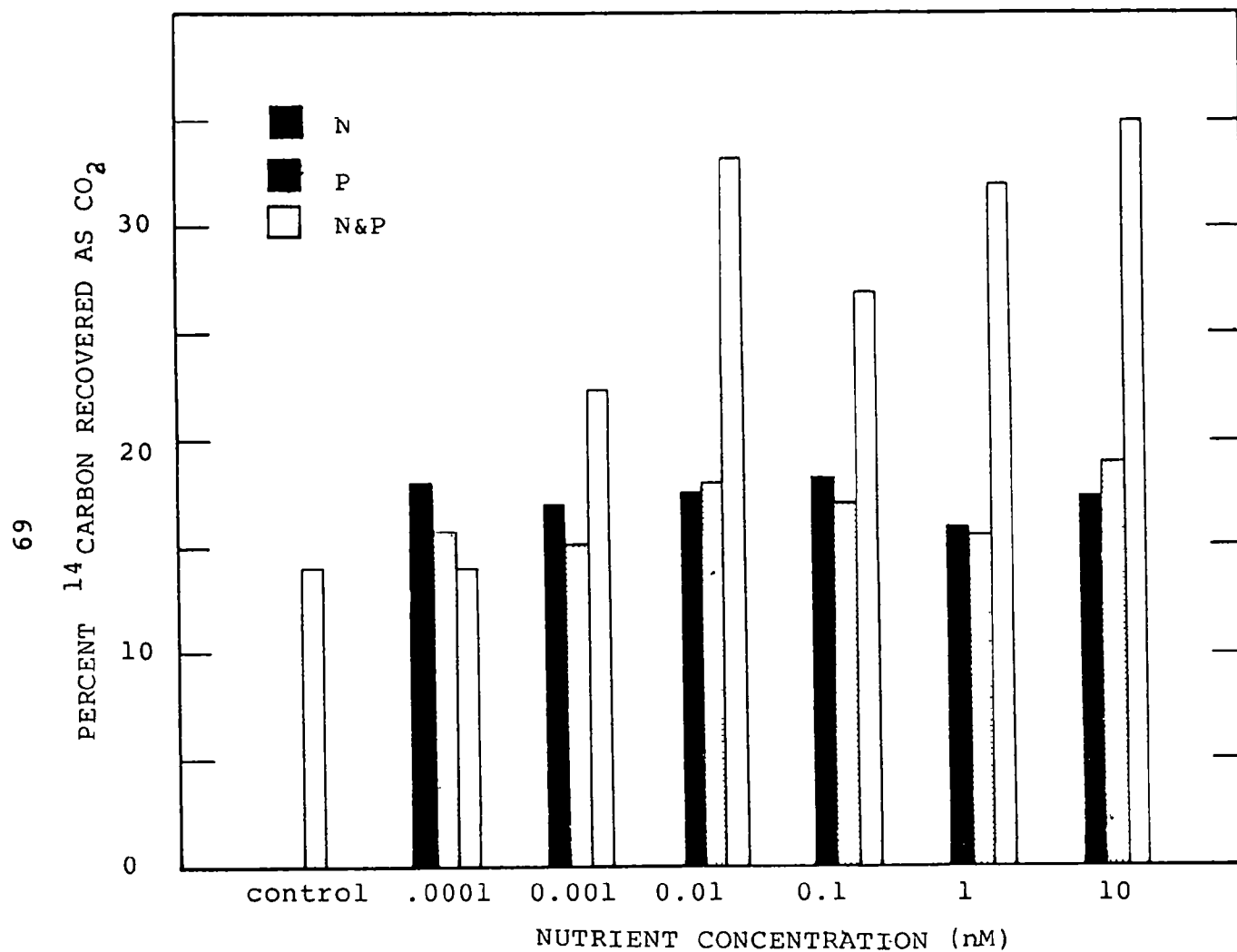
Figure 20. Mineralization of <u>Carex</u> $^{14}$C-cellulose labeled lignocellulose in response to different concentrations of added $NH_4NO_3$ (N), $Na_2HPO_4$ (P), and $NH_4NO_3$ plus $Na_2HPO_4$ (N&P) by Toolik Lake sediment after 16 days during the summer of 1979 (Federle and Vestal, 1980).

supported by the observation that phosphorous enrichment of oxygen-depleted treatments resulted in reversal of the inhibition caused by the lack of oxygen. These results indicate that nutrient availability is a significant factor in controlling cellulose biodegradation.

Addition of nitrogen had no effect on the amount of Carex lignin mineralized. However, addition of phosphorous resulted in mineralization which was significantly lower than the control (Figure 21). Phosphorous concentrations as low as 0.1μM caused this inhibition with the greatest inhibition observed at the highest phosphorous concentration (10μM). The antagonistic role of phosphorous in lignin mineralization may be of significance in understanding the increased proportion of lignin relative to cellulose in decomposing litter.

## Dichloroaniline (DCA)

Hsu and Bartha (1973) investigated the effects of glucose and $NH_4NO_3$ on the mineralization of chloroaniline residues. Table 4 shows that addition of a nitrogen source alone (50 mg $NH_4NO_3$/50g soil) had little effect on either total $CO_2$ or $^{14}CO_2$ evolution from radio-labeled DCA, but a massive amount of glucose (250 mg/50g soil) with a source of nitrogen (50 mg $NH_4NO_3$/50g soil) severely inhibited $^{14}CO_2$ production, though total $CO_2$ production increased greatly. It is not known whether this phenomenon reflects the decreased availability of $O_2$ in the soil due to the rapid oxidation of glucose.

TABLE 4.  $^{14}CO_2$ AND $CO_2$ PRODUCTION IN SOIL CONTAINING RADIO LABELED DCA-SOIL ORGANIC MATTER COMPLEX UNDER VARIOUS NUTRIENT CONDITIONS IN 21 DAYS (Hsu and Bartha, 1973)

| Conditions | $^{14}CO$: (mmol) | Total $CO_2$ (mmol) |
|---|---|---|
| Nutrients added | | |
| 30 mg glucose | $2.68 \times 10^{-3}$ | 3.82 |
| 250 mg glucose | $5.25 \times 10^{-3}$ | 7.02 |
| 50 mg $NH_4NO_3$ | $2.36 \times 10^{-3}$ | 3.29 |
| 250 mg glucose + 50 mg $NH_4NO_3$ | $3.74 \times 10^{-3}$ | 8.13 |

## Dimethylamine (DMA)

Tate and Alexander (1976) conducted a study to determine the effect of the C/N ratios on the accumulation of DMA during the biodegradation of trimethylamine (TMA), as shown in Table 5. This
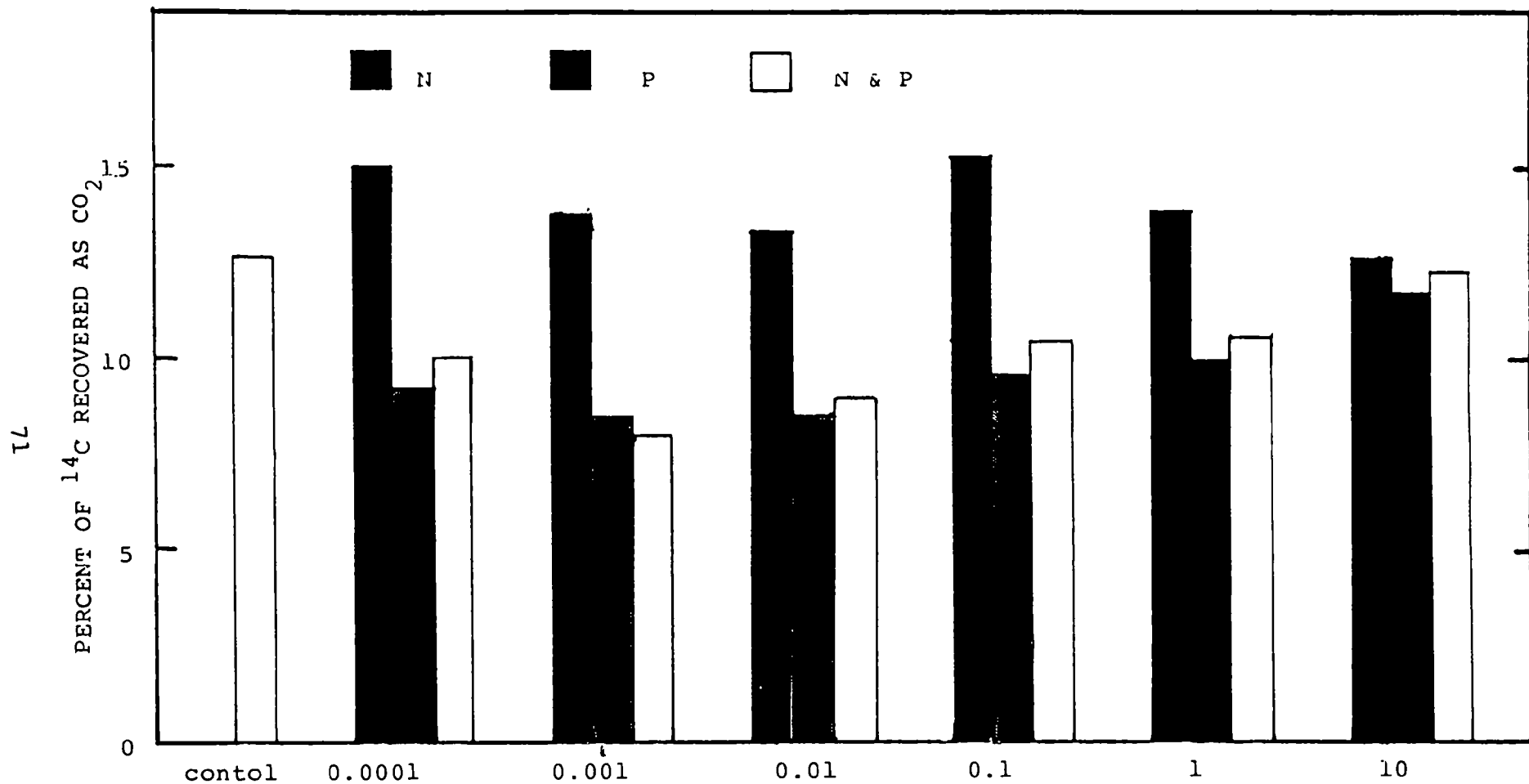
Figure 21. Mineralization of <u>Carex</u> $^{14}$C-lignin-labeled lignocellulose in response to different concentrations of added $NH_4NO_3$ (N), $Na_2HPO_4$ (P), and $NH_4NO_3$ plus $Na_2HPO_4$ (N&P) by toolik Lake sediments after 16 days during the summer of 1979 (Federle and Vestal, 1980).

study was performed using a laboratory culture and, although not necessarily directly applicable to natural ecosystems, suggest that the prevailing C/N ratio may not affect the accumulation of DMA resulting from the biodegradation of TMA. These results are surprising in view of the fact that the accumulation of DMA in cultures of Micrococcus sp. provided with TMA depended on the nitrogen sources available to the bacterium. It is speculated that nitrogen levels were not limiting in this study.

TABLE 5. EFFECT OF CARBON-NITROGEN RATIO ON DIMETHYLAMINE ACCUMULATION BY MICROCOCCUS SP. (Tate and Alexander, 1976).

| C-N ratio | Dimethylamine (μg/ml) |
|-----------|------------------------|
| 60:1 | 831 |
| 24:1 | 882 |
| 12:1 | 726 |
| 6:1 | 814 |
| 3:1 | 955 |
| 1:1 | 838 |

## Hexadecane and Mineral Oil

In studies designed to identify critical environmental parameters affecting hydrocarbon biodegradation in freshwater, Ward and Brock (1976) investigated nutrient limitation on mineral oil and hexadecane oxidation rates. Indigenous rates of hydrocarbon oxidation were found to be lower in lake water samples without addition of 300 μg of N per liter as $KNO_3$ and 100 μg of P per liter as $KH_2PO_4$. In fact, enrichment of lake water samples with nitrogen and phosphorus resulted in an increase in the rate of mineral oil and hexadecane oxidation of 2.7 and 27.7 times the indigenous rates, respectively. When either nitrogen or phosphorous was provided in excess, biodegradation rates were limited by the amount of the other nutrients, as indicated in Figure 22.

Figure 23 shows the correlation between mineral oil oxidation rates and dissolved inorganic phosphate levels. The data resemble a saturation relationship of the Michaelis-Menten type, and the line drawing in Figure 22 is a "statistical fit of the linear regression of a double-reciprocal plot of the same data." The authors suggest that, although quite high amounts of nitrogen and phosphorus may be necessary to balance the carbon used when substantial hydrocarbon degradation occurs, the rate of degradation by natural communities only becomes limited at quite low nutrient levels. In fact, substantial decreases in hydrocarbon oxidation rates did not occur until the indigenous nutrient levels decreased to levels near the limits of detection.

Atlas and Bartha (1972b) and Soli and Bens (1972) suggested that nitrogen and phosphorous would limit biodegradation of

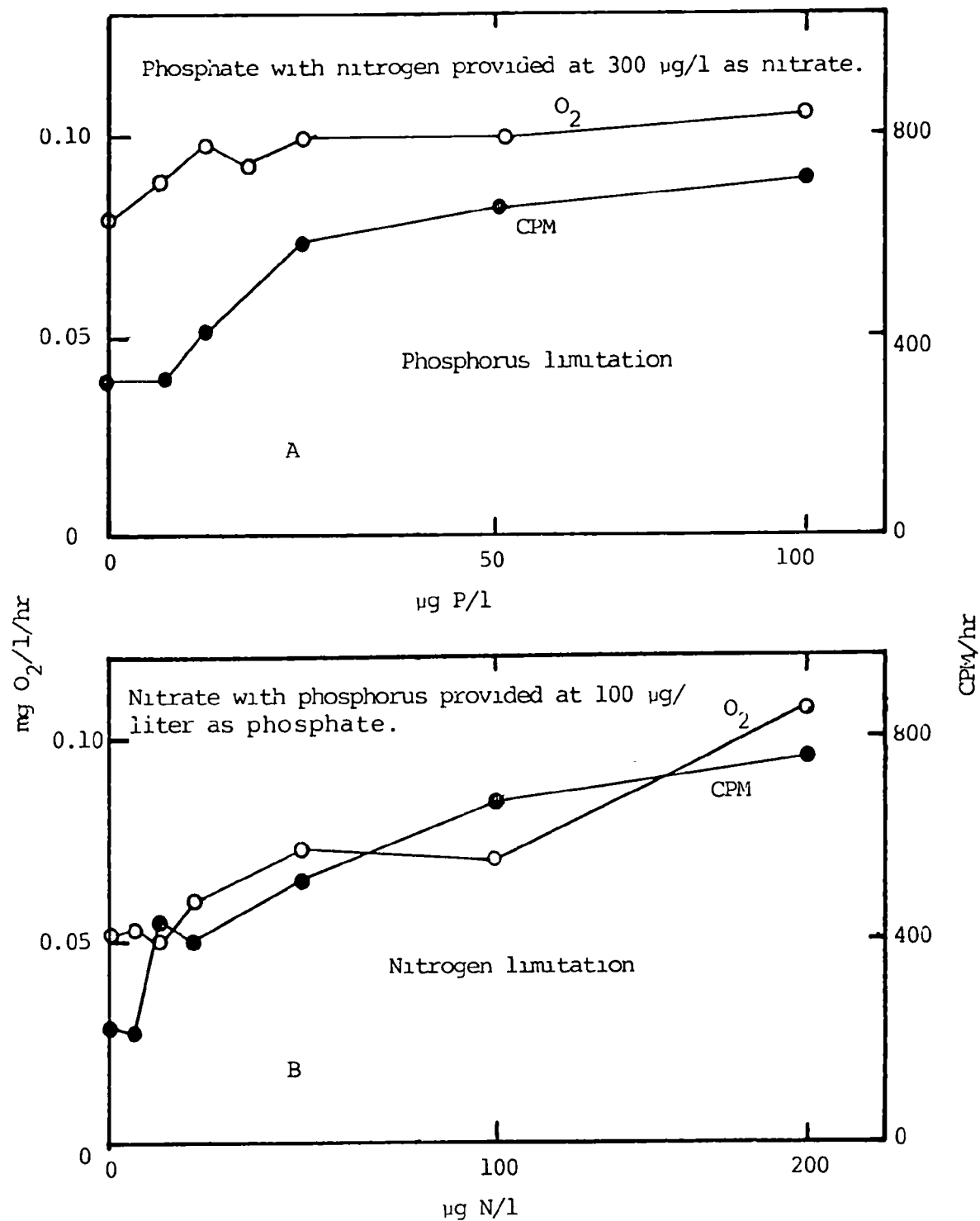Figure 22. Rates of oxygen uptake during mineral oil oxidation (O) and [14]$CO_2$ production during hexadecane oxidation (●) by Lake Mendota surface water samples. (Ward and Brock, 1976).
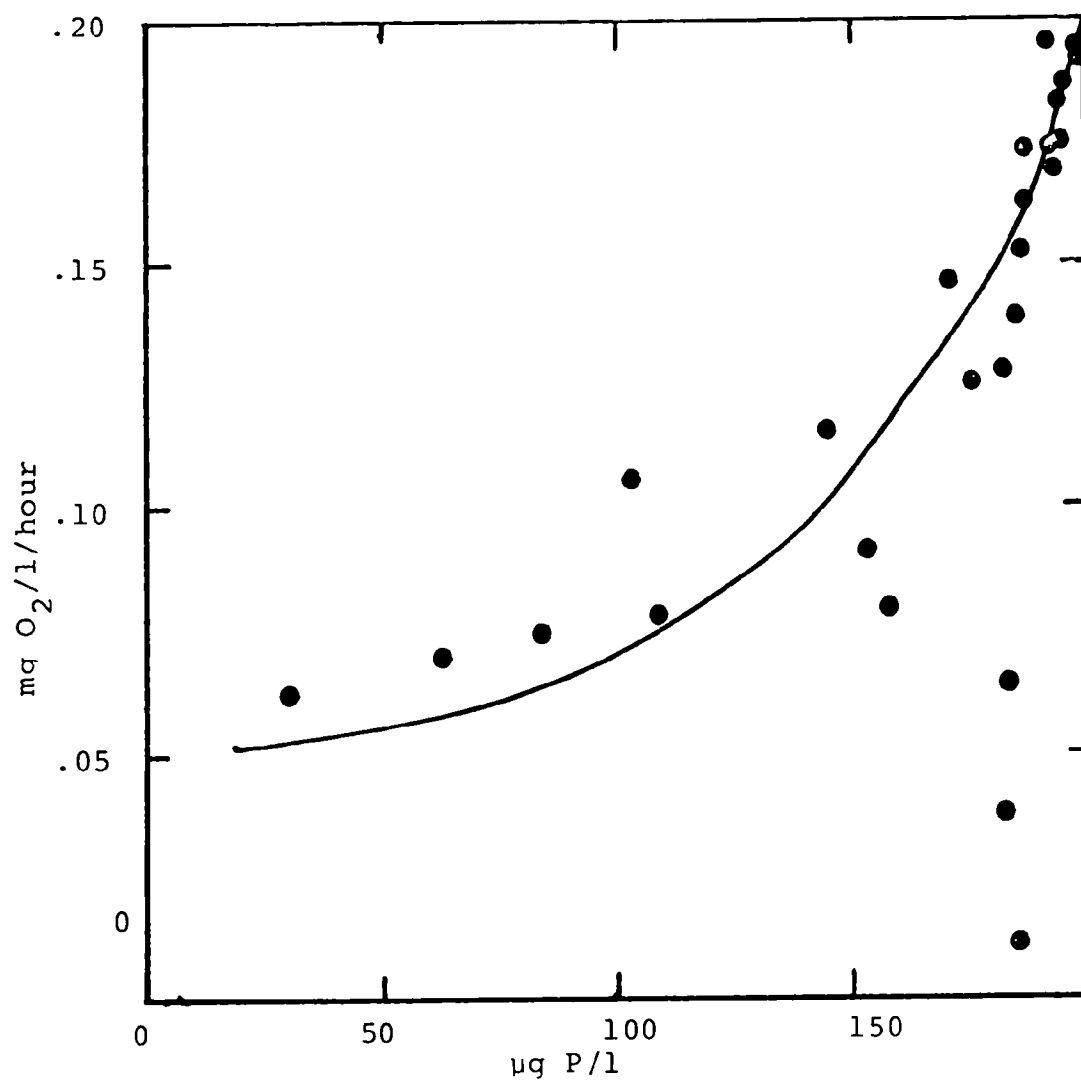
Figure 23. Correlation between the indigenous rates of oxygen uptake during mineral oil oxidation and the indigenous dissolved inorganic phosphate concentrations less than 2.5 µg of P per liter (Ward and Brock, 1976).

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hydrocarbons in a marine area. However, in a study by Mulkins-Phillips and Stewart (1974), the concentration of nitrogen occurring naturally in the marine environment did not significantly affect the rate of degradation of hexadecane. From the straight line portion of the curve in Figure 24, representing hexadecane disappearance, it was deduced that approximately 0.05 mg of elemental nitrogen was required to bring about the disappearance of 1 mg of hexadecane in the presence of adequate supplies of phosphorous. Growth of the organism followed a course similar to the hexadecane disappearance.

In contrast to the effect of nitrogen on the growth rate, Mulkins-Phillips and Stewart (1974) found that decreased phosphorous concentrations increased the generation time of Nocardia sp. (Table 6). The log of phosphorous concentration versus generation time yields a straight-line relationship (exponential) which indicates a more complex situation than that shown for nitrogen. The maximal population size was also affected by the concentration of phosphorous, indicative of a depletion of the available nutrient. The results indicate that the rate of natural biodegradation of oil in marine environments is limited by phosphorous concentration, but suggest that the concentration of nitrogen occurring in sea water (approximately 0.875 mg/l) is probably not rate-limiting.

TABLE 6. EFFECT OF PHOSPHOROUS CONCENTRATION ON THE GENERATION TIME (G) AND MAXIMAL POPULATION OF NOCARDIA SP. (PER mL) GROWN AT 15C FOR 14 DAYS ON 1% HEXADECANE (Mulkins-Phillips and Stewart, 1974)

| Mg of P/liter[*] | G (h) | Maximal Population |
|---|---|---|
| 970.0 | 10.1 | $8.8 \times 10^7$ |
| 97.0 | 11.3 | $4.8 \times 10^7$ |
| 9.7 | 13.5 | $7.6 \times 10^6$ |
| 0.97 | 15.0 | $6.4 \times 10^6$ |
| 0 | no growth | $4.0 \times 10^4$ |

[*] Phosphorus was added as $K_2HPO_4$ and $KH_2PO_4$

## Malathion

Merkel and Perry (1977) studied the effect of cosubstrate enrichment on the biodegradation of malathion. Their results showed that the addition of 0.1% n-heptadecane as cosubstrate increased the malathion production of $^{14}CO_2$ from radio-labeled to nearly three times that produced in the "control" culture. Glucose, glycerol, glycerophosphate, and a mixture of amino acids and peptides did not have an appreciable effect on the rate of
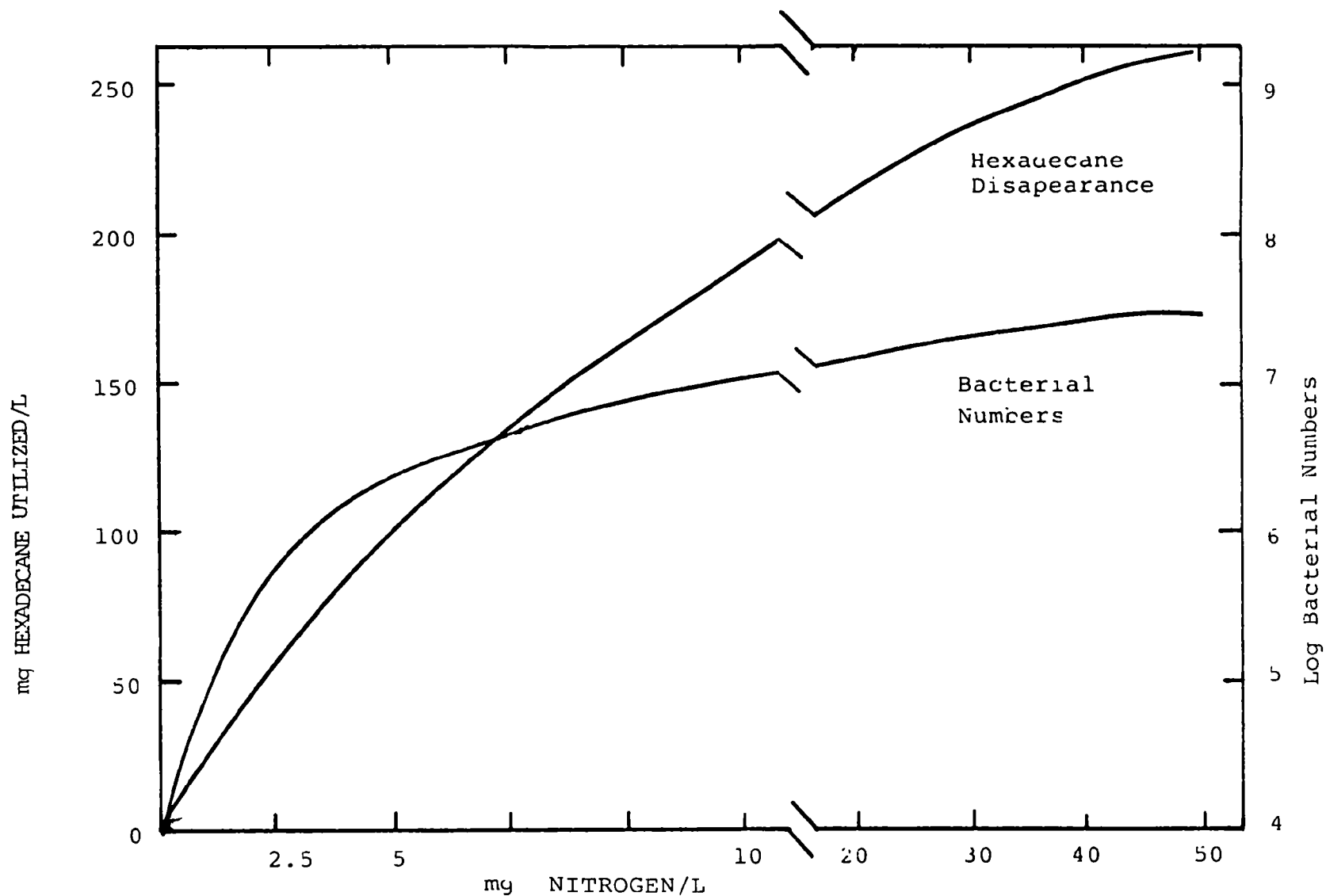
Figure 24. Effect of nitrogen concentration (source $NH_4NO_3$) on the disappearance of hexadecane brought about by <u>Nocardia</u> sp. at 15°C. Trials in triplicate (Mulkins-Phillips and Stewart, 1974).

$^{14}CO_2$ evolved from malathion. These results indicate that the rate at which recalcitrant compounds are biodegraded may depend also on the presence of selected organic compounds.

## Naphthalene

Strawinski and Stone (1955) conducted studies on the effects of the presence of copper sulfate and calcium chloride on the biodegradation of napthalene in a basal medium by a pure culture of the Pseudomonas group. It was found that the omission of either calcium chloride or copper sulfate, or both, tested after three subcultures in the deficient medium, resulted in a significant loss in yield (Table 7), demonstrating that both are necessary under these conditions for increasing the biodegradation of naphthalene .

Aranha and Brown (1981) studied the effects of nitrogen sources (i.e., $NH_4Cl$ and $KNO_3$) on the biodegradation of naphthalene in soil cultures. The results obtained indicate that additions of $NH_4Cl$ had a greater stimulatory effect on the biodegradation of naphthalene than was observed for $KNO_3$. This study suggests that the mineral nitrogen source may have a major influence on naphthalene degradation. However, the relevance of these findings to the biodegradation of other aromatic hydrocarbons remains to be determined. Confirmation of these results would be an important factor in obtaining a better understanding of hydrocarbon biodegradation as affected by the nitrogen source.

TABLE 7. EFFECT OF CALCIUM CHLORIDE AND COPPER SULFATE ON THE ACCUMULATION OF ETHER-SOLUBLE ACIDS DURING NAPHTHALENE OXIDATION[*] (Strawinski and Stone, 1955)

| Medium | Final pH | Yield | |
| --- | --- | --- | --- |
| | | g | % |
| No $CaCl_2$ | 5.8 | 0.35 | 14.0 |
| No $CuSO_4$ | 6.2 | 0.40 | 16.0 |
| No $CaCl_2$ or $CuSO_4$ | 5.9 | 0.03 | 1.2 |
| Control | 6.2 | 0.69 | 27.0 |

[*] 2.5 g. naphthalene in 1-liter flasks aerated.

## Parathion

Organic matter, either native or applied, is known to influence the persistence of pesticides applied to soil. By stimulating microbial activity in soil, the effect of organic carbon sources on

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the degradation of parathion via nitrogroup reduction and hydrolysis in flooded alluvial soil was investigated by Rajaram and Sethunathan (1975). The total carbon and nitrogen contents of organic sources used in this study are given in Table 8.

TABLE 8. CARBON AND NITROGEN CONTENTS OF ORGANIC SOURCES USED IN THE EXPERIMENT (Rajaram and Sethunathan, 1975).

| Organic Matter | Carbon (%) | Nitrogen (%) | C/N ratio |
|---|---|---|---|
| Glucose | 40.0 | -- | -- |
| Rice Straw | 34.0 | 0.59 | 58 |
| Farmyard manure | 24.1 | 1.51 | 16 |
| Algal crust | 12.8 | 1.37 | 9 |

The degradation of parathion via nitrogroup reduction was enhanced when the soil containing parathion was incubated with 0.5%-glucose, rice straw, algal crust, and farmyard manure under flooded conditions. The degradation followed the order glucose > rice straw > algal crust > farmyard manure > unamended. The greatest degradation occurred with glucose, with 99 percent of the insecticide being lost in 3 days (Table 9). During the same period, only 56 percent of the added insecticide was decomposed in unamended soil. At 18 days, the insecticide was not detected in all the amended soils, whereas 9 percent of applied insecticide still persisted in the unamended soil. It is suggested that organic amendments to the flooded soil apparently lowered the Eh of the soil and, thereby, permitted the rapid biodegradation of parathion.

TABLE 9. STIMULATORY EFFECT OF ORGANIC SOURCES ON THE DEGRADATION OF PARATHION VIA NITRO-GROUP REDUCTION IN FLOODED ALLUVIAL SOIL (Rajaram and Sethunathan, 1975)

| Incubation (days) | µg Parathion/20g soil recovered | | | | |
|---|---|---|---|---|---|
| | Unamended | Farmyard/ manure | Algal crust | Rice Straw | Glucose |
| 0 | 628 | 625 | 594 | 630 | 630 |
| 3 | 276 | 189 | 155 | 89 | 7 |
| 6 | 240 | 163 | 83 | 30 | 0 |
| 12 | 99 | 39 | 10 | 0 | 0 |
| 18 | 57 | 0 | 0 | 0 | 0 |

## Polychlorinated Biphenyl

Polychlorinated biphenyls (PCBs) are among the most per-sistent toxic substances found in the environment and are of great concern because of their widespread occurrence (Peakall, 1975). Extensive scientific literature addresses the metabolism of PCBs in living organisms and several excellent works concern-ing microbial degradation of PCBs have been published. Ahmed and Focht (1973) described the degradation of PCBs by two species of Achromobacter and the kinetics of PCB degradation by some fresh water bacteria have been investigated by Wong and Kaiser (1975). Tucker et al. (1975) and Baxter et al. (1975) reported that with certain multi-component commercial products, some PCB isomer mixtures were biodegraded more rapidly than if present as a single compound.

Liu (1980) investigated the stimulation of PCB biodegradation by sodium ligninsulfonate. It was reported that the rate of bio-degradation of monochlorinated Aroclor 1221 could be greatly enhanced by growing Pseudomonas sp. 7509 in a stable PCB-ligninsulfonate emulsion. The importance of using lignin-sulfonate to stabilize the PCB emulsion was reflected by its ability to stimulate the growth of Pseudomonas sp. 7509 growth rate as shown in Figure 25. It can be seen that Pseudomonas sp. 7509 rapidly used the stable Arochlor 1221-sodium ligninsulfonate emulsion as the sole carbon and energy source for growth. With-out ligninsulfonate, the emulsion tended to break down with consequent decline in both the growth rate and the rate of PCB biodegradation. However, sodium ligninsulfonate, itself, did not appear to be utilized in the earlier stage of biodegradation and it supported little microbial growth by itself. Liu (1980) concluded that the stimulation of PCB degradation by sodium ligninsulfonate was due to its inherent resistance to microbial attack, which enables a stable PCB emulsion to be maintained. This helped the cells to overcome the substrate-surface area limitation which might otherwise have been a major limiting factor governing the rate of subsequent PCB degradation.

Liu and Strachan (1981) investigated the effect of nitrogen enrichment in the growth medium on anaerobic PCB biodegradation (Table 10). The data show that yeast extract stimulated PCB degradation, in agreement with other studies (Watanabe, 1973; Suzuki, 1977). Peptone, however, tended to have some inhibitory effect on PCB biodegradability. Ammonium sulfate, ammonium nitrate and urea did not exhibit any significant stimulation or inhibition when compared with sodium nitrate, which was the nitrogen source in the basal medium. These results are not in general agreement with those obtained by Aranha and Brown (1981) in which hydrocarbon biodegradation was observed to be affected by the nitrogen source.
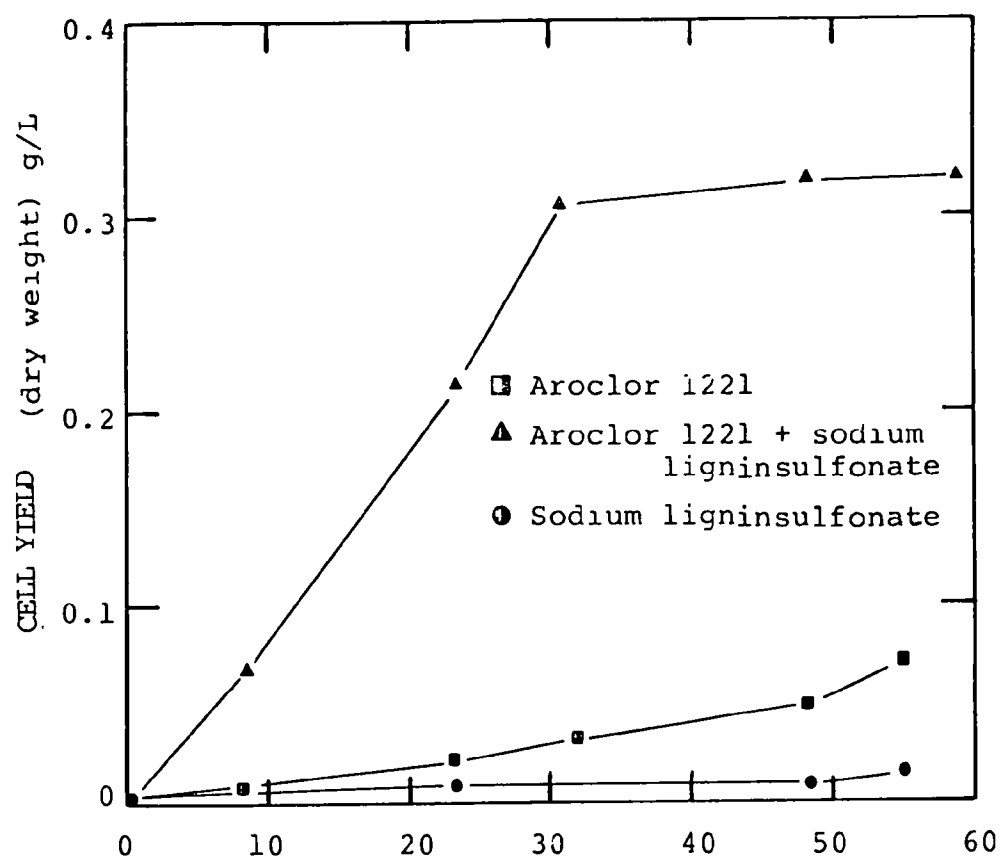
Figure 25. Effect of sodium ligninsulfonate on the growth of
Pseudomonas sp. 7509 (Liu, 1980).

TABLE 10.    DEGRADATION OF PCB IN FERMENTORS (Liu and Strachan, 1981)

| Reaction Conditions | Induction Period (days) | Degradation | |
|---|---|---|---|
| | | $t\frac{1}{2}$ (day) | $k(h^{-1}) \times 10^2$ |
| Aerobic metabolism PCB | 2.1 | 0.36 | 7.4 |
| Aerobic co-metabolism PCB | 2.5 | 0.52 | 5.1 |
| Anaerobic metabolism PCB | 13.0 | 190 | 0.014 |
| Anaerobic co-metabolism PCB | 20.0 | >200 | -- |
| Nitrogen source* | | | |
| $NaNO_3$ | 1.2 | 0.63 | 4.2 |
| $(NH_4)_2SO_4$ | 1.2 | 0.75 | 3.5 |
| $NH_4NO_3$ | 1.2 | 0.85 | 3.1 |
| Urea | 1.2 | 0.61 | 1.9 |
| Peptone | 1.9 | 1.2 | 2.3 |
| Yeast extract | 1.2 | 0.36 | 7.3 |

*  Experiments were carried out under anerobic metabolism conditions.

Trichlorobenzene (TCB)

Early studies (Chamber et al., 1963; Malaney and McKinney, 1966) concluded that trichlorobenzene (TCB) biodegradation is negligible.  Garrison (1969) suggested volatilization as the major mechanism of TCB removal from water, but Gaffney (1976) found di- and trichlorobenzenes in river sediments, indicating that TCBs can persist in such environments.  Simmons et al. (1976) reported mineralization of 1,2,4-TCB in activated sludge, with reduction in TCB volatility in the high-organic sludge environment. The biodegradation rates of the individual TCB isomers, and the influence of nutrient additions on TCB degradation were investigated by Marinucci and Bartha (1979).

Glucose and benzene caused a 20 to 50% increase in the bio-degradation rate compared with a control.  Dichlorohexane and dichlorophenol, used primarily to enrich for dechlorinase activity, did not appreciably effect the biodegradation rate.  The biodegrada-tion rates in the dichlorobenzene and forest litter treatments were slightly lower than the control rate.

Soil organic matter (compost) had little direct influence on TCB mineralization, but a significant abiotic effect of high organic matter content was the reduction in volatility of both TCBs.  In a field disposal situation, this would reduce volatility losses and allow the relatively slow microbial degradation processes a longer time period to act.

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The kinetics of TCB biodegradation suggest rate-limiting reactions of the cometabolic type. However, in these experiments none of the substrates added to soil increased TCB biodegradation to a significant extent.

## Hydrilla sp. Weed

Due to the lack of sound quantitative information on plant decay rates, a study was performed to derive a rate function for plant degradation by bacteria (Waite and Kurucz, 1977). Bacteria capable of degrading plant material were isolated and introduced to samples of the rooted hydrophyte Hydrilla sp. Figure 26 shows the dependency of the degradation rate constant on nitrate concentration. It can be seen that the rate appears to be inversely proportional to nitrate concentration, at least in the concentration range utilized in these experiments. It is not clear why this occurred, as one should expect an increase in biodegradation activity with increasing nitrate. It is speculated that nitrate levels were high enough to adversely affect the degradation process. The model proposed shows good agreement with observed data, but is limited to nitrate-nitrogen levels lower than 1 mg $l^{-1}$.

## Summary

The concentration of available nutrients is an important environmental factor affecting the extent and rate of biodegradation. Nutrients may or may not be limiting depending on the availability of readily biodegradable carbonacous substrates. Nutrients may also exhibit inhibitory effects on biodegradation.

Several studies suggested that nitrogen and phosphorus may limit biodegradation of hydrocarbons in marine and fresh water environments. However, one study demonstrated that the concentration of nitrogen occurring naturally in sea water would not affect the rate of biodegradation of a petroleum hydrocarbon. In general, the available data indicate that low concentrations of phosphate in marine environments are the principal factors limiting petroleum biodegradation, providing adequate aeration and favorable temperatures are provided.

Several studies have shown that PCB isomers are biodegraded more rapidly in a mixture than by themselves. It has also been observed that the addition of biphenyl enhances the biodegradation of some PCB's. The stimulation of PCB biodegradation in the presence of ligninsulfonate has been demonstrated, and it was indicated that this enhancement is due to the PCB-ligninsulfonate emulsion's increased susceptibility to microbial attack and its support of microbial growth. It has also been shown that nitrogen enrichment stimulates the biodegradation of PCBs.

A study on the effect of cosubstrate enrichment for malathion degradation by addition of n-heptadecane indicated a significant increase in the biodegradation rate. This study lends support to
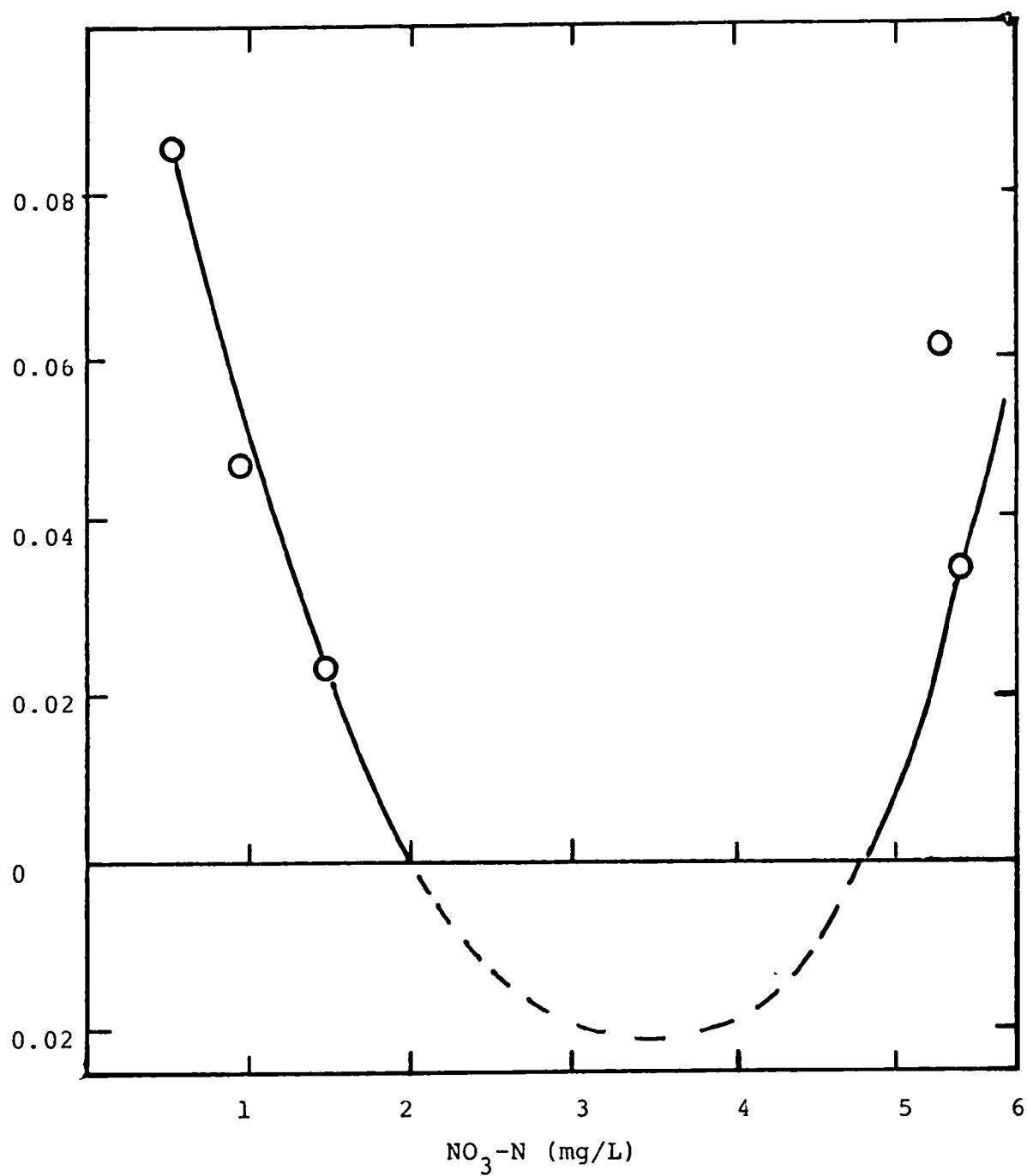
82

Figure 26, Dependence of decay rate on $NO_3$-N concentration (Waite and Kurucz, 1977).

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the supposition that the biodegradation of recalcitrant compounds may be influenced by the addition of selected organic compounds. It has also been shown that cosubstrate enrichment for biodegradation of other pesticides may either inhibit or stimulate biodegradation. Several studies indicate that nitrogen sources may have a significant effect on the biodegradation of certain hydrocarbons.

TOXINS/INHIBITORS

The toxic and inhibitory effects of heavy metals and organic toxins on specific microorganisms have been described in the literature. However, definitive information on the effect of inorganic and organic toxins on biodegradation is greatly lacking.

Even though many heavy metals are essential for growth, they are also reported to have toxic effects on cells, mainly as a result of their ability to denature protein molecules. As a result of the chemical and biological factors that affect the toxicity of a heavy metal, a given concentration of that metal may be inhibitory under a given set of conditions and non-inhibitory under other conditions. It is not surprising, therefore, that a significant amount of data in the literature is apparently contradictory, in that certain concentrations are reported to be harmless. In many cases, these differing effects of concentration are due to specific environmental conditions of the microbial community.

A substantial body of literature exists that describes toxic, antagonistic, and synergistic effects of heavy metals, inorganic anions, and organic chemicals on conventional biological treatment processes. Biological treatment can be accomplished in a number of ways, but the basic characteristic of the system is the use of a mixed (heterogeneous) bacterial culture for the biodegradation of organic materials. By defining the effects of inorganic and organic toxins on the efficiency of common biological treatment processes (or biodegradation processes), one can better speculate on the effects of such toxins on microbial communities and biodegradation processes in other natural systems. A summary of the major inhibitory effects of specific inorganic and organic elements and compounds is given in Tables 11 and 12. The following summarizes the currently available data on the inhibitory effects of specific elements and compounds on biological treatment.

Summary of Inhibitory Properties of Inorganic Substances

Ammonia--
At excessively high levels (480 mg/l), ammonia exhibits inhibitory effects on the activated sludge process (EPA, 1973). At concentration levels of 1500 to 3000 mg/l, ammonia is inhibitory to anaerobic digestion (Pohland and Kang, 1971).

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TABLE 11.  THRESHOLD CONCENTRATIONS OF INORGANIC POLLUTANTS
          THAT ARE INHIBITORY TO BIOLOGICAL TREATMENT PROCESSES.
          (U.S. EPA, 1977)

| POLLUTANT | CONCENTRATION (mg/l) | | |
|---|---|---|---|
| | ACTIVATED SLUDGE PROCESSES | ANAEROBIC DIGESTION PROCESSES | NITRIFICATION PROCESS |
| Ammonia | 480 | 1500 | |
| Arsenic | 0.1 | 1.6 | |
| Borate (Boron) | 0.05-100 | 2 | |
| Cadmium | 10-100 | 0.02 | |
| Calcium | 2500 | | |
| Chromium (Hexavalent) | 1-10 | 5-50 | 0.25 |
| Chromium (Trivalent) | 50 | 50-500 | |
| Copper | 1.0 | 1.0-10 | 0.005-0.5 |
| Cyanide | 0.1-5 | 4 | 0.34 |
| Iron | 1000 | 5 | |
| Lead | 0.1 | | 0.5 |
| Manganese | 10 | | |
| Magnesium | | 1000 | 50 |
| Mercury | 0.1-5.0 | 1365 | |
| Nickel | 1.0-2.5 | | 0.25 |
| Silver | 5 | | |
| Sodium | | 3500 | |
| Sulfate | | | 500 |
| Sulfide | | 50 | |
| Zinc | 0.08-10 | 5-20 | 0.08-0.5 |

Note:  Concentrations shown represent influent to the unit processes
       in dissolved form.

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TABLE 12.   THRESHOLD CONCENTRATIONS OF ORGANIC POLLUTANTS THAT
            ARE INHIBITORY TO BIOLOGICAL TREATMENT PROCESSES
                          (U.S. EPA, 1977)

| | CONCENTRATION (mg/l) | | |
|---|---|---|---|
| POLLUTANT | ACTIVATED SLUDGE PROCESSES | ANAEROBIC DIGESTION PROCESSES | NITRIFI- CATION PROCESSES |
| Alcohols | | | |
| Allyl | | 100 | 19.5 |
| Crotonyl | | 500 | |
| Heptyl | | 500 | |
| Hexyl | | 1000 | |
| Octyl | | 200 | |
| Propargyl | | 500 | |
| Phenols | | | |
| Phenol | 200 | | 4-10 |
| Creosol | | | 4-16 |
| 2-4 Dinitrophenol | | | 150 |
| Chlorinated Hydro- carbons | | | |
| Chloroform | | 10-16 | |
| Carbon Tetrachloride | | 10-20 | |
| Methylene Chloride | | 100-500 | |
| 1-2 Dichloroethane | | 1 | |
| Dichlorophen | | 1 | |
| Hexachlorocyclohexane | | 48 | |
| Pentachlorophenol | | 0.4 | |
| Tetrachloroethylene | | 20 | |
| 1,1,1,-Trichloroethane | | 1 | |
| Trichloroethylene | | 20 | |
| Trichlorofluoromethane | | 0.7 | |
| Trichlorotriflouroethane (Freon) | | 5 | |
| Allyl Chloride | | | 180 |
| Dichlorophen | | | 50 |
| Organic Nitrogen Compounds | | | |
| Acrylonitrile | | 5 | |

(Continued)

TABLE 12.   (Continued)

| POLLUTANT | CONCENTRATION (mg/l) | | |
| --- | --- | --- | --- |
| | ACTIVATED SLUDGE PROCESSES | ANAEROBIC DIGESTION PROCESSES | NITRIFI-CATION PROCESSES |
| Organic Nitrogen Compounds (Continued) | | | |
| Thiourea | | | 0.075 |
| Thioacetamid | | | 0.14 |
| Analine | | | 0.65 |
| Trinitrotoluene (TNT) | 20-25 | | |
| EDTA | 25 | | 300 |
| Pyridine | | | 100 |
| Surfactants | | | |
| Nacconol | 200 | | |
| Ceepryn | 100 | | |
| Miscellaneous Organic Compounds | | | |
| Benzidine | 500 | 5 | |
| Thiosemicarbazide | | | 0.18 |
| Methyl isothiocyanate | | | 0.8 |
| Allyl isothiocyanate | | | 1.9 |
| Dithio-oxamide | | | 1.1 |
| Potassium thiocyanate | | | 300 |
| Sodium methyl dithiocarbamate | | | 0.9 |
| Sodium dimethyl dithiocarbamate | | | 13.6 |
| Dimethyl ammonium dimethyl ι dithiocarbamate | | | 19.3 |
| Sodium cyclopentamethylene dithiocarbamate | | | 23 |
| Piperidinium cyclopentamethylene dithiocarbamate | | | 57 |
| Methyl thiuronium sulphate | | | 6.5 |
| Benzyl thiuronium chloride | | | 49 |

(Continued)

TABLE 12. (Concluded)

| POLLUTANT | CONCENTRATION (mg/l) | | |
| --- | --- | --- | --- |
| | ACTIVATED SLUDGE PROCESSES | ANAEROBIC DIGESTION PROCESSES | NITRIFI-CATION PROCESSES |
| Miscellaneous Organic Compounds (Contd.) | | | |
| Tetramethyl thiuram momosulphide | | | 50 |
| Tetramethyl thiuram disulphide | | | 30 |
| Diallyl Ether | | | 100 |
| Dimethyl-paranitrosoaniline | | | 7.7 |
| Guanidine carbonate | | | 19 |
| Skatole | | | 16.5 |
| | | | 7.0 |
| Strychnine hydrochloride | | | 175 |
| 2 chloro–6 trichloro-methyl-pyridine | | | 100 |
| Ethyl urethane | | | 250 |
| Hydrazine | | | 58 |
| Methylene blue | | | 100 |
| Carbon disulphide | | | 35 |
| Acetone | | | 840 |
| 8-hydroxyquinoline | | | 73 |
| Streptomycin | | | 400 |

Arsenic--
     A level of 0.1 mg/l sodium arsenate (arsenic concentration
0.04 mg/l) showed no effect on oxygen uptake, while levels of
1.0 mg/l sodium arsenate and 0.1 mg/l arsenic trichloride
depressed oxygen uptake about 50% (Goss, 1969).

Cadmium--
     Cadmium had no adverse effect on the activated sludge pro-
cess up to a concentration of about 1 mg/l.  In the range of
10 to 100 mg/l, a decrease in BOD removal efficiency and re-
duction in oxygen uptake was observed (Goss, 1969).  Synergestic
effects have been reported for cadmium and zinc, as well as
cadmium and manganese.  other heavy metals may also show syn-
ergistic effects with cadmium.

Chromium--
     Chromium has a stimulatory effect on growth at a concen-
tration level of 0.005 to 0.05 mg/l (Rudolphs et al., 1950).
Interference with biological processes is reported at a concen-
tration level of 1 mg/l (Rudolphs, et al., 1979) to 10 mg/l
(Reid, 1968).  The published literature is contradictory with
respect to the toxicity of chromium in the concentration range
of 1 to 50 mg/l, ranging from serious interference to insignifi-
cant effects (U.S. Dept. of HEW, 1965).  In the range of 50 to
500 mg/l, synergistic effects of chromium with acidity, iron,
and copper have been reported (Rudolphs et al., 1950; Goss, 1969;
U.S. Dept. of HEW, 1965).

Copper--
     Synergism of copper with cyanide, acidity, and other heavy
metals has been reported.  Antagonism of copper with sulfide,
high pH, and certain chelating agents such as EDTA have been
identified (Loveless and Painter, 1968).  Over the range of
copper concentration of 0.1 to 10 mg/l, digestor problems
attributed to the presence of copper were reported that may
have been due to antagonistic or synergestic effects (Rudolphs
et al., 1950).

Cyanide--
     Cyanide concentrations of 5 mg/l have been found to inter-
fere with the activated sludge process (Rudolphs et al., 1950).
It has been reported that the toxicity of copper and nickel
are enhanced by the presence of cyanide (U.S. Dept. of HEW, 1965).

Iron--
     Iron is a necessary element for microbiological growth and
its absence causes a reduction in metabolic activity (Pfeifer
and White, 1964).  It is reported that 1000 mg/l stops oxygen
uptake (Goss, 1969).  A specific effect of iron synergism with
chromium has been reported (EPA, 1976).  Antagonistic effects
may be anticipated with sulfide and hydroxyl ions.

Lead--
    Moderate toxicity of lead to microorganisms has been reported
for concentrations ranging from 0.1 to 1 mg/l.  A significant
effect on oxygen uptake in the presence of lead is noted at a
concentration level of 10 to 29 mg/l (Rudolphs et al., 1950).

Manganese--
    Synergestic effects of manganese with zinc and cadium have
been reported.  Oxygen uptake was completely inhibited at 50 mg/l
of manganese (Goss, 1969).

Mercury--
    Mercury at a concentration of 0.1 mg/l was reported to re-
duce the oxygen uptake by 10% in the activated sludge process
(Brinsko, 1974).  For concentrations ranging from 1 to 200 mg/l,
there are numerous reports of different degrees of inhibitory
effects on the activated sludge process (Zugger and Ghosh, 1972;
Goss, 1969).  In experiments performed by Marinucci and Bartha
(1979) involving the biodegradation of trichlorobenzene (TCB)
in a soil environment, consistently more $^{14}CO_2$ was evolved from
radio-labeled TCBs when incubated in normal as compared with
HgCl or $NaN_3$ poisoned soil (Table 13).  A comparison of 1,2,3-
and 1,2,4-TCB biodegradation rates show that 1,2,4-TCB was degrad-
ed two to three times as fast as 1,2,3-TCB in active soil.

TABLE 13.  MINERALIZATION OF TCBs IN BIOLOGICALLY ACTIVE VERSUS
           POISONED SOIL

| TCB | Study | Mineralization rate (nmol/day per 20 g of soil) | | |
| | | Active soil | Poisoned soil | Poison (1%) |
|---|---|---|---|---|
| 1,2,3- | A | 0.33 ± 0.13 | 0.09 ± 0.12 | $HgCl_2$ |
| | B | 0.38 ± 0.35 | 0.16 ± 0.35 | $NaN_3$ |
| 1,2,4- | A | 1.09 ± 0.12 | 0.05 ± 0.12 | $HgCl_2$ |
| | B | 0.93 ± 0.35 | 0.20 ± 0.35 | $NaN_3$ |
| | C | 1.37 ± 0.13 | 0.04 ± 0.13 | $NaN_3$ |

Nickel--
    Various adverse effects on oxygen uptake for the activated
sludge process have been reported for nickel concentrations of
2.5 to 200 mg/l (Rudolphs and Amberg, 1952; Barth et al., 1965;
Goss, 1969).

Silver--
Silver is extremely toxic to microorganisms. A concentration of 5 mg/l causes a 84% inhibition of the activated sludge process. At the 25 mg/l level, inhibition is complete (Goss, 1969).

Sulfide--
Excessive levels of sulfide (25 to 50 mg/l) interfere with the activated sludge process by depleting the oxygen supply (EPA, 1973).

Zinc--
Adverse inhibitory effects have been reported for zinc for 0.08 to 0.5 mg/l range (EPA, 1973). Synergistic effects have been observed for zinc and cadmium, and zinc and manganese (Goss, 1969).

Summary of Inhibitory Properties of Organic Substances

Phenols--
Phenol slug doses of 200 mg/l can deactivate activated slug and other aerobic treatment plants by killing the biomass (Beychok, 1967).

Chloroform--
Continuous doses of chloroform at 16 mg/l or more in raw sludge feed are reported to cause inhibition of anaerobic digestion. Doses at concentrations of between 10 and 15 mg/l produced a noticeable drop in gas yield during anaerobic digestion (Stickley, 1970; Ghosh, 1972). Another investigation (Swanwich and Foulkes, 1971) found a 50% gas reduction due to 0.96 mg/l of chloroform.

Carbon tetrachloride--
Carbon tetrachloride can inhibit anaerobic digestion at levels of 10 mg/l (Jackson and Brown, 1970). In fact, a 50 percent reduction in methane production was reported at 2.2 mg/l (Swanwick and Foulkes, 1971).

Methylene Chloride--
Methane production in anaerobic digestion was reduced by 50 percent in the presence of 100 mg/l of methylene chloride (Swanwick and Foulkes, 1971).

Pesticides--
One laboratory test revealed that two pesticides, aldrin and simazine, were not inhibitory to the growth of nitrifying bacteria, Nitrobacter, whereas five other pesticides including chlordane, heptachlor, lindane, CIPC, and DDD prevented growth. Heptachlor was the most deleterious compound (Winely and Clemente, 1970).

The inhibition of linuron degradation by diazinon was reported by Keckes and Cserhati (1977). A concentration of 30 ppm applied to carbonaceous soil containing 30 ppm linuron decreased the decomposition rate by 90% compared to a control sample.

Acrylonitrile--

Inhibitory effects of acrylonitrile on anaerobic digestion are reported at 5 mg/l (Jackson and Brown, 1970). Another investigation (Lank and Wallace, 1970) reports that more than 20 mg/l of acrylonitrile in sludge is not harmful to anaerobic digestion.

Surfactants--

Laboratory tests with the anionic surfactant Nacconol (at 100 mg/l) showed a stimulatory effect on the activated sludge process. At concentration levels greater than 200 mg/l, inhibitory effects were noted. These effects were worse at low pH (about 5) and low sludge loadings (Manganelli, 1948).

In another laboratory test with the cationic surfactant Ceepryn, 100 mg/l of this material suppressed oxygen uptake. The effect was more deleterious at high pH (About 9) (Manganelli, 1948).

## Summary

The inhibitory, antagonistic and synergistic effects of inorganic and organic substances on biological treatment processes have been described. Inhibitory concentrations of specific elements and compounds reported in the literature are often contradictory. However, in consideration of the many chemical and biological factors that may affect inhibition, it is not surprising that certain concentrations of a given substance have been reported as being inhibitory, while higher concentrations have been reported as harmless.

## WATER AVAILABILITY

The effects of water availability on biodegradation in aqueous and non-aqueous environments are discussed in this section.

## Aqueous Environment

Water availability in the aqueous environment is usually expressed in terms of water activity, osmotic pressure, ionic strength or salinity. In early work, the water content of the environment was the widely used measure of water availability. As Mossell (1975) and Corry (1978) have pointed out, this is not very satisfactory, as the properties of, for example, food systems, can vary significantly though being of similar water content. Another measure popular with early workers was osmotic

pressure. This has been discussed by Brown (1976), who commented on the difficulty of accurately measuring the osmotic pressure in many systems. Scott (1977) suggested that a suitable measure of water availability was the water activity, $a_w$, of the equilibrium system. The effect of ionic strength and salinity on water availability is usually measured in terms of their influence on $a_w$ values.

The water requirements of microorganisms can be expressed quantitatively in the form of the water activity ($a_w$). Water has an $a_w$ value of 1.000; this value decreases when solutes are dissolved in water. Microorganisms can grow in media with $a_w$ values between 0.99 and about 0.63. Bacteria generally require media of higher $a_w$ value (0.99-0.93) than either yeast or molds (Rose, 1976). The range of $a_w$ from just below 1.00 to 0.96 covers the normal fresh water, brackish water, and sea water environments in which most microorganisms exist.

A variety of microorganisms exist that have their growth optima at an $a_w$ close of 0.99, but are capable of growth at a low $a_w$, i.e., 0.60. These are classified as xenotolerant organisms (Brown, 1976).

An organism's response to a low $a_w$ can depend on whether the reduction in $a_w$ in the external environment is due to high salt levels or high non-electrolyte levels. This is not surprising as the ionic strength of the external medium would be expected to affect the charge distribution on the cell surface (Reid, 1980).

For any one organism, the important values are the optimum and minimum $a_w$ values. These have been determined for a number of microorganisms, and they seem to be remarkably constant for a particular species. As other environmental factors (e.g., temperature, pH, $pO_2$, ionic and nutritional composition) deviate from their optimum, the range of tolerance of organisms decreases. The general effect of lowering the $a_w$ value of the medium below the optimum is to increase the length of the lag phase and to decrease the growth rate. Water activity exerts its most obvious effects on biodegradation when it falls below a critical $a_w$ value. Water activity $a_w$ values may be applied as a predictive tool to indicate if biodegradation by specific microorganisms is possible or unlikely. A large volume of literature exists on the effects of water activity ($a_w$) on the growth of microorganisms; however, data are lacking on the effect of this environmental factor on biodegradation.

The cell-water interface is essentially an ionic one, and small changes in the ionic composition induce large changes in microbial physiology. Ionic strength affects osmotic pressure, or differences in ionic or solute concentrations on opposite sides of the microbial cell membrane.

In addition to affecting osmotic pressure, high salt con-
centrations tend to denature proteins, i.e., disrupt the terti-
ary structure which is essential for enzymatic activity.
Changes in ionic composition are directly influenced by other
physiochemical factors (e.g., pH, Eh, temperature, atmospheric
composition) as well as the metabolic activity of the microbes
themselves. In addition to the overall ionic strength of the
environment, the type, charge, valence, and size of the pre-
dominant ions probably influence microbial events and thus
biodegradation. Data on the affects of ionic strength on bio-
degradation were not found in the literature reviewed.

There is also a scarcity of information available on the
effect of salinity on biodegradation. A pertinent study by
Bourquin and Przybyszewski (1977) reported on the effect of
salinity on the biodegradation of nitrilotriacetate (NTA) by
a estuarine bacteria. A known NTA-degrading bacterium, isolated
from freshwater streams, Pseudomonas sp., was grown on 0.1%
NTA as a sole carbon source in the presence of various NaCl
concentrations. Figure 27 shows that NTA degradation was in-
versely related to salinity under these conditions. Biodegrad-
ation of NTA by the organisms was completely inhibited at 20%
salinity. The organism grew readily during the first 24 hours
of incubation in freshwater medium, but a 48-hr and 72-hr lag
occurred in 10 and 15% salinity media, respectively. It is im-
possible to speculate on the effect of salinity on the biodegrad-
ation of other compounds because of lack of pertinent data.

## Non-Aqueous Environment

Water availability may be more critical in non-aqueous
environments than in aqueous environments. In addition to the
water quality (e.g., ionic strength, salinity, etc.) concern
for aqueous environments, water availability in non-aqueous
environments (such as soils) can also be affected by secondary
factors such as medium structure (especially porosity and parti-
cle size), weather conditions (e.g., temperature, humidity,
precipitation, etc.), and composition of the medium (Gray et
al., 1968).

The pore space in a non-aqueous medium is either filled
with air, as in dried soils, or filled with water, as in water-
logged soils. In the first case all life processes practically
cease, in the latter case anaerobic processes predominate. The
air-water ratio in the pore space is of utmost importance for
determining the type of biological processes occurring in non-
aqueous environments.

Water in the soil environment can be classified as gravita-
tional, capillary, osmotic, and hygroscopic. Not all of
these fractions of water are available for microbial activities.
In order to utilize water, a growing organism must expend the
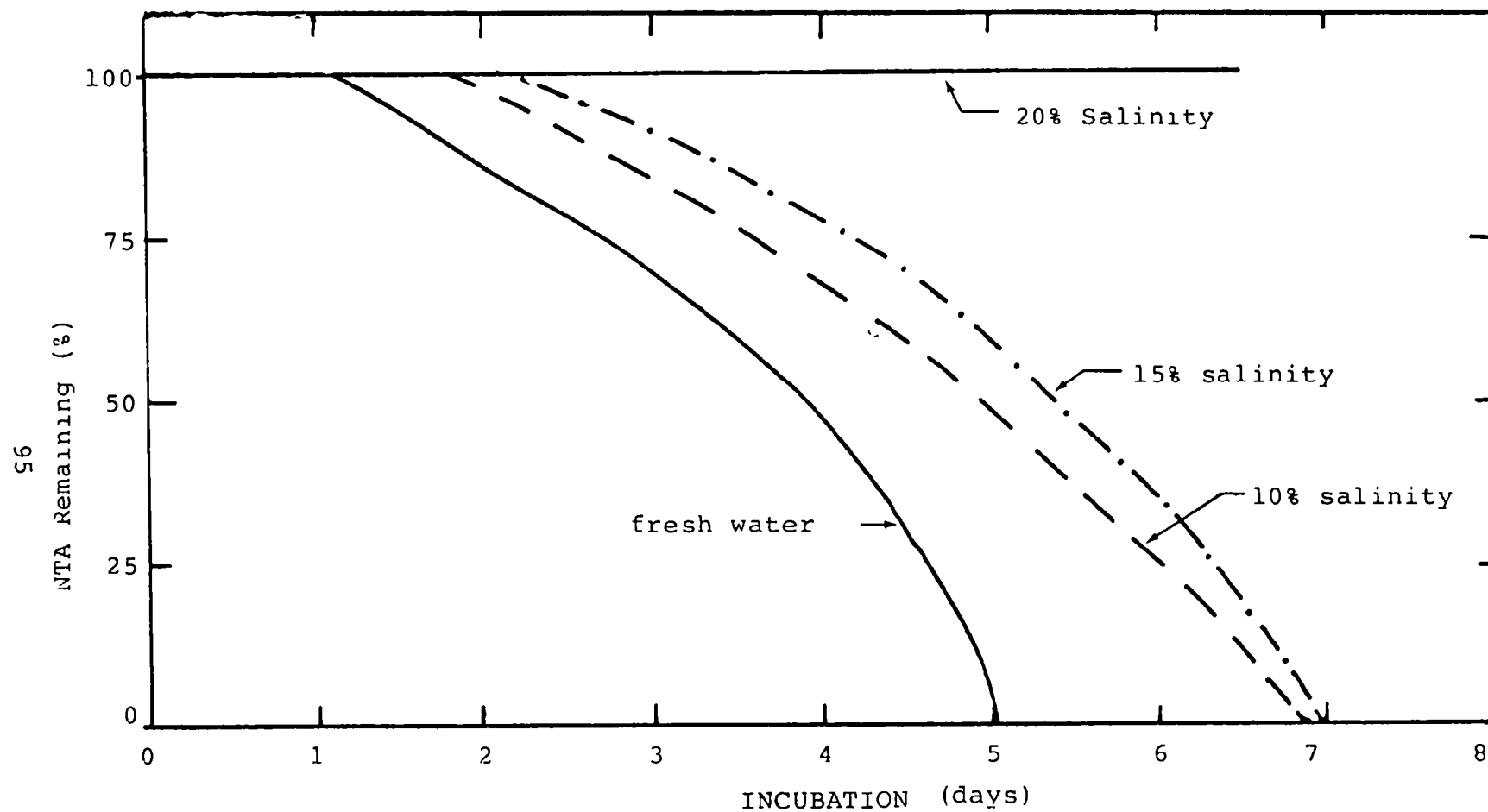
Figure 27. Effect of salinity on the rate of NTA degradation by Pseudomonas sp. All cultures were incubated in BS medium plus 0.1% NTA with CaCl added to the desired salinity (Bourquin and Przybyszewski, 1977).

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energy or pressure required to remove water from soil. Much depends on the types and sizes of the soil particles, the characteristics of the aggregates, and on the concentration of the ions in the water phase. Gray et al. (1968) showed that the pressure required for removing gravitational water is relatively low, in the range of 0 to 0.3 atm. But for capillary, osmotic, and hydroscopic waters, the pressures required are as high as 0.3 to 15, 15 to 150, and above 150 atm, respectively.

A review of the literature revealed that no information is available on the availability of the above-mentioned water fractions for microorganisms. In soil environments, water availability is usually measured in one of two ways: total moisture content or percent field capacity.[*][†] Gravitational water, as mentioned previously, is equivalent to water content that exceeds the field capacity.

In general, biodegradation rates increase with increasing moisture content up to a certain moisture level. Walker (1974), in his biodegradation study of napropamide, found that the biodegradation rate increased exponentially with soil moisture content, as can be expressed by the following equation:

$$k = mM^n$$

where k is the biodegradation rate in terms of $day^{-1}$; M is the percent moisture content by weight; and m and n are constants. For napropamide, the m and n values are 0.0037 and 0.550, respectively. A soil moisture effect study was conducted by Lay et al. (1975) for the biodegradation of propanil. The authors found that when the soil moisture content was about 1.5% (by weight), the residual propanil was 27 to 30% of that originally present. But when the soil moisture content increased to 5.5 and 12.6% (by weight), residual propanil was reduced to 18 and 9.6%, respectively. Similar trends in the effects of soil moisture content on the biodegradation of parathion were also found by Gerstle et al. (1979). The authors found that with three soil moisture contents (14, 24, and 34%, by volume), the rate of parathion biodegradation rose with increasing water content.

Moisture exerts its most obvious effect when it falls below some critical value. Howard et al. (1979) reported that for the biodegradation of litter such a critical value may be as high as 27 to 100% moisture content (by weight). Above the critical value, the effects of moisture on biodegradation are

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[*] Field Capacity: The maximum moisture content that a soil or a solid material can retain in a gravitational field without producing continuous downward percolation.

[†] Percent moisture content or field capacity can be expressed based on either weight or volume.

insignificant. A formula similar to the Michaelis-Menten Equation was suggested as a means of expressing the effects of moisture on biodegradation rates (Flanagan et al., 1976; Bunnell et al., 1977; and Howard et al., 1979):

$$c = \frac{M}{a_1 + M} \times \frac{a_2}{a_2 + M}$$

where c is the correction factor for the biodegradation rate; M is the moisture content; $a_1$ is the moisture content at which biodegradation is half its "optimal" value; and $a_2$ is the moisture content at which gas exchange is half its "optimal" value.

In contrast to the above-mentioned trend in the effect of moisture content on biodegradation, Nakas et al. (1979) found that in field testing, the decomposition of bacterial and fungal cell walls demonstrated an inverse relationship with soil water content (Figure 28). However, because several other critical environmental factors associated with the tests were undisclosed, the correct interpretation of such data is indeed difficult. It is speculated that other factors (such as temperature, pH, etc.) might have had a more pronounced influence on biodegradation than that of moisture content. Thus, the results of Nakas et al. (1979) may not reflect the effects of water content as indicated.

Other researchers have selected field capacity (or water-holding capacity) as an indicator of water availability in soil environments. Gray et al. (1968) found that microbial activity increased with increasing water content to the critical values of about 60 to 80% field capacity.* Zimdahl et al. (1977) reported that at zero percent field capacity, trifluralin did not appear to degrade. However, the extent of biodegradation was positively correlated with soil water content. After three weeks, biodegradation of trifluralin was more extensive at 100% of field capacity than at lower levels. After 3 months, the ranking of the effects of water content on biodegradation was 100% > 50% > 20% = 0% of field capacity. At 6 months, the ranking was 100% > 50% > 25% > 0% of field capacity. Helweg (1979) reported that the biodegradation of 2-aminobenzimidazole increased exponentially from 28% to 94% of field capacity. He also found that when the water content was too high, biodegradation rates decreased. Marinucci et al. (1979) found that a lowering of the soil moisture level from 66% to 40% of field capacity did not affect significantly 1,2,3-TCB biodegradation, but decreased the biodegradation rate of 1,2,4-TCB from 1.09 to

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* Unless otherwise indicated, percent of field capacity mentioned in this report is on a weight basis.
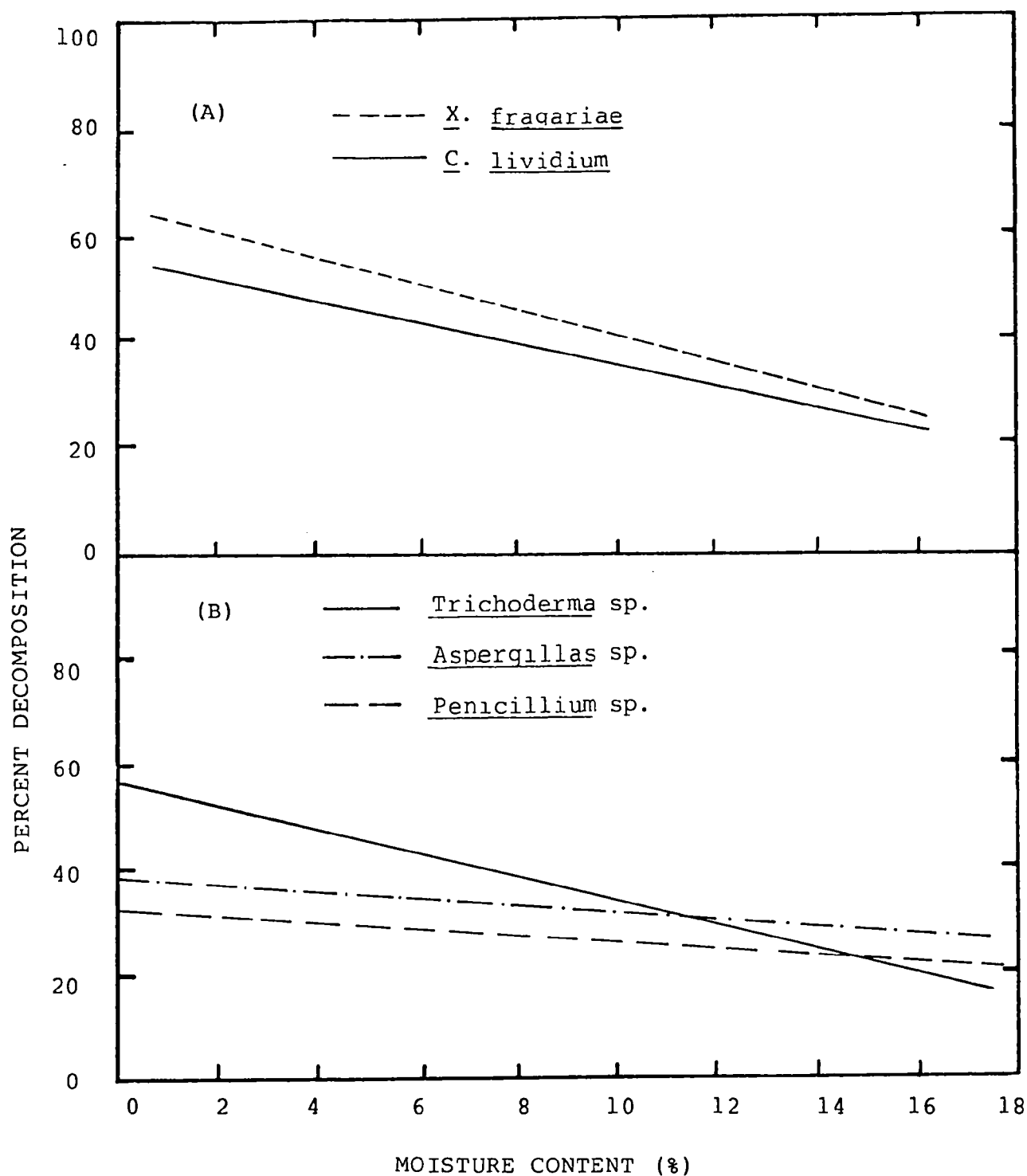
Figure 28. Biodegradation of (A) bacterial cell wall, and
(B) fungal cell wall in a semi-arid grassland
soil (Nakas et al., 1979).

0.31 nmol/day. Helweg (1981), in a study of maleic hydrazide biodegradation, reported that at soil moisture contents between the wilting point and 80 to 90% of field capacity, the degradation rate doubled with an increase in moisture content of 50% of field capacity. Above field capacity, the degradation rate was either unchanged or decreased. Below the wilting point of the soil, the degradation rate was extremely low.

Although water availability in the non-aqueous environment is an essential factor for biodegradation, researchers have yet to derive a theoretically based model. Detailed knowledge of the influence of important secondary factors, such as soil structure, particle size, or reduction of the water availability through osmotic and adsorption processes, is greatly lacking. Another concern is the choice of indicators of water availability. Measurements of the moisture content of soils do not relate to the ability of organisms to absorb and utilize soil water (Gray et al., 1968). An energy or pressure indicator, such as vapor pressure, or terms representing other characteristics of the medium, water composition, and organism types may have to be incorporated into the quantitative evaluation of the effects of water availability on biodegradation of chemicals.

# SECTION 5

## BIOLOGICAL ENVIRONMENTAL VARIABLES

### OVERVIEW

In addition to the constraints imposed upon biodegrading populations by the physical and chemical environment, the activity of neighboring populations acts as an additional external factor affecting the dynamics of growth and physiological behavior (Gause, 1934). In theory (Slater and Bull, 1978), for microbial populations in a continuous culture acting upon a growth-limiting substrate, one organism can competetively displace all others since the chance of two organisms having identical maximum specific growth rates, substrate affinities, energy requirements, and growth yields is exceedingly small. However, the real world does not ordinarily behave as a chemostat with a single, growth limiting substrate. According to Slater and Bull (1978) it is common, especially where xenobiotics are being studied, to find communities of microorganisms with two types of populations: primary and secondary. Primary species are those which metabolize the primary substrate, whereas secondary species cannot grow on the primary substrate but rely on metabolites of the primary species and/or lytic products to sustain their growth.

The importance of microbial interactions in affecting chemical biodegradation is obvious, e.g., xenobiotics which can be metabolized by a single microbial species will persist if the species is eliminated by another organism. Yet despite the wealth of literature on microbial interactions, relatively little is known about interactions between microbes in situ. Most of the information has been derived from laboratory studies on simple mixed populations, which precludes an evaluation of higher order interactions between multiple microbial species and also between the microbiota and the various physicochemical environmental factors. However, because of the apparent importance of interactions between microbes to their ecology and population dynamics, a brief discussion of various types of interactions is presented.

In some instances, mathematical relationships have been developed in attempts to model basic microbial interactions. The models are, as yet, useful only for specific sets of circumstances (e.g., specific microbial populations, substrates, and environmental conditions), and describe only population dynamics.

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Because of the complexity of microbial interactions, it is difficult to be certain that simple interactions have not been obscured by other interactions. For these reasons, mathematical expressions describing microbial interactions which can be used in explaining biodegradation phenomena are generally unavailable.

## MICROBIAL INTERACTIONS

### Basic Types of Interactions

The association of two or more microbes is usually defined in terms of their nutritional needs. These associations can be described by a few basic types of interactions between hypothetical populations A and B, coexisting in a natural environment. There are, in fact, only three possible responses a growing population, e.g., A, can make in the presence of population B. These are (Slater and Bull, 1978):

1.  The growth of population B may have a beneficial or positive effect on the growth of population A;

2.  The presence of organism B could have a detrimental or negative effect on organism A; and

3.  The growth of population B may have no effect on the growth of population A. A neutral response of this kind would be shown by similar growth patterns for population A whether or not population B was present.

These basic responses by A concerning B are, of course, reciprocal, inasmuch as the presence of organism A can evoke a similar or different response from organism B. The various combinations between the two populations may be summarized in the simple matrix indicated below (Table 14).

TABLE 14.  MATRIX OF INTERACTION OF TWO MICROBIAL POPULATIONS A
           AND B (Slater and Bull, 1978)

| | | The effect on the growth of A by the activity of organism B | | |
| --- | --- | --- | --- | --- |
| | | +<br>(positive) | 0<br>(neutral) | −<br>(negative) |
| The effect on the growth of organism B by the activity of organism A | +<br>(positive) | ++ | +0 | +− |
| | 0<br>(neutral) | 0+ | 00 | 0− |
| | −<br>(negative) | −+ | −0 | −− |

For two membered mixtures, Table 14 illustrates nine different interactions between the organisms, and indicates a maximum of six basic types of interactions, defined as follows:

1. Neutralism (oo)

2. Mutualism (++)

3. Commensalism (+0 or 0+)

4. Amensalism (-0 or 0-)

5. Prey-predator relationships (+- or -+)

6. Competition (--)

In reviewing the above list, it is initially apparent that neutralism or non-interaction between component species can be excluded from the list because of its insignificance with regard to microbial interaction. Though a limited number of studies (e.g., Lewis, 1967) have indicated that neutralism does occur, the growth of any microbial population will induce changes in that environment which will likely affect the growth of the second population. The remaining interactions listed above will be described in this section.

Mutualism (++)--
In this type of interaction, each member in a mixture derives some benefit from the other's presence, either in the form of increased growth rate or increased population size. The types of relationships from which both species benefit are particularly varied. Interactions can range from a loose cooperation which is not essential for the survival of the interacting species, to an obligate association on which both species depend for their continued survival (symbiosis). Alternatively, two organisms may interact to degrade a compound or produce a product that either was incapable of degrading or producing independently. Such a relationship is termed synergism. Observed interactions often do not fall nearly into these categories and usually show the characteristics of more than one type of interrelationship.

Reviewed literature indicated that few studies have positively identified a symbiotic or synergistic microbial degradation of xenobiotic compounds. Gunner and Zuckermann (1968) demonstrated the synergistic microbial degradation of diazinon, but the utilization of diazinon for growth was not demonstrated. Daughton and Hsieh (1977) maintained growth of a multispecies community for more than 2 years in a continuous culture with parathion as the sole substrate. Since competition, if the only interaction between species, would necessarily lead to mutual exclusion of all but one species (Jannasch and Mateles, 1974), the authors assumed a symbiotic interaction existed. This assumption proved correct when it was shown that parathion

was hydrolyzed by a strain of Pseudomonas stutzeri and the resultant p-Nitrophenol was utilized by a strain of P. aeruginosa. Neither of the species could perform each other's role, but they could grow symbiotically on parathion in batch and continuous culture. Therefore, P. stutzeri probably grew on metabolic products from P. aeruginosa.

A mutualistic relationship demonstrated by Yeoh et al. (1968) involved a two-membered mixed culture of Bacillus polymyxa and Proteus vulgaris grown in a carbon-limited chemostat in a simple growth medium which could not sustain either population alone. That the populations were able to exist together under these conditions indicated a dependence of one population on the other to complement its minimum growth requirements. But instead of organisms existing in steady-state in the continuous culture, regular oscillations in the populations were observed, which were attributed to a third interaction between the two populations. Evidently P. vulgaris produced a proteinaceous compound which inhibited the growth of B. polymyxa and caused a decrease in its population size. This in turn reduced the rate of biotin addition to the environment and, as its concentration declined, it could not maintain the original Proteus population size which also went into decline. At a later state the concentration of the inhibiting protein was lowered sufficiently to cause a resurgence of the B. polymyxa population, completing the cyclical changes of the two populations, which were then repeated. This example illustrates one of the major difficulties in population interaction studies; i.e., it is difficult to be certain that all the contributing interactions have been recognized or that the postulated simple interactions have not been obscured by other interactions. These difficulties become particularly acute in attempting to construct quantitative models with mathematical formulations of the interactive forces between different populations.

Commensalism (+0 or 0+)--
Many bacteria produce substances that promote the growth of other species. Such a phenomenon is probably the most widespread form of commensalism. In these situations, the microorganism producing the substances that others use as food may or may not have received benefit (generally means growth) from the substance it degraded or transformed. If no benefit was received by the degrading species, the species is said to have cometabolized the degraded substance.

A number of studies have appeared to demonstrate cometabolic degradation mechanisms that ultimately lead to complete degradation of the subject chemical. In such cases, one or more microorganism carries out an initial transformation of the chemical, with the product of this reaction serving as a growth substrate for the other population(s). Thus a microorganism unable to use a given substrate may live commensally because it obtains

assimilable products that the heterotrophic associate(s) generate during the degradation of the substrate in question. Extensive research on the microbial degradation of DDT exemplifies the cometabolic degradation of xenobiotics. Barker et al. (1965), Johnson et al. (1967), and Ledford and Chen (1969) have demonstrated that microorganisms can catalyze modest changes in the DDT molecule, converting it to 1, 1-dichloro-2, 2-bis (p-chlorophenyl) ethene (DDD) and bis (p-chlorophenyl) methane (DDM). Research by Focht and Alexander (1970) disclosed that DDM could be further degraded by a strain of Hydrogenomonas. Pfaender and Alexander (1972) further demonstrated that the action of two different bacteria resulted in the extensive biodegradation of DDT, the enzymes of Hydrogenomonas sp. converting DDT to PCPA, and those of Arthrobacter sp. degrading PCPA.

There are other ways by which the growth of one population may stimulate the growth of a cohabitant species. For example, one section of the population may remove a substance which is toxic to another or one species may convert an otherwise unavailable substrate into a form which is useful to its commensalistic partner. Alteration in the physicochemical properties of the microhabitat (e.g., changes in pH, Eh, osmotic pressure, ionic strength) during growth of one or more unrelated species can enhance development of the other species. The transfer of plasmids and genetic recombination in bacteria can be considered a type of commensalism, as the recipient may derive an ecological advantage, such as resistance to inhibitors and greater nutritional capabilities. Although such transfers have not been demonstrated unequivocably in soil, this form of commensalism would require close proximity between the commensal and the donor. In many other forms of commensalism (e.g., nutritional, detoxification, changes in environment), the actions of the independent species probably influence commensals in relatively distant microhabitats, especially if the chemical factors involved are volatile. Although mathematic expressions of the population dynamics of commensal relationships have been developed, the expressions are highly specific and merely serve to explain experimental results.

Amensalism (-0 or 0-)--
An amensal relationship occurs when one population is restricted by the presence of a second, which is unaffected by the metabolism of the inhibited populations. This kind of harmful interrelationship may be caused either by the removal of essential nutrients or by the formation of toxic products, or through non-specific effects, such as the lowering of the dissolved oxygen tension or changes in the hydrogen ion concentration (Slater and Bull, 1978). Although there may be no direct effect on the inhibiting population, there may well be an indirect advantage because, by limiting the assimilation of growth resources by the affected populations, a greater proportion

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of these materials can be made available for growth of the inhibiting population.

Predator-Prey Relationships (+- or -+)--

These relationships cause one member of a mixed culture (predator) to benefit at the expense of a second member (prey), the latter representing nutrition for the predator. In open-growth systems or continuous culture experiments, oscillations of the two populations are typically established, wherein the growth of the predator population lags behind increases in the prey population. A number of mathematical models have been constructed to describe population fluctuations. However, other laboratory studies have demonstrated that steady-state conditions can be achieved in prey-predator interactions, which acts to discredit the modelling efforts or limit their applicability. Dent et al. (1976) have concluded that oscillations in predator-prey populations, as predicted by the Lotka-Volterva model, are apparently not observed because the prey-predator mixed culture is "a rather more complex microbial ecosystem composed of a variety of physiological and ecological interrelationships." More recently, Yaron (1981) demonstrated that the interaction of Bdellovibrio with its prey can be affected by the presence of other microorganisms regardless of whether they serve as prey for Bdellovibrio. Different bacteria affected the predator-prey interaction in different ways: some "competed" with the original prey for the predator; others enhanced the activity of the predator toward the original prey, and still others inhibited it. This case again illustrates the major difficulties in modelling population interactions: it is difficult to know whether all contributing interactions have been recognized or that simple interactions have not been obscured by other interactions.

The effects of predation, as well as other microbial interactions, extend beyond the population dynamics of the microbes involved. Predation, despite the effect of depression of microbial populations, apparently stimulates mineralization (Barsdate et al., 1974), and can accelerate decomposition by releasing nutrients tied up on microbial biomass (Stout, 1974). Hunt et al. (1977) developed a model which simulated the effect of predation on bacteria in a continuous culture. The mcdel was fitted to data from chemostats on the chemical composition of bacteria growing in C-, N-, and P-limiting media. The model's performance was reasonably successful, and suggested (Figure 29) that predation effects major changes in the C/N/P (carbon/nitrogen/phosphorus) ratios of media, thus influencing the availability of the nutrients for microbial activity and biodegradation. Notice in Figure 29, however, that the glucose substrate level increased substantially as the predation rate increased and biomass decreased.

More recent attempts have been made to examine the effects of bacterial predation in ecosystems without distinguishing the
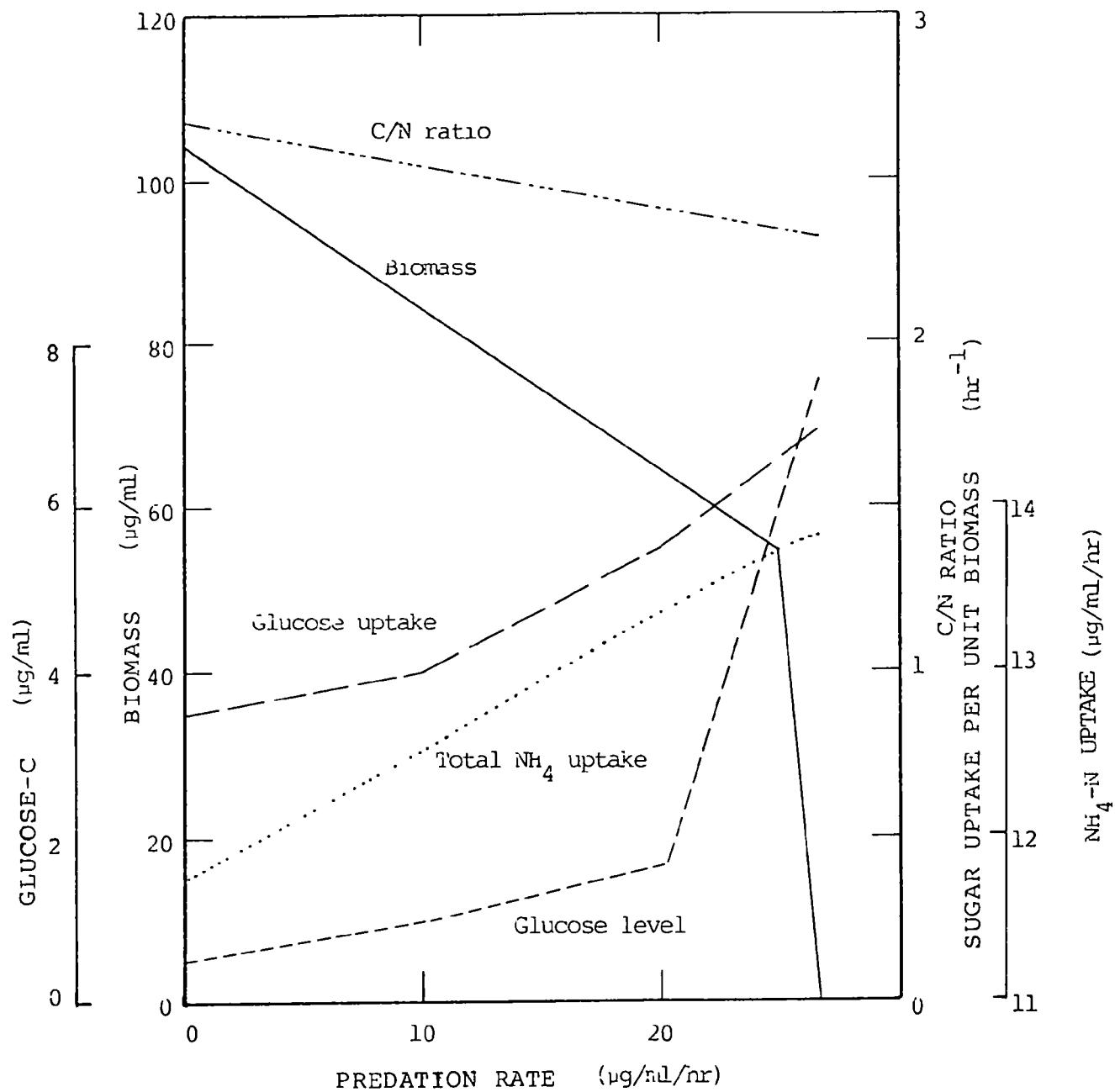
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Figure 29. Simulated effect of predation on C-limited bacteria in a chemostat at a dilution rate of 0.45. The predator returns no nutrients to the bacteria (Hunt et al., 1977)

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specific activities of individual microbes.  In developing mathematical models of biodegradation, practical approaches examining the bulk impact of the total microfauna can eliminate complex microbial interactions.  DeLeval and Menacle (1979) estimated the extent of microfaunal predation by batch culture and continuous culture techniques, without distinguishing the specific activities of microorganisms.  Bottles of river water were prepared with and without predators.  In the bottles without predators, productivity was estimated by:

$$P = B_t - B_o \text{-----------------------------------------------} (5)$$

Where:

$P$ = bacterial productivity (mg C $\ell^{-1}$ $t^{-1}$)

$B$ = bacterial biomass (mg C $\ell^{-1}$) of the water filtered through a 8 μm filter

$B_o$= bacterial biomass where T = O

$B_t$= bacterial biomass where T = t(h)

While in bottles with predators

$$P = N_t - N_o + G \text{----------------------------------} (6)$$

$N$ = bacterial biomass (mg C $\ell^{-1}$) of the river water

$N_o$= bacterial biomass when T = O

$N_i$= bacterial biomass where T = t(h)

$G$ = predation (mg C $\ell^{-1}$ $t^{-1}$)

Thus, combining results from bottles with and without predators, i.e., equations (5) and (6):

$$G = B_t - B_o - N_t + N_o$$

Results indicated that the bacterial population is not greatly influenced by predation but should be limited by other environmental factors.

Competition (- -)--
    Competition may be defined as a situation in which the populations of two species are mutually limiting because of a joint dependence on a common factor or factors external to them.  The effects of competition on microbial population dynamics have been demonstrated in a number of studies (Powell, 1958; Tempest et al., 1967; Brunner et al., 1968), the majority of which suggest that a

rapid growth rate can give one species the competitive advantage over another.

Carbon substrates, mineral nutrients, growth factors, $O_2$, water, light and possibly space can become limiting factors for which species within the same microhabitat compete (Alexander, 1971). The extent of competition probably varies between microhabitats, depending on their structure, available nutrients, and other physical and chemical properties. Many environmental factors (e.g., pH, Eh, temperature) probably alter the competitive ability of species, primarily by influencing their metabolic rates and pathways. For example, van Gemerden (1974) found that the relative abundance of large photosynthetic bacterium (Chromatium weissei) in natural habitats, normally unable to compete with a smaller species (Chromatium vinosum), was attributable to variations in light: in continuous light, with sulfide as the growth rate-limiting substrate, the specific growth rate of C. vinosum exceeds that of C. weissei, and the latter is unable to compete successfully and is washed out in continuous cultures. With intermittent light-dark illumination, the organisms showed balanced coexistence when grown in continuous cultures. The "steady-state" abundance of C. vinosum was found to be positively related to the length of the light period, and that of C. weissei to the length of the dark period.

A number of kinetic models have been developed which describe competitive interactions between microbes in an open or continuous culture environment (growing populations are continuously supplied with a restricted quantity of a required nutrient). In such a system, the organism with the lower growth rate is eventually eliminated from the culture. The growth rates of the component species are influenced, of course, by the affinity of an organism for the limiting substrate. In most cases the kinetic models can have a general application to any competitive situation occurring between two or more organisms under nutrient-limited conditions in an open (continuous culture) environment.

Although there is little doubt that competition is a major phenomenon in soil and aquatic ecosystems, conclusive evidence for most of the mechanisms, in situ, is not available. Many of the data have been derived from simple laboratory experiments in which most environmental factors have been kept constant. In such studies, it is often difficult to distinguish between competition and amensalism.

ADAPTATION

An important characteristic of heterotrophs and autotrophs is their ability to acquire new physiological and/or morphological traits which enable them to operate under a new set of environmental conditions or metabolize a previously unmetabolizable substrate. This process of adaptation permits the microorganims

to survive in a habitat which has been significantly altered and in which it could not have survived if its physiological or morphological needs had not changed. Adaptation may be expressed subtly, such as in the synthesis of a new enzyme or set of enzymes, or may be linked to the formation of additional morphological features. Adaptive capacity is of considerable ecological significance. For in the event that populations are eliminated or relegated to a minor position when environmental conditions are altered, adaptable organisms can acclimate to the prevailing temperature, salinity, light intensity, or newly available organic substrate.

The occurrence of adaptation in nature and in laboratory-scale experiments involving xenobiotics cannot be disputed. Massive oil pollution of the sea has resulted in extensive research on hydrocarbon biodegradation, particularly bacterial adaptation to oily substrates. These studies have shown not only that bacteria can degrade oils (Miget et al., 1969; ZoBell, 1969; and Soli and Bens, 1972), but that biodegradation can proceed rapidly when microbial cultures have been previously exposed to oils (ZoBell, 1969; Soli and Bens, 1972; Mulkins-Phillips and Steward, 1974). This effect has led some investigators to suggest seeding oil spills with enriched (or adapted) bacteria as a means of cleaning up accidental spills (Liu and Dutka, 1972). Spain et al. (1980) devised experiments to determine whether exposure to xenobiotics would cause microbial populations to degrade the compounds more rapidly during subsequent exposures. Water/sediment systems were tested for adaptation to the compounds methyl parathion and p-nitrophenol. River populations preexposed to methyl parathion and p-nitrophenol degraded the nitrophenol much faster than did control populations. However, salt marsh populations did not adapt to degrade the xenobiotics. The results thus indicated that the ability of organisms to adapt depends on the presence of specific microorganisms.

The role of adaptation in chemical biodegradation is also suggested in literally hundreds of studies showing lag periods prior to the onset of biodegradaton. For example, Tiedje (1977) showed that initial degradation of EDTA was limited, but after 9 weeks incubation the rate accelerated, indicating the adaptation of thermo-tolerant EDTA-degrading populations. Ward and Broch (1976) note a lag phase preceding mineral oil oxidation, the length of which depended on population density or on factors influencing growth rate. Tucker et al. (1975) found that the degradation rates of PCB mixtures in activated sludge units were most rapid after the sludge units had been acclimated for about 5 months to the appropriate PCB.

The question of whether a culture highly adapted to degrade a given compound possesses an enzyme system capable of degrading related compounds has been explored by various researchers. For example, Chambers et al. (1963) examined the degradation of 104

chemical compounds in laboratory flasks containing a mineral salt medium by a phenol-adapted culture. Results showed that 65.5 percent of the compounds were degraded at rates 1.5 to over 12 times the endogenous rate (rate in flasks seeded, but without a phenol-adapted culture). In another study, Mulkins-Phillips and Steward (1974) found that a Nocardia sp., isolated from a culture enriched on Bunker C oil, grew on Venezulan crude oil, hexadecane, and other hydrocarbon mixtures. These studies suggest that enzyme systems capable of degrading a given compound can, in fact, degrade related compounds.

Evidence indicates that the role of adaptation in chemical biodegradation is clearly significant. However, few natural systems are analogous to batch or continuous culture laboratory systems because of their spatial and temporal heterogeneity. In light of this, one is forced to ask the following highly relevant question: does the adaptation commonly observed in laboratory studies really occur in nature? Laboratory studies have tended to use concentrations of chemicals in the ppm range, though environmental levels are often in the ppb range or less than 1 ppb. Because biodegradation is substantially reduced or pre-vented at concentrations below some critical levels (Section 3, Physical Evnrionmental Variables, "Concentration"), adaptation may not occur at such concentrations. It seems logical to think that compounds at ppm levels would result in higher numbers of degraders, and hence greater ability to degrade the chemical upon subsequent exposure.

CONCLUSIONS

Because of the complexity of microbial systems, interactions between microorganisms and their influence on biodegradation are difficult to define. Attempts have been made to develop math-ematical models, based on the biological factors involved, which describe and predict the behavior of one or more populations in their natural environment. The use of such models is limited by the problems involved in determining the biological parameters, and by environmental stresses which alter these relationships. Although interactions may be well-defined for two species under specific and controlled conditions, changes in temperature, salinity, nutrient concentrations, etc. can effect major changes in species interactions. Though the effects of microbial inter-actions on biodegradation cannot be neglected, the effects in most cases can only be defined qualitatively.

The ability of microbial populations to acquire new physio-logical and/or morphological characteristics has profound ecological significance. The adaptive potential appears to be influenced by the microbial system or medium.

# SECTION 6

## BIODEGRADATION ALGORITHMS

### OVERVIEW

An extensive body of literature dealing with biodegradation rates of chemicals is available; however, there are relatively few studies treating biodegradation algorithms. A complete generic algorithm(s) which include the relationships between biodegradation rates and various environmental factors (e.g., chemical concentration, temperature, pH, moisture, pressure, adsorption, diffusion, mixing, etc.) is still lacking. It seems unlikely that significant progress can be made in modelling biodegradation until more is known about the response of microbial populations to the controlling factors, whatever they may be (Shamat, 1978; Howard et al., 1979; Lo et al., 1978; and Smith, 1979a and b). At present, such information is sparse, and the modeller is forced to invent relationships. This approach would be acceptable if the assumptions were then checked experimentally.

In this literature review, the following criteria were used to evaluate the biodegradation algorithms:

- Algorithms of a generic nature that can be used to represent various environmental conditions;

- Algorithms that have been proven by experimental data to have reasonable accuracy;

- Algorithms that are not chemical specific and can indicate the common type of reaction constants. The biodegradation rates of various chemicals or chemical groups can thereby be compared; and

- Algorithms including important variables, especially environmental factors, as discussed in Sections 3 to 5.

In this section, the advantages and limitations of the existing algorithms are also assessed. Research needs and problem areas are noted.

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Studies of biodegradation rates are usually conducted under one of two conditions: batch and continuous. Both conditions have been used in laboratory and field environments. The majority of the biodegradation algorithms surveyed were employed under laboratory environments. Few field applications of batch biodegradation algorithms were found, probably because of the complexity of the natural environment, which precludes the use of the batch models. Many field studies, however, were performed for continuous flow conditions, especially in the area of wastewater treatment (such as the activated sludge process).

The derivation of biodegradation algorithms, whether batch or continuous, or laboratory or field studies, is usually based on one of two basic approaches: decay or enzymatic reactions. Algorithms may also be derived by strict data fitting to polynomial power functions. Table 15 shows some examples of the basic biodegradation algorithms (i.e., expressions of the rate of disappearance of a growth substrate as a function of the substrate concentration) used in various studies. These algorithms can then be used as the basis for the incorporation of other environmental variables, as will be discussed later in this section.

## Decay Algorithms

The use of decay equations (details will be discussed later in this section) for biodegradation was applied as early as 1925. Streeter and Phelphs (1925) used a first-order decay equation to express the bio-oxidation of organic matter in river water. Since then, the same concept has been used by many researchers either for specific or non-specific organics. For examples, zero-order decay kinetics have been used for 2,4-D (Hemmett and Faust, 1969). First-order kinetics have been observed for urea herbicides (Hill et al., 1955), 2-4-D (Burschel and Freed, 1960), triazine and simazine (Zimhahl et al., 1970), dichlobenil (Montgomery et al. 1972), SAN 6706* and SAN 9789* (Rahn and Zimdahl, 1973), napropamide (Walker, 1974), azinphosmethyl (Yaron et al., 1974), metribuzin (Hyzak and Zimdahl, 1974), picloram (Miekle et al., 1974), aquatic weed (Waite and Kurucz, 1977), and 2,4-D (Parker, 1979). Results of these studies all showed that the decay algorithms could satisfactorily approximate the observed patterns of degradation.

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* SAN 6706 = 4-chloro-5-(dimethylamino)-2-(a,a,a-trifluoro-m-tolyl)-3(2H)-pyridazinone.

SAN 9789 = 4-chloro-5-(methylamino)-2-(a,a,a-trifluoro-m-tolyl)-3(2H)-pyridazinone.

TABLE 15  EXAMPLES OF BASIC BIODEGRADATION ALGORITHMS USED IN VARIOUS STUDIES[*]

| REFERENCE | COMPOUND | MEDIUM | MICRO-ORGANISM | TYPE OF MODEL | MODEL | RESULTS/COMMENTS |
|---|---|---|---|---|---|---|
| Walker (1974) | Herbicide (napropamide) | Soil | N/A | Decay reactions | (1) 1st-order rate equation $\frac{\partial C}{\partial t} = -KC$ where C = concentration, t = time, K = rate constant | (1) $t_{\frac{1}{2}}$ for napropamide· |

(1) $t_{\frac{1}{2}}$ for napropamide·

| Temper-ature | Herbicide concen-tration | Half-life at soil moisture content (%) | | |
|---|---|---|---|---|
| °C | kg/ha-5cm | 10.0 | 7.5 | 3.5 |
| | | days | | |
| 28 | 4 50 | 54 | 63 | 90 |
| 14 | 4 50 | 102 | 112 | -- |
| 28 | 2 25 | 56 | -- | -- |

(2) Half-life $(t_{\frac{1}{2}})$· $t_{\frac{1}{2}} = \frac{0.6932}{K}$

(3) Effect of moisture content: $t_{\frac{1}{2}} = aM^{-b}$ where a & b = constants, M = moisture content

(2) a & b for napropamide at 28°C·
a = 189 3
b = -0 550

(4) Effect of temperature: $\log \frac{t_{\frac{1}{2}}}{t'_{\frac{1}{2}}} = \frac{\Delta E}{2.303R}\left(\frac{1}{T} - \frac{1}{T'}\right)$ where T & T' = absolute temperature

(3) ΔE for napropamide·

| Moisture (%) | ΔE ($^{Kcal}/_{mole}$) |
|---|---|
| 10 | 7 85 |
| 7.5 | 7.80 |

(4) Use of the models gave good approximations to the observed patterns of biodegradation of napropamide

(Continued)

TABLE 15. (CONTINUED)

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| REFERENCE | COMPOUND | MEDIUM | MICRO-ORGANISM | TYPE OF MODEL | MODEL | RESULTS/COMMENTS |
|---|---|---|---|---|---|---|
| Yaron, Heuer, and Birk (1974) | Azinphos-methyl (pesticides) | Soil | N/A | Decay reactions | (1) Rate: $$C = C_0 e^{-kt}$$ (1st-order kinetics) | (1) Degradation follows two steps. firstly, the initial concentration remains constant; secondly, the concentration decreases following the first-order kinetics. |

(2) Half-life $(T_{\frac{1}{2}})$:

$$T_{\frac{1}{2}} = t_o + t_{\frac{1}{2}}$$

where· $t_o$ = lag period

$t_{\frac{1}{2}}$ = half-life of the 1st-order reactions.

$C_o$ = initial concentration

(2) Degradation affected by temperature and moisture. Half-lifes $(T_{\frac{1}{2}})$ are shown as follows:

| Temp. °C | Moisture | | | |
|---|---|---|---|---|
| | Sterile Soil | | Natural Soil | |
| | 3% | 50% | 3% | 50% |
| 6 | 484[a] | 88 | 484 | 64 |
| 25 | 135 | 29 | 88 | 13 |
| 40 | 36 | 6 | 32 | 5 |

[a] Half-life in days.

(Continued)

TABLE 15. (CONTINUED)

| REFERENCE | COMPOUND | MEDIUM | MICRO-ORGANISM | TYPE OF MODEL | MODEL | RESULTS/COMMENTS |
|---|---|---|---|---|---|---|
| Waite and Kurucz (1977) | Aquatic weed (Hydrilla sp ) | Aqueous solution | Gram-negative non-motile rod shaped bacterium | Decay reactions | $y = y_o e^{-kt}$<br><br>where<br><br>$y$ = dry weight<br>$y_o$ = initial y<br>$k$ = rate constant<br>$t$ = time (day)<br><br>$C = C_o e^{-kt}$ | (1) The first order decay model was found to fit the degradation data. (The second order model was not as good as the first order model)<br><br>(2) Biodegradation rate constants were affected by nitrate levels. k varied between 0.0239/day and 0 0907/day for $NO_3$-N within the range of 0.5 to 6 mg/l. |

Parker (1979) — 2,4-D — Soil — Coryne-form group — Decay reactions

(1) Biodegradation:

$$\frac{dS}{dt} = kS$$

(2) Temperature effect·

(Arrhenius eq )

$$\log (k \times 10^3) = \frac{-E_A}{2\ 303RT} + A$$

where: S = 2,4-D conc.

k = rate constant $(day^{-1})$

$E_A$ = activation energy (Kcal/mole)

A = Constant $(10^3/day)$

R = Gas Constant

T = Absolute Temperature (°K)

(1) Two first order reactions were observed-slow phase and fast phase

(2) Results of k:

| 2,4-D Conc. (mg/g soil) | k for slow phase | k for fast phase |
|---|---|---|
| 1 | 0 062 | 0.103 |
| 5 | 0.031 | 0.094 |
| 10 | 0.020 | 0.069 |
| 25 | 0.010 | 0 089 |

(3) Results of temperature and moisture effects·

| Moisture (%, volume basis) | temp.<27°C $E_A$ | temp.<27°C A | temp.>27°C $E_A$ | temp.>27°C A |
|---|---|---|---|---|
| 22 | 22.54 | 17 66 | -38 03 | 2.8 |
| 13.9 | 20.14 | 18 19 | 1.16 | 0.12 |
| 10.7 | 19.28 | 14 69 | - 4.60 | 0.50 |
| 6.8 | 25.40 | 19.01 | -53.40 | 3 66 |

(Continued)

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TABLE 15. (CONTINUED)

| REFERENCE | COMPOUND | MEDIUM | MICRO-ORGANISM | TYPE OF MODEL | MODEL | RESULTS/COMMENTS |
|---|---|---|---|---|---|---|
| Grady and Williams (1975) | COD (multi-component substrate, containing glucose, galactose, fructose, sorbitol, and lysine) | Aqueous solution (mineral salts medium) | (heterogeneous microorganisms from sewage treatment plant) | Enzymatic reactions (Effects of substrate concentration in a continuous biological reactor) | (1) Effect of $S_o$ on K· $$\frac{1}{K} = K'S_o$$ where: $$K = \frac{\mu_m}{K_s}$$ $\mu_m$= max specific growth rate constant $K_s$= substrate saturation constant $$\mu = \frac{\mu_m S}{K_s + S}$$ (Monod eq ) $$= \frac{\mu_m}{K_s} S$$ (where S is low) (2) Effect of $S_o$ and D on flow reactor effluent S· $$S = K'S_o D + K'' S_o$$ where· D= dilution rate K' & K''=Constants | (1) The effects of influent substrate concentration can be modeled by an extension of the linear approximation of the Monod model in which the proportionality constant is a function of the influent concentration. (2) Results· |

(2) Results table:

| Substrate | Culture | K' | K" |
|---|---|---|---|
| glucose | A Aero-genes | 0 0633 | 0 0030 |
| glucose | mixed culture | 0.261 | 0.0135 |
| mixed substrate | mixed culture | 0.174 | 0.0014 |

(Continued)

TABLE 15. (CONTINUED)

| REFERENCE | COMPOUND | MEDIUM | MICRO-ORGANISM | TYPE OF MODEL | MODEL | RESULTS/COMMENTS |
|---|---|---|---|---|---|---|
| Borighem and Vereecken (1978) | Phenol | River water, distilled water, and tap water | N/A | Enzymatic reactions | (1) Haldane model: $$K = \cfrac{k_o}{1 + \cfrac{K_m}{S} + \cfrac{S}{K_i}}$$ (2) Hill and Robinson $$K = \frac{k_o S}{S + K_m} \exp\left(\frac{-S}{K_i}\right)$$ (both equations based on substrate inhibition) | (see table below) |

| Model | Organism | $k_o$ ($h^{-1}$) | $K_m$ (mg.l) | $K_i$ (mg/l) |
|---|---|---|---|---|
| (1) | Bacterium NCIB 8750 | 0.29 | – | 110 |
| (1) | Trichosporan cutaneum | 0.464 | 1.66 | 380 |
| (1) | Pseudomonas putida | 0.567 | 2.38 | 106 |
| (1) | Pseudomonas putida | 0.534 | <1 | 470 |
| (2) | Pseudomonas putida | 0.481 | – | 840 |

| REFERENCE | COMPOUND | MEDIUM | MICRO-ORGANISM | TYPE OF MODEL | MODEL | RESULTS/COMMENTS |
|---|---|---|---|---|---|---|
| Lo, Marchildon, Lakshmanan, and Garceau (1978) | N/A | Solution (From activated sludge reactor) | N/A | Enzymatic reactions (Continuous flow, stirred) | (1) Substrate removal: $$\frac{dC_s}{dt} = -k_1 C_s C_x$$ (2) $\bar{C}_x = R_F + (y/\beta)\,(\alpha\beta K - \bar{D})/(K + \bar{D})$ (3) $1/\bar{C}_s' = \left\{ R_F/d + A/d + K + [R_F/d + A/d + K]^2 - 4AK/d]^{0.5} \right\}/2$ where: $C_s$ = concentration of substrate, mg/l. $k_1$ = rate constant for substrate utilization $C_x$ = concentration of microorganisms, mg/l $\bar{C}_x = C_x/C_{so}$ $C_{so}$ = initial conc. of substrate | The overall effect of mixing, in terms of $\beta$, can greatly affect the biodegradation. The larger the $\beta$, the higher the biodegradation rate. |

TABLE 15. (CONTINUFD)

| RFFFRENCF | COMPOUND MEDIUM | MICRO-ORGANISM | TYPE OF MODEL | MODEL | RESULTS/COMMENTS |
|-----------|-----------------|----------------|---------------|-------|------------------|
| | | | | | |

$$R_F = C_{xo}/C_{so}$$

$C_{xo}$ = initial conc of microorganisms in feed

$y$ = $1 - C_s$

$\bar{C}_s = C_s/C_{so}$

$\beta$ = average frequency of contact between micro-organisms and limiting substrate per unit of biomass

$\alpha$ = yield coefficient, grams of microorganisms produced per gram of substrate removal

$K = k_2/C_{so}k_1$

$k_2$ = forward rate constant for decomposition of intermediate, $h^{-1}$

$\bar{D} = D/C_{so}k_1$

$D$ = dilution rate, $h^{-1}$

$\bar{C}_s' = C_s k_1/k_2$

$d = D/k_2$

$A = \dfrac{(1/B)\ (\alpha B - d)}{1 + d}$

(Continued)

TABLE 15. (CONTINUED)

| REFERENCE | COMPOUND | MEDIUM | MICRO-ORGANISM | TYPE OF MODEL | MODEL | RESULTS/COMMENTS |
|---|---|---|---|---|---|---|

Shamat (1978) — Chlorinated organics (eight compounds were studied: 2,4-dichloro-phenoxyacetic acid; m-, p-, and O-chloro-benzoic acids; and 2,4-, 2,5-, 3,5-, and 2,6-dichloro-benzoic acids) — Aqueous solution — From activated sludge — Enzymatic reactions (Monod kinetics)

(1) Batch System:

$$-\frac{K_s}{S_o + \frac{X_o}{Y}} \ln \frac{S\left(-\frac{X_o}{Y}\right)}{S_o\left[S - S_o - \left(\frac{X_o}{Y}\right)\right]}$$

$$+\ln\left[\frac{S - S_o\left(\frac{X_o}{Y}\right)}{\frac{-X_o}{Y}}\right] = \mu_m t$$

(2) Chemostat:

$$\frac{dx}{dt} = \mu X - DX$$

where steady state:
$$\mu = D$$

$$\frac{1}{S} = \frac{\mu_m}{K_s} \frac{1}{D} - \frac{1}{K_s}$$

where:
$K_s$ = saturation constant
$S$ = substrate conc.
$S_o$ = initial S
$X$ = microbial conc.
$X_o$ = initial X
$Y$ = microbial yield coeff.
$\mu$ = specific growth rate
$\mu_m$ = max. $\mu$
$D$ = dilution rate
$t$ = time

RESULTS/COMMENTS:

(1) Batch System:

| Substrate | $\mu_m$ ($d^{-1}$) | $K_s$ (mg/l) | Y |
|---|---|---|---|
| 2,4-D | 2.30 | 5.40 | 0 14 |
| M-CB | 0.57 | 2.03 | 0.14 |
| P-CB | 1.22 | 1.12 | 0.25 |
| O-CB | 1.02 | 0.98 | 0.22 |
| 2,5-DCB | 0.65 | 1.52 | 0 16 |
| 3,5-DCB | 0.051 | 25.3 | -- |

(2) Continous flow:

| Substrate | $\mu_m$ ($d^{-1}$) | $K_s$ (mg/l) |
|---|---|---|
| 2,4-D | 2.18 | 2.68 |
| O-CB | 1.13 | 1.53 |
| P-CB | 1.12 | 0.99 |

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TABLE 15. (CONTINUED)

| REFERENCE | COMPOUND | MEDIUM | MICRO-ORGANISM | TYPE OF MODEL | MODEL | | RESULTS/COMMENTS |
|---|---|---|---|---|---|---|---|
| Edeline and Lamber (1979) | Organic pollutants (COD) | River | N/A | Two phases enzymatic reactions | (1) | External (Exogenous) substrate degradation: <br> (a) Organism growth-- <br><br> $$u = \frac{\bar{u}S}{K_s + S} \;\approx\; \frac{\bar{u}}{K_s} S$$ <br><br> (b) Substrate degradation-- <br><br> $$\frac{dS}{dt} = \frac{-\bar{u}}{YK_s} SB$$ <br><br> $$\frac{dS}{dt} = \frac{-\alpha\beta e^{-\beta t}}{(1+\gamma e^{-\beta t})^2}$$ <br> (sigmoidal curve) <br> (c) Oxygen uptake-- <br> $$\frac{dy}{dt} = (1-Y)\frac{dS}{dt}$$ <br><br> (2) Endogenous substrate utilization: <br><br> $$v_e = \frac{dS_e}{dt} = C_1' S_e (C_2' - S_e)$$ <br> or <br> $$v_e = K_1 S_e (K_2 - S_{exo})$$ <br> where· <br> $u$ = growth constant $(h^{-1})$ <br> $\bar{u}$ = max $u$ <br> $S$ = substrate concentration <br> $K_s$ = half-saturation constant <br> $B$ = bacterial biomass (mg/l) <br> $Y$ = bacterial cell yield <br> $y$ = dissolved oxygen (mg/l) <br> $\alpha = S_0 (1+\frac{YS_0}{B_0})$ <br><br> $\beta = \frac{\bar{u}}{K_s}(S_0+\frac{B_0}{Y})$ <br><br> $\gamma = Y\frac{S_0}{B_0}$ <br><br> $v_e$ = endogenous substrate utilization rate <br> $S_e$ = endogenous substrate conc. <br> $S_{exo}$ = exogenous substrate conc <br> $C_1', C_2', K_1, K_2$ = constants | Two-phase model accounts for up to 95% of the total oxygen consumption in the river. The two phases are characterized by a logistic model. The first corresponds to a destruction of about 2/3 of the substrate, and is accompanied by the formation of cellular reserves. The second is the consumption of the stored substances. |

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TABLE 15. (CONTINUED)

| REFERENCE | COMPOUND | MEDIUM | MICRO-ORGANISM | TYPE OF MODEL | MODEL | RESULTS/COMMENTS |
|---|---|---|---|---|---|---|
| Gerstl, Nye and Yaron (1979) | Parathion | Soil | N/A | (1) Enzymatic reactions<br><br>(2) Diffusion | (1) Biodegradation | (1) Biodegradation of parathion showed two distinct stages as shown by equation listed. In the first stage parathion degraded at an increasing rate. The initial rate was roughly proportional to the initial concentration except at very low concentrations. The second stage was characterized by a declining rate<br>(2) Biodegradation is affected by time, moisture content and initial concentration. Results as follows: |

(1) Biodegradation:

$$\ln \frac{C_o^* - C_o}{C_o} + \ln \frac{C}{C_o - C} = -C_o^* kt$$

(2) Diffusion:

$$\frac{\delta C}{\delta t} = D \frac{\delta^2 C}{\delta x^2} - R_{xt}$$

where:
C = parathion conc. (µg/ml)

$C_o^* = C_o + M_o$

$C_o$ = initial C

$M_o$ = initial microbial activity (µg/ml)

k = rate constant

D = diffusion coeff.

$R_{xt}$ = rate of microbial decomposition

(i e $\frac{dC}{dt}$ = kCM)

| | $\theta=0.34$[†] | | $\theta=0.24$ | | $\theta=0.14$ | |
|---|---|---|---|---|---|---|
| $C_o$ | $m_o$ | k | $m_o$ | k | $m_o$ | k |
| µg/ml | | | | | | |
| 28 | 0.325 | 0.0363 | 0 724 | 0.0229 | 1.066 | .0 0164 |
| 14 | 0.214 | 0.0629 | 0.386 | 0.0450 | 0.910 | 0.0264 |
| 7 | 0.290 | 0.090 | 0.372 | 0.0643 | 0.577 | 0.0421 |
| 4 4 | 0.150 | 0.203 | 0.426 | 0.0621 | 0.482 | 0.0473 |

[†] $\theta$ = moisture content

(3) Combine biodegradation and diffusion models, and rate of parathion decomposition at any distance, time, and local concentration can be calculated The proposed model satisfactorily fits the experimental results.

TABLE 15 . (CONTINUED)

| REFERENCE | COMPOUND | MEDIUM | MICRO-ORGANISM | TYPE OF MODEL | MODEL | | RESULTS/COMMENTS |
|---|---|---|---|---|---|---|---|
| Beltrame, Beltrame, Carniti, and Pitea (1979) | phenol | Activated Sludge | N/A | Enzyme reactions | Monod equation: $$k = \dfrac{k_o}{1 - K_m \over S}$$ (batch reactor) | (1) (2) | $k_o = 0.140\ \mathrm{hr}^{-1}$ $K_m = 245\ \mathrm{mg/l}$ Kinetic data collected from a batch reactor are of limited value for the design of a continuous biodegradation process. |
| Mason, Anderson, and Shariat (1979) | Methyl-mercuric chloride | Solution | (1) Enterobacter aerogenes (2) Serratia marcescens | Enzymatic reactions | Michaelis-Menten Kinetics: $$\dfrac{1}{V_o} = \dfrac{1}{V_m} + \dfrac{K_m}{V_m S_o}$$ | (1) (2) | Degradation is affected by pH and the initial concentration Results of $K_m$ (in mg/l) and $V_m$ (in µg/l/d) are as follows· For Enterobacter aerogenes: |

For Enterobacter aerogenes:

| pH | 6 | 7 | 8 |
|---|---|---|---|
| $K_m$ | 4.09 | 4.2 | 3.9 |
| $V_m$ | 192 | 143 | 92 |

For Serratia marcescens:

| pH | 6 | 7 | 8 |
|---|---|---|---|
| $K_m$ | 8.4 | 8.5 | 38.3 |
| $V_m$ | 122 | 139 | 661 |

TABLE 15 . (CONTINUED)

| REFERENCE | COMPOUND | MEDIUM | MICRO-ORGANISM | TYPE OF MODEL | MODEL | RESULTS/COMMENTS |
|---|---|---|---|---|---|---|
| Shamat and Maier (1980) | Chlorinated Organic Compounds (Benzoic acid, o-chlorobenzoate, m-chlorobenzoate, p-chlorobenzoate, 2,4-dichloro-benzoate, 2,5-dichloro-benzoate, 2,6-dichloro-benzoate, 3,5-dichloro-benzoate Phenoxyacetic acid, 2,4-dichloro-phenoxyacetic acid) | Liquid | N/A | Enzymatic reactions | $k = \dfrac{k_o S}{K_m + S} - k_d$ <br><br> (Both continuous-flow reactors and batch reactors were tested) | (see below) |

(1)  For batch reactor:

| Compound | $k_o$ (day$^{-1}$) | $K_m$ (mg/l) | Y (mg/mg) |
|---|---|---|---|
| 2,4-Dichloro-phenoxyacetate | 2.3 | 5.4 | 0.14 |
| m-Chlorobenzoate | 0.6 | 2.0 | 0.14 |
| p-Chlorobenzoate | 1.2 | 1.1 | 0.25 |
| o-Chlorobenzoate | 1.0 | 2.4 | 0.22 |
| 2,5-Dichloro-benzoate | 0.6 | 1.5 | 0.16 |
| 3,5-Dichloro-benzoate | 0.05 | 25.3 | -- |

(2)  For continuous-flow reactor:

| Compound | $k_o$ (day$^{-1}$) | $K_m$ (mg/l) |
|---|---|---|
| 2,4-Dichlorophenoxy-acetate | 2.2 | 2.7 |
| o-Chlorobenzoate | 1.1 | 1.5 |
| p-Chlorobenzoate | 1.1 | 1.0 |

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(Continued)

TABLE 15. (CONCLUDED)

| REFERENCE | COMPOUND | MEDIUM | MICRO-ORGANISM | TYPE OF MODEL | MODEL | RESULTS/COMMENTS |
|---|---|---|---|---|---|---|
| Baskir and Spearing (1980) | Carbo-hydrates | Liquid (sugar, sodium alginate, and nutrients) | N/A | Enzymatic reactions | (1) For Substrate: (Monod kinetics) $$k = \frac{k_o S}{K_m + S}$$ (Steady-state continuous-culture) (2) For Product· (Luedeking and Piret Hypothesis) $$P = \alpha Y (S_o - S) + \frac{\beta\, Y\, (S_o - S)}{k}$$ where $\alpha$ and $\beta$ are constants. | (1) where COD is the growth-limiting factor, the Monod equation is not applicable to long activated sludge ages. (2) The Luedeking and Piret hypothesis can be used to estimate the amount of the product and undegraded substrate present at any sludge age. |
| Pal and Broadbent (1975) | Rice Straw | Clay soil | N/A | power function | $$C = kt^m$$ where: C=carbon loss k&m=constants t=time | (1) The carbon loss data could not be described by the first order kinetics. Neither did they conform well to Michaelis-Menten kinetics. (2) The carbon loss can be expressed by a power function as shown. k and m were affected by loading rate of rice straw. |

* Explanation of symbols are shown below, unless otherwise explained in the table:

$k$ = kinetic rate constant

$k_o$ = maximum growth rate constant

$K_e$ = equilibrium constant in terms of concentration

$K_s$ = solubility product

$K_i$ = inhibition constant

$K_m$ = substrate saturation constant

$S$ = substrate concentration

$X$ = biomass concentration

The general form of the decay algorithm can be written as follows:

$$\frac{dS}{dt} = -k\ \phi\ (S) \quad\text{------------------------------------ (7)}$$

where S represents, directly or indirectly (e.g., organic carbon disappearance or oxygen demand), the concentration of the substance at time t; k is a rate factor or specific reaction-rate constant, and $\phi(S)$ is a function of the concentration of the substance remaining. In many cases, $\phi(S)$ for biodegradation can be expressed in terms of $S^n$, where n is a dimensionless factor representing the order of the reaction. Therefore, Equation (7) becomes:

$$\frac{dS}{dt} = -kS^n \quad\text{--------------------------------- (8)}$$

As discussed above, many of the biodegradation studies showed a first-order reaction (i.e., n=1). In this case, Equation (8) is simplified as:

$$\frac{dS}{dt} = -kS \quad\text{----------------------------------- (9)}$$

$$\text{or } S = S_o e^{-kt} \quad\text{----------------------------- (10)}$$

where $S_o$ = original concentration of the test substance. The biodegradation rate constant, k, can then be calculated:

$$k = \frac{1}{t} \ln \frac{S_o}{S} \quad\text{--------------------------- (11)}$$

In a complex system, the reaction order may be a fractional order or higher than first order (Parker, 1979). The following table shows biodegradation rate constants at several selected reaction orders:

| Reaction Order (n) | Biodegradation Rate Constant (k) |
|---|---|
| 0 | $\frac{1}{t}(S_o - S)$ |
| 1 | $\frac{1}{t} \ln \frac{S_o}{S}$ |
| 2 | $\frac{1}{t}\frac{S_o - S}{S_o S}$ |
| 3 | $\frac{1}{t}\frac{(S_o + S)(S_o - S)}{2S_o^2 S^2}$ |

Basically, the decay algorithm is a direct expression of the changes of concentration versus time, in which no other factors are incorporated. Such factors or variables are incorporated into the k value, that is:

$$k = f \text{ (other environmental factors)} \text{------------(12)}$$

The factors affecting k values will be discussed later in this section.

## Enzymatic Algorithms

Decay algorithms often fail to express the complicated microbial interactions and population dynamics. Many biodegradation kinetic studies introduce a biological factor, B (i.e., concentration of organism(s)), to modify the model. Because the enzyme(s) is the source or mediator for the uptake of organic substances by microorganism(s), B can be replaced by the enzyme concentration, E. The simplified enzymatic algorithm can be developed as follows:

$$E + S \underset{k_{-1}}{\overset{k_{+1}}{\rightleftharpoons}} ES \underset{k_{-2}}{\overset{k_{+2}}{\rightleftharpoons}} E + P \text{---------------------------(13)}$$
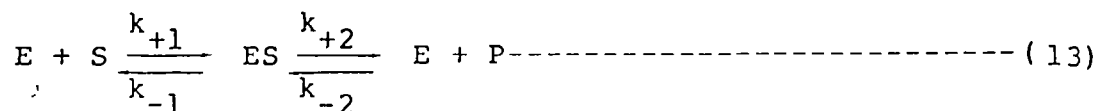
Where:

S = substrate;

E = enzyme;

$k_{+1}$, $k_{-1}$, $k_{+2}$, and $k_{-2}$ = rate constants;

ES = substrate-enzyme complex; and

P = product.

The rate of substrate biodegradation can then be expressed as:

$$\frac{dS}{dt} = - k_{+1} S \cdot E + k_{-1} ES \text{---------------------------(14)}$$

The rate of ES changes can be expressed as:

$$\frac{dES}{dt} = k_{+1} S \cdot E - (k_{-1} + k_{+2}) ES + k_{-2} E \cdot P \text{-----------------(15)}$$

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The mass balance relationships:

$$E_o = E + ES \text{----------------------------------------} (16)$$

$$S_o = S + P + ES \text{----------------------------------} (17)$$

Where $E_o$ and $S_o$ represent the initial concentration of E and S, respectively. Equations (14) through (17) contain four variables, S, E, ES and P, and can be solved by the simultaneous solution of four equations. If it is assumed $S_o \gg ES$, $k_{-2} = 0$, and equilibrium condition exists for Equation (13) (i.e.,

$\frac{dES}{dt} = 0$), then the biodegradation rate of a substance can be simplified to the following form:

$$\frac{dS}{dt} = -\frac{V_m S}{K_m + S} \text{----------------------------------} (18)$$

Equation (18) is the well known Michaelis-Menten kinetics model, where $K_m$ is the Michaelis-Menten or substrate saturation constant and equal to $(k_{-1} + k_{+2})/k_{+1}$; $V_m$ depends on the initial concentration of microorganism, B:

$$V_m = k_o B \text{-----------------------------------------} (19)$$

where $k_o$ = maximum specific growth rate constant (unit in time$^{-1}$)

Therefore: $$\frac{dS}{dt} = -\frac{k_o BS}{K_m + S} \text{--------------------------} (20)$$

When: $K_m \gg S$, Equation (20) becomes

$$\frac{dS}{dt} = -\left(\frac{k_o}{K_m}\right) BS \text{------------------------------} (21)$$

By comparing Equation (21) to decay equation (Equation 9), it can be seen that the enzyme model incorporates the biological factor, B, into the decay kinetic model. Equation (21) becomes first order for substrate and biomass concentrations, or alternatively, second order overall.

When $S \gg K_m$, the reaction kinetics (Equation 20)) are first order for the biomass concentration and independent of the the substrate concentration. When $S = K_m$, Equation (20) shows that the reaction rate is one-half of its maximum value.

If $S_o$ and ES are assumed to be constant, Equation (17) becomes:

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$$\frac{dS}{dt} = -\frac{dP}{dt} \qquad \text{------------------------------------(22)}$$

If P is assumed to be equal to B, Equation (22) becomes:

$$\frac{dB}{dt} = \frac{k_o BS}{K_m + S} \qquad \text{--------------------------------(23)}$$

If $P \neq B$ and the growth yield, Y, is a constant ratio between cell

yield and substrate utilized, Equation (23) becomes:

$$\frac{dS}{dt} = -\frac{dB}{Y \cdot dt} = -\frac{k_o BS}{Y(K_m + S)} \qquad \text{------------------------(24)}$$

Equations (23) or (24) are usually referred to as the Monod equation. The specific growth rate constant or the biodegradation rate constant can be solved from equation (23) or (24):

$$k = \frac{dB}{dt} /B = \frac{k_o S}{K_m + S} \qquad \text{----------------------------(25)}$$

The Michaelis-Menten and Monod equations or their derivatives have been used extensively to model the biodegradation of various organic substances in either aqueous or soil environments. Examples of such applications are shown in Table 15. As for the decay reaction algorithms, enzymatic reaction algorithms were also used for both specific or nonspecific organics under field or laboratory conditions. Results as discussed in Table 15 indicate that enzymatic reaction algorithms can model the biodegradation rates with satisfactory accuracy. However, if kinetic expressions are derived from the Monod equation, they should be applied with caution under unsteady-state conditions, since they were usually derived assuming steady-state concentration of metabolic intermediates.

Many enzymatic reaction algorithms considered the effects of inhibition caused by complex formation between substrate and enzyme (called substrate inhibition), enzyme and other chemicals (competitive inhibition), or substrate and other chemicals (noncompetitive inhibition). Whenever inhibition is present, the rate of biodegradation is decreased. Table 16 gives examples of some common biodegradation algorithms based on enzymatic reaction kinetics.

## Power Rate Algorithms

The power rate algorithms were derived mainly from curve fitting. Theoretically, a curve (or equation) can be derived for a set of data involving two variables. Examples of such equations are presented by Hamaker (1972), Pal et al. (1975) and Parker (1979):

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TABLE 16. COMMON BIODEGRADATION ALGORITHMS
BASED ON ENZYMATIC REACTION KINETICS[*]

| Algorithm | Name |
|---|---|
| $k = \dfrac{k_o}{1 + K_m/S}$ | Monod |
| $k = k_o \,(1 - e^{-S/K_t})$ | Teissier |
| $k = \dfrac{k_o}{1 + (K_c X/S)}$ | Contois |
| $k = \dfrac{k_o}{1 + (K_S S^{-\lambda} M)}$ | Moser |
| $k = \dfrac{k_o}{1 + (K_m/S) + (S/K_1)}$ | Haldane |
| $k = \dfrac{k_o}{1 + (K_m/S) + I K_m/K_1}$ | |
| $k = \dfrac{k_o}{[1 + (K_m/S)][1 + (1/K_1)]}$ | |
| $k = \dfrac{k_o}{1 + \dfrac{K_m}{S} + (\dfrac{S}{K_1})^2}$ | |
| $k = \dfrac{k_o S}{S + K_m} \; \exp\,(-S/K_1)$ | |
| $k = \dfrac{k_o S (1 + S/K)}{1 + K_m/S + S/K_i}$ | |
| $k = \dfrac{k_o}{1 + K/S + (S/K_1)(1 + S/K)}$ | |

[*] References: Sundstrom et al. (1979)
Der Yang et al. (1975)

$k$ = kinetic rate constant                     $K_r$ = Teissier constant
$k_o$ = maximum growth rate constant
$K_c$ = equilibrium constant in terms of concentration
$K_S$ = solubility product                        $K_m$ = substrate saturation
$K_i$ = inhibition constant                              constant
$\lambda_M$ = Moser constant                          S = substrate concentration
                                                  X = biomass concentration
 I = concentration of inhibitive chemicals

$$\frac{S}{S_o} = a + bt + ct^2 + \ldots it^n \text{ -------------------- (26)}$$

and:

$$S_o{}^n = S^n + nkt \text{ ----------------------------------- (27)}$$

where   S = substrate concentration;

a,b,c,i,m,n = constants; and

k = rate constant.

However, the power rate algorithms have very limited value because of their non-generic nature and in some cases no biodegradation rate is expressed.  Very few researchers have used the power rate expressions for biodegradation studies.  Hamaker (1972) suggested that fractional order reaction rates might be beneficial in predicting pesticide degradation.  His model is as follows:

$$S_o{}^{(1-n)} = S^{(1-n)} + (1-n) kt \text{ ----------------------- (28)}$$

Pal et al. (1975) stated that, in their study, the organic carbon degradation data could not be described by first-order kinetics. Also, their data did not conform well to Michaelis-Menten kinetics.  However, a good fit was obtained by using the power rate algorithm shown below:

$$S = kt^m \text{ --------------------------------------- (29)}$$

where S = substrate concentration in terms of carbon;

k and m = constants; and

t = time.

## Effects of Environmental Variables

Biodegradation algorithms as discussed above describe, mainly, the relationships between two factors:  concentration of organic substances (S), and time (t).  Many of the environmental variables have been neglected in the biodegradation rate algorithms.  Existing literature, however, does show some algorithms for the quantitative expression of the environmental variables. Such algorithms can be incorporated into the basic algorithms as discussed previously.

Temperature Variable--
Unlike physical or chemical reactions, the temperature range

in which biological reactions occur is small. The range for survival usually extends from -5 to close to 100°C. When growth rate is plotted as a function of temperature, the optimum temperatures (which give higher biodegradation rates) usually lie within the range for survival but toward the higher end of the range. Biodegradation rates approach zero towards the lower and higher ends of the survival range (Stanier et al., 1976).

Three mathematical algorithms have been used by biologists to describe relationships between biodegradation rates and temperature:

- Simple linear regression of biodegradation vs. temperature;

- $Q_{10}$ model; and

- Arrhenius equation.

The first approach (i.e., linear regression) assumes that in a certain temerature range, the biodegradation rate, k, is directly proportional to the temperature, T (°C), (Howard et al., 1979). That is,

$$k = k_t T \tag{30}$$

where: $k_t$ = constant .

or:
$$k_1 = k_2 \cdot \frac{T_1}{T_2} \tag{31}$$

where: $k_1$, $k_2$ = biodegradation rates at temperature $T_1$ and $T_2$, respectively.

The second approach ($Q_{10}$ mdoel) is based on the Van't Hoff's empirical rule that the ratio of reaction rates at an interval of 10°C is of the order of 2 to 3. This ration is the $Q_{10}$ value. The relationships between biodegradation rates and temperature can then be expressed as:

$$\ln k = C + T \left( \frac{\ln Q_{10}}{10} \right) \tag{32}$$

or:
$$k_1 = k_2 \cdot Q_{10}^{\left( \frac{T_1 - T_2}{10} \right)} \tag{33}$$

where: C = constant; and

T = temperature, °C .

This $Q_{10}$ model assumes that the logarithm of the biodegradation rate, $k$, is a linear function of T. The $Q_{10}$ approach has been used by Howard et al. (1979) and Flanagan et al. (1976) for non-specific organics.

Most biological constants fit an Arrhenius type of temperature relationship (the third approach):

$$k = Ae^{-E/RT} \qquad \text{------------------------------------} \quad (34)$$

where:   k = biodegradation rate constant at temperature T°K;

E = activation energy;

A = constant;  and

R = gas constant in cal/g-mol-°K.

$$\text{or:} \quad k_1 = k_2 \cdot \exp\left[\frac{-E}{R}\left(\frac{T_2-T_1}{T_1T_2}\right)\right] \qquad \text{--------------------} \quad (35)$$

The Arrhenius equation has been used extensively to describe the effect of temperature on biodegradation (e.g., Zimdahl et al., 1970; Hamaker, 1972; Walker, 1974; Stanier et al., 1976; Parker, 1979; Howard et al., 1979; and Sandstrom et al., 1979).

When biodegradation rates as a function of temperature are plotted, no matter which approach is used, the results are usually satisfactory only over a certain part of the temperature range. The deviation near the maximum temperature is interpreted as due to the thermal denaturation of cell proteins (Stanier et al., 1976). At the minimum temperature, the deviation may be caused by the regulatory machinery of the cell (Stanier et al., 1976). Howard et al. (1979) stated that information on the factors affecting the temperatures permitted for growth is meagre, and a precise temperature-biodegradation model can not be derived using existing limited knowledge.

Contact Opportunity--
As discussed previously, biodegradation rates may be affected by contact opportunity, in terms of several physical (e.g., mixing, diffusion, etc.) and physical-chemical (e.g., sorption, etc.) mechanisms. Few algorithms correlating such mechanisms with biodegradation rates were found.

Lo et al. (1978) suggested complicated algorithms to correlate the overall effect of mixing, in terms of β, and the biodegradation rate for a continuous biological reactor:

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$$\frac{dC_s}{dt} = -k_1 C_s\, C_x \quad\text{---------------------------------------------------- (36)}$$

$$\bar{C}_x = R_F + \frac{(y/\beta)\,(\alpha\beta K - \bar{D})}{K + \bar{D}} \quad\text{--------------------------------------------- (37)}$$

and
$$\frac{1}{\bar{C}'_s} = \left\{ \frac{R_F}{d} + \frac{A}{d} + K + \left[ \left(\frac{R_F}{d} + \frac{A}{d} + K\right)^2 - \frac{4AK}{d} \right]^{0.5} \right\} \frac{1}{2} \quad\text{------- (38)}$$

where:

$C_s$ = concentration of substrate, mg/l;

$k_1$ = biodegradation rate constant for substrate utilization;

$C_x$ = concentration of microorganisms, mg/l;

$\bar{C}_x = C_x/C_{so}$;

$C_{so}$ = initial concentration of substrate;

$R_F = C_{xo}/C_{so}$;

$C_{xo}$ = initial concentration of microorganisms in feed;

$y = 1 - \bar{C}_s$;

$\bar{C}_s = C_s/C_{so}$;

$\beta$ = average frequency of contact between microorganism and limiting substrate per unit of biomass;

$\alpha$ = yield coefficient, grams of microorganisms produced per gram of substrate removal;

$K = k_2/C_{so}k_1$;

$k_2$ = forward rate constant for decomposition of intermediate, $h^{-1}$;

$\bar{D} = D/C_{so}k_1$;

$D$ = dilution rate, $h^{-1}$;

$\bar{C}'_s = C_s k_1/k_2$;

$d = D/k_2$; and

$A = \frac{(1/\beta)\,(\alpha\beta - d)}{1 + d}$

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As shown by the above equations, the larger the β values, the higher the biodegradation rates.

Diffusion was also found to have a profound influence on the rate of biodegradation. This is because diffusion tends to move the substrate away from or toward the microorganisms, and so influences the availability (or concentration levels) of the substrate. In order to correlate such an effect to the biodegradation rate, the diffusion coefficient (D) of a substrate in a specific medium should be quantified. The following algorithm can then be used to calculate the diffusion effect (Gerstl et al., 1979):

$$\frac{\delta C}{\delta t} = D_x \frac{\delta^2 C}{\delta x^2} - R_x t \quad\text{------------------------------------}(39)$$

where

C = substrate concentration at time t;

$R_x$ = biodegradation rate; and

x = the x direction.

Zimdahl et al. (1977) suggested an algorithm to express the effects of sorption on biodegradation rates of herbicides in the soil environment. The authors found that the clay and organic contents are the most important sorbents influencing the biodegradation rate:

$$-\frac{d[C]}{dt} = k [C] [clay] [OM] \quad\text{---------------------------}(40)$$

where [C] = substrate concentration;

[clay] = clay content;

[OM] = organic content; and

k = biodegradation rate constant.

Nutrients/Inhibitors/Toxins--
Microorganisms cannot degrade a chemical substance without a balanced supply of other essential chemical substances ("nutrients", as defined previously). Most studies, however, have failed to evaluate the effects of these nutrients on the biodegradation of the substrate of interest. Misleading conclusions may be reached if such effects are not taken into consideration. For example, in the biodegradation of carbohydrates in a low

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nitrogen environment, the specific biodegradation rate may be-
come (assuming Monod equation applies):

$$k = \frac{k_o S_N}{K_m + S_N} \qquad \text{------------------------------------------------ (41)}$$

instead of:

$$k = \frac{k_o S_C}{K_m + S_C} \qquad \text{---------------------------------------- (42)}$$

where: $S_N$ = nitrogen concentration;

$S_C$ = carbon concentration.

In the above example, the biodegradation rate obtained will be
limited by the concentration of utilizable nitrogen compounds
instead of the carbon compound of interest (Sundstrom et al.,
1979). It is quite possible that, in a natural environment (or
even in a laboratory controlled environment), the observed
biodegradation rate(s) are influenced by certain unknown nu-
trient(s) (such as certain unknown growth factors or inorganic
ions and do not accurately portray the biodegradation rate of
the compound of interest. Measured biodegradation rates for the
chemical compound of interest are only representative of true
rates when the studied chemical becomes the limiting growth
factor.

The biodegradation rate is also affected by inhibitors.
Inhibitors may be the substrate of interest (e.g., when the
substrate itself is present at very high levels) or other chemi-
cals. Such effects have been discussed previously under the
heading of "Enzymatic Algorithms". Whenever an inhibitor is
present, the biodegradation rate is decreased (Sundstrom et al.,
1979). Mathematically, this lower rate is caused by the addition
of more terms to the denominator of the biodegradation rate
algorithms. Examples of such algorithms are presented in Table
16.

Quantitative expressions of the effects of toxins on bio-
degradation rates are still lacking. Due to the wide variety
of toxins and complex relationships between different toxin con-
centration levels and types of microorganisms, derivation of a
generic model appears to be beyond the present knowledge.

Water Availability--

In an aqueous environment, the water requirements of micro-organisms can be expressed quantitatively in the form of the water activity $(a_w)$ of the environment (Rose, 1976):

$$a_w = \frac{p}{p_o} \text{------------------------------------------- (43)}$$

$$\text{or } a_w = \frac{-vm\phi}{55.5} \text{------------------------------------------- (44)}$$

where p = vapor pressure of the solution;

$p_o$ = vapor pressure of pure water;

v = number of ions formed by each solute molecule;

m = molar concentration of solute; and

$\phi$ = molar osmotic coefficient.

Qualitative effects of $a_w$ on biodegradation rates have been reported (as discussed in Section 4). No quantitative correlatiions are available, however, for the $a_w$ and the biodegradation rates.

In a non-aqueous environment, water availability will exert its effect on biodegradation when moisture content falls below some critical value (Howard et al., 1979). Three types of algorithms have been suggested for the quantitative expression of the effect of moisture content on biodegradation rate:

$$\frac{dS}{dT} = \gamma + mM^n \text{----------------------------------- (45)}$$

$$k = mM^n \text{----------------------------------------- (46)}$$

$$\text{and: } c = \frac{M}{a_1 + M} \times \frac{a_2}{a_2 + M} \text{----------------------- (47)}$$

where: S = concentration of organic substances;

$\gamma$ = degradation rate at M = O;

M = moisture content;

m and n = constants (for Equation (45), n <1);

$a_1$ = moisture content at which activity is half its "optimal value";

$a_2$ = moisture content at which gas exchange is half its "optimal value";

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c = correction constant; and

k = degradation rate constant.

Equation (45) was devised by Hamaker (1972) based on the Freundlich equation. The constant, m, in Equation (45) is dependent on chemical, soil, and temperature factors. Equation (46) was modified from the half-life equation used by Walker (1974). Equation (47) was suggested by Flanagan et al. (1976) and Bunnell et al. (1977) based on the Michaelis-Menten equation. To date, no theoretical model has been derived. The first two algorithms have been checked by experimental results. However, the third algorithm was not tested experimentally. It is suggested that additional studies are needed for the confirmation of the above algorithms or for the derivation of a more generic algorithm.

Other Variables--
Algorithms for other variables, such as hydrostatic pressure, pH, Eh, and microbial interactions, are still lacking. Only very limited data have been reported in the literature for general trends of the effects of the above variables. Apparently, mathematical expressions of the effects of these variables on biodegradation rates cannot be derived using the limited experimental results available.

## ALGORITHMS MODIFICATION

Evaluation of the literature reveals that most algorithms reported in biodegradation studies were used to explain experimental results involving specific environmental conditions. Generic models that can be used universally to describe the effects of various environmental variables are not available. If biodegradation is to be evaluated on a common basis, the effects of all significant environmental variables should be included. Algorithms should be modified by incorporating various environmental factors.

One of the schemes that can be used to modify biodegradation algorithms is to incorporate correction factors for all important environmental variables into the basic biodegradation algorithms, i.e., substitute Equation (49) into Equation (48):

$$\frac{dS}{dt} = - k \, \phi \, (S) \, \text{---------------------------------------} \, (48)$$

$$k = f \text{ (other environmental variables)}$$

$$= k_o \prod_{i=1}^{i} F_i \, \text{-----------------------------------} \, (49)$$

where

        $S$ = substrate concentration;

        $k$ = biodegradation rate;

        $k_o$ = biodegradation rate obtained under certain given environmental conditions;

        $F_i$ = correction factor for environmental variable i; and

        $\phi(S)$ = function of substrate concentration.

As discussed in Sections 3 to 5, a wide variety of environmental variables can affect biodegradation rates. Practically, it is impossible to include all the correction factors ($F_i$) for environmental variables. However, it is generally known that, in any given situation, only one or some limited number variables are of significance. For example, in applying herbicides to a given soil environment, the important variables would be temperature and soil moisture content, inasmuch as other variables (e.g., clay content, soil organics, pH, nutrients, etc.) would be relatively constant, and only temperature and moisture content would show significant variation during a specified time interval. Under such a situation, and if the Haldane equation (Table 16), the Arrhenius equation (Equation (35)), and the equation suggested by Flanagan et al. (1976) (i.e., Equation 47)) apply, then the degradation could be estimated by:

$$\frac{dS}{dt} = - k_o \left[ \exp\left( \frac{E}{R} \cdot \frac{T - To}{T\ To} \right) \right] \left[ \frac{M}{a_1 + M} \cdot \frac{a_2}{a_2 + M} \right]$$

$$\left[ \frac{1}{1 + (K_m/S + (S/K_i)} \right] \ \text{---------------------} \ (50)$$

(refer to the mentioned equations for meaning of symbols)
It should be noted that the derivation of such modified algorithms relies on the availability of algorithms for each important individual variable.

SUMMARY AND DISCUSSION

    Basic biodegradation algorithms are usually based on one of two basic approaches: decay or enzymatic reactions. These algorithms only address the rate of disappearance of a growth substrate as a function of the substrate concentration; no environmental variables are included in the models. Among the decay algorithms, the first order decay algorithm is used more widely for expressing the biodegradation of chemicals. However, decay algorithms often fail to express the complicated microbial interactions and population dynamics. Many enzymatic algorithms have thus been developed. Numerous studies have modified the

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enzymatic algorithms by including inhibition effects. Such modified algorithms are believed to be superior to the decay algorithms.

Three algorithms, i.e., simple linear regression, the $Q_{10}$ model, and the Arrhenius equation, are used to describe temperature effects on biodegradation rates. Among them, the Arrhenius equation is more extensively used, and in most cases fits the data satisfactorily. The $Q_{10}$ model also found to fit the data quite well for some organic species. Comparing between the $Q_{10}$ and the Arrhenius equations, $Q_{10}$ provides a readily comprehensible term descriptive of the overall effect of temperature, and has the advantage over E (activation energy) in that it offers less temptation to enter into an oversimplified interpretation. The main disadvantage in using $Q_{10}$ is that $Q_{10}$ drifts more with temperature than does E. The disadvantage of the Arrhenius equation is that several types of reactions are known which do not give a straight line when log k is plotted against 1/T. These deviations from the Arrhenius equation have been reviewed by Hulett (1964). Biological processes, including microbial growth and enzyme reactions studied in vivo or in vitro, characteristically give non-linear plots (Farrell and Rose, 1967) and several attemps have been made to explain this non-linearity for enzyme activity (Ingraham and Bailey, 1959; Hulett, 1964; Farrell and Rose, 1967; Hamaker, 1972; Howard et al., 1979). Three factors likely to be important are: (a) susceptibility of enzyme proteins to certain temperatures; (b) the complexity of reaction mechanisms of enzyme catalysis and the biochemical organization of the organisms; and (c) changes in population size, distribution or metabolic processes influencing the activity of the population and, therefore, biodegradation rates.

Diffusion effects on biodegradation rates may be quantifiable if diffusion coefficients in complicated (usually nonhomogenous) systems are available. It is suggested that many of the models used in geohydrology or for pollutant migration in environmental media can be applied.

Determination of the effects of nutrients on biodegradation rates may use the same algorithms as that of substrate biodegradation (i.e., decay or enzymatic algorithms). Apparently, these models are applicable when nutrients become the limiting factor for the growth of microorganisms.

There are numerous algorithms available describing inhibition effects on biodegradation rates (Table 16). Many of the algorithms are so complicated that their practicality may be limited. Unlike inhibition effects, quantitative expressions of the effects of toxins are greatly lacking. It is believed that such effects may be beyond the enzyme-substrate complexation algorithms as derived for most of the inhibition effects.

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No algorithms are available for quantifying the effect of water availability on biodegradation rates in aqueous environments. For non-aqueous environments, algorithms based on the exponential relationships (e.g., Equations (45) and (46) have been derived based on experimental results. Algorithms similar to the Michaelis-Menten equation (e.g., Equation (47) have also been derived for describing the effects of moisture on biodegradation. However, these types of models need to be validated experimentally.

Algorithms for other environmental variables (e.g., pH, Eh, hydrostatic pressure, microbial interactions, etc.) have not been reported in the literature. Due to the complexity of environmental factors affecting biodegradation rates, it is unlikely that useful mathematical expressions relating changes in those factors to effects on biodegradation rates will become available in the near future.

The literautre review also revealed that biodegradation studies have encompassed a wide variety of environmental conditions. Some studies were conducted without the control or measurement, of environmental variables. It is virtually impossible to derive generic algorithms based on experimental results in which environmental conditions were not controlled or were unknown. If generic algorithms describing effects of environmental variables on biodegradation rates are to be derived, controlled environmental conditions and more experiments to cover wide varieties of chemicals and environmental variables are urgently needed.

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