AQUATIC HETEROTROPHIC BACTERIA ACTIVE IN THE BIOTRANSFORMATION OF ANTHRACENE AND PENTACHLOROPHENOL

THESIS

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By

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Dominant genera of bacteria were isolated from three river waters during anthracene and pentachlorophenol biotransformation studies. The genera Pseudomonas, Acinetobacter, Micrococcus, Chromobacterium, Alcaligenes, Azomonos, Bacillus, and Flavobacterium were capable of biotransforming one or both of these compounds. These isolates were subjected to further biotransformation tests, including river water and a basal salt medium with and without additional glucose.

The results of these experiments were evaluated statistically. It was concluded that only a limited number of the bacteria identified were able to transform these chemicals in river water. The addition of glucose to the growth medium significantly affected the biotransformation of these chemicals. It was also determined that the size of the initial bacterial population is not a factor in determining whether biotransformation of anthracene or pentachlorophenol can occur.

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CHAPTER I

INTRODUCTION

A large variety of synthetic organic chemicals are currently produced for use in industry, agriculture, the home, and elsewhere (King, 1981, Alexander, 1965a). Production of these chemicals increased dramatically in the years immediately following World War II. If they are not removed or degraded by biological or non-biological means they will eventually reach the environment where they may present a hazard to man and/or animals (King, 1981).

Early investigators were primarily concerned with determining which degradative processes were important in establishing the stability of these molecules in the environment. When it was realized that microorganisms played an important role in the degradation of these xenobiotics, biochemical and microbiological investigations were initiated to determine the basic principles of microbial metabolism. Thus, throughout the 1950s and 1960s investigators were involved with associating microbial metabolic activities with synthetic chemical transformations. It was found that microbes had an astounding capability for degrading these newly synthesized, unique chemicals, and some scientists claimed that microbes were infallible and could

degrade any synthetic molecule. Others were more skeptical and believed that certain molecules could not be metabolized and were therefore considered recalcitrant (Alexander, 1965b; Horvath, 1972).

This debate tremendously invigorated research on organic chemical metabolism, and led to a better understanding of the basic biochemistry of synthetic chemical metabolism. This argument also helped to establish the fact that the persistence and fate of xenobiotics in the environment were strongly affected by microbial activity, and in many situations microbes were more instrumental in an organic chemical's degradation than were physical or chemical processes.

In the 1970s, industries manufacturing these synthetic organic chemicals began to recognize the damages that large-scale use of chemicals could have on the environment as well as on public health. Pesticide usage increased from 1.1×10^9 lb/year in 1971 to 1.4×10^9 lb/year in 1977, and to 1.5×10^9 lb/year by the end of the decade (Storck, 1980). Problems of chronic exposure, of improper waste disposal (Murry, 1976; Munnecke et al., 1976), environmental damage, and many more adverse responses to pesticides required that federal laws such as the Federal Insecticide, Fungicide, and Rodenticide Act of 1972 and the Federal Water Pollution Control Act of 1972 be promulgated to govern the manufacture, distribution, and use of agriculture

chemicals (Munnecke, 1979a). These laws were aimed at restricting the use of extremely toxic, environmentally dangerous chemicals and reducing the undesirable discharge of chemicals into the environment. In developing these laws, the microbial metabolism studies from the 1950s to 1960s were very helpful in determining which chemicals, due to their environmental persistence and inherent toxicity, truly represented a danger to society. These new federal laws were enacted to control these potentially hazardous chemicals by requiring better technologies for control of industrial waste discharges and for chemical cleanup.

The diversity in the types of chemicals to be detoxified mandated that many different approaches be considered. Physical methods of incineration, entrapment, and burial were developed, as well as chemical methods involving oxidation, reduction, and hydrolysis (Scurlock et al., 1975; Bouwer, 1976; Ottinger and Blumental, 1974; Baughman and Lassiter, 1978). However, heavy use of biological treatment systems persisted, and this technology was improved.

There are two main routes by which a chemical can reach natural waters: (1) via discharges following wastewater treatment (point sources), or (2) from nonpoint sources (i.e., urban and/or agricultural runoff). Once in the aquatic environment, biological degradation can contribute to the removal of synthetic organic chemicals. Thus, assessment of biotransformation is essential in determining

the extent to which chemicals can be assimilated by a receiving water (King, 1981).

Microorganisms in soil and water are able to convert many synthetic organic chemicals to inorganic products. These microbial processes may lead to environmental detoxification, the formation of new toxicants, or the biosynthesis of persistent products. Several different types of reaction mechanisms have been proposed for major categories of microbial transformation of synthetic chemicals in soil, natural waters, and sewage. However, some organic molecules are resistant to microbial attack (Odeyemi and Alexander, 1977). Using the knowledge gained from research in the 1960s, efforts in the late 1970s led to the demonstration of the potential use of bacterial action for a variety of detoxification applications in industrial wastewater, and agricultural water treatment, and in natural waters after accidental chemical spills (Munnecke, 1979b; Munnecke, 1980).

Concern about the microbial degradation of synthetic molecules, which was the purpose of this study, is of practical importance for several reasons.

1. Criteria and standards for water quality refer to maximum acceptable levels of many organic pollutants that are below 100 ng/mL (Alexander, 1973, 1985).

2. Numerous toxicants are harmful at levels in the part per billion range (Powers et al., 1977; Batterton et al., 1978) and the risk assessments suggest that many others are probably injurious even in such trace amounts.
3. A substance may be nontoxic in the amounts that exist free in the water or outside the microbial cell in soil, but if the chemical is subjected to bioconcentration, species at higher trophic levels may be harmed (Alexander, 1985). Although the toxic chemical affecting the species at the higher trophic level is at a high concentration within the organism, the chemical is not subjected to microbial decomposition because it is within the tissues of the animal or plant and not free in water, soil, or sediments.
4. Undesirable tastes and odors in water may result from the presence of certain compounds at the ng/mL level (Faust and Aly, 1964).
5. Organic chemicals introduced into water or soil, either inadvertently or deliberately, are subject to non-biological and biological changes. Although significant alteration in structure and properties of organic molecules may result from nonbiological processes, the major and often the only mechanism by which such compounds are

converted to inorganic products is biological (Alexander, 1980). Moreover, the chief and possibly the sole biological agents for the total conversion of organic compounds to inorganic products in water and soil appear to be microorganisms.

6. Incomplete degradation is frequently of environmental concern because the products of these partial reactions may be (a) more toxic than the original substance, (b) toxic, whereas the parent molecule was non-toxic at environmental concentration, (c) more persistent and able to endure longer than the original substance, or (d) subject to bioconcentration or other biological changes which are different from those undergone by the precursor molecule. However, the microorganisms of waters and soils may bring about one or more of the following transformations: mineralization (ultimate biodegradation), detoxication, co-metabolism, activation (Alexander, 1980). This will be more substantively discussed in a later part of this section. Characteristics of aquatic microflora are evident in their remarkable capacity for mineralization or, maybe co-metabolism (Alexander, 1980). A necessary consequence of mineralization of toxicants is detoxication. With a few exceptions, mineralization in marine and inland waters

probably is the result solely or largely of microbial activity.

Given the tens of thousands of compounds that are now potentially subject to regulation under the Toxic Substances Control Act of 1976, it is surprising that the mineralization of remarkably few of the environmentally important synthetic chemicals have been investigated. In only a small number of instances is there information indicating that these synthetic products are destroyed in natural waters. Much of the limited information that is available is derived from studies of pesticides, but pesticides make up a small percentage of the total number of compounds that are introduced deliberately or inadvertently into natural waters. Hence, a critical area for research is the establishment of the susceptibility to mineralization of synthetic organic compounds. In a number of instances, micro organisms fail to mineralize synthetic organic compounds and has led to considerable research on a phenomenon termed co-metabolism or sometimes co-oxidation (Alexander, 1979; Golab et al., 1980).

Researchers assumed that if a compound was mineralized, co-metabolized, or resistant to microbial conversion at the levels normally used for biodegradation tests, it would be similarly mineralized, co-metabolized, or resistant in nature. It was also assumed that the product would be the same regardless of substrate concentration and that the

kinetics would be unchanged except that rates would decline in direct proportion to substrate concentration (Kuznetsov et al., 1979; Kooij et al., 1980; Suflita et al., 1983; Simkins and Alexander, 1984; Paris et al., 1981; Alexander, 1985).

The primary goal of this research was to isolate and identify aquatic heterotrophic bacteria active in the biotransformation of anthracene and pentachlorophenol (PCP). Attempts were also made to assess whether or not the bacteria used anthracene and PCP as sole carbon source or possibly co-metabolized these compounds.

Biotransformation

Biotransformation can be defined as biologically mediated change in the composition or structure of a particular chemical. It is an encompassing term including addition to the parent chemical or biodegradation which involves biologically induced reduction in the complexity of a particular chemical by splitting off one or more constituent groups of the components (Dickson et al., 1981; Alexander, 1980). Biotransformation is the only way in which chemicals are mineralized in natural environments. Furthermore, biotransformation is a major fate process for many, but not all, chemicals. Biotransformation of a compound may reduce its toxicity, or in some cases increase the toxicity. Some metabolites have been shown to be more toxic than their

parent compounds (Alexander, 1981). For many compounds, biotransformation influences how persistent the compound will be in the environment (Kobayashi and Rittman, 1982). Consequently, biotransformation of a compound is important in making hazard assessment decisions because of requirements set forth by the Toxic Substances Control Act of 1976 (Alexander, 1981).

Several variables may affect the biotransformation of a chemical in the aquatic environment. These variables can be divided into two main categories: those that determine the availability and concentration of the compound in question and those that directly influence the reaction rate. Some variables that may affect the apparent biotransformation are (Scow, 1982):

oxygen availability
redox
sediments (packed vs. suspended)
temperature
pH
chemical concentration
physico-chemical properties of the chemical
acclimation of the organisms to the chemical
number of organisms present
type of organisms present
chemical mixtures
carrier solvents
water volume
flask size
mixing rate
nutrient, and numerous other parameters.

However, if a xenobiotic compound is exposed to a bacterium there are several possibilities for its transformation or inactivation.

1. Ultimate biodegradation (mineralization or complete biodegradation) - The compound can serve as a substrate for growth and energy, and in this case the compound is degraded to (a) water, (b) carbon dioxide, and (c) inorganic compounds (if elements other than carbon, hydrogen and oxygen are present) (Water Pollution Control Federation, 1967; Bunch and Chambers, 1967; U.S. EPA, 1979a).
2. Detoxication - Detoxication refers to the conversion of a toxicant to innocous metabolites.
3. Co-metabolism - The microbial transformation of an organic compound that does not provide the active organisms with a source of nutrients or energy is known as co-metabolism (Pfaender and Alexander, 1973), or if the reaction involves an oxidative process, co-oxidation (Stuart et al., 1980). Co-metabolism commonly leads to the accumulation of organic products structurally similar to the initial substrate (Loos et al., 1967; Brown, 1978). However, some of the products of microbial metabolism in culture do not appear to accumulate in soil or natural water, possibly because other microorganisms are able to mineralize these products (Rosenberg and Alexander, 1980; Jacobson et al., 1980). Co-metabolism of a toxicant may also result in detoxication, and certain synthetic

compounds that appear to be co-metabolized in nature are converted to products that are not of ecological concern (Mick and Dahn, 1970; Rosenberg and Alexander, 1980).

4. Conjugation or Polymerization - A xenobiotic molecule or a corresponding intermediate can be conjugated or polymerized with naturally occurring compounds (U.S. EPA, 1979a).
5. Bioaccumulation - the increase in concentration of a particular chemical in (or on) microorganisms by direct contact with the chemical in water (U.S. EPA, 1979a).
6. Activation - When a nontoxic molecule is converted to one that is toxic, or a molecule with low potency is made into a product of greater activity against some species it is said to be activated (Alexander, 1980).

No attempts have been made to cover all aspects of biotransformation of anthracene and PCP. However, there are several excellent reviews available covering the topic of biotransformation (Evans et al., 1971; Hegeman, 1972; Gibson, 1971; Omorit and Alexander, 1978; Watanabe, 1973; Dagley, 1972; Stanlake and Finn, 1982; Southworth, 1977; Stark, 1969; Pierce et al., 1978; Oloffs et al., 1972; Paris et al., 1981; Scow, 1982; Lee and Jones, 1980; Staples et al., 1983; Pritchard, 1984; Alexander, 1985).

Test Compounds

The major objectives of this study were to isolate, identify, and characterize the dominant organisms that biotransform anthracene or PCP under various conditions. Two compounds, anthracene and PCP, were chosen to study for several reasons: (1) the compounds are on EPA's 129 priority pollutant list, (2) used as agricultural or manufacturing products or intermediates, (3) have been potentially identified as carcinogens, (4) have been found in public water supplies, and (6) biotransformation is a major fate process. A brief summary of anthracene and PCP characteristics and literature review follows.

Anthracene

Anthracene is a polynuclear aromatic hydrocarbon (PAH). It is present in the environment from anthropogenic and natural sources. It is widely distributed in the environment, having been detected in animal and plant tissue, sediment, soils, air, and surface water (Radding et al., 1976).

Shackelford and Keith (1976) report that anthracene has been detected in finished drinking water, industrial effluents, and ambient river water. According to studies conducted with bacteria and mammals, as summarized by the National Academy of Sciences (1972) and Radding et al.

(1976) the degradation and metabolism of anthracene and the identification of its metabolites are not well known.

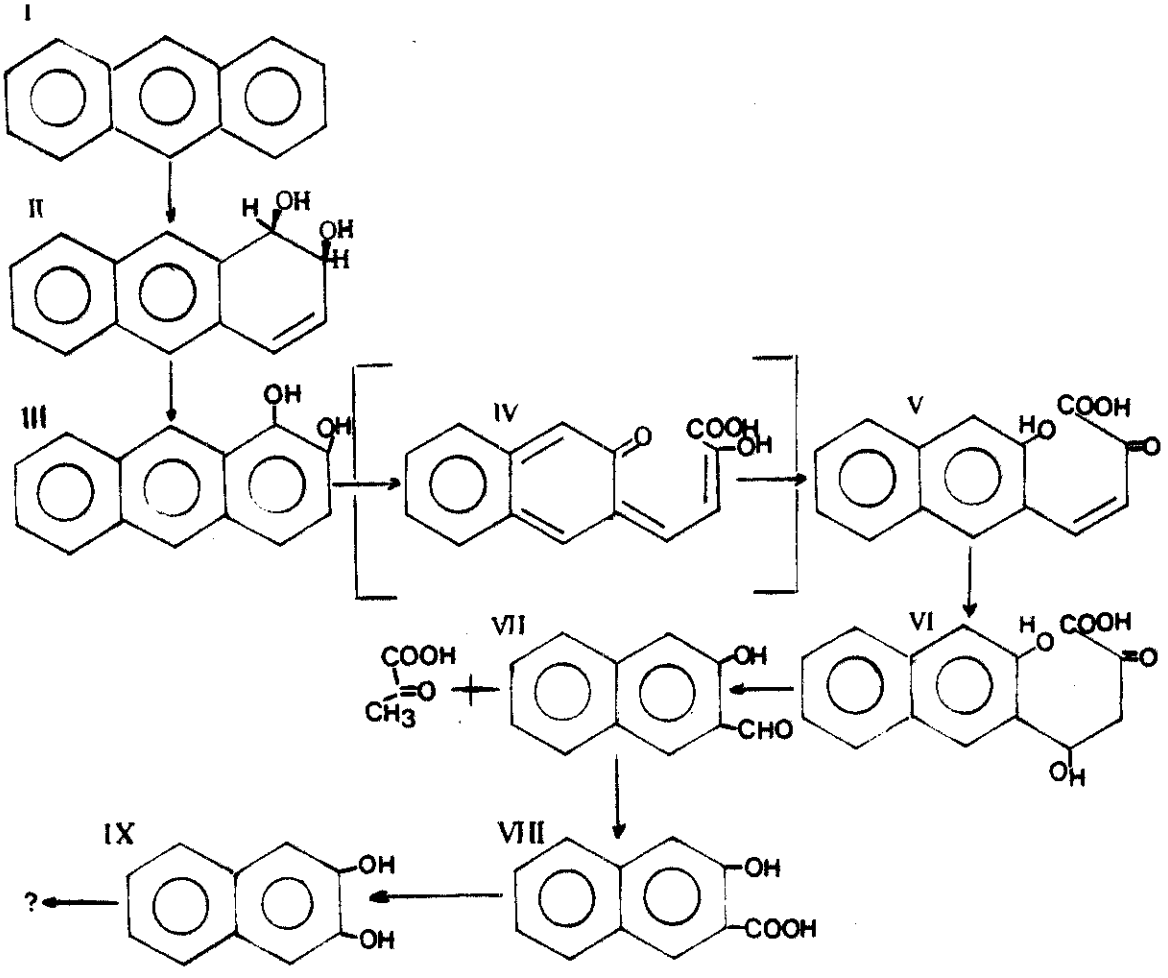
Bacteria have been shown to utilize some polycyclic aromatic hydrocarbons as a sole carbon source for growth. Evidence summarized by Radding et al. (1976) suggests that bacteria can degrade such compounds much more completely than mammals. Evidence for bacterial degradation comes from studies conducted on only a few compounds. Data specific to anthracene are limited. Herbes and Schwall (1978) determined the microbial transformation of several polycyclic aromatic hydrocarbons including naphthalene, anthracene, benzo[a]anthracene and benzo[a]pyrene in both pristine and petroleum contaminated sediment. They reported that half-lifes for degradation ranged from 5 hours for naphthalene to 280 hours for anthracene, 7,000 hours for benzo[a]anthracene and 21,000 hours for benzo[a]pyrene in petroleum contaminated sediments. It has also been reported that for PAHs the transformation even by acclimated microorganisms is inversely related to the number of cyclic rings that a compound possesses, with some such as benzo[a]pyrene being almost totally resistant to biotransformation (Herbes and Schwall, 1978). Since no microorganisms have been isolated that are capable of using 4 or 5 ringed compounds as a sole carbon source, it is most likely that these types of compounds are being co-metabolized along with simpler and relatively more degradable

compounds (U.S. EPA, 1979a).

The microbial degradation of polycyclic aromatic hydrocarbons was also studied by Groenewegen and Stolp (1975). Their data showed that some of tricyclic aromatic hydrocarbons are capable of being degraded by microbes. The authors stated that not much is known about anthracene degradation. They further report that organic compounds which are difficult to degrade are sometimes co-oxidized by microbes. This is based on the observation that many bacteria have the ability to oxidize a variety of organic compounds even though they do not use them as growth substrates. Thus, co-oxidation in this case refers to the oxidation of aromatic hydrocarbons when they are present as co-substrates in a medium in which one or more different organic compounds are furnished for growth (Raymond and Jamison, 1971; Stuart et al., 1980). However, little is known about the co-oxidation of anthracene.

Figure 1 is the usual catabolic pathway described for anthracene, but it should be regarded with some uncertainty (Rogoff and Wender, 1957). 3-Hydroxy-2-naphthoate has been isolated as a metabolite of anthracene with cultures of Pseudomonas aeruginosa and Flavobacterium sp. (Rogoff and Wender, 1957). P. aeruginosa grown on media containing anthracene readily oxidized salicylate and catechol, but it is not known if this was because of the non-specific induction of a naphthalene pathway, a pathway by which

Fig. 1--Proposed catobolic pathway for anthracene catabolism (Rogoff and Wender, 1957).


I
II
H
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III
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COOH
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V
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COOH
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VI
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VII
COOH
C=O
CH3
OH
CHO
VIII
OH
COOH
IX
OH
OH
?

anthracene has been shown to degrade. 3-Hydroxy-2-naphthoate was proposed to be the precursor of 2,3-dihydroxynaphtalene, a compound isolated by Fernley (1959) from culture filtrates of P. aeruginosa grown on media containing anthracene. In turn, this compound was reported to be a precursor of salicylate and catechol, but there is no strong evidence for this claim. From a review of the literature it is evident that much remains to be learned about the bacterial degradation of anthracene and its analogs. In Table I some physical properties of anthracene are presented. Table II gives a summary of the known environmental fate of anthracene (U.S. EPA, 1979a).

Pentachlorophenol (PCP)

Chlorinated aromatic hydrocarbons are widely used as pesticides and herbicides in the agricultural industry and as precursors in chemical synthesis by the chemical industry. Pentachlorophenol is a general biocide. It was first introduced into the United States during the 1930s, and originally was employed as a wood preservative (Pignatello et al., 1983; Arsenault, 1976). It is still used for that purpose, but its uses have expanded to include applications as a fungicide, bactericide, herbicide, molluscicide, algicide, and insecticide. This compound may present a potential threat to human health through its wide use in agricultural and industrial applications. Pollution of

TABLE I

GENERAL PHYSICAL PROPERTIES OF ANTHRACENE

Molecular weight.	178.23*
Melting point	216°C*
Vapor pressure (20°C)	1.95×10^{-4} torr*
Solubility in water (25°C).	0.045 mg/L** 0.073 mg/L***
Log octanol/water partition coefficients	4.45*

*Radding et al. (1976)

**May and Wasik (1978)

***Mackay and Shiu (1977)

TABLE II

SUMMARY OF AQUATIC FATE OF ANTHRACENE

Environmental Process	Summary Statement	Rate	Half-Life ($t_{\frac{1}{2}}$)	Confidence of Data
Photolysis	Dissolved portion may undergo rapid photolysis.	-	∿35 minutes	Medium
Oxidation	Oixdation of anthracene by RO_2 radical is slow; not a significant process.	50 liter mol^{-1} sec^{-1}	1600 days	Medium
Hydrolysis	Anthracene does not contain groups amenable to hydrolysis.	0	-	Medium
Volatilization	May be competitive with adsorption.	0.002 to 0.179 hr^{-1}	18-300 hours	Low
Sorption*	Measured adsorption coefficients for anthracene and suspended solids are high; movement via sediment is considered to be an important transport process.	-	-	Medium
Bioaccumulation	A short-term process; anthracene is readily metabolized and long-term partitioning into biota is not a significant fate process.	-	-	Medium
Biotransformation/ Biodegradation	Anthracene can be degraded by microbes and is readily metabolized by multicellular organisms; biodegradation is probably the ultimate fate process.	<0.0612 hr^{-1}	>11.3 hours	Medium

*Because the solubility of this compound is relatively high, 50 percent or more may exist in true solution under conditions of normal sediment loading. U.S. EPA (1979a).

water by PCP may be a serious problem because of its high toxicity to fish (Ellis, 1937; Doudoroff et al., 1953; Statham et a. , 1976; Cleveland et al , 1982). However, PCP is not very persistent in the aqueous medium itself. It is sorbed by the organic matter of solids and sediments in a freshwater ecosystem (U.S. EPA, 1979a; Delaune, 1983).

Worldwide production of PCP is about $5x10^8$ kg per year, of which about $2.3x10^6$ kg is produced in the United States (Crosby, 1981). About 80% of U.S. production is used for commercial wood treatment. PCP is also widely used in rice farming. Rice paddy soil has been shown to contain PCP levels ranging from 5 to 100 ppb which corresponds to one percent or less of the applied amount (Watanabe, 1973). Pentachlorophenol was used as a major herbicide in rice fields in Japan from 1950 to 1970. The annual production or usage of the active ingredient in 1970 was about 15,000 tons, which was about one fourth of the total amount of all pesticides used in Japan. Because of its recently recognized ecotoxicity, its use has decreased dramatically since 1971 (Kuwatsuka and Igarashi, 1975).

It has also been reported that PCP may arise from environmental biotransformation of other commercial chemicals such as hexachlorobenzene and lindane (Pignatello et al., 1983). The relative contribution of these sources to the overall biospheric burden of PCP was not reported. Pentachlorophenol has been found in the atmosphere, even in

pristine areas (~0.25 ng m^3 in an uninhabited mountainous area). Air concentration can be as high as 160 $\mu g/m^3$ in rooms containing PCP-treated wood or paint (Crosby, 1981). Pentachlorophenol also contaminates rain and snow (Bevenue et al., 1972). Streams commonly contain PCP concentrations in the micrograms per liter range (Buhler et al., 1973), and ground water or drinking water contamination by PCP is fairly common in the United States (Buhler et al., 1973).

As Liu et al. (1981) points out, very little information is available regarding PCP biotransformation in aquatic environments. Most of the studies that are available involve laboratory models of aquatic systems (Kirsch and Etzel, 1973; Boyle et al., 1980; Liu et al., 1981). Pierce et al. (1978) studied the fate of PCP accidentally spilled into a freshwater stream-lake system. They were primarily interested in determining PCP persistence and the formation of PCP transformation products in contaminated lake sediments, and not necessarily the response of the microbial flora to the sudden presence of PCP. It has been reported that natural freshwater microorganisms may adapt to the presence of PCP and become effective at PCP mineralization (Watanabe and Hayashi, 1972; Watanabe, 1973). The microbial degradation of PCP, however, has been observed in several laboratory investigations in which the compound was used as the sole carbon source provided to microorganisms. Chu and Kirsch (1972) observed the oxidation of PCP by

bacteria obtained from a continuous flow enrichment culture. Watanabe and Hayashi (1972) reported the ability of Pseudomonas sp., isolated from PCP-saturated soil cultures, to grow in the presence of 40 ppm PCP as the sole carbon source. Microbial decomposition of PCP in soil and axenic cultures has been studied by several investigators (Kuwatsuka, 1972; Kirsch and Etzel, 1973; Heidman et al., 1967). They have reported the presence of two decomposition products, a tri- and a tetrachlorophenol that were relatively stable to further decomposition. Pierce et al. (1978), in one of the few studies to investigate the fate of PCP in natural aquatic environments, documented the presence of pentachloroanisole and the 2,3,5,6- and 2,3,4,5-tetrachlorophenol isomers as major degradation products. Cserjesi and Johnson (1972) also observed the methylation of PCP to pentachloroanisole by fungi. They noted, however, that the formation of pentachloroanisole did not account for the total reduction in the concentration of PCP in the growth medium and concluded that methylation is either the first step in the metabolism of this compound or a reaction parallel to degradation. Despite these studies, many of the details regarding biodegradation of PCP in soil and water are left unanswered.

Pentachlorophenol has been shown to be detoxified by fish and other aquatic organisms in several laboratory investigations. Akitake and Kobayashi (1975) found that

the goldfish, Carassium auratus, transformed PCP to a pentachlorphenyl sulfate, which was identical to that found in the littleneck clam (Kobayashi et al., 1970). Lu and Metcalf (1975) also noted that conjugation at the phenolic hydroxy group was the most important detoxification mechanism among the organisms they studied in their model aquatic ecosystem. Studies indicate that the primary route for removal of PCP in mammals, including man, is urinary excretion of a conjugate form (Jacobson and Yelner, 1971; Braum and Sauerhoff, 1976).

By 1984, investigations relating the biotransformation of PCP had been carried out by Young (1951), Tsunoda (1965), Suzuki and Nose (1971a), Aso and Sakamoto (1972), Watanabe (1973)), Watanabe and Hayashi (1972), Boyle et al. (1980), Crosby (1981), Montagna (1982), Stanlake and Finn (1982), and Pignatello et al. (1983). These studies, however, did not provide much information on the fate and behavior of PCP in soil and water with regard to the microbial degradation. Table III gives some physical properties of PCP and Table IV gives a summary of the environmental fate of PCP.

Summary of Literature Review

The removal of aromatic hydrocarbons from water is essential because of their direct or indirect toxic effects on plants, animals, and man. The literature review on the

TABLE III

GENERAL PHYSICAL PROPERTIES OF PENTACHLOROPHENOL

Molecular weight (Verschueren 1977)	266.35
Melting point. (Verschueren 1977)	190°C
Boiling point at 760 torr. (Verschueren 1977)	310°C
Vapor pressure at 20°C (Bevenue and Beckman 1967)	0.00011 torr
Solubility in water at 20°C. (Verschueren 1977)	14 mg/L*
Log octanol/water partition coefficient. (Leo et al. 1971)	5.01
pK_a (Drahonovsky and Vacek 1971)	4.74

*Windholz (1976) lists the solubility as 80 mg/L. In a natural water system this pollutant will be present primarily as an anion and its solubility, therefore, will be dependent on the cationic composition of the water. The anion, however, will be much more soluble than the undissociated compound under any circumstances.

TABLE IV

SUMMARY OF AQUATIC FATE OF PENTACHLOROPHENOL

Environmental Process	Summary Statement	Rate	Half-Life ($t_{\frac{1}{2}}$)	Confidence of Data
Photolysis*	Photolysis of pentachlorophenol is reported to occur under natural conditions and it is probably an important process near the water surface.	3.4×10^{-4} sec^{-1}**	4.75 hours** 1.5 days***	High
Oxidation	No specific information found; is thought not to be an important process.	-	-	Low
Hydrolysis	No specific information found; is thought not to be an important process.	-	-	Low
Volatilization	Probably does not affect the aquatic fate of this pollutant.	-	-	High
Sorption	Definitely sorbed by the leaf litter and other organic matter in the soil and sediments of the watershed of a fresh-water lake.	-	-	High
Bioaccumulation	Has been shown to bioaccumulate in numerous aquatic organisms.	-	-	High
Biotransformation/ Biodegradation*	In a well-documented study of a fresh-water lake, two accidental spills were metabolized to pentachloroanisole and a mixture of chlorinated phenols.	-	-	High

*Both photolysis and biodegradation appear to be important fate processes.

**The rate was determined at pH 7 with a light intensity of 0.04 watts/cm^2 between 290 and 330 nm. The half-life was calculated for a depth of 300 cm on a midsummer day at the latitude of Cleveland, Ohio (Hiatt et al. 1960).

***The half-life was estimated from the data of Wong and Crosby (1978) for photolytic destruction by sunlight in an aqueous solution at Davis, California.

fate of anthracene and PCP indicated that biotransformation is an important fate process for aromatic hydrocarbons with four or less aromatic rings, and for chlorinated hydrocarbons (Rubin and Alexander, 1983). Biodegradation is probably much more important in those aquatic systems which are chronically affected by aromatic hydrocarbon contamination as a result of microbial acclimation. In general, very little is known regarding the types of bacteria that can use PCP and anthracene molecules as their sole source of carbon. Likewise, breakdown of these compounds as a result of co-metabolism is also not well understood. Information is needed on the identity of microorganisms which can biotransform these organic molecules in water and soil.

Hazard assessment requires knowledge of a wide range of topics such as biotransformation, transport, hydrolysis, volatilization, photolysis, dose-response interaction, among others. It is not feasible to cover all of the necessary elements of the hazard assessment process in one study. Hence, the area of my research deals with one area of hazard assessment, biotransformation.

Objectives and Hypothesis

The objectives of my thesis research were

1. To isolate and identify the five most dominant bacteria (to the genus level) present in tests assessing the influence of three different aquatic

water/sediment systems on biotransformation rates of anthracene and PCP;

2. To determine the ability of the two most dominant bacteria isolated from each aquatic system to grow in basal medium, with anthracene and PCP, as a sole source of carbon and energy, and in experimental water with the chemical as an additional carbon source;
3. To determine the ability of the two most dominant bacteria isolated from each aquatic system to biotransform anthracene or PCP in the experimental waters via co-metabolism.

The following hypotheses were formulated.

1. H_0: Bacteria associated with the three different aquatic sediments used in biotransformation tests respond differently in their growth (CFUs/mL) with each chemical and each aquatic system.

   Ha: The bacteria associated with three different aquatic sediments used in the biotransformation tests respond similarly in their growth (CFUs/mL) with each chemical and each aquatic system.

2. H_0: Bacteria present in three different river systems and associated suspended solids are not able to metabolize anthracene or PCP.

   Ha: Bacteria present in water and associated suspended solids are able to metabolize anthracene or

PCP.

3. H_0: Bacteria present in the three river systems and associated suspended solids are not able to biotransform anthracene or PCP when the growth medium is enriched with 1 g/L glucose.

   Ha: Bacteria present in the three river systems and associated suspended solids are able to biotransform anthracene and PCP when the growth medium is enriched with 1 g/L glucose.

4. H_0: Bacterial concentration has no effect on the biotransformation of anthracene and/or PCP.

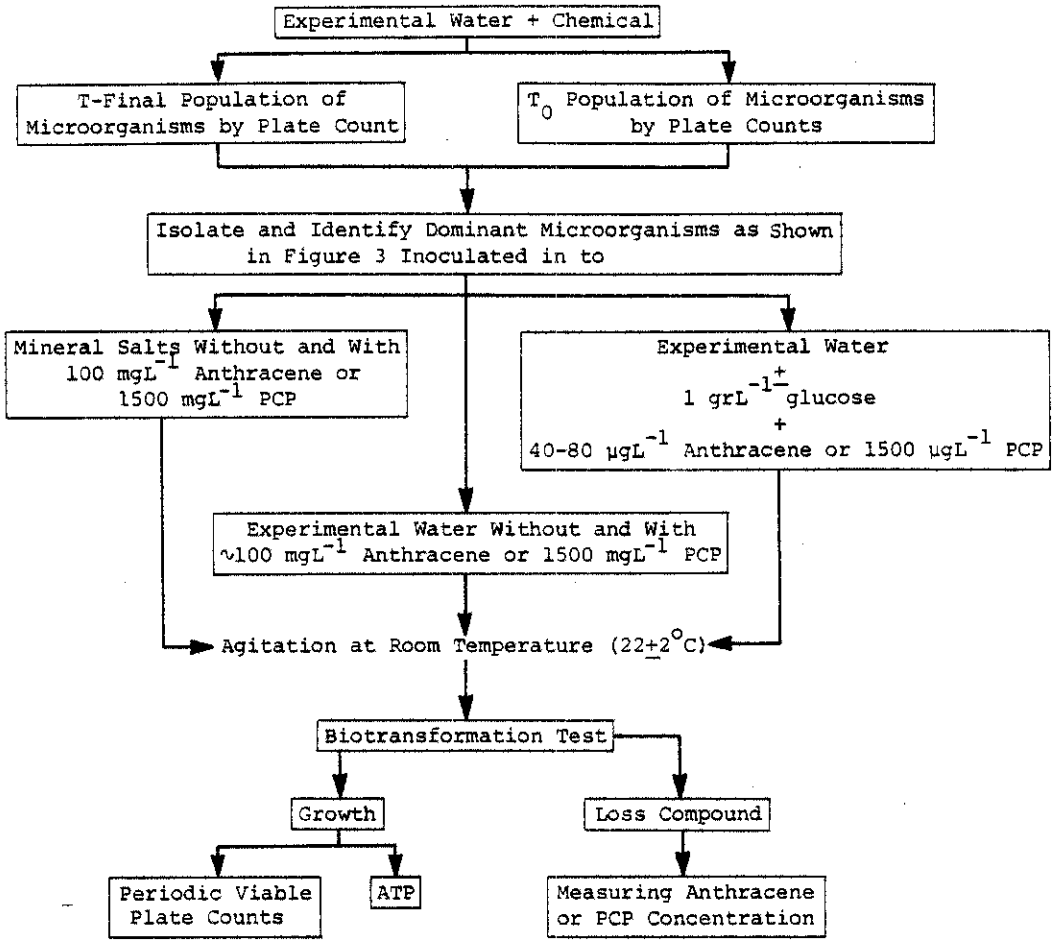
   Ha: Bacterial concentration has an effect on the biotransformation of anthracene and/or PCP.

5. H_0: Bacteria type has no effect on the biotransformation of anthracene and/or PCP.

   Ha: Bacteria type has an effect on the biotransformation of anthracene and/or PCP.

The general experimental approach used to test these hypothesis is outlined in Figure 2.

Fig. 2--To test hypothesis - Dominant organisms isolated from each water/sediment rate kinetic experiment can biotransform anthracene and pentachlorophenol (PCP).


Experimental Water + Chemical
T-Final Population of Microorganisms by Plate Count
T_0 Population of Microorganisms by Plate Counts
Isolate and Identify Dominant Microorganisms as Shown in Figure 3 Inoculated in to
Mineral Salts Without and With 100 mgL^{-1} Anthracene or 1500 mgL^{-1} PCP
Experimental Water
1 grL^{-1} ± glucose
+
40-80 μgL^{-1} Anthracene or 1500 μgL^{-1} PCP
Experimental Water Without and With ∿100 mgL^{-1} Anthracene or 1500 mgL^{-1} PCP
Agitation at Room Temperature (22±2°C)
Biotransformation Test
Growth
Loss Compound
Periodic Viable Plate Counts
ATP
Measuring Anthracene or PCP Concentration

CHAPTER II

MATERIALS AND METHODS

This research considered the effect of different types of bacteria from different aquatic systems on the biodegradation of anthracene and pentachlorophenol (PCP). The methods and materials used are discussed under the following sections:

- Test Chemicals
- Experimental Water Systems
- Test Organisms and Isolation Procedures
- Experimental Design
- Analytical procedures used for:
  (a) determination of bacteria growth and
  (b) determination of chemical concentration (biotransformation).
- Statistics and other methods of data evaluation.

Test Chemicals

Anthracene

Anthracene was chosen as a test chemical for several reasons. It is one of EPA's 129 Priority Water Pollutants (U.S. EPA, 1979a). It is representative of a large group of environmental pollutants (polynuclear aromatic hydrocarbons) as previously discussed, is a widely used industrial

chemical, and it may occur as a waste product of fossil fuel production. In addition, biotransformation has been identified as an important fate process for this chemical (U.S. EPA, 1979a and 1979b). Anthracene was obtained from the Matheson Coleman & Bell (MC & B) Chemical Company.

Pentachlorophenol (PCP)

Pentachlorophenol (PCP) was chosen as a test chemical because it is also included on EPA's 129 Priority Water Pollutant list (U.S. EPA, 1979a). It represents a widely used group of compounds (chlorinated pesticides) with uses in agriculture and industry (Beren and Beckman, 1967). PCP is used as a wood treatment. A 1974 survey disclosed that only 60% of the wood treatment plants surveyed met water pollution standards, and 17% of the plants released their wastewater without any treatment (Thompson, 1975). By 1983, all plants were required by the EPA to be in compliance with regulation restricting the release of chlorinated phenol in wastewater (Mulligan and Fox, 1976). One method of removing chlorinated phenols (including PCP) from water and wastewater is by means of microbial degradation (U.S. EPA, 1976). To date, information regarding microbial mediated breakdown of PCP in water is scarce.

Experimental Water Systems

Mississippi River

This system receives many types of industrial chemicals, by-products and agricultural runoff. The sampling site was at Memphis, Tennessee.

Red River

At the sampling site there has been little industrial by-product discharge into the systems. The sampling site was below Lake Texoma near Sherman, Texas.

Trinity River

This system receives extensive urban and agricultural runoff. Before reaching Dallas, Texas, the system receives little industrial waste. The sampling site was at Lamar Street Crossing, Dallas, Texas.

The differences in water characteristics between these three aquatic systems should allow for the detection of differences in microbial action with respect to biotransformation, if any, between the three river systems.

Basal Salt Medium (BM)

Mineral salts media was made up of the following constituents (in gram per liter) (Stanlake and Finn, 1982) $K_2H PO_4$, 1.73; KH_2PO_4, 0.68; NH_4NO_3, 1.0; $MgSO_4$ $7H_20$, 0.1; $CaCL_2$ $2H_2O$, 0.02; $FeSO_4$ $7H_20$, 0.003; and 0.002 trace metal (Table V).

TABLE V

BASAL SALTS MEDIUM

	X (gm/L)	Stock Solution 10X (gm/L)
Solution A. Phosphate Buffer (pH=7.4)		
K_2HPO4	1.73	17.30
KH_2PO4	0.68	6.80
Solution B. Major Elements		
$NH_4\ NO_3$	1.0	10.0
mg $SO_4 \cdot 7H_2O$	0.10	1.0
Solution C. Minor Elements		
$CaCL_2 \cdot 2H_2O$	0.02	0.20
$FeSO_4 \cdot 7H_2O$	0.003	0.03
Trace Metal		
Solution D. Major Elements		
$MnCL_2$	0.416	4.16
BO_3H_3	0.186	1.86
$ZnCl_2$	0.327	0.327
Solution E. Minor Elements		100X (gm/L)
$CoCl_2$	0.00143	0.143
$CuCl_2$	0.00011	0.011

Make stock solution and autoclave at 121°C for 15 minutes. After cooling, at room temperature, mix stock solution at a ratio of A:B:C:ofl:1 and D:E:ofl:10 separately (by volume). All ingredients were dissolved in distilled water.

Test Organisms and Isolation Procedures

The bacteria that were used in my thesis research were isolated from each of the three different natural water systems. The following procedures were used for isolation and identification of the various bacteria studied.

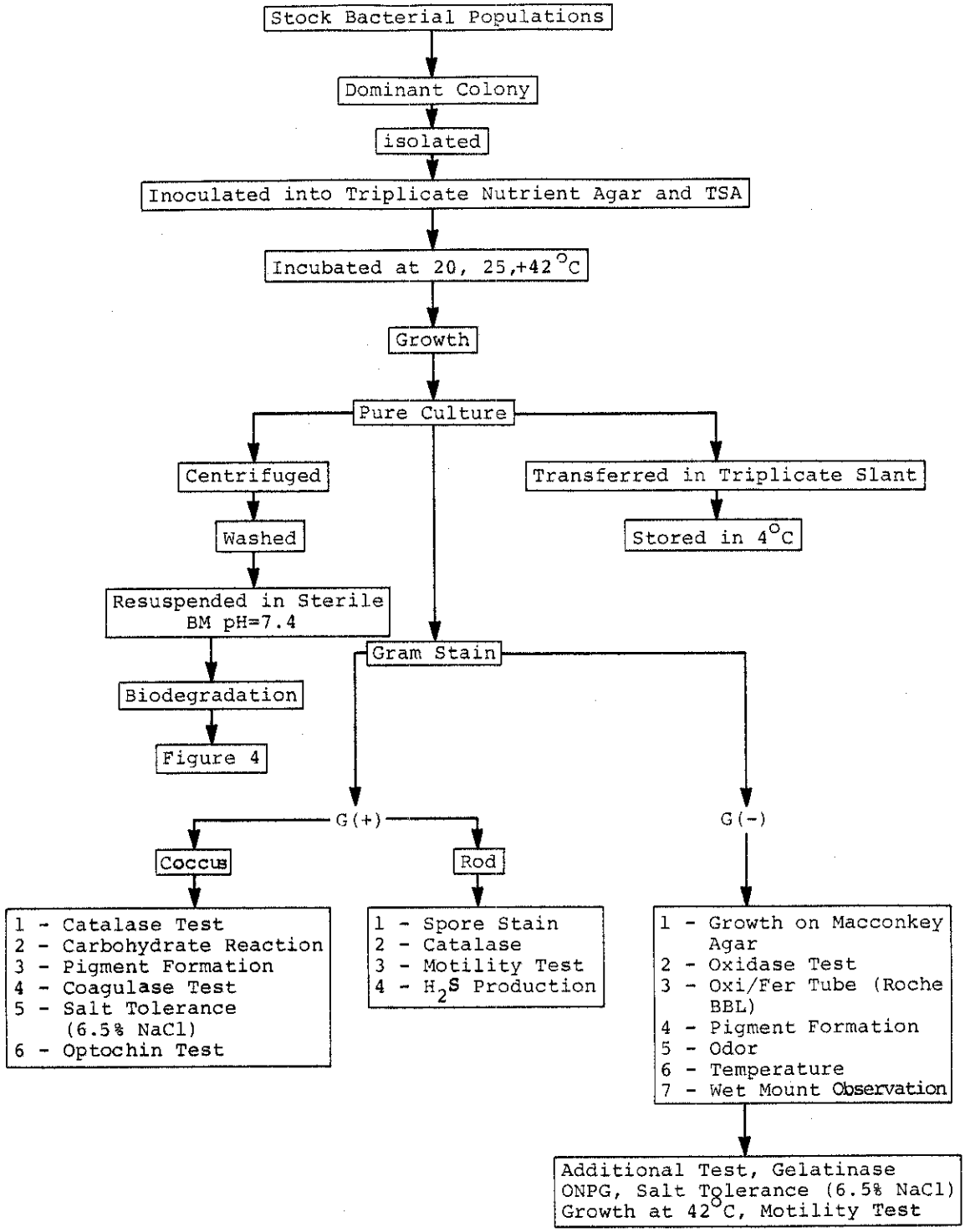
Initial samples of bacterial population for subsequent microbial analysis, both quantitative (Colony Forming Units CFUS) and qualitative (isolation and identification) were obtained from Tim J. Leslie's biotransformation experiments. These experiments were designed to determine the influences of suspended solids on biotransformation rates of anthracene and PCP. The experimental systems consist of river water samples containing 100 mg/L suspended solids. The Red River, Trinity River, and Mississippi River water/sediment sources were chosen in order to determine if laboratory generated biotransformation rate data varied between river systems.

Anthracene was added by Tim Leslie to each of his experimental flasks using acetone as the co-solvent when anthracene was the test chemical. The correct dilution was made to add 0.1 ml of the acetone-anthracene mixture to each flask to give a resulting concentration of approximately 40 μgL^{-1}. The acetone was then evaporated in a fume hood overnight. PCP was made up as a concentrated stock solution in the appropriate river water, then diluted with

the river water to give a working concentration of approximately 500 μgL^{-1}. One hundred mls of experimental water, either water alone or water with an additional 100 mgL^{-1} equivalent dry weight of sediment, were added to individual 125 ml Erlenmeyer screw cap flasks with a specially fitted teflon cap liner, and the test chemical was allowed to resolubilize in the water. Controls were prepared in the same manner and then autoclaved for 15 min at 121°C. Standard pour plates in duplicate were made using 0.1% plate count agar and 1% agar from control and test flasks. Bacteria on these duplicate plates were used for isolation and identification, in my experiments, using the following procedures.

The bacterial colonies formed on the plates were examined under a Quebec Colony Counter to determine the dominant colonial types. The five dominant colonies, from time zero and termination of biotransformation anthracene or PCP experiments, were inoculated onto triplicate nutrient agar plates (Difco) and/or trypticase soy agar plates (TSA) (Difco) and then incubated at 20°C, 25°C, 37°C, and 40°C for 24-96 hrs. Isolates were then transferred until pure cultures were obtained. After obtaining pure cultures, the isolates were transferred into nutrient agar and TSA slants and maintained at 4°C until needed for experiments. All isolates were Gram stained and identified using the basic determinative scheme outlined in Figure 3.

Fig. 3--Bacterial determination scheme


Stock Bacterial Populations
Dominant Colony
isolated
Inoculated into Triplicate Nutrient Agar and TSA
Incubated at 20, 25,+42 °C
Growth
Pure Culture
Centrifuged
Washed
Resuspended in Sterile BM pH=7.4
Biodegradation
Figure 4
Transferred in Triplicate Slant
Stored in 4°C
Gram Stain
G(+)
G(-)
Coccus
Rod
1 - Catalase Test
2 - Carbohydrate Reaction
3 - Pigment Formation
4 - Coagulase Test
5 - Salt Tolerance (6.5% NaCl)
6 - Optochin Test
1 - Spore Stain
2 - Catalase
3 - Motility Test
4 - H2S Production
1 - Growth on Macconkey Agar
2 - Oxidase Test
3 - Oxi/Fer Tube (Roche BBL)
4 - Pigment Formation
5 - Odor
6 - Temperature
7 - Wet Mount Observation
Additional Test, Gelatinase
ONPG, Salt Tolerance (6.5% NaCl)
Growth at 42°C, Motility Test

Identification to the generic level was facilitated by using: (1) Bergey's Manual of Determinative Bacteriology (Buchanan and Gibson, 1974), (2), Practical Clinical Microbiology and Mycology Technique and Interpretation (Wolf et al., 1975), (3) Manual of Clinical Microbiology (Lennete et al., 1974), (4) Computer Coding and Identification Systems for OXI/FERM Tube (Roche Diagnostics, Division of Hoffman-Laroche Inc., Nutley, J.J., 1974) and (5) Manual for the Identification of Gram-negative Nonfermentative Organisms (Weaver, 1976). Bergey's manual was the final arbitrator for all identifications of bacteria.

Experimental Design

Three different water systems were used in this study in addition to basal salt medium (BM) (Bergman et al., 1980; Oloffs et al., 1972). The three water systems included Red River, Mississippi River, and Trinity River waters. All waters used for the biotransformation study were stored, after collection, until used at 4^{o}C in 20 L Nalgene containers.

The two most dominant bacteria, as determined from the pour plating technique, from the river water/sediment systems were evaluated for their ability to utilize anthracene and/or PCP as a sole carbon source and to co-metabolize these compounds. This research project was done in three stages.

Stage 1 (long term incubation, 0-21 days)

The initial focus of this experiment was to assess the ability of a high population (10^6 - 10^{10} CFU mL^{-1}) of bacteria to grow in basal medium and in Red River water, Trinity River water, and Mississippi River water (experimental water) with one of the two chemicals added (anthracene, PCP).

Stage 2 (short term incubation, 0-7 days)

In order to obtain additional information concerning the significance of bacterial metabolism of anthracene and PCP a second part of the study was conducted. This experiment was started with lower and initial population of bacteria (~ 10 CFUs mL^{-1}) which were found from the long term study to biotransform anthracene or PCP in basal medium.

Stage 3

This experiment addressed biotransformation of anthracene or PCP in experimental waters enriched with glucose.

To assess the abilities of the two most dominant bacteria isolated from each water/sediment system to metabolize or co-metabolize anthracene or PCP the experimental protocol outlined in Figures 4 and 5 were followed for experimental waters and BM.

This protocol was designed to answer the following questions:

Fig. 4--Experimental protocol for measuring microbial growth in the basal medium and/or experimental water with addition of anthracene or pentachlorophenol (PCP).

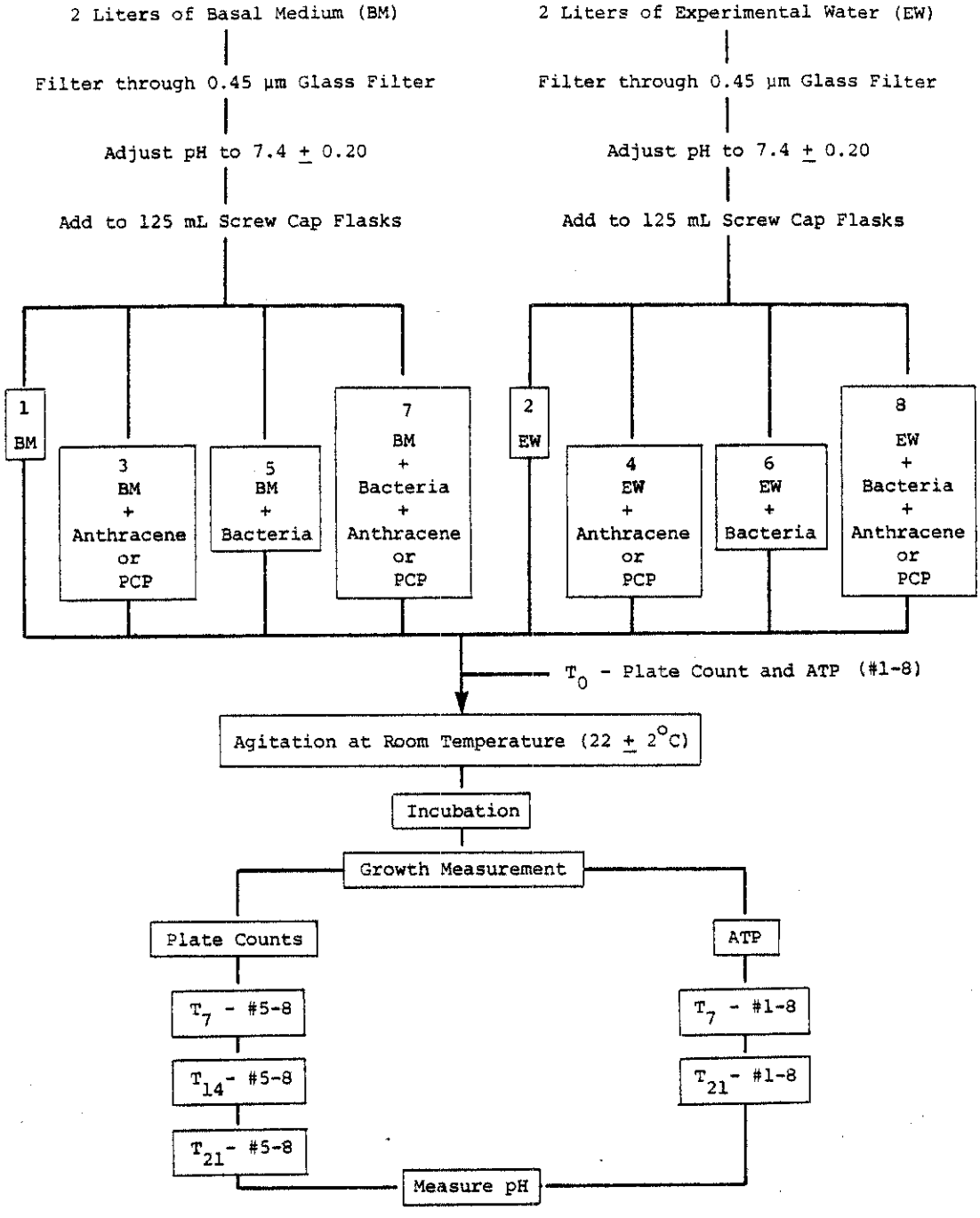
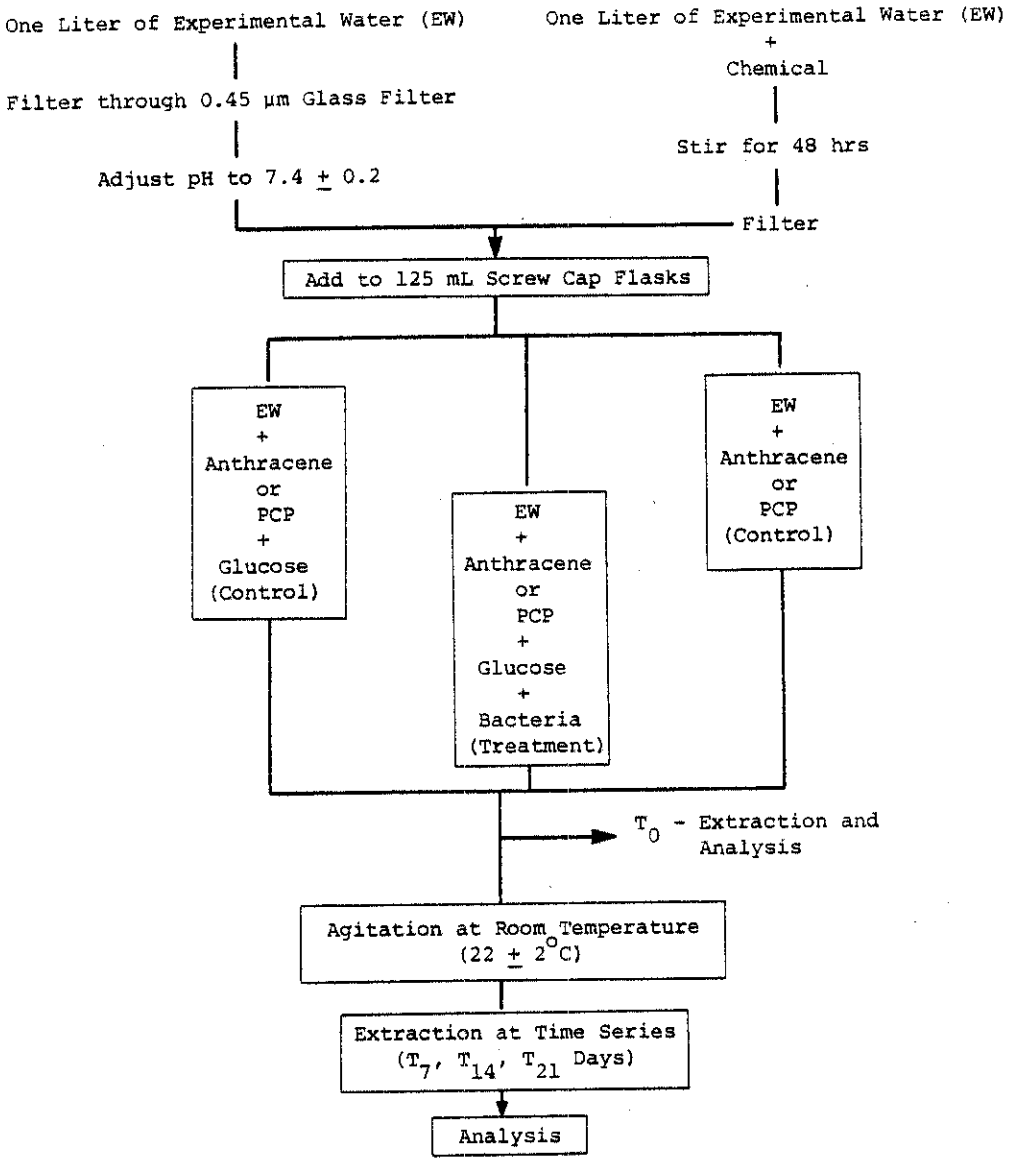

2 Liters of Basal Medium (BM)
2 Liters of Experimental Water (EW)
Filter through 0.45 µm Glass Filter
Filter through 0.45 µm Glass Filter
Adjust pH to 7.4 ± 0.20
Adjust pH to 7.4 ± 0.20
Add to 125 mL Screw Cap Flasks
Add to 125 mL Screw Cap Flasks
1 BM
3 BM + Anthracene or PCP
5 BM + Bacteria
7 BM + Bacteria + Anthracene or PCP
2 EW
4 EW + Anthracene or PCP
6 EW + Bacteria
8 EW + Bacteria + Anthracene or PCP
T0 - Plate Count and ATP (#1-8)
Agitation at Room Temperature (22 ± 2°C)
Incubation
Growth Measurement
Plate Counts
ATP
T7 - #5-8
T14 - #5-8
T21 - #5-8
T7 - #1-8
T21 - #1-8
Measure pH

Fig. 5--Experimental protocol for analyzing anthracene or pentachlorophenol (PCP) in the experimental water with addition of glucose.


One Liter of Experimental Water (EW)
Filter through 0.45 µm Glass Filter
Adjust pH to 7.4 ± 0.2
One Liter of Experimental Water (EW)
+
Chemical
Stir for 48 hrs
Filter
Add to 125 mL Screw Cap Flasks
EW + Anthracene or PCP + Glucose (Control)
EW + Anthracene or PCP + Glucose + Bacteria (Treatment)
EW + Anthracene or PCP (Control)
T_0 - Extraction and Analysis
Agitation at Room Temperature (22 ± 2°C)
Extraction at Time Series (T_7, T_{14}, T_{21} Days)
Analysis

1. Can the isolated dominant bacteria metabolize anthracene or PCP as a sole carbon source in basal medium?
2. Can the isolated dominant bacteria metabolize anthracene or PCP in the experimental waters?
3. Can the isolated dominant bacteria co-metabolize anthracene or PCP in the experimental water enriched with another carbon source (1 gL^{-1} glucose).

The experiments were conducted in triplicate 125-ml screw cap flasks with a specially fitted teflon cap liner. One hundred milliliters of a natural water or basal medium, which had been filtered through 0.45 micron glass fiber filters for sterilization, were added to each flask. All pH determinations were made with a Markson Electromark Analyzer at time zero and termination of the experiment. The pH of experimental waters and basal salts medium at initial time were adjusted to 7.2 $\pm$ .2 with concentrated ammonium hydroxide. Bacterial innocula were grown for 24-96 hrs in nutrient broth or TSB. The cultures were then centrifuged, washed three times with sterile basal medium, and resuspended in a sterile buffer with (pH=7.4) and held at room temperature for several hours to allow utilization of endogenous materials. These cultures were then added aseptically to appropriate sterile flasks according to the protocol outlined in Figures 4 and 5.

A series of flasks with BM (#1) and each experimental water (#2) were used as controls for checking for contamination. Flasks #3 and #4 were used as controls and contained BM (#3) or experimental water (#4) plus ∿100 mgL^{-1} anthracene or 1.5 mgL^{-1} of PCP. These uninoculated flasks were used as a control to check for possible chemical or physical changes of the chemicals in the medium. Flask #5 contained BM plus isolated bacteria. Flask #6 contained experimental water and bacteria and served as a control for bacterial growth occurring in the absence of either chemical.

Flasks #7 and #8 represented the actual experimental treatment and contained basal medium (#7) and each of the experimental waters (#8) plus isolated bacteria and chemical.

Anthracene was added aseptically into these autoclaved flasks while PCP was added before autoclaving. This was necessary due to the poor stability of anthracene at elevated temperature. Anthracene or PCP was used as the only additional carbon source for these experimental tests. As a result, this series of flasks were used for investigation of biotransformation which may be ultimate biodegradation in the BM. In addition to these tests, another series of flasks were set up for assessing co-metabolism. Experimental Series #9, #10, and #11 were also conducted in replicate (5) 125-ml screw cap flasks. Each flask

contained 100 ml of experimental water in final volume.

Flask #11 contained water plus chemical (anthracene or PCP) and accounted for reduction in chemical concentration via non-biological means. Flask #10 was a control which contained water, chemical, and 1 gL^{-1} glucose. Glucose served as a carbon and energy source. It was aseptically added into Flasks #9 and #10.

Flasks #9 was used as the experimental treatment which contained water plus chemical, and 1 gL^{-1} glucose and isolated bacteria. This second set of flasks were used to study biotransformation by co-metabolism. For this study, anthracene and PCP were allowed to resolubilize in water for ∿48 hrs. Then filter sterilized anthracene was aseptically added into previously autoclaved flasks. The desired PCP concentration was added into each flask before they were autoclaved. At the initiation of an experiment, time zero, the concentration of anthracene in each flask was ∿45-80 μgL^{-1} while PCP was ∿1500 μgL^{-1}. Experimental Series #9, 10, and 11 were monitored for loss of parent compound by sampling at 0, 7, 14, and 21 days following inoculation. Measurement of microbial growth was not made on Series #9, 10, and 11. The inoculated flasks (#1-11) were incubated for 21 days in the dark at 22 $\pm$ 2^{o}C (room temperature) on a shaker table (Flo Gyrotory Shaker, New Brunswick Scientific Co., Inc., New Brunswick, NJ) with continuous agitation (100 cycles/min). Figure 4

illustrates the experimental approach.

Analytical Procedures

The ability of the bacteria to biotransform the two chemicals was determined by two methods:

a. Bacterial Growth Measurements (comparisons between treatment and controls)

1. Colony Counts (Spread Plates)
2. The Measurement of Adenosine Triphosphate (ATP)

b. Measurement of Chemical Concentration Through Time.

All experiments were conducted using time series analyses of these parameters (i.e., T_0, T_7, T_{14}, and T_{21}).

Bacterial Growth Measurements

Colony Counts by Spread Plating--One of the methods chosen for bacterial transformation testing was the spread plate technique (Bousfield et al., 1973; Young, 1979). Samples were serially diluted in sterile basal medium to obtain the appropriate concentrations for plating, then 0.1 milliliter of a diluted sample was pipetted onto each of two sterile pre-dried plates containing plate count agar plus 1.5% agar (Hungate, 1962) and spread evenly over the surface with a sterile bent glass rod (alcohol-flamed). All inoculated duplicate plates were inverted and incubated at 22 $\pm$ 2°C for 7 days before the colonies were counted

(Young, 1979).

At the end of the incubation period, the bacterial colonies were counted using a Quebec Colony Counter. Acceptable ranges for counts were 30-300 colonies per plate.

Heterotrophic bacteria concentration for spread plate methods were obtained by multiplying the number of colonies (in the acceptable range) by the appropriate dilution factor. Viable microbial populations were enumerated by spread plate count technique (Young, 1979) at various time intervals (0,1,2,3,4,5,6,7 days). All counts were extrapolated from the most countable duplicate plates and expressed as the number of colony-forming unit (CFUs) per ml of sample. In the second study of biotransformation, a standard curve based on optical density measurements for serial dilutions of cell suspensions was derived using a Beckman spectronic 20 spectrophotometer (λ = 600 nm). This relationship was then employed to determine bacterial concentrations in the test vessels.

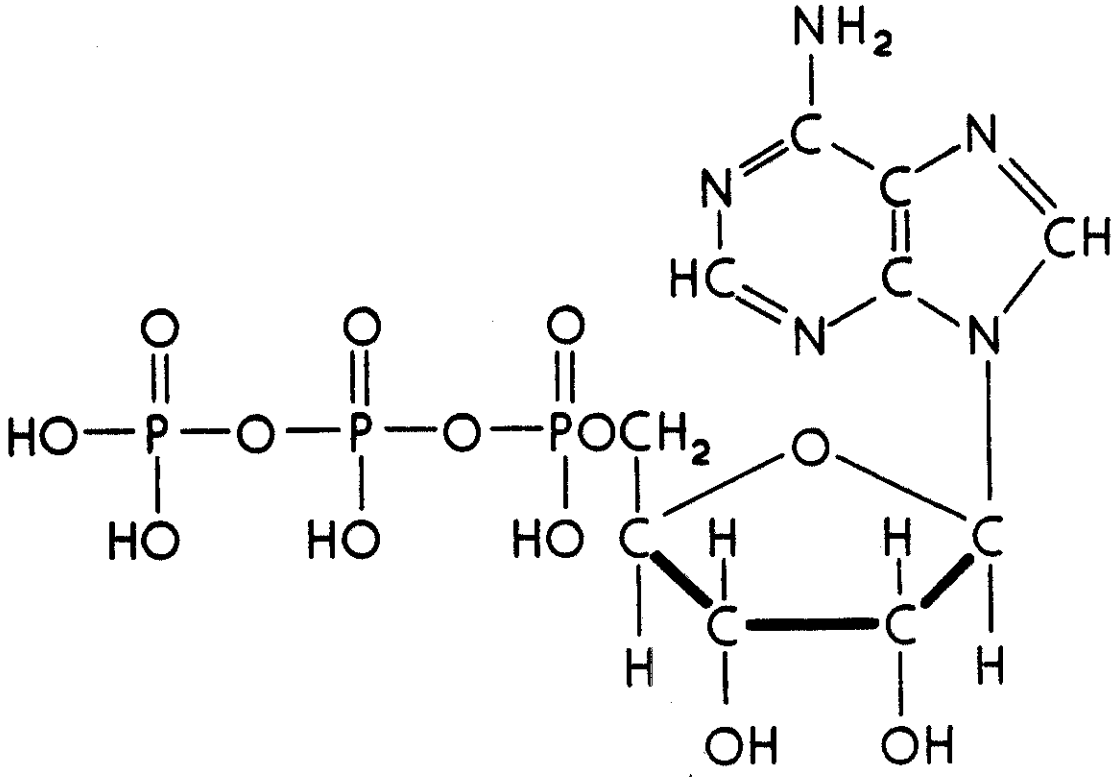
The Measurement of Adenosine Triphosphate (ATP).-- Adenosine triphosphate (ATP) has long been recognized as one of the most important low molecular weight compounds in living organisms. Since its isolation from muscle by Lohmann (1929), the role ATP plays in energy transfer in metabolic processes has become well documented, with substantial evidence that it is present in the cells of all

animals, plants, and microorganism (Lehniger, 1965).

More recently, ATP has received interest as an estimater of living microbial biomass (Hansen, 1970; Hansen and Booth, 1966), providing a valuable tool in ecological studies. Basing their method on that of Strehler and McElroy (1957), Hansen and Booth demonstrated that ATP can be used to estimate microbial biomass in the ocean and lakes. Since then, the method has found use in a variety of studies of freshwater (Rudd and Hamilton, 1973; Tobin et al., 1978). The method has also been adapted for use in studies of microbial biomass in soil (Doxtader, 1969; Ausmus, 1973).

Adenosin-5'triphosphate is a nucleotide, consisting of adenylic acid (adenosine-5'-monophosphate of AMP) linked by a high energy ester bond to a pyrophosphate group (Figure 6). The latter is very susceptible to cleavage in acid solution, or by enzymatic activity, being dephosphorylated to ADP or AMP, with release of energy. For this reason, ATP is important as an energy source in many metabolic processes, including glycolysis, fatty acid synthesis, and oxidation, protein and nucleic acid synthesis and in such organ functions as muscle contraction and nerve impulse generation. It is also responsible for energy transfer processes concerned with light production in bioluminescent organisms, as for example, the 'lantern' of Photinus pyralis. It is this property which has resulted in a sen-

Fig. 6--The molecular structure of Adenosine-5'-triphosphate (ATP)


NH2
C
N
C
N
CH
HC
C
N
N
O
O
O
HO—P—O—P—O—POCH2
HO
HO
HO
C
H
H
C
C
H
H
C
H
OH
OH

sitive and specific analytical method (Bulleid, 1977; McElroy and Strehler, 1949; Strehler and Totter, 1952).

The details of the light producing reaction were first described by McElroy and Strehler (1949) and later by Moyer and Henderson (1983). They were able to identify the compound luciferin which, in the presence of the enzyme luciferase, reacts with ATP to produce light. The reaction has been described in several stages. First, the cleavage of a pyrophosphate group and secondly, the formation of an activated complex which undergoes oxidation, producing light. The reaction also requires the presence of the magnesium ion, or certain other divalent metal ions.

$$E + L\,H_2 + ATP \xrightarrow{Mg^{++}} E \cdot L\,H_2 \cdot AMP + P\text{-}P$$

$$E \cdot LH_2 \cdot AMP + 1/2\ O_2 \longrightarrow P - E^* \cdot AMP + CO_2$$

$$P - E^* - AMP \longrightarrow P + E + AMP + h\nu$$

The optimum temperature of the reaction is about $25^{o}C$ (Strehler, 1968) and the optimum pH about 7.7

where:

E = firefly luciferase enzyme

LH = Reduced Luciferin

ATP = Adenosine triphosphate

AMP = Adenosine monophosphate

PP = Pyrophosphate

$h\nu$ = Light (550 nm)

P = Product (oxyluciferin)

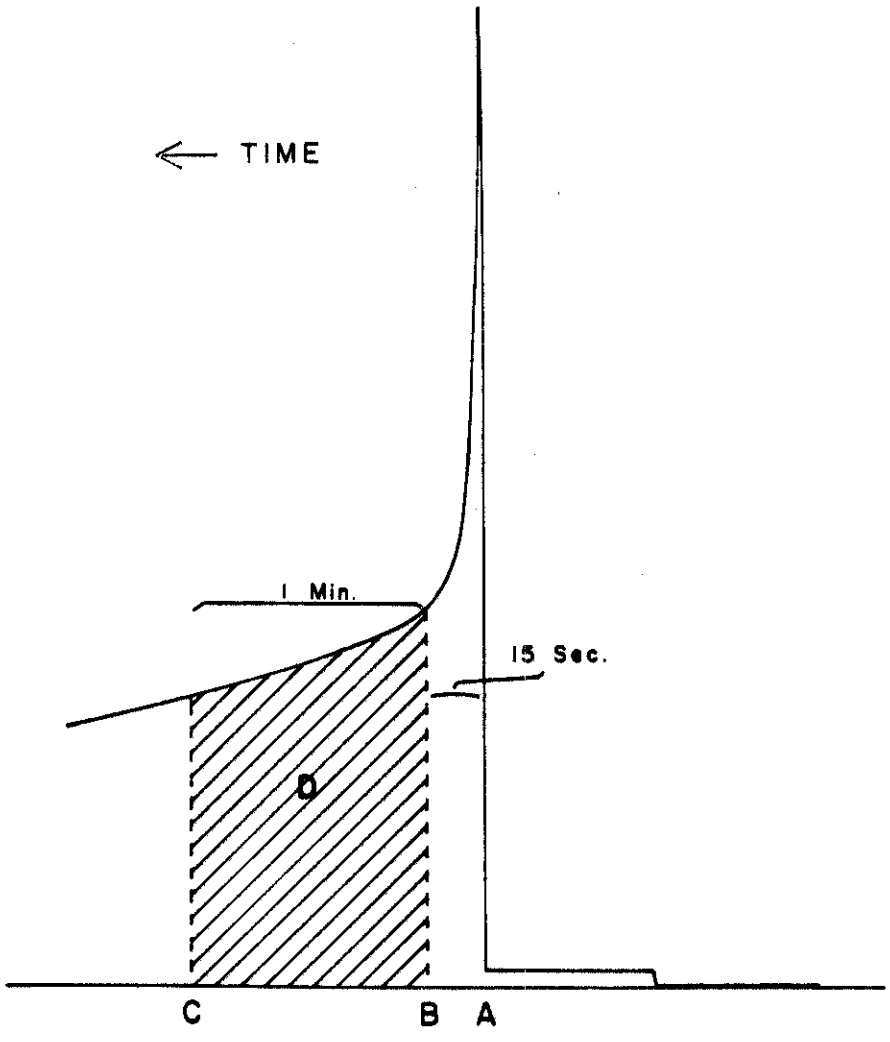
The reaction is specific to ATP and is actually more complicated than shown above (White et al., 1971). When all other reactants are in excess, the rate of the reaction is proportional to the ATP concentration, with one photon of light emitted for each molecule of ATP hydrolyzed. Figure 7 shows a typical light-emission curve for a sample containing ATP when added to the enzyme preparation. A measure of the light flux can thus be used to calculate the ATP concentration in a sample.

Method of Analysis ATP.--The following section describes the methods of extraction and ATP analysis used in my thesis research:

1 - Reagents

A - Buffer (McIlvaine buffer).--This buffer is more stable at high temperatures and is more efficient at extracting ATP than others (Bulleid, 1977). It was made up as follows: 5.68 g of Na_2HPO_4 was diluted to one liter with distilled water to give an 0.04 molar solution. 1.9 g of citric acid was dissolved in 500 mls of distilled water to give a 0.02 molar solution. The phosphate solution was adjusted to pH 7.70 by adding citric acid solution in a ratio of approximately 19:1 (phosphate:citrate).

Fig. 7--Light emission curve when a sample containing ATP is injected into the Luciferine-Luciferase enzyme preparation. A = time of injection of sample; B = end of 15 second mixing period; C = end of 1.0 minute light-integration period; D = area which is electronically integrated.


TIME
1 Min.
15 Sec.
D
C
B
A

B - ATP Standards.--Standard solutions were made by using disodium ATP $3 \cdot 5H_2O$, M W 614·2 with 99% purity (Sigma Chemical Co., St. Louis, Missouri). ATP was kept as a concentrated stock solution, dispensed into vials, and frozen at below $0^{o}C$. A stock solution 10 mM per ml in McIlvaine buffer was diluted with the same buffer to a working strength of between 0.1 nM and 10 nM. The standard used during the assay was also made up from this stock solution. After preparation, this working strength standard was stored in an ice bath.

C - Enzyme.--The luciferin-luciferase enzyme solution was prepared from commercially available vials of lantern extract (Sigma Chemical Co., FLE, 50). This extract typically contains 50 mg of lyophilized crude firefly lantern extract, Mg^{++} and arsenate buffer at pH 7.4. These vials were stored at $0^{o}C$. For an experiment the required number of vials were then rehydrated. The contents of each vial was hydrated with 5 ml of distilled water and then left for 3-5 hrs at $4^{o}C$ to reduce endogenous ATP, at which time the contents of the vial were centrifuged at 4000 rpm for 20 minutes to remove particulate material. The supernatant was then decanted and allowed to stand an additional 18-24 hours at $4^{o}C$ to "age." It was necessary to age the firefly enzyme for this period of time in order to decrease its background noise to insure reproducibility. The enzyme solutions were stored in an ice bath until used.

2 - Procedures

Extraction of Adenosine Triphosphate from Sample.--ATP analysis was performed on all triplicate samples at T_0, T_7, T_{21} days. One-ml of the culture sample was rapidly injected into 1 ml of hot ethanol by a sterile pipet. The sample was then incubated in a hot-water bath (90°C) for 10 min. The sample was then cooled in an ice bath. The volume was readjusted to 2 ml with cold sterile water, and the denatured protein was removed by centrifugation. The supernatant was then transferred to a labeled test tube and frozen until assayed.

Enzymatic Assay Procedure.--A SAI Technology model 2000 ATP Photometer was used to assay extracted samples. A blank determination was first performed to check on the residual activity of the extract. Frozen samples were removed from the freezer and allowed to reach room temperature and were then held in an ice bath until used. Stock ATP standards were prepared prior to analysis for development of a standard curve. 200 µL McIlvaine buffer was autopipetted into a scintillation vial. The vial was then mixed on a vortex stirrer and, during mixing, 200 µL of enzyme preparation was added. The vial was then placed into the counting chamber of the photometer, and the chamber's cap replaced and the shutter was opened. Fifteen seconds after the sample and enzyme were mixed, the instru-

ment starts a 60 sec counting period. At the end of the counting period the total count were recorded for the enzyme blank. The same method was used for standard ATP and samples. Final ATP concentrations were calculated from the predetermined standard curves. This method was used to quantify the bacterial concentration in samples grown in the presence of anthracene or PCP in Basal Medium or in the water systems.

Analytical Methods for Anthracene and Pentachlorophenol

Anthracene concentrations were determined using an Amico Bowman Ratio Spectrophotometer. Excitation and emission wavelengths were 345 and 401 nm, respectively. Anthracene standards of 100, 80, 50, 40, 25, 12.5, 5 μgL^{-1} were made in pesticide grade hexane (L.T. Baker Co.) from a 1000 μgL^{-1} stock solution. New standards and standard curves were developed every time anthracene was assayed. Anthracene concentrations were determined after extraction of 5 ml of sample with an equal volume of hexane. All extractions were done in 20 ml glass vials with teflon-lined screw top lids. Thorough extractions were achieved by mixing all samples for two minutes using a Thermoclyne model M-16715 mixer.

Pentachlorophenol (PCP) concentrations were determined using a Tracor model 560 gas chromatograph equipped with an ^{63}Ni electron capture detector. A 50 cm glass column

packed with 5% DEGS (Diethylene glycol succinate) was utilized. Column temperature was 186^{o}C and the carrier gas was P-10 mix of argon-methane at a flow rate of 40 ml/ minute. Injection and detection temperatures were 213^{o}C and 226^{o}C, respectively. New standards were prepared and a standard curve developed every time PCP was analysed. PCP concentrations were determined from the standard curve using linear regression analyses. PCP concentrations in experimental samples were determined after extraction of 5 ml of sample with an equal volume of each PCP standard in benzene after acidifying to pH<2 with 2 ml of concentrated HCl (Pignatello et al., 1983). All extractions were done in 20 ml glass vials with teflon-lined screw top lids. Extraction was completed by mixing for 2 min on a Thermo-clyne maxi-mix.

Data Analysis

All data analyses were performed using a National Advanced System (NAS) 5000 computer. Statistical Analysis System (SAS) (Helwing and Council, 1982) and MUSIC (IBM, 1981) interactive programs were used for all statistical computations, such as t-tests, analyses of covariance, and probit analyses. The statistical tables in Zar (1974) were consulted for statistical significance.

CHAPTER III

RESULTS AND DISCUSSION

The results of this research are presented on the following pages with special emphasis given to:

1. Results of identification of five dominant bacteria from anthracene and pentachlorophenol (PCP) rate kinetic experiments.
2. Results of the biotransformation tests for the Red River, Mississippi River, Trinity River waters, and Basal Medium.
3. Results of the biotransformation tests for the Red River, Mississippi River, and Trinity River waters with addition of glucose.

Characteristics of Water/Sediment Systems

The water chemistry for the Red River, the Trinity River, and the Mississippi River water samples are presented in Table VI. The chemical characteristics of the three waters are not identical. The pH values for the three waters are similar with that of the Red River water being slightly more basic than the other two waters. The hardness of the Red River water is very low, yet the hardness of the other two river waters is moderate to hard. The alkalinity of the Red River water and the Mississippi River water is

TABLE VI

RESULTS OF WATER CHEMISTRY ANALYSES OF MISSISSIPPI, RED, AND TRINITY RIVER WATERS

Parameter	Mississippi River	Red River	Trinity River
PH	7.3	7.8 ± 0	7.2 ± 0.03
Alkalinity (mg $CaCO_3$/l)	102.1 ± 16.5*	126.7 ± 5.8	76.7 ± 2.9
Hardness (mg $CaCO_3$/l)	150 ± 20.9*	8.5 ± 0	159.7 ± 2
Orthophosphates (mg PO_4^- P/l)	0.23 ± 0.07*	$6.9 \times 10^{-4} \pm 9 \times 10^{-5}$	NO**
Total Phosphates	0.7 ± 0.3*	$2.7 \times 10^{-3} \pm 4 \times 10^{-4}$	NO
Ammonia (mg NH_3 N/l)	0.075 ± 0.07*	BDL***	2.6 ± 0.2
Nitrate (mg NH_3^- N/a)	0.025 ± 0.02*	0.03 ± 0.04	8.4 ± 0.06
Total Suspended Solids (mg/l)	170.8 ± 131.1	33.3 ± 16	50 ± 23
Chlorides (mg/l)	16.9 ± 4.1	312 ± 56	72.6 ± 0.3
Total Carbon (mg/l)	29.6	32 ± 1.7	33 ± 1.1
Inorganic Carbon (mg/l)	21.0	31 ± 0.49	30.4 ± 0.6
Organic Carbon (mg/l)	8.6 ± 4.2	1 ± 1.9	2.6 ± 0.6
Calcium (mg/l)	33.0 ± 0.1	101 ± 0.6	40.5 ± 0.1
Sodium (mg/l)	11.2 ± 1.3	126 ± 1.8	101 ± 0.6
Iron (mg/l)	0.49 ± 0.02	0.03 ± 0.02	1.2 ± 0.02

*Values from STORET (1971).

**NO = Not available.

***BDL = Below Detectable Limit for Ammonia BDL = .03 mg NH_3 N/l.

almost identical. The alkalinity of the Trinity River water is somewhat lower than the Mississippi and Red River waters. In considering the nutrient content of the waters, as indicated by nitrate, phosphate, and ammonia, the Trinity River water has greater than an order of magnitude higher concentration than the others; the Red River water has lowest nutrient concentration. The concentrations of nutrients in the Mississippi River water falls between the other two waters. Suspended solids in the Red and the Trinity River waters are similar. Background suspended solids in the Mississippi River water are three to five times higher. Carbon analysis of the three waters shows similarities in the concentrations of total carbon with the highest concentration of organic carbon in the Mississippi River water.

Sediment characteristics are presented in Table VII. Of the three systems, the particle size data, nutrient data, and the percent volatile matter is of particular interest. The particle size data show that the Red River has the most sand, over 86%, followed by the Mississippi, with over 62% and the Trinity River with only 42% sand. The Trinity River sediment data show that this sediment contains almost 40% clay and over 20% silt. The Mississippi River sediments contain less than 18% clay and 20% silt. The silt and clay content of the Red River sediments are 0% and 14%, respectively. Particle size data indicate that if any of the sediments are going to affect the sorption of the anthracene

TABLE VII

SEDIMENT CHARACTERISTICS OF THE MISSISSIPPI, RED, AND TRINITY RIVERS

Parameter	Mississippi River	Red River	Trinity River
pH	7.0	7.3	6.7
% Sand	62.4	86.4	41.8
% Silt	19.8	0	21.2
% Clay	17.8	13.6	37.0
CFU/gr	5.2×10^6	1.9×10^7	1.6×10^7
Cation Exchange (meq/100 g)	NA	41	427.2
Nitrogen (mg NH_3 N/g wet wt)	0.0632	0.0529	0.198
Total Phosphate (PO_4 P/g wet wt)	3.7	0.88	6.01
Volatile Matter (mg/kg)	60533 $\pm$ 932	5456 $\pm$ 196	5429 $\pm$ 191

or PCP, it should be the sediment of the Trinity River because of its high clay and silt content. In considering the nutrient content of the sediments, as indicated by nitrate and total phosphate concentrations, the Trinity River has greater than an order of magnitude higher concentrations than the Red River sediments. The sediments of the Mississippi River fall between the sediments of the Red and the Trinity in nutrient content. The levels of the nutrients in the sediments is of the same order as the nutrients of the waters. Volatile matter data for the three sediments indicate that the Trinity and the Red River sediments have almost the same volatile matter (5,400 mg/kg). The Mississippi River has over an order of magnitude greater volatile matter (60,000 mg/kg). The volatile matter can be used as an indicator of the carbon content of the sediments. From these data it would be predicted that the Mississippi River sediments may sorb more anthracene and PCP therefore affecting the biotransformation results of the compounds.

Identification of Dominant Bacteria from Anthracene and Pentachlorophenol Rate Kinetic Biotransformation Experiments

Experiments were conducted by Tim Leslie to determine the rate of anthracene and PCP biotransformation. At the initiation and at the conclusion of each experiment, standard pour plates were prepared to determine the numbers and most dominant type of bacteria present. Since a serial

dilution was made, it was possible to enumerate the dominant bacteria present. One objective of my research was to identify and enumerate the five (5) dominant types of bacteria present in these kinetic experiments.

The rate kinetic experiments utilized three river systems (Red River water, Trinity River water, and Mississippi River water) as the water/sediment source in addition as the source of naturally occurring bacteria. I determined the five (5) dominant bacteria types present at the initiation and termination of the anthracene and PCP biotransformation experiments for each of water/sediment systems amended with 100 mgL^{-1} suspended solids.

The five dominant bacterial types at the initiation and termination of the biotransformation experiments are shown in Table VIII. The most dominant bacteria found from all systems with PCP or anthracene were all common soil and water bacteria. Most can be characterized as being: (1) saprophytic chemoorganotrophs, (2) gram negative rods, (3) aerobic, (4) nonfermentative, and (5) forming yellow or pink colonies.

Genera isolated from the anthracene biotransformation experiments included Pseudomonas, Alcaligenes, Acinetobacterium, Flavobacterium, Azomonas, Corynebacterium, Bacillus, and Chromobacterium. Pseudomonas was the most abundant genus in the anthracene biotransformation experiments using Red River water/sediments and in the experiment using

TABLE VIII

COLONY-FORMING UNITS (CFUs mL^{-1}) AND CHARACTERIZATION OF FIVE DOMINANT BACTERIA ISOLATED FROM EACH WATER SYSTEM AT INITIATION (T_0) AND TERMINATION TIME (T_{FINAL}) OF ANTHRACENE OR PENTACHLOROPHENOL (PCP) RATE KINETIC EXPERIMENTS

Source *	Chemical	Genera of Bacteria	CFUs mL^{-1}		Characterization by:						
			T_o $\bar{X}$	T_{Final} $\bar{X}$	Gram Stain	Cellular Morphology	Motility**	Pigmentation	O F Test ***	Catalase	Cytochrom-oxidase
RR	Anthracene	Pseudomonas sp.	$3x10^4$	$5x10^4$	neg	Rods	+	Yellow	O	+	+
		Alcaligenes sp.	$4.2x10^3$	$8.2x10^3$	neg	Rods	+	Red	NO	+	+
		Acinetobacter sp.	$3.2x10^3$	$2.3x10^3$	neg	Rods	-	White	NO	+	-
		Flavobacterium sp.	$4x10^3$	$3.4x10^3$	neg	Rods	-	Yellow	F	+	-
		Azomonas sp.	$3.8x10^3$	$4.3x10^3$	neg	Rods&Cocci	+	White	O	+	-
RR	PCP	Pseudomonas sp.	$9.5x10^3$	$8.2x10^3$	neg	Rods	+	Pink	O	+	+
		Bacillus sp.	$8.7x10^3$	$7.5x10^3$	pos, neg	Rods	-	Cream-Red	O	+	-
		Flavobacterium sp.	$4.8x10^3$	$4.4x10^3$	neg	Rods	-	Yellow	NO	+	-
		Acinetobacter sp.	$7x10^3$	$6x10^3$	neg	Rods	-	White	NO	+	-
		Corynebacterium sp.	$4.8x10^3$	$4x10^3$	pos, neg	Rods	-	Milky	NO	+	-
MR	Anthracene	Flavobacterium sp.	$2.9x10^4$	$2.6x10^4$	neg	Rods	-	Yellow	NO	+	-
		Acinetobacter sp.	$1.3x10^4$	$2.9x10^4$	neg	Rods	-	-	O	+	-
		Pseudomonas sp.	$6x10^3$	$8x10^3$	neg	Rods	+	Yellow	O	+	+
		Corynebacterium sp.	$4x10^3$	$1x10^3$	pos, neg	Rods, cocoid	-	Yellow	NO	+	-
		Chromobacterium sp.	$8x10^3$	$6x10^3$	neg	Rods	+	Violet	O	+	+
MR	PCP	Pseudomonas sp.	$8x10^3$	$9x10^4$	neg	Rods	+	Orange	NO	+	+
		Corynebacterium sp.	$5x10^3$	$7x10^4$	pos	Rods	-	Yellow	NO	+	-
		Flavobacterium sp.	$3x10^3$	$2x10^3$	neg	Rods	-	Yellow	NO	+	-
		Acinetobacter sp.	$3x10^3$	$1.0x10^4$	neg	Rods	-	-	NO	+	-
		Alcaligenes sp.	$2x10^3$	$2x10^4$	neg	Rods	+	Red	NO	+	+

TABLE VIII--Continued.

Source *	Chemical	Genera of Bacteria	CFUs mL^{-1}		Characterization by:						
			T_o $\bar{X}$	T_{Final} $\bar{X}$	Gram Stain	Cellular Morphology	Motility**	Pigmentation	O-F Test ***	Catalase	Cytochrom-oxidase
TR	Anthracene	Pseudomonas sp.	$2.8x10^4$	$6.4x10^4$	neg	Rods	+	Yellow	O	+	+
		Corynebacterium sp.	$1.9x10^4$	$5.6x10^4$	pos, neg	Rods	-	Cream	NO	+	-
		Bacillus sp.	$1x10^4$	$1x10^3$	pos, neg	Rods	+	Yellow	NO	+	-
		Flavobacterium sp.	$7x10^3$	$8x10^3$	neg	Rods	-	Yellow	F	+	-
		Alcaligenes sp.	$5x10^3$	$3.8x10^3$	neg	Rods	+	Pink	NO	+	+
TR	PCP	Pseudomonas sp.	$5x10^4$	$6x10^4$	neg	Rods	+	Yellow	O	+	+
		Micrococcus sp.	$3x10^4$	$5x10^4$	pos	Spherical	-	Yellow	O	+	+
		Flavobacterium sp.	$9x10^3$	$6x10^3$	neg	Rods	+	-	NO	+	-
		Corynebacterium sp.	$6x10^3$	$4x10^3$	pos, neg	Rods	-	-	NO	+	-
		Acinetobacter sp.	$6x10^3$	$4x10^3$	neg	Rods	-	-	O	+	-

*RR = Red River water.
MR = Mississippi River water.
TR = Trinity River water.

**Motility was determined by phase-contrast examination of wet mounts prepared from TSA or Nutrient Agar Culture, or by Flagella Stain.

***Oxidation-Fermentation Test. O = Oxidative; F = Fermentative; NO = No Reaction.

\+ = Positive Reaction.
\- = No Reaction.
neg = Negative.
pos = Positive.

Trinity River water/sediments. The most abundant genus in anthracene biotransformation experiments using Mississippi water/sediment was Flavobacterium. In general the five dominant bacteria were similar for each of the three water/sediment systems examined in the anthracene biotransformation experiments. The dominant bacteria isolated from PCP biotransformation experiments included: Pseudomonas, Bacillus, Flavobacterium, Acinetobacter, Corynebacterium, Micrococcus, and Alcaligenes. In all these water/sediment systems Pseudomonas was present in highest numbers.

Figures 8-13 show the change in numbers of the five dominant bacteria from the initiation of the anthracene and PCP biotransformation experiments to their termination. The biotransformation experiments were terminated when a chemical was lost from the system to an extent equal to the lowest detectable limits. Thus, the duration of the biotransformation experiments was variable depending on the chemical and the water/sediment system. The numbers of bacteria generally increased during the biotransformation experiments indicating that sufficient energy sources were present to support growth as shown in Figures 8-13. These results suggest that some of the dominant bacteria present may have been involved in the observed biotransformations of anthracene and PCP. However, just because they were dominant and in general increased in numbers does not confirm that they were involved in the biotransformation of the

Fig. 8--Graphical representation of mean CFUs mL^{-1} for the five dominant bacteria isolated from Red River water at initiation (zero) and termination time (final) of anthracene rate kinetic experiment.

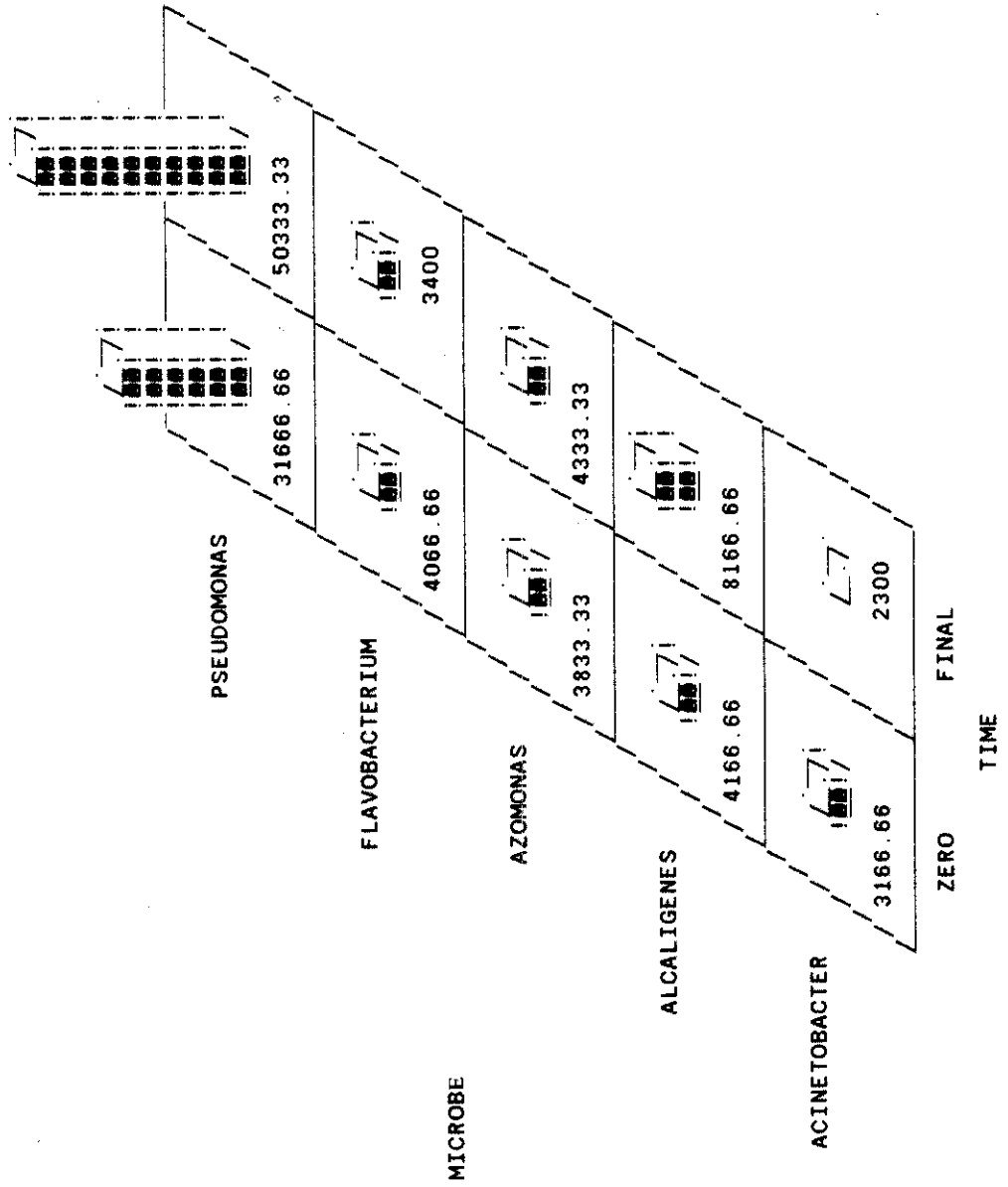

PSEUDOMONAS
FLAVOBACTERIUM
AZOMONAS
ALCALIGENES
ACINETOBACTER
MICROBE
31666.66
50333.33
4066.66
3400
3833.33
4333.33
4166.66
8166.66
3166.66
2300
ZERO
FINAL
TIME

Fig. 9--Graphical representation of mean CFUs mL^{-1} for the five dominant bacteria isolated from Red River water at initiation (zero) and termination time (final) of pentachlorophenol (PCP) rate kinetic experiment.

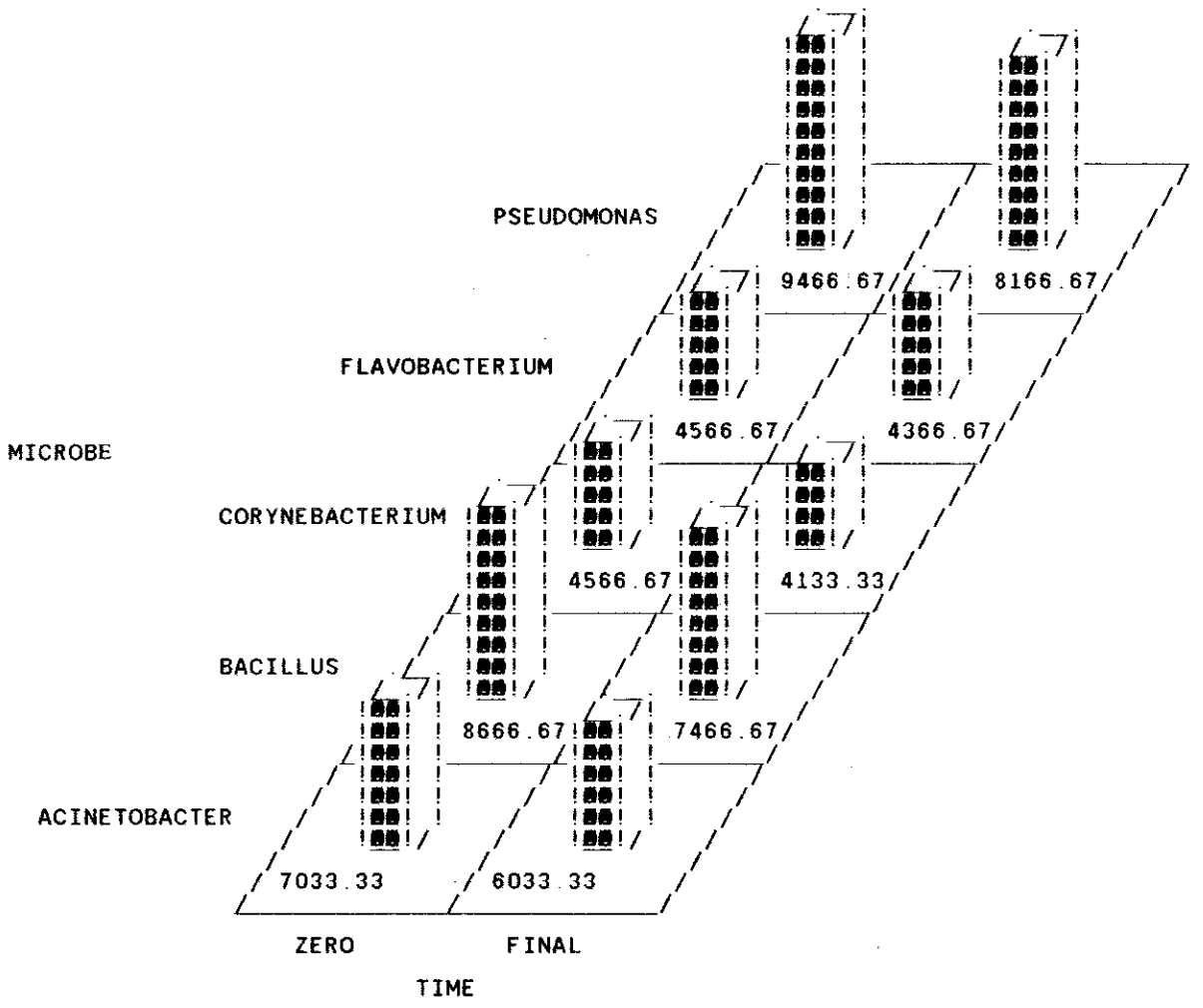

PSEUDOMONAS
9466.67
8166.67
FLAVOBACTERIUM
4566.67
4366.67
MICROBE
CORYNEBACTERIUM
4566.67
4133.33
BACILLUS
8666.67
7466.67
ACINETOBACTER
7033.33
6033.33
ZERO
FINAL
TIME

Fig. 10--Graphical representation of mean CFUs mL^{-1} for the five dominant bacteria isolated from Mississippi River water at initiation (zero) and termination time (final) of anthracene rate kinetic experiment.

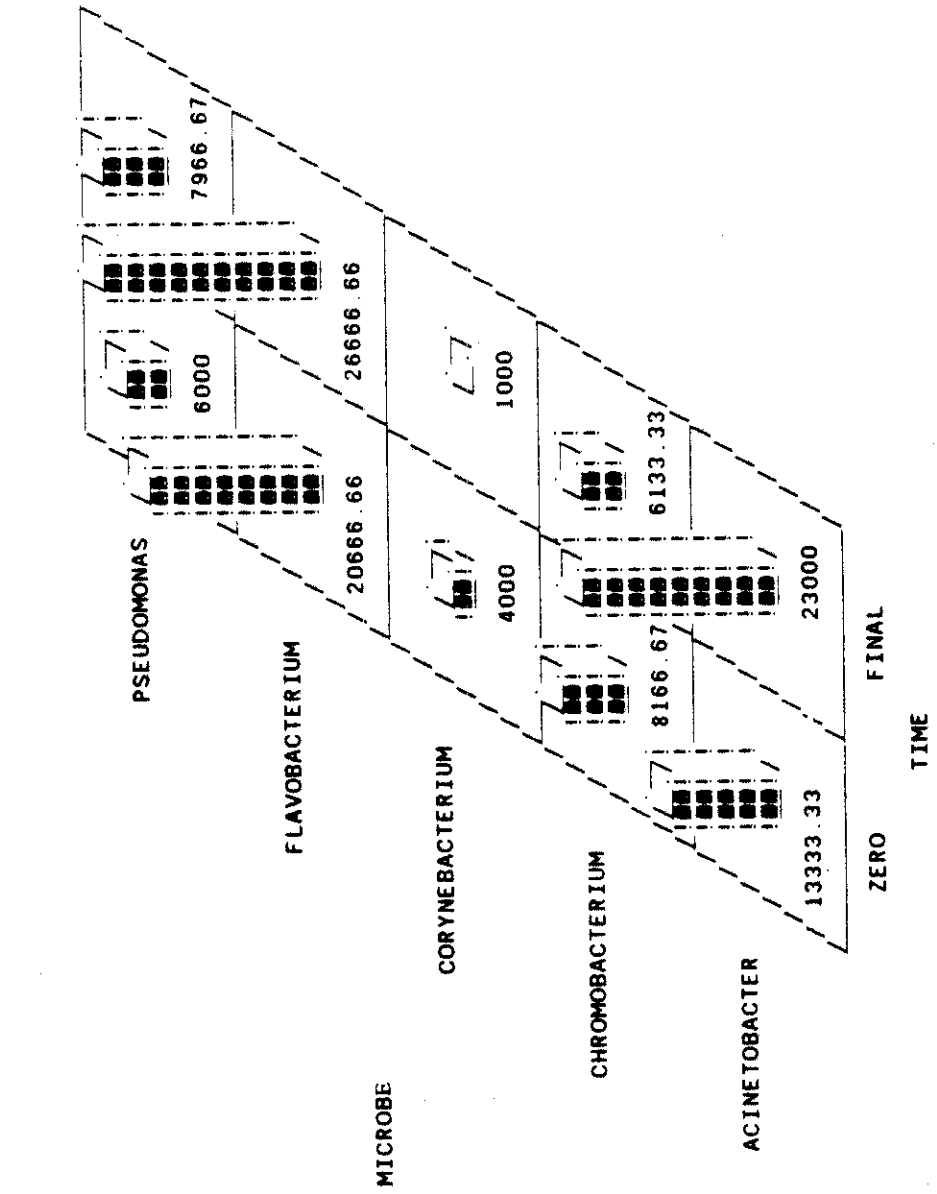

PSEUDOMONAS
FLAVOBACTERIUM
CORYNEBACTERIUM
CHROMOBACTERIUM
ACINETOBACTER
MICROBE
6000
7966.67
20666.66
26666.66
4000
1000
8166.67
6133.33
13333.33
23000
ZERO
FINAL
TIME

Fig. 11--Graphical representation of mean CFUs mL^{-1} for the five dominant bacteria isolated from Mississippi River water at initiation (zero) and termination time (final) of pentachlorophenol (PCP) rate kinetic experiment.

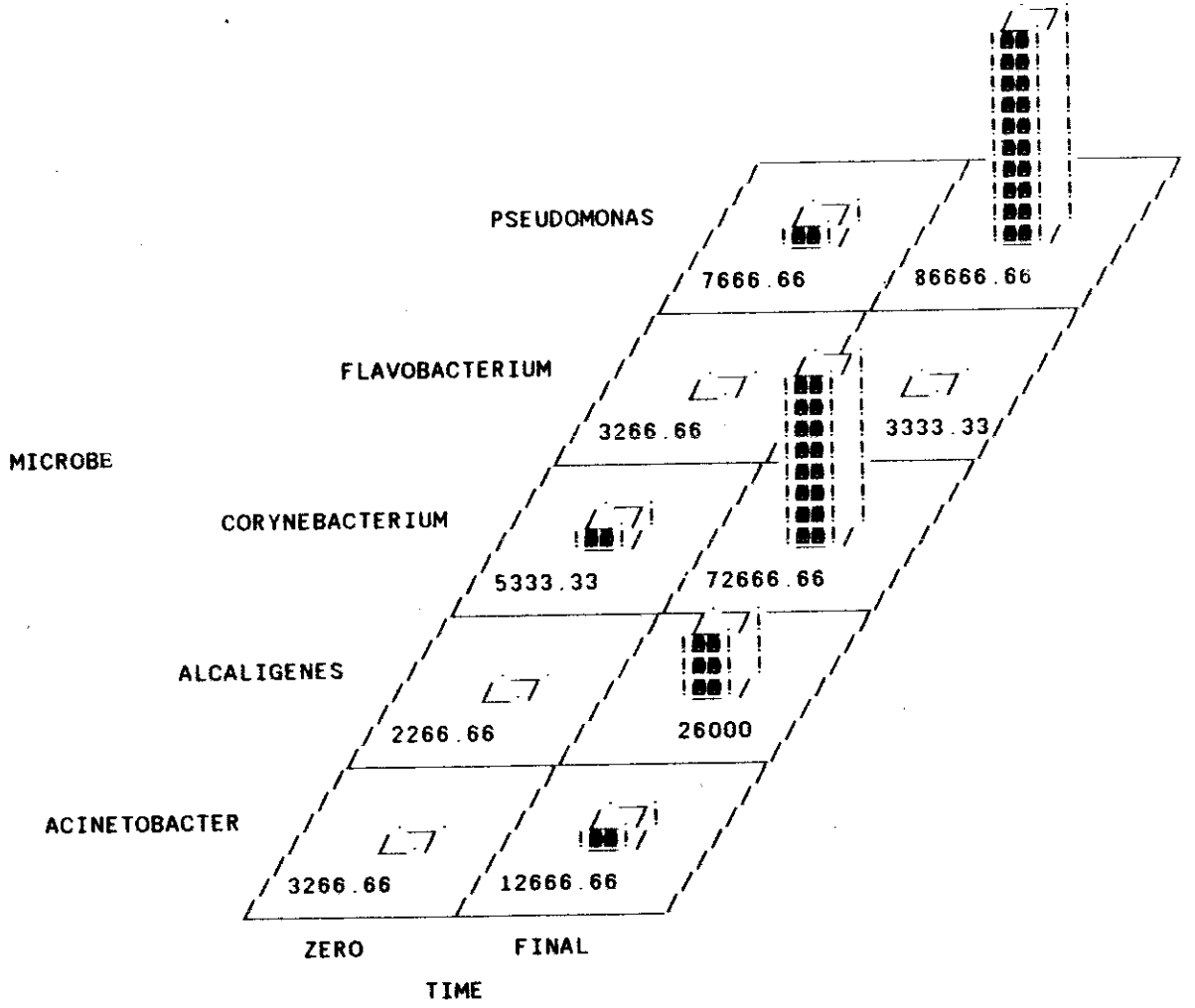

PSEUDOMONAS
7666.66
86666.66
FLAVOBACTERIUM
3266.66
3333.33
MICROBE
CORYNEBACTERIUM
5333.33
72666.66
ALCALIGENES
2266.66
26000
ACINETOBACTER
3266.66
12666.66
ZERO
FINAL
TIME

Fig. 12--Graphical representation of mean CFUs mL^{-1} for the five dominant bacteria isolated from Trinity River water at initiation (zero) and termination time (final) of anthracene rate kinetic experiment.

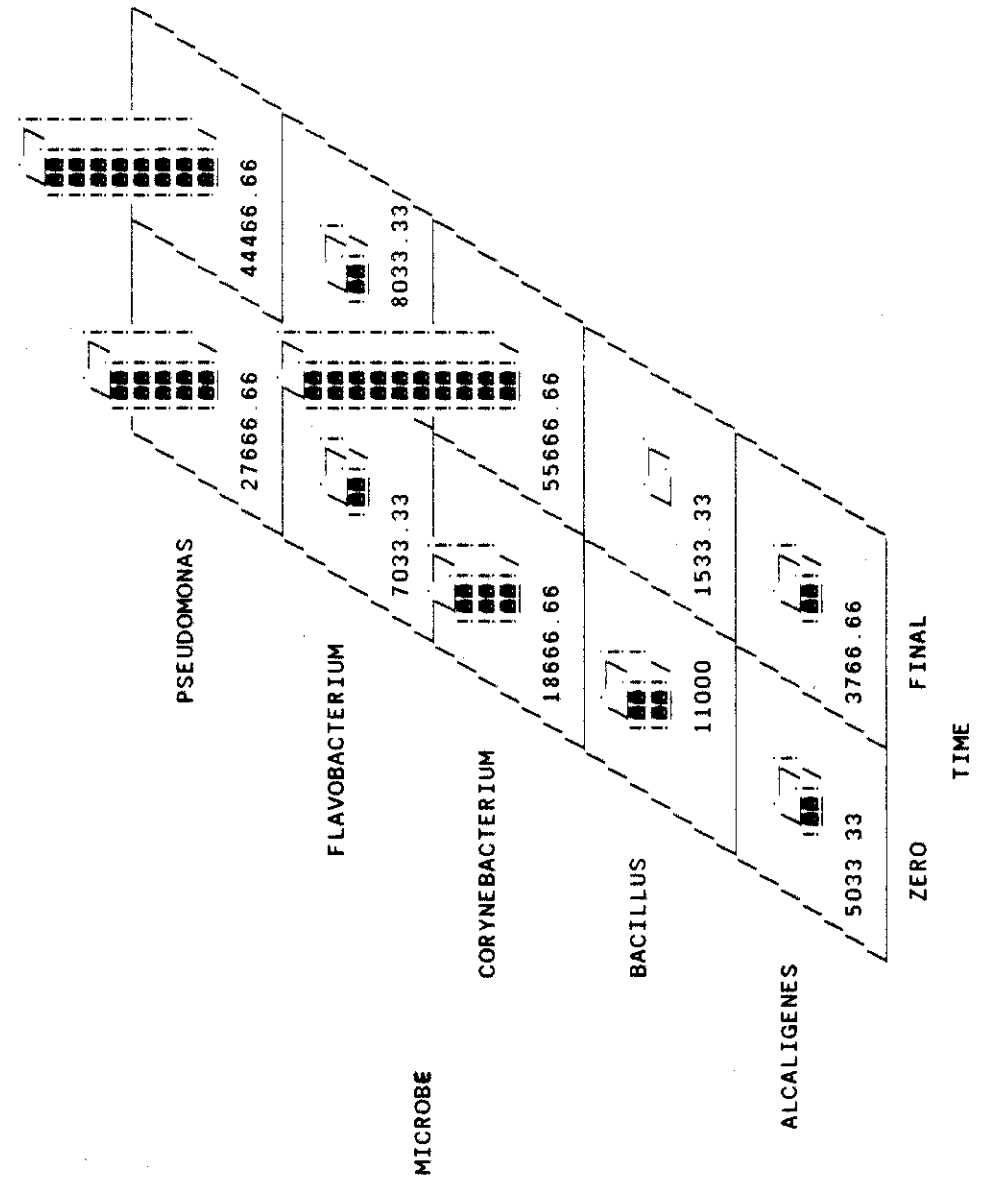
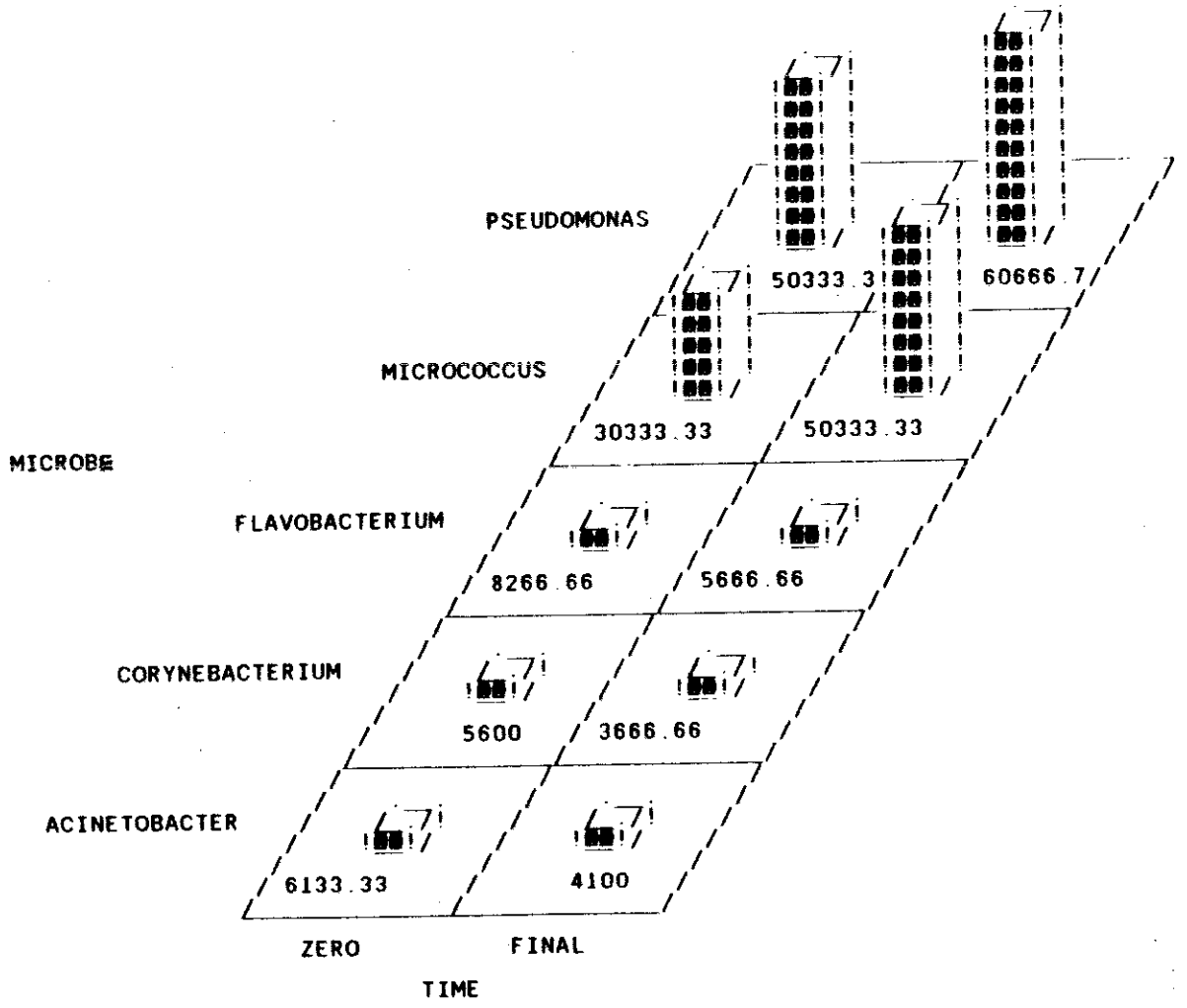

PSEUDOMONAS
FLAVOBACTERIUM
CORYNEBACTERIUM
BACILLUS
ALCALIGENES
MICROBE
27666.66
44466.66
7033.33
8033.33
18666.66
55666.66
11000
1533.33
5033.33
3766.66
ZERO
FINAL
TIME

Fig. 13--Graphical representation of mean CFUs mL^{-1} for the five dominant bacteria isolated from Trinity River water at initiation (zero) and termination time (final) of pentachlorophenol (PCP) rate kinetic experiment.


PSEUDOMONAS
MICROCOCCUS
MICROBE
FLAVOBACTERIUM
CORYNEBACTERIUM
ACINETOBACTER
50333.3
60666.7
30333.33
50333.33
8266.66
5666.66
5600
3666.66
6133.33
4100
ZERO
FINAL
TIME

chemicals. In the sections which follow, their role in biotransformation of anthracene and PCP is further assessed.

Biotransformation Tests

Results of selected biotransformation tests are discussed below. The following results will indicate:

1. The effects that the source of water (Red River water, Trinity River water, or Mississippi River water) has on the biotransformation of anthracene and PCP as compared with the Basal Medium (BM).
2. The effect that the type of bacteria has on biotransformation of anthracene and PCP.
3. The effects that initial bacterial population size has on biotransformation of anthracene or PCP.
4. The effects that the presence of additional glucose has on biotransformation of anthracene and PCP.

The results of experiments to assess bacterial ability to biotransform anthracene and PCP are discussed in two sections.

1. Biotransformation of anthracene and PCP in the water systems and BM. This section shows the results of bacterial growth measurements in each of the three water systems and in BM containing anthracene or PCP. These results help to determine the effects of water type on biotransformation of the chemicals. Therefore, these results indicate

whether or not bacteria are able to use anthracene or PCP as the sole source of carbon and energy, in the BM, or as a source of carbon in the river waters.

2. Results of the effect of glucose addition on biotransformation of anthracene and PCP in the three river waters.

Results of Bacterial Growth Measurements in Media Containing Anthracene or PCP

Bacterial growth results from long-term incubation of microorganisms with anthracene or PCP were measured as colony-forming units per one mL sample (CFUs mL^{-1}) at time zero, 7, 14, and 21 days, and as ATP concentration (nM/mL) at various time intervals (0, 7, 21 days) as indicated in the Materials and Methods section. ATP data were not obtained for the Red River water, nor for BM with anthracene at times 7 and 14 days. Bacteria grown in media containing anthracene or PCP as a carbon source or sole source of carbon should show an increase in CFUs mL^{-1} and ATP level if the bacteria present can use these chemicals as an energy source. This phenomenon should be shown in the experiment treatment (bacteria + chemical + BM, or water source) as compared to the control (bacteria + BM, or water source). For this comparison, the Student t-test was performed. The Student t-test (Zar, 1974) was used to compare the mean of CFUs mL^{-1} and ATP concentration of three replicate samples

obtained from the treatments and the controls at each sampling time. The results of the microbial growth during incubation determined by colony forming units and ATP concentration are summarized in table form for each experimental study. These tables show mean values of three replicate samples of treatment and control tests. Also listed in these tables are results of the Student t-test comparison between treatment and control tests. Graphical depiction of the bacterial growth as measured by colony-forming units (CFUs) and ATP concentration are presented for each experiment. These figures plot the logarithm (log 10) of the mean of CFUs mL^{-1} and the mean of ATP concentration versus time.

During long-term incubation studies, if the statistical analysis (t-test) indicated there was a significant difference between mean of CFUs mL^{-1} and ATP concentration of the treatment and the control at the 0.05 level, then the experiment was repeated in the BM in short-term incubation (0-7 days) to better gain an understanding of the behavior of xenobiotic chemicals biotransformation at a lower bacterial biomass. Analysis of covariance was performed to determine whether or not slopes of bacterial growth from the treatment and the control based on CFUs mL^{-1} from 0-7 days are significantly different at the $p=0.05$ level. The results of the microbial growth studies on the CFUs mL^{-1} at time zero through 7 days are summarized in table form. These tables

demonstrate the actual values in CFUs mL^{-1} for the three replicate samples of treatment and control tests. These tables also indicate the results of Analysis of Covariance. Graphical depiction of the bacterial growth are presented for each experiment. These figures depict the actual values of bacterial colony forming units versus time for the treatment and control tests. Results of bacterial activities for anthracene and PCP from the short-term incubation studies are demonstrated in Figure 27. This figure plots the logarithm of CFUs mL^{-1} for each isolate in BM versus time.

Biotransformation of Anthracene in the Red River Water by Pseudomonas sp. and Alcaligenes sp.--The results of the bacterial growth obtained from the Red River water with anthracene inoculated with pure cultures of Pseudomonas sp. and Alcaligenes sp. are presented in Table IX and Figure 14. The results of the Student t-test for these experiments are also indicated in Table IX. The experimental results in Table IX and Figure 14 show that Pseudomonas sp. growth is accompanied by an appreciable enhancement of the CFUs. The increases in CFUs of Pseudomonas sp. in the control in 7 days probably results from the type and amount of nutrient present in the Red River water and is responsible for this phenomenon as well as for treatment. However, this observation can also be explained by the fact that some nutrients within the bacteria were retained, although, they were

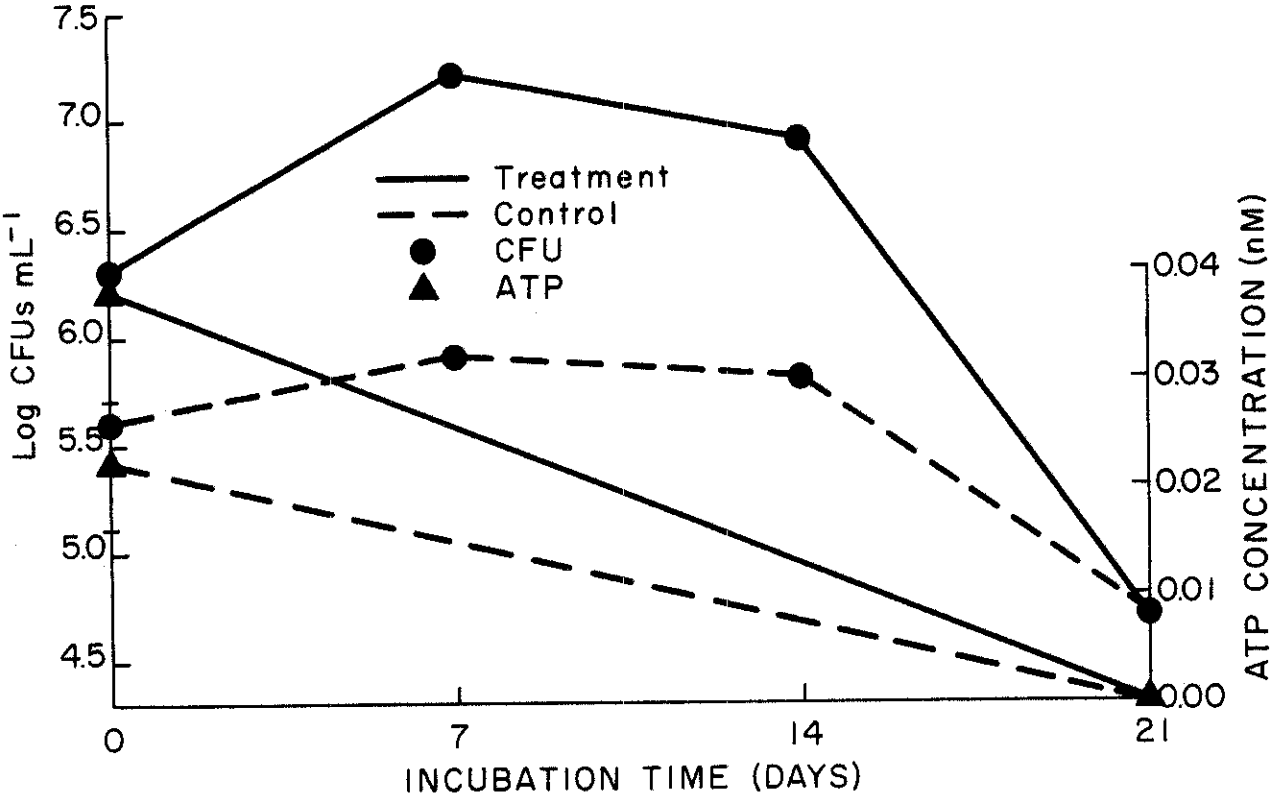
TABLE IX

MEAN VALUES AND STATISTICAL COMPARISONS OF CFUS AND ATP CONCENTRATION BETWEEN TREATMENT AND CONTROL SAMPLES FOR *ALCALIGENES* SP. AND *PSEUDOMONAS* SP. ISOLATED FROM RED RIVER WATER, GROWN IN MEDIUM SATURATED WITH ANTHRACENE

Medium	Isolates	Days	ATP (nM)		CFUs mL^{-1}		Student t-test	ANCOV Analysis
			Treatment $\bar{x}$	Control $\bar{x}$	Treatment $\bar{x}$	Control $\bar{x}$		
Red River water	*Alcaligenes* sp.	0	1.5×10^{-2}	2.2×10^{-2}	1.41×10^{6}	1.62×10^{6}	NS	
		7	NO	NO	1.77×10^{6}	2.2×10^{6}	NS	
		14	NO	NO	2.46×10^{6}	2.2×10^{6}	NS	-*
		21	1.03×10^{-1}	1.07×10^{-1}	1.90×10^{6}	1.96×10^{6}	NS	
BM	*Alcaligenes* sp.	0	7.60×10^{-2}	3.74×10^{-2}	1.26×10^{6}	1.0×10^{6}	NS	
		7	NO	NO	1.67×10^{6}	4.50×10^{5}	NS	
		14	NO	NO	1.70×10^{6}	6.83×10^{5}	NS	-*
		21	5.37×10^{-2}	3.25×10^{-2}	4.5×10^{5}	6.00×10^{5}	NS	
Red River water	*Pseudomonas* sp.	0	3.7×10^{-2}	2.10×10^{-2}	2.16×10^{6}	3.66×10^{5}	NS	
		7	NO	NO	1.43×10^{7}	7.5×10^{5}	S	-*
		14	NO	NO	7.86×10^{6}	6.06×10^{5}	S	
		21	2.5×10^{-4}	2.8×10^{-4}	4.56×10^{4}	5.06×10^{4}	NS	
BM	*Pseudomonas* sp.	0	2.77×10^{-2}	2.54×10^{-2}	4.53×10^{5}	4.73×10^{5}	NS	
		7	NO	NO	2.12×10^{7}	3.33×10^{5}	S	
		14	NO	NO	1.62×10^{7}	6.33×10^{4}	S	S
		21	5.33×10^{-1}	1.84×10^{-4}	3.36×10^{4}	3.0×10^{4}	NS	

*Data was not analyzed due to the result of the Student t-test.
NS = Not significant (Student t-test, ANCOV analysis), $p \geq 0.05$.
S = Significant, $p < 0.05$.
NO = ATP was not measured at this time.

Fig. 14--Log mean value of CFUs mL^{-1} and mean ATP concentration of Pseudomonas sp. grown in the Red River water containing anthracene as additional carbon source (treatment). Control = isolate and water, without anthracene.


7.5
7.0
6.5
6.0
5.5
5.0
4.5
Log CFUs mL⁻¹
Treatment
Control
CFU
ATP
0.04
0.03
0.02
0.01
0.00
ATP CONCENTRATION (nM)
0
7
14
21
INCUBATION TIME (DAYS)

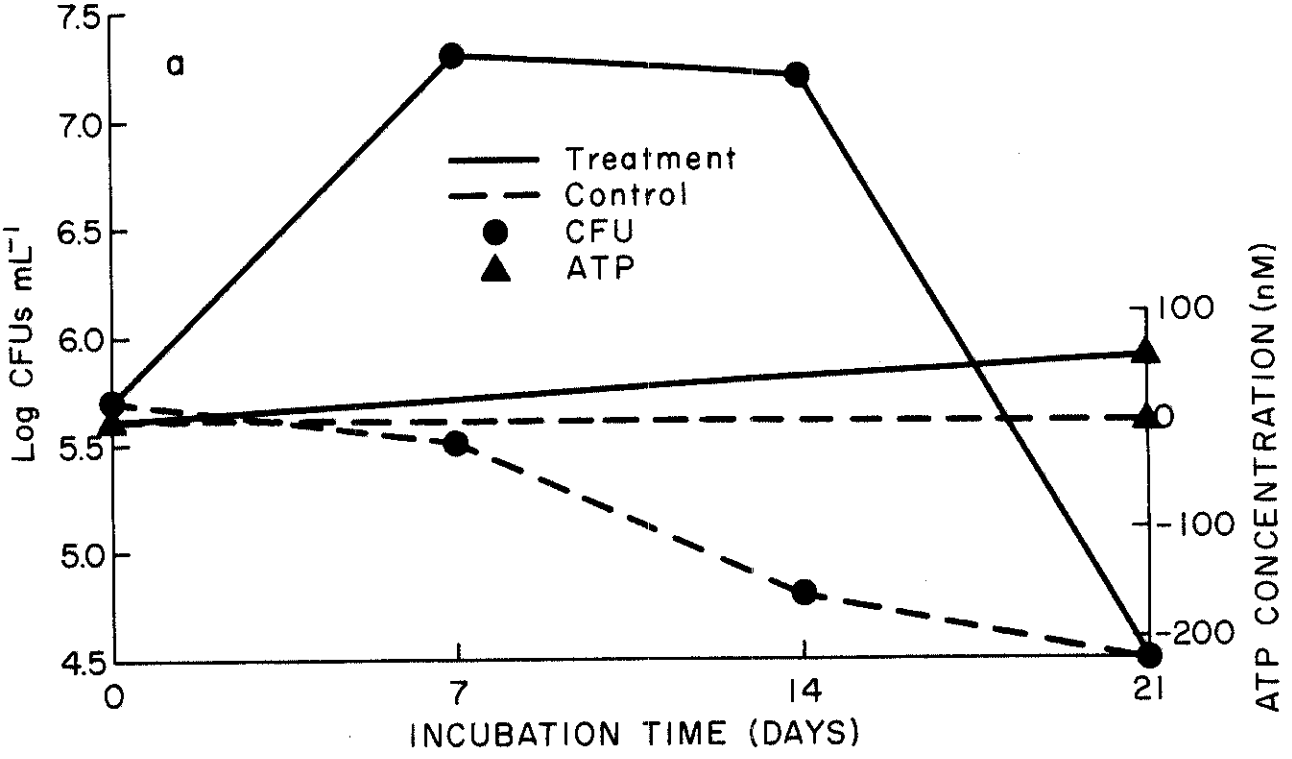
harvested by centrifugation at room temperature and starved by incubation in sterile BM for several hours to reduce the endogenus level of nutrients. The Student t-test analysis also showed that there is a significant difference between mean of CFUs in treatment (Red River water, Anthracene, and Pseudomonas sp.) and the control (Red River water, Pseudomonas sp.) at the $p=0.05$ level at 7 and 14 days.

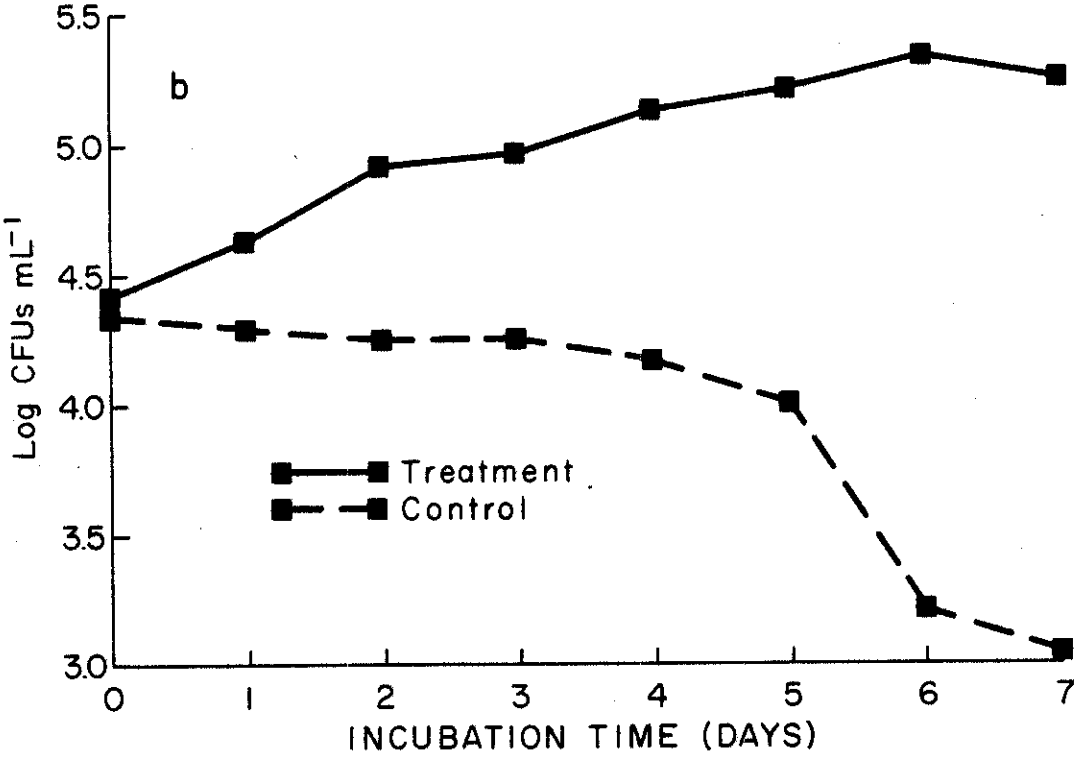
The results of bacterial growth studies in Red River water containing anthracene inoculated with Alcaligenes sp. is presented in Table IX. Based upon the Student t-test for this study, no significant difference ($p \geq 0.05$) was found for Alcaligenes sp. growth as measured by CFUs and ATP levels between the treatment and the controls. However, there was a relative increase in the CFUs in both treatment and the controls which may be attributed to the some nutrients present in the Red River water.

Biotransformation of Anthracene in The Basal Medium by Pseudomonas sp. and Alcaligenes sp.--The results obtained from the BM with anthracene, as the sole carbon source, and inoculated with Pseudomonas sp. and Alcaligenes sp. are presented in Table IX and Figure 15a. As can be seen from Table IX and Figure 15a, the Pseudomonas sp. growth (CFUs) in the treatment flasks, containing BM and anthracene is significantly greater than the bacterial growth in the control flasks containing BM and Pseudomonas sp. These

Fig. 15a--Log mean value of CFUs mL^{-1} and mean ATP concentration of Pseudomonas sp. grown in the basal medium containing anthracene as the sole source of carbon (treatment). Control = isolate and basal medium, without anthracene.

Fig. 15b--Log value of CFUs mL^{-1} of Pseudomonas sp. grown in the basal medium containing anthracene as the sole source of carbon (treatment). Control = isolate and basal medium, without anthracene.


a
Treatment
Control
CFU
ATP
Log CFUs mL⁻¹
7.5
7.0
6.5
6.0
5.5
5.0
4.5
0
7
14
21
INCUBATION TIME (DAYS)
100
0
-100
-200
ATP CONCENTRATION (nM)


b
Log CFUs mL⁻¹
5.5
5.0
4.5
4.0
3.5
3.0
Treatment
Control
0
1
2
3
4
5
6
7
INCUBATION TIME (DAYS)

differences have been shown at 7 and 14 days. Following these results, the experiment was repeated with lower bacterial biomass in the short-term incubation experiments (0-7 days). The results of short-term studies are presented in Table X and Figure 15b. Significant differences ($p \leq 0.05$) were shown by Analysis of Covariance (ANCOV) between the bacterial growth in the treatment (BM, anthracene, and Pseudomonas sp.) and the control (BM and Pseudomonas sp.). However, the results of the biotransformation of anthracene by Pseudomonas sp. in the Red River water and BM demonstrate that Pseudomonas sp. are able to utilize anthracene as a sole carbon and energy source for their growth in a pure culture. Also from Figures 14 and 15 it can be seen that the Pseudomonas isolate had exponential growth until day 7, then this phase was followed by the stationary phase (7-14 days). The number of viable cells then sharply decreased. These results can be explained by exhaustion of essential nutrients or toxic products accumulation. These results also indicate that physical and chemical properties of the Red River water and the initial bacterial population did not have significant effects on the Pseudomonas sp. growth.

The results of the growth of Alcaligenes sp. using anthracene as the sole source of carbon are presented in Table X. The results of the Student t-test showed that there is no significant difference ($p \geq 0.05$) between the CFUs in the treatment and the control. The results obtained from

TABLE X

CFUS AND ANALYSIS OF COVARIANCE (ANCOV) RESULTS FOR PSEUDOMONAS SP. ISOLATED FROM RED RIVER WATER, GROWN IN BASAL MEDIUM SATURATED WITH ANTHRACENE

Time (Days)	Replicate	CFUs mL^{-1} Treatment Actual Value	CFUs mL^{-1} Control Actual Value	ANCOV Analysis
0	1	2.40×10^4	2.30×10^4	
	2	2.0×10^4	2.20×10^4	S
	3	2.10×10^4	2.20×10^4	
1	1	4.60×10^4	2.0×10^4	
	2	4.20×10^4	1.80×10^4	
	3	4.50×10^4	1.80×10^4	
2	1	8.40×10^4	1.90×10^4	
	2	8.60×10^4	1.95×10^4	
	3	8.0×10^4	1.92×10^4	
3	1	9.0×10^4	1.90×10^4	
	2	9.40×10^4	1.90×10^4	
	3	9.50×10^4	1.94×10^4	
4	1	1.40×10^5	1.60×10^4	
	2	1.30×10^5	1.50×10^4	
	3	1.20×10^5	1.60×10^4	
5	1	1.80×10^5	1.0×10^4	
	2	1.80×10^5	1.0×10^4	
	3	1.40×10^5	1.0×10^4	
6	1	2.30×10^5	1.60×10^3	
	2	2.20×10^5	1.80×10^3	
	3	2.20×10^5	1.60×10^3	
7	1	9.0×10^4	1.0×10^3	
	2	1.80×10^5	1.0×10^3	
	3	2.20×10^5	1.20×10^3	

S = Significant (Analysis of Cavariance) $p < 0.050$

the biotransformation of anthracene in the Red River water and the BM indicate that in contrast to Pseudomonas sp., the Alcaligenes isolate was unable to utilize anthracene as its sole carbon source.

Biotransformation of Pentachlorophenol by Pseudomonas sp. and Bacillus sp. in the Red River Water.--Statistical analysis (Student t-test) performed on the data obtained from the Red River water and PCP, inoculated with Pseudomonas sp. and Bacillus sp. can be found in Table XI and Figure 16. Table XI and Figure 16 show that the Pseudomonas isolate had more growth in the treatment flasks (Red River water + PCP + Pseudomonas sp.) as compared to the control flasks (Red River water + Pseudomonas sp.). This figure also shows that Pseudomonas sp. was actively growing until day 7 after which the number of viable cells then sharply decreased. This result may suggest a possibility of toxicity of PCP by-product. The Student t-test also showed a significant difference ($p<0.05$) in the concentration of Pseudomonas cells and ATP concentration between the treatment and the control at day 7. The same statistical test conducted on data obtained from the Red River water and PCP inoculated with Bacillus sp. show that there was no significant differences ($p\geq0.05$) between the concentration of ATP and CFUs of treatments and controls (Table XI).

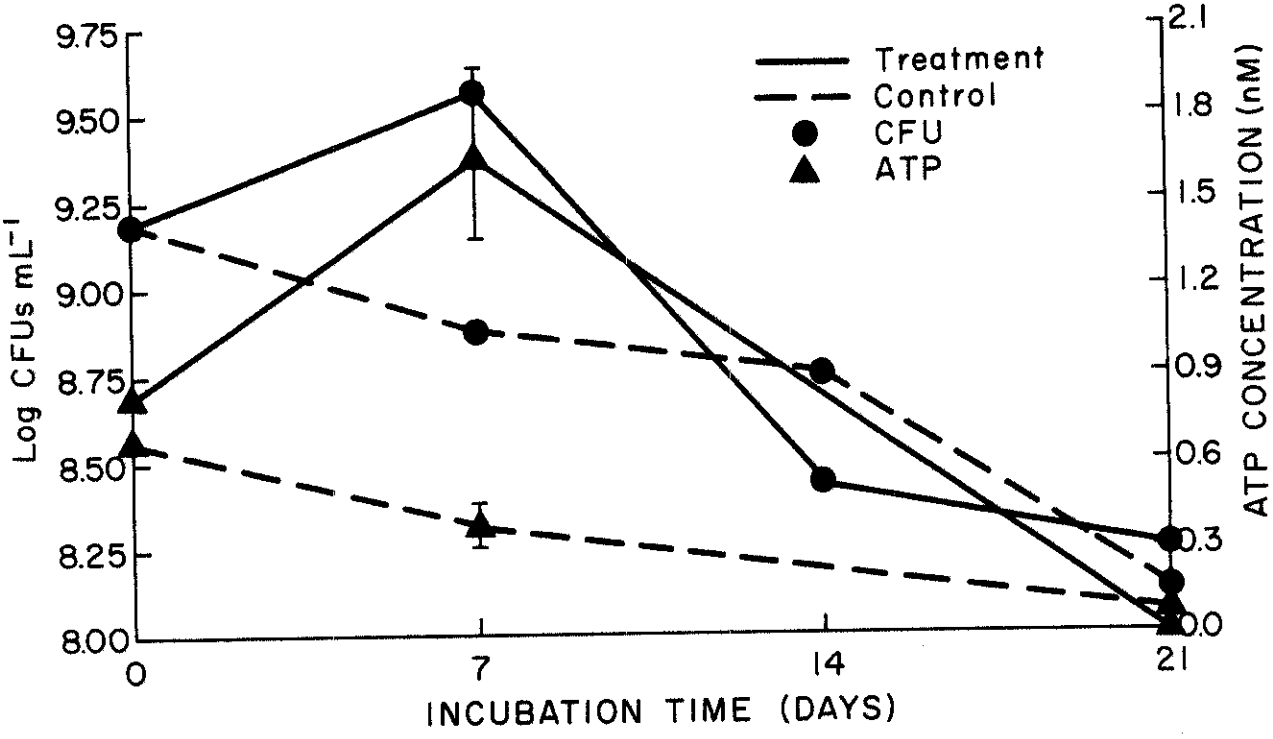
TABLE XI

MEAN VALUES AND STATISTICAL COMPARISONS OF CFUS AND ATP CONCENTRATIONS BETWEEN TREATMENT AND CONTROL SAMPLES FOR ALCALIGENES SP. AND PSEUDOMONAS SP. ISOLATED FROM RED RIVER WATER, GROWN IN MEDIUM CONTAINING 1500 µg L^{-1} OF PENTACHLOROPHENOL (PCP)

Medium	Isolates	Days	ATP (nM) Treatment $\bar{x}$	ATP (nM) Control $\bar{x}$	CFUs mL^{-1} Treatment $\bar{x}$	CFUs mL^{-1} Control $\bar{x}$	Student t-test	ANCOV Analysis
Red River water	Pseudomonas sp.	0	8.1×10^{-1}	6.70×10^{-1}	1.58×10^{9}	1.54×10^{9}	NS	
		7	1.65×10^{0}	3.67×10^{-1}	3.40×10^{9}	7.5×10^{8}	S	-*
		14	NO	NO	2.70×10^{8}	5.72×10^{8}	NS	
		21	3.0×10^{-3}	4.0×10^{-2}	1.73×10^{8}	1.36×10^{8}	NS	
BM	Pseudomonas sp.	0	7.9×10^{-1}	7.60×10^{-1}	1.58×10^{9}	1.46×10^{9}	NS	
		7	9.0×10^{-1}	3.30×10^{-1}	1.82×10^{9}	6.43×10^{8}	S	NS
		14	NO	NO	2.70×10^{8}	2.75×10^{8}	NS	
		21	1.23×10^{-1}	1.90×10^{-1}	1.90×10^{8}	2.30×10^{8}	NS	
Red River water	Bacillus sp.	0	1.50×10^{-2}	1.63×10^{-2}	7.90×10^{7}	7.63×10^{7}	NS	
		7	6.5×10^{-3}	7.10×10^{-3}	3.44×10^{7}	3.13×10^{7}	NS	-*
		14	NO	NO	2.70×10^{7}	1.49×10^{7}	NS	
		21	3.50×10^{-3}	6.7×10^{-4}	1.63×10^{7}	3.33×10^{6}	NS	
BM	Bacillus sp.	0	4.0×10^{-3}	8.7×10^{-3}	4.53×10^{7}	4.40×10^{7}	NS	
		7	2.50×10^{-3}	7.7×10^{-3}	1.36×10^{7}	3.83×10^{7}	NS	-*
		14	NO	NO	1.13×10^{7}	2.16×10^{7}	NS	
		21	2.1×10^{-3}	4.0×10^{-3}	1.13×10^{7}	1.26×10^{7}	NS	

*Data was not analyzed due to the result of the Student t-test.
NS = Not significant (Student t-test, ANCOV analysis), $p \geq 0.05$.
S = Significant, $p < 0.05$.
NO = ATP was not measured at this time.

Fig. 16--Log mean value of CFUs mL and mean ATP concentration of Pseudomonas sp. grown in the Red River water containing pentachlorophenol (PCP) as additional carbon source (treatment). Control = isolate and water, without PCP.


9.75
9.50
9.25
9.00
8.75
8.50
8.25
8.00
Log CFUs mL⁻¹
2.1
1.8
1.5
1.2
0.9
0.6
0.3
0.0
ATP CONCENTRATION (nM)
Treatment
Control
CFU
ATP
0
7
14
21
INCUBATION TIME (DAYS)

Biotransformation of Pentachlorophenol by Pseudomonas sp. and Bacillus sp. in the Basal Medium.--Mean values of three replicates obtained from BM and PCP inoculated with Pseudomonas sp. and Bacillus sp. are presented in Table XI. Also listed in Table XI are results of the Student t-test between the treatments and the controls. Statistical significance ($p<0.05$) was found at day 7 for Pseudomonas sp. This difference between treatment and control can be seen in Figure 17. Following this result, the experiment was repeated with lower concentrations of bacteria for short-term incubation (0-7 days). No significant difference ($p\geq0.05$) between the treatment and the control in CFUs was shown by an Analysis of Covariance (Table XII). The difference in these results from long-term incubation and short-term incubation of Pseudomonas sp. can probably be attributed more to the initial concentration of bacteria rather than any environmental factors since no other parameter was changed during this study. The results from the Student t-test in CFUs of Bacillus sp. in BM indicated that there was no significant difference in CFUs mL^{-1} obtained from the treatments and the controls. Results from experiments examining the biotransformation of PCP inoculated with Pseudomonas sp. or Bacillus sp. indicate that Pseudomonas sp. growth and activity is occurring in the systems containing PCP. The experimental results for Bacillus sp. indicate that it is unable to utilize PCP as a sole carbon

TABLE XII

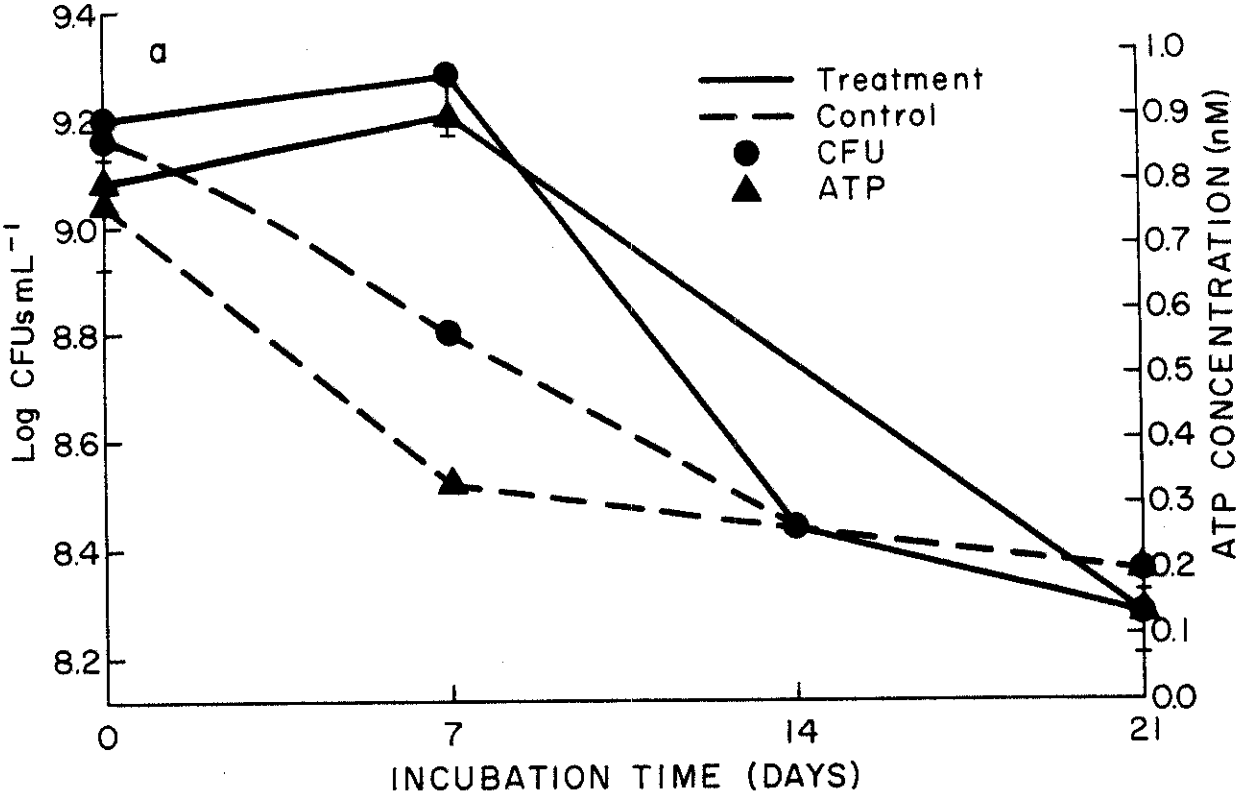
CFUS AND ANALYSIS OF COVARIANCE (ANCOV) RESULTS FOR PSEUDOMONAS SP. ISOLATED FROM RED RIVER WATER, GROWN IN BASAL MEDIUM CONTAINING 1500 μg^{-1} OF PENTACHLOROPHENOL (PCP)

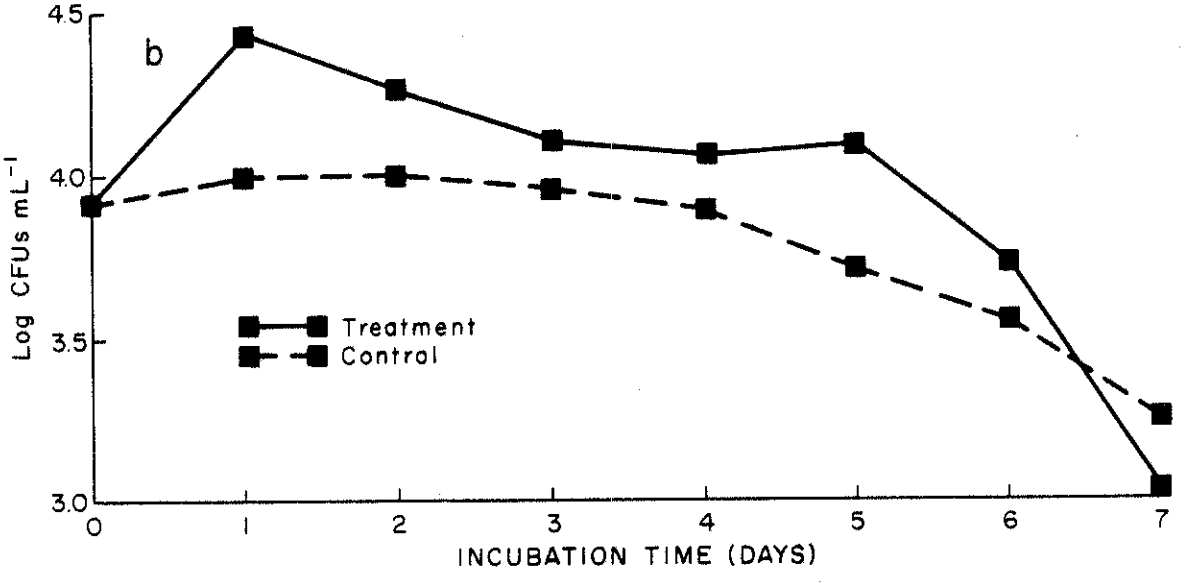
Time (Days)	Replicate	CFUs mL^{-1} Treatment Actual Value	CFUs mL^{-1} Control Actual Value	ANCOV Analysis
0	1	1.40×10^4	1.0×10^4	
	2	1.30×10^4	1.40×10^4	S
	3	1.0×10^4	1.20×10^4	
1	1	2.80×10^4	1.80×10^4	
	2	2.80×10^4	1.70×10^4	
	3	2.90×10^4	1.60×10^4	
2	1	2.0×10^4	1.90×10^4	
	2	1.40×10^4	1.80×10^4	
	3	1.60×10^4	1.70×10^4	
3	1	1.40×10^4	1.50×10^4	
	2	1.20×10^4	1.40×10^4	
	3	1.30×10^4	1.30×10^4	
4	1	1.20×10^4	4.0×10^3	
	2	1.30×10^4	8.0×10^3	
	3	1.20×10^4	8.0×10^3	
5	1	1.40×10^4	4.0×10^3	
	2	1.40×10^4	4.6×10^3	
	3	1.60×10^4	4.5×10^3	
6	1	5.20×10^3	2.80×10^3	
	2	5.30×10^3	2.70×10^3	
	3	5.30×10^3	2.80×10^3	
7	1	1.20×10^3	2.40×10^3	
	2	1.0×10^3	2.0×10^3	
	3	1.4×10^3	2.30×10^3	

NS = Not Significant (Analysis of Covariance) $p \geq 0.050$

Fig. 17a--Log mean value of CFUs mL^{-1} and mean ATP concentration of Pseudomonas sp. grown in the basal medium containing pentachlorophenol (PCP) as the sole source of carbon (treatment). Control = isolate and basal medium.

Fig. 17b--Mean value of CFUs mL^{-1} of Pseudomonas sp. grown in the basal medium containing pentachlorophenol (PCP) as the sole source of carbon (treatment). Control = isolate and basal medium, without PCP.


a
Treatment
Control
CFU
ATP
Log CFUs mL⁻¹
ATP CONCENTRATION (nM)
9.4
9.2
9.0
8.8
8.6
8.4
8.2
1.0
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0.0
0
7
14
21
INCUBATION TIME (DAYS)


b
Log CFUs mL⁻¹
4.5
4.0
3.5
3.0
Treatment
Control
0
1
2
3
4
5
6
7
INCUBATION TIME (DAYS)

source.

Biotransformation of Anthracene by Acinetobacter sp. and Flavobacterium sp. in the Mississippi River Water.--The results obtained from the Mississippi River water and anthracene inoculated with Acinetobacter sp. and Flavobacterium sp. are shown in Table XIII. These results indicate that CFUs and ATP concentrations of the Acinetobacter sp. in the treatments are greater than in the control at days 7 and 14. The Student t-test showed that the magnitude of this differences was not significant at the p=0.05 level. Experimental results from Flavobacterium sp. also presented in Table XIII and Figure 18 show that Flavobacterium sp. elicited appreciable enhancement of the CFUs and ATP concentrations in the treatments during the first 7 days (0-7 days) as compared with the controls and then sharply decreased. The Student t-test showed that a significant difference existed between treatment and the control only at day 7 of incubation.

Flavobacterium sp. growth ceases after 7 days. This cessation of growth might be related to metabolites of anthracene which may be toxic to Flavobacterium sp. A second possibility may be that the Flavobacterium sp. can only partially metabolize the anthracene. After 7 days the chemical may be metabolized to an extent where no further energy can be obtained from the molecule.

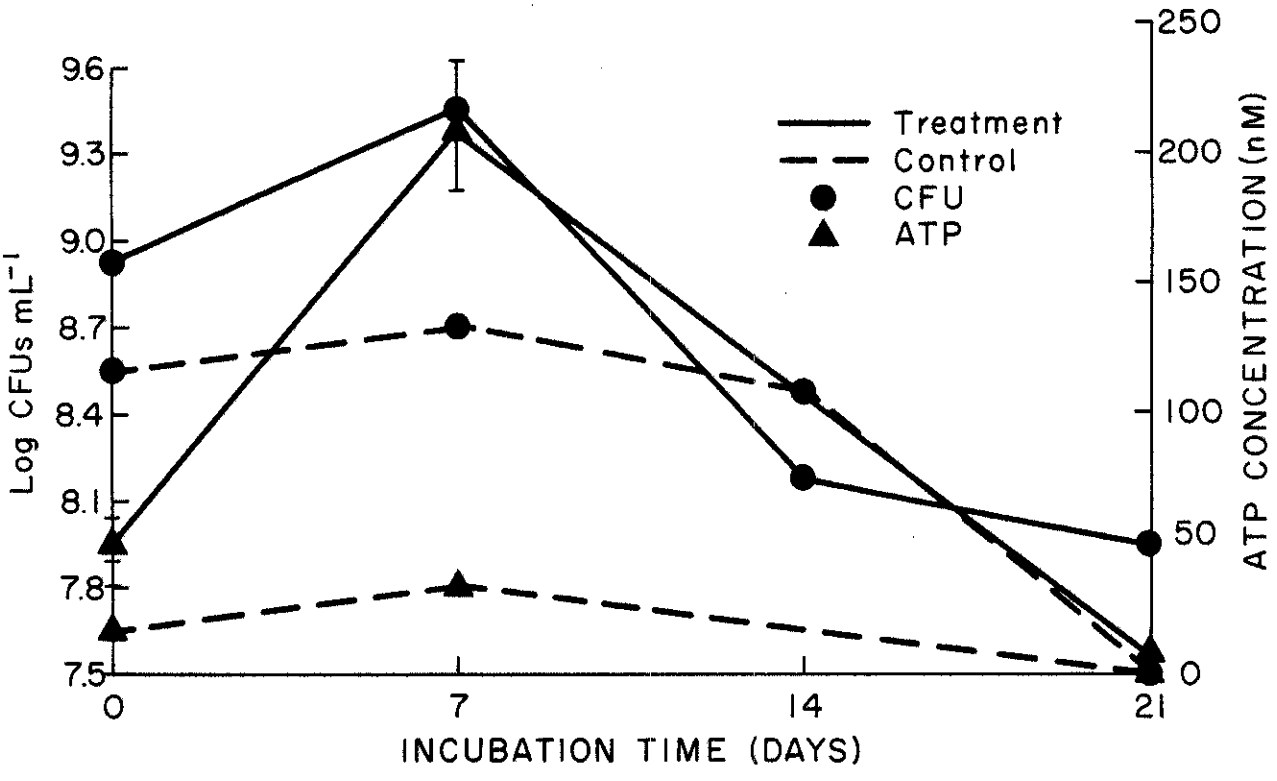
TABLE XIII

MEAN VALUES AND STATISTICAL COMPARISONS OF CFUS AND ATP CONCENTRATIONS BETWEEN TREATMENT AND CONTROL SAMPLES FOR *ACINETOBACTER* SP. AND *FLAVOBACTERIUM* SP. ISOLATED FROM MISSISSIPPI RIVER WATER, GROWN IN MEDIUM SATURATED WITH ANTHRACENE

Medium	Isolates	Days	ATP (nM)		CFUs mL^{-1}		Student t-test	ANCOV Analysis
			Treatment $\bar{x}$	Control $\bar{x}$	Treatment $\bar{x}$	Control $\bar{x}$		
Mississippi River water	*Acinetobacter* sp.	0	1.20×10^{2}	1.87×10^{2}	3.06×10^{10}	3.57×10^{10}	NS	NS
		7	2.61×10^{2}	1.60×10^{2}	4.76×10^{10}	3.36×10^{10}	NS	
		14	NO	NO	3.40×10^{10}	1.40×10^{10}	NS	
		21	8.07×10^{1}	5.33×10^{1}	2.43×10^{10}	3.0×10^{9}	NS	
BM	*Acinetobacter* sp.	0	1.41×10^{2}	4.10×10^{2}	3.56×10^{10}	3.96×10^{10}	NS	NS
		7	1.77×10^{2}	4.72×10^{2}	4.43×10^{10}	4.66×10^{10}	NS	
		14	NO	NO	2.33×10^{10}	1.83×10^{10}	NS	
		21	5.1×10^{1}	3.96×10^{1}	1.30×10^{10}	4.10×10^{10}	NS	
Mississippi River water	*Flavobacterium* sp.	0	5.16×10^{1}	1.86×10^{1}	7.80×10^{8}	3.66×10^{8}	NS	S
		7	2.06×10^{2}	3.53×10^{1}	3.06×10^{4}	5.03×10^{8}	S	
		14	NO	NO	1.40×10^{8}	3.13×10^{8}	NS	
		21	6.23×10^{0}	1.83×10^{0}	8.33×10^{7}	3.30×10^{7}	NS	
BM	*Flavobacterium* sp.	0	3.30×10^{1}	3.53×10^{1}	4.43×10^{8}	4.76×10^{8}	NS	S
		7	1.47×10^{2}	2.20×10^{1}	3.63×10^{4}	3.30×10^{8}	S	
		14	NO	NO	3.16×10^{8}	2.83×10^{8}	NS	
		21	4.7×10^{0}	5.63×10^{1}	3.30×10^{7}	7.06×10^{7}	NS	

NS = Not significant (Student t-test, ANCOV analysis, $p \geq 0.05$.
S = Significant, $p < 0.05$.
NO = ATP was not measured at this time.

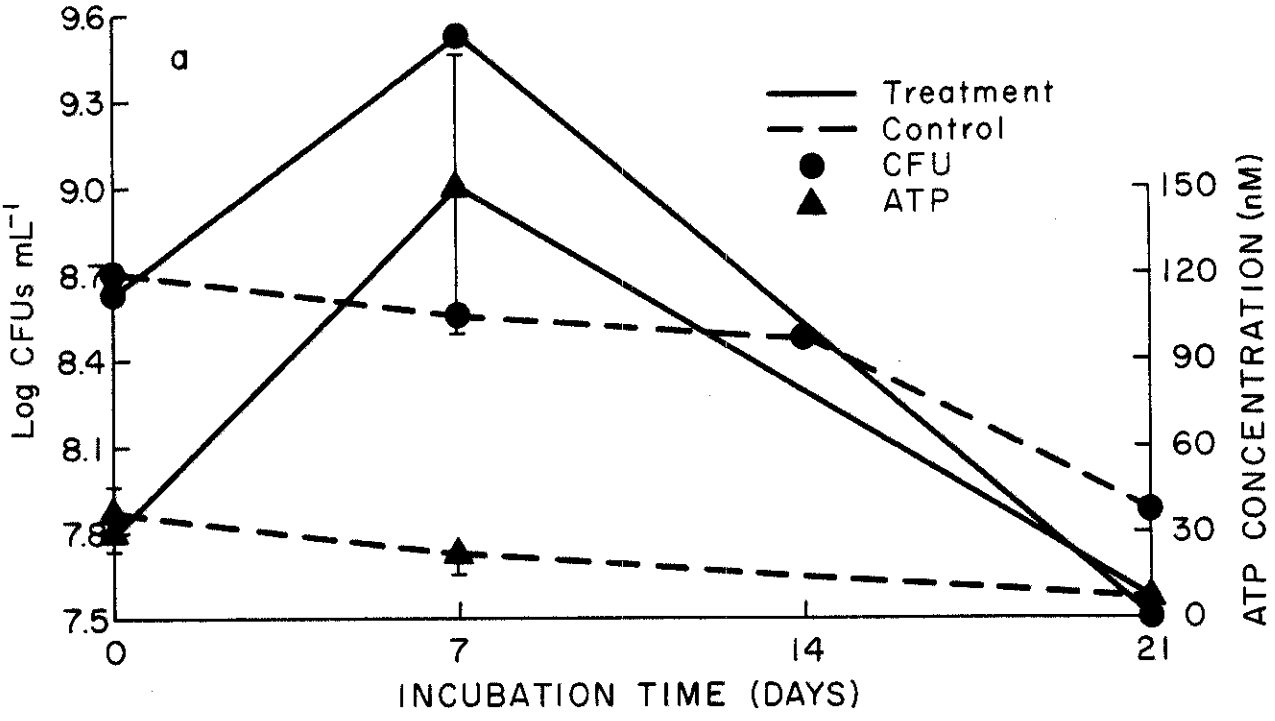
Fig. 18--Log mean value of CFUs mL^{-1} and mean ATP concentration of Flavobacterium sp. grown in the Mississippi River water containing anthracene as additional carbon source (treatment). Control = isolate and water, without anthracene. Bars show the two standard deviations on both sides of the ATP means.

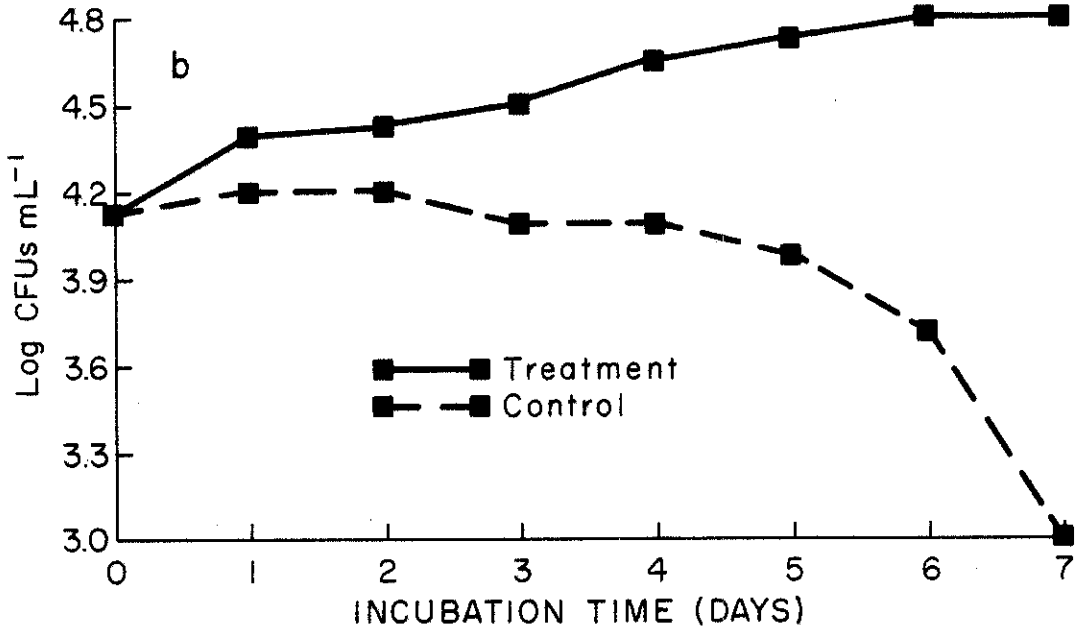

9.6
9.3
9.0
8.7
8.4
8.1
7.8
7.5
Log CFUs mL⁻¹
250
200
150
100
50
0
ATP CONCENTRATION (nM)
Treatment
Control
CFU
ATP
0
7
14
21
INCUBATION TIME (DAYS)

Biotransformation of Anthracene by Acinetobacter sp. and Flavobacterium sp. in the Basal Medium.--Statistical analysis performed on the data from the BM and anthracene inoculated with Acinetobacter sp. yielded results similar to the Mississippi River water (Table XIII). There was no significant difference between the treatments and the controls at the p=0.05 level. Results of this study show that Acinetobacter sp. is unable to utilize anthracene as the sole carbon source. Also from Table XIII it can be seen that Acinetobacter sp. growth in the Mississippi River water is greater than in the BM. The high nutrient content in the Mississippi River may possibly explain this result. However, these nutrients were not enough for Acinetobacter sp. growth to be significantly different compared with control. Results from the long-term study of Flavobacterium sp. in the BM containing anthracene as the sole carbon source are shown in Table XIII and Figure 19a. From the table and figure it can be seen that rapid growth occurred during the 7 days of incubation. This growth is presumably from the utilization of the anthracene since no similar growth is seen in the control. However, numbers of viable cells and ATP concentration after 7 days decreased. This observation was in agreement with results obtained for the biotransformation of anthracene by Flavobacterium sp. in the Mississippi River water. Following these results, an experiment was repeated

Fig. 19a--Log mean value of CFUs mL^{-1} and mean ATP concentration of Flavobacterium sp. grown in the basal medium containing anthracene as the sole source of carbon (treatment). Control = isolate and water, without anthracene. Bars show the two standard deviation on both sides of the ATP means.

19b--Log value of CFUs mL^{-1} of Flavobacterium sp. grown in the basal medium containing anthracene as the sole source of carbon (treatment). Control = isolate and basal medium, without anthracene.


a
Treatment
Control
CFU
ATP
Log CFUs mL⁻¹
9.6
9.3
9.0
8.7
8.4
8.1
7.8
7.5
ATP CONCENTRATION (nM)
150
120
90
60
30
0
0
7
14
21
INCUBATION TIME (DAYS)


b
Log CFUs mL⁻¹
4.8
4.5
4.2
3.9
3.6
3.3
3.0
Treatment
Control
0
1
2
3
4
5
6
7
INCUBATION TIME (DAYS)

with BM using short-term incubation (0-7 days). The data in Table XIV and Figure 19b show a greater increase of CFUs mL^{-1} in the treatment than in the control. In addition, a subsequent ANCOV analysis also showed a significant difference ($p<0.05$) between the treatments and the controls. These results indicate that Flavobacterium sp. was able to use anthracene for growth under these experimental conditions.

Biotransformation of Pentachlorophenol by Pseudomonas sp. and Corynebacterium sp. in the Mississippi River Water.-- The results obtained from the Mississippi River water and PCP inoculated with Pseudomonas sp. and Corynebacterium sp. are shown in Table XV. As can be seen from Table XV the number of viable cells and ATP concentration of Pseudomonas sp. in the treatment are not significantly greater than the control ($p\geq0.05$). As shown in Table XV and Figure 20 the same statistical test conducted on the data from the Mississippi River water and PCP with Corynebacterium sp. show that no significant difference ($p\geq0.05$) existed between the treatments and the controls.

Biotransformation of Pentachlorophenol by Pseudomonas sp. and Corynebacterium sp. in the Basal Medium.--The results of experiments using BM and PCP inoculated with Pseudomonas sp. and Corynebacterium sp. are shown in Table XV. From Table XV it can be seen that there is no

TABLE XIV

CFUS AND ANALYSIS OF COVARIANCE (ANCOV) RESULTS FOR FLAVOBACTERIUM SP. ISOLATED FROM MISSISSIPPI RIVER WATER, GROWN IN BASAL MEDIUM SATURATED WITH ANTHRACENE

Time (Days)	Replicate	CFUs mL^{-1} Treatment Actual Value	CFUs mL^{-1} Control Actual Value	ANCOV Analysis
0	1	1.50×10^4	1.50×10^4	
	2	1.30×10^4	1.20×10^4	S
	3	1.30×10^4	1.40×10^4	
1	1	2.60×10^4	1.70×10^4	
	2	2.20×10^4	1.60×10^4	
	3	2.30×10^4	1.60×10^4	
2	1	2.90×10^4	1.60×10^4	
	2	2.80×10^4	1.50×10^4	
	3	2.60×10^4	1.60×10^4	
3	1	2.90×10^4	1.40×10^4	
	2	3.40×10^4	1.20×10^4	
	3	3.50×10^4	1.30×10^4	
4	1	4.80×10^4	1.20×10^4	
	2	4.50×10^4	1.30×10^4	
	3	4.70×10^4	1.20×10^4	
5	1	5.60×10^4	1.40×10^4	
	2	5.50×10^4	1.40×10^4	
	3	5.40×10^4	1.40×10^4	
6	1	6.60×10^4	5.10×10^3	
	2	6.80×10^4	5.0×10^3	
	3	6.60×10^4	5.30×10^3	
7	1	6.50×10^4	1.0×10^3	
	2	6.80×10^4	1.0×10^3	
	3	6.70×10^4	1.0×10^3	

S = Significant (Analysis of Covariance) $p < 0.050$

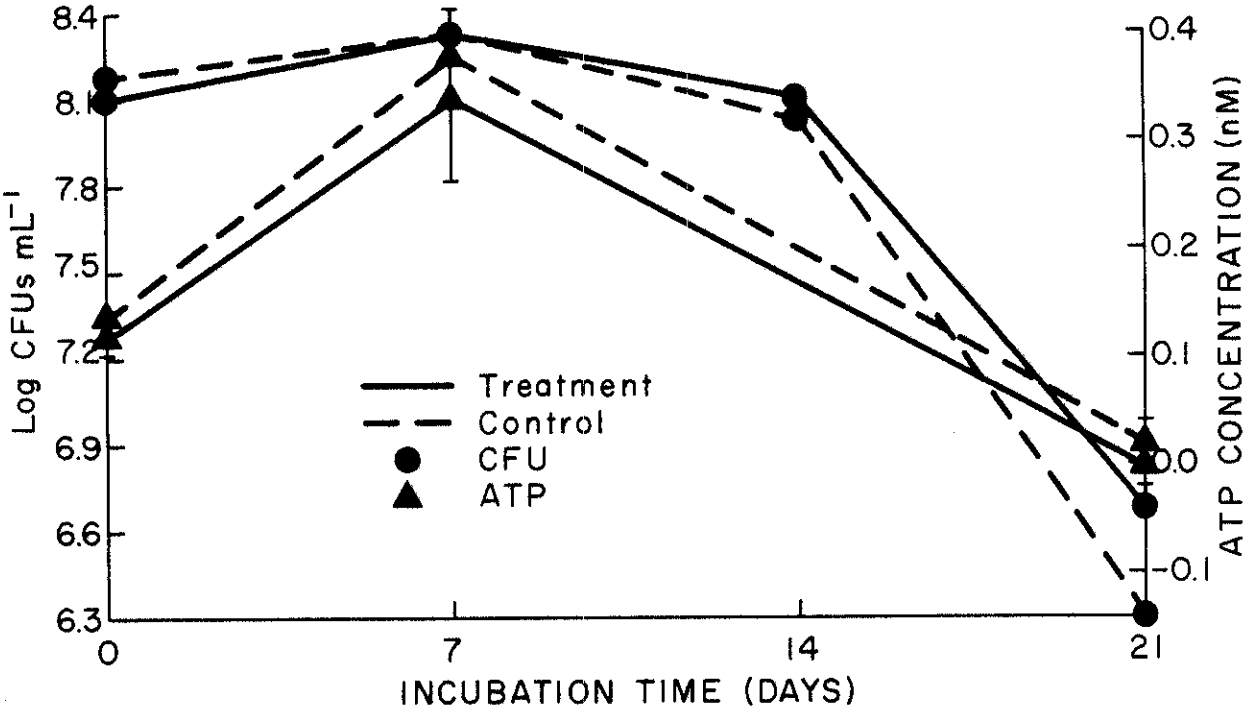
TABLE XV

MEAN VALUES AND STATISTICAL COMPARISONS OF CFUS AND ATP CONCENTRATIONS BETWEEN TREATMENT AND CONTROL SAMPLES FOR PSEUDOMONAS SP. AND CORYNEBACTERIUM SP. ISOLATED FROM MISSISSIPPI RIVER WATER, GROWN IN MEDIUM CONTAINING 1500 $\mu g\ L^{-1}$ OF PENTACHLOROPHENOL

Medium	Isolates	Days	ATP (nM) Treatment $\bar{x}$	ATP (nM) Control $\bar{x}$	CFUs mL^{-1} Treatment $\bar{x}$	CFUs mL^{-1} Control $\bar{x}$	Student t-test	ANCOV Analysis
Mississippi River water	Pseudomonas sp.	0	2.8×10^{-2}	2.70×10^{-2}	8.37×10^{8}	7.96×10^{8}	NS	
		7	1.9×10^{-2}	1.80×10^{-2}	1.41×10^{8}	2.23×10^{8}	NS	-*
		14	NO	NO	1.16×10^{7}	1.16×10^{7}	NS	
		21	3.27×10^{-3}	1.87×10^{-4}	3.36×10^{6}	1.9×10^{6}	NS	
BM	Pseudomonas sp.	0	4.8×10^{-2}	6.5×10^{-2}	8.37×10^{8}	8.34×10^{8}	NS	
		7	3.0×10^{-2}	2.40×10^{-2}	2.26×10^{8}	1.54×10^{8}	NS	-*
		14	NO	NO	3.79×10^{7}	1.49×10^{8}	NS	
		21	6.60×10^{-4}	2.43×10^{-4}	3.43×10^{5}	2.33×10^{5}	NS	
Mississippi River water	Corynebacterium sp.	0	4.53×10^{-1}	3.90×10^{-1}	3.50×10^{8}	3.56×10^{8}	NS	
		7	3.37×10^{-1}	1.90×10^{-1}	2.30×10^{8}	2.30×10^{8}	NS	-*
		14	NO	NO	8.50×10^{7}	3.72×10^{7}	NS	
		21	9.0×10^{-3}	3.03×10^{-3}	8.33×10^{6}	3.28×10^{6}	NS	
BM	Corynebacterium sp.	0	1.30×10^{-1}	1.37×10^{-1}	1.27×10^{8}	1.40×10^{8}	NS	
		7	3.47×10^{-1}	3.77×10^{-1}	2.14×10^{8}	1.96×10^{8}	NS	-*
		14	NO	NO	1.15×10^{8}	1.14×10^{8}	NS	
		21	4.23×10^{-3}	1.26×10^{-2}	4.5×10^{6}	1.95×10^{6}	NS	

*Data was not analyzed due to the result of the Student t-test.
NS = Not significant (Student t-test, ANCOV analysis), $p \geq 0.05$.
NO = ATP was not measured at this time.

Fig. 20--Log mean value of CFUs mL^{-1} and mean ATP concentration of Corynebacterium sp. grown in the basal medium containing pentachlorophenol (PCP) as the sole source of carbon (treatment). Control = isolate and basal medium, without PCP.


8.4
8.1
7.8
7.5
7.2
6.9
6.6
6.3
Log CFUs mL⁻¹
0.4
0.3
0.2
0.1
0.0
-0.1
ATP CONCENTRATION (nM)
Treatment
Control
CFU
ATP
0
7
14
21
INCUBATION TIME (DAYS)

significant difference in the colony forming units and ATP concentration of the system containing BM, anthracene, and Pseudomonas sp. or Corynebacterium sp. (Treatments) and the system that contains BM and Pseudomonas sp. or Corynebacterium sp. (Controls). Similar results were obtained based on the Student t-test at the p=0.05 level (Table XV). It is interesting to note, in contrast to the behavior of Pseudomonas sp. in the Red River water study with PCP, this system (Mississippi River water) containing anthracene had no effect on the colony forming units and ATP concentration of Pseudomonas sp. this result may be explained by the possibility that the Pseudomonas sp. might be a different species type than those isolated from previous studies.

Biotransformation of Anthracene by Pseudomonas sp. and Corynebacterium sp. in the Trinity River Water.--The results of Pseudomonas sp. and Corynebacterium sp. growth in the Trinity River water containing anthracene are presented in Table XVI and Figures 21 and 22. Also listed in Table XVI are the results of the Student t-test. The experimental results show appreciable growth by the isolated Pseudomonas sp. and Corynebacterium sp. during the 7-21 days in the treatment. The increase of CFUs in the treatments and controls during the time interval 0-7 days might be explained by the fact that the Trinity River water is nutrient rich (Table XVII). The Student t-test for Pseudomonas sp. and

TABLE XVI

MEAN VALUES AND STATISTICAL COMPARISONS OF CFUS AND ATP CONCENTRATIONS BETWEEN TREATMENT AND CONTROL SAMPLES FOR *PSEUDOMONAS* SP. AND *CORYNEBACTERIUM* SP. ISOLATED FROM TRINITY RIVER WATER, GROWN IN MEDIUM SATURATED WITH ANTHRACENE

Medium	Isolates	Days	ATP (nM) Treatment $\bar{x}$	ATP (nM) Control $\bar{x}$	CFUs mL^{-1} Treatment $\bar{x}$	CFUs mL^{-1} Control $\bar{x}$	Student t-test	ANCOV Analysis
Trinity River water	*Pseudomonas* sp.	0	4.67×10^{-2}	3.90×10^{-3}	2.36×10^{7}	1.90×10^{7}	NS	-*
		7	2.67×10^{0}	2.5×10^{-1}	2.13×10^{9}	1.20×10^{7}	NS**	
		14	NO	NO	8.50×10^{9}	2.06×10^{9}	S	
		21	5.40×10^{0}	4.70×10^{-2}	2.93×10^{9}	2.53×10^{8}	S	
BM	*Pseudomonas* sp.	0	3.78×10^{-1}	1.93×10^{-2}	8.43×10^{7}	9.20×10^{7}	NS	S
		7	5.30×10^{-1}	7.30×10^{-2}	3.26×10^{9}	4.83×10^{7}	S	
		14	NO	NO	2.52×10^{10}	2.96×10^{7}	S	
		21	9.30×10^{-1}	2.0×10^{-2}	5.0×10^{10}	1.76×10^{7}	S	
Trinity River water	*Coryne-bacterium* sp.	0	4.90×10^{-2}	5.3×10^{-3}	1.76×10^{7}	1.70×10^{7}	NS	-*
		7	3.8×10^{-1}	2.2×10^{-1}	1.23×10^{9}	6.96×10^{8}	NS	
		14	NO	NO	4.6×10^{10}	8.06×10^{7}	S	
		21	2.9×10^{0}	5.1×10^{-3}	1.03×10^{10}	1.56×10^{7}	S	
BM	*Coryne-bacterium* sp.	0	8.8×10^{-3}	7.40×10^{-3}	3.26×10^{7}	2.60×10^{9}	NS	S
		7	1.61×10^{0}	7.50×10^{1}	6.20×10^{9}	2.3×10^{9}	NS	
		14	NO	NO	5.3×10^{10}	5.0×10^{9}	S	
		21	8.0×10^{0}	3.12	2.90×10^{10}	1.02×10^{9}	S	

*Significant for ATP $p<0.05$.
**Data was not analyzed due to the result of the Student t-test.
NS = Not significant (Student t-test, ANCOV analysis), $p \geq 0.05$.
S = Significant, $p<0.05$.
NO = ATP was not measured at this time.

Fig. 21--Log mean value of CFUs mL and ATP concentration of Pseudomonas sp. grown in the Trinity River water containing anthracene as additional carbon source (treatment). Control = isolate and water, without anthracene.

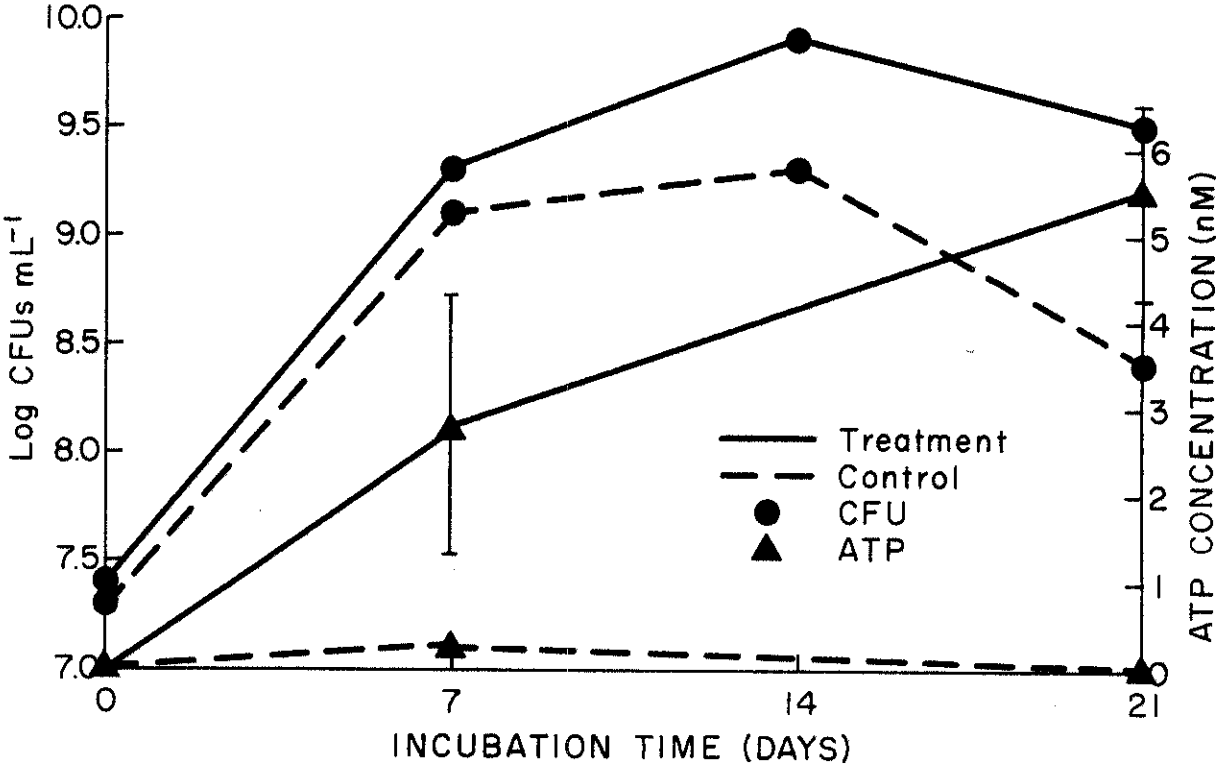

10.0
9.5
9.0
8.5
8.0
7.5
7.0
Log CFUs mL⁻¹
0
7
14
21
INCUBATION TIME (DAYS)
6
5
4
3
2
1
0
ATP CONCENTRATION (nM)
Treatment
Control
CFU
ATP

Fig. 22--Log mean value of CFUs mL and mean ATP concentration of Corynebacterium sp. grown in the Trinity River water containing anthracene as additional carbon source (treatment). Control = isolate and water, without anthracene.

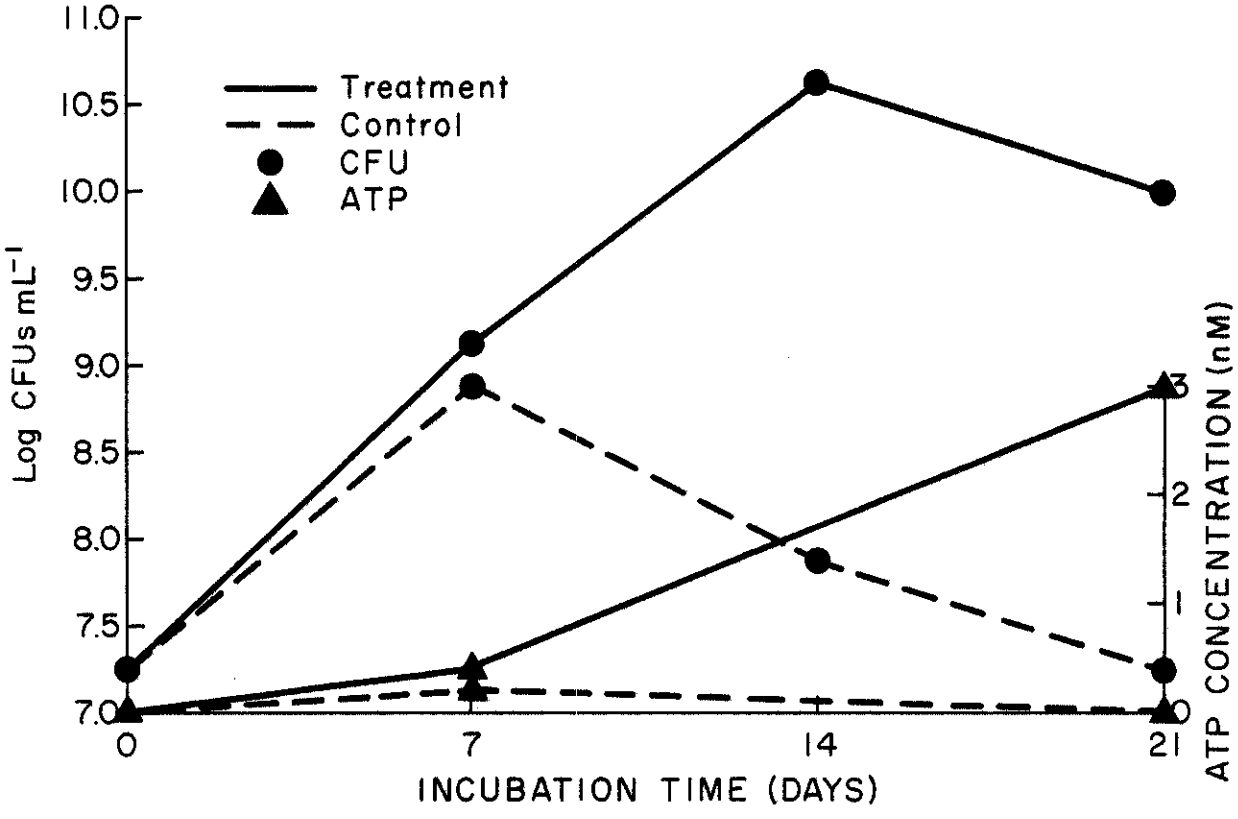

11.0
10.5
10.0
9.5
9.0
8.5
8.0
7.5
7.0
Log CFUs mL⁻¹
Treatment
Control
CFU
ATP
0
7
14
21
INCUBATION TIME (DAYS)
3
2
1
0
ATP CONCENTRATION (nM)

TABLE XVII

CFUS AND ANALYSIS OF COVARIANCE (ANCOV) RESULTS FOR PSEUDOMONAS SP. ISOLATED FROM TRINITY RIVER WATER, GROWN IN BASAL MEDIUM SATURATED WITH ANTHRACENE

Time (Days)	Replicate	CFUs mL^{-1} Treatment Actual Value	CFUs mL^{-1} Control Actual Value	ANCOV Analysis
0	1	2.0×10^4	2.0×10^4	
	2	2.30×10^4	2.20×10^4	S
	3	2.20×10^4	2.10×10^4	
1	1	5.0×10^5	2.60×10^4	
	2	4.80×10^5	2.80×10^4	
	3	4.80×10^5	2.50×10^4	
2	1	7.0×10^5	2.60×10^4	
	2	7.30×10^5	2.70×10^4	
	3	7.10×10^5	2.30×10^4	
3	1	8.80×10^5	2.60×10^4	
	2	8.90×10^5	2.50×10^4	
	3	9.0×10^5	2.20×10^4	
4	1	9.30×10^5	2.0×10^4	
	2	9.20×10^5	1.80×10^4	
	3	9.10×10^5	1.60×10^4	
5	1	9.60×10^5	1.0×10^4	
	2	9.70×10^5	1.30×10^4	
	3	1.0×10^6	1.20×10^4	
6	1	1.20×10^6	1.0×10^4	
	2	1.0×10^6	1.10×10^4	
	3	1.0×10^6	1.0×10^4	
7	1	2.50×10^6	6.0×10^3	
	2	2.70×10^6	6.20×10^3	
	3	2.40×10^6	6.0×10^3	

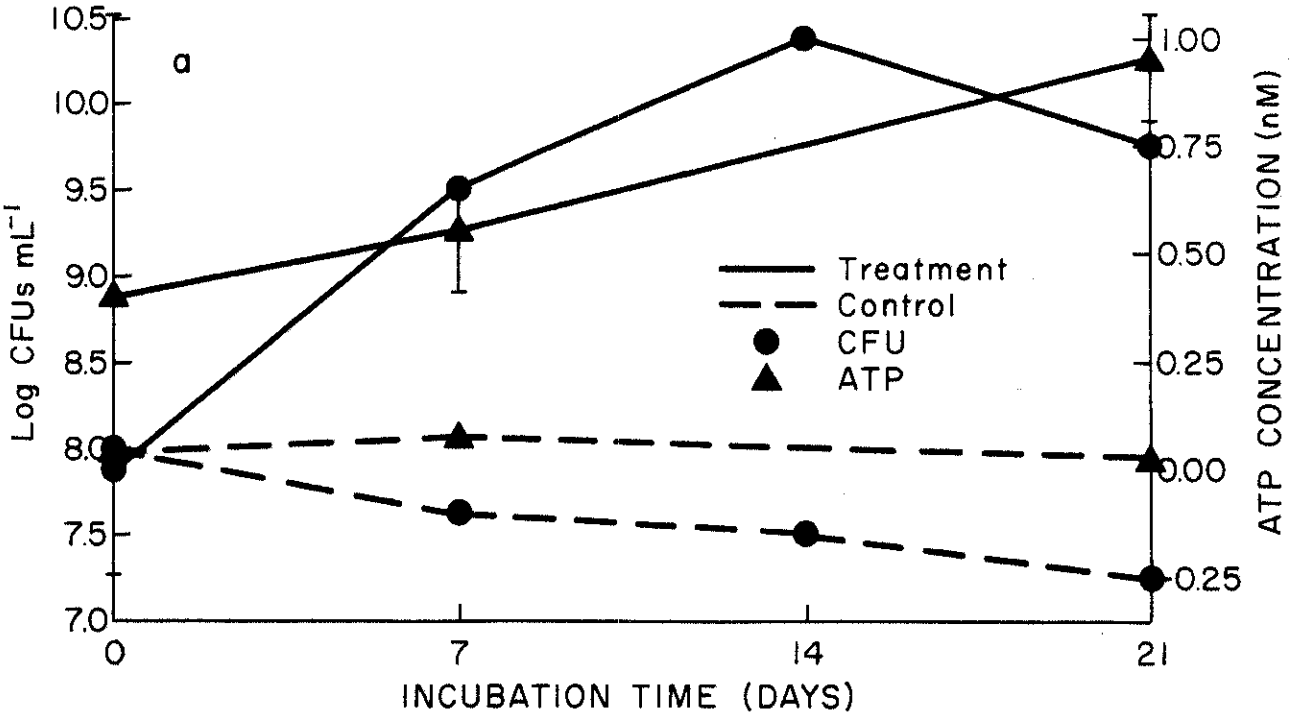
S = Significant (Analysis of Covariance) $p < 0.050$

Corynebacterium sp. based on CFUs and ATP concentration show that the treatment tests produced significantly higher number of CFUs and ATP than the controls at 14 and 21 days ($p<0.05$). However, in contrast to days 14 and 21, a significant difference was found between the treatment and the control ATP concentration at day 7 for the Pseudomonas sp. This different relationship between CFUs and ATP was also reported by Hamilton and Hansen (1967). The reason why Pseudomonas sp. has a lower ATP concentration than would be indicated from CFUs is not reported, and also was not further investigated in this study.

Biotransformation of Anthracene by Pseudomonas sp. and Corynebacterium sp. in the Basal Medium.--The results of the growth tests incorporating sterile BM and anthracene inoculated with Pseudomonas sp. or Corynebacterium sp. are presented in Table XVI and Figures 23a and 24a. The results of Student t-tests are also summarized in Table XVI. In contrast to the results from the Trinity River water with Pseudomonas sp. (Figure 21), there is no relative increase shown in CFUs of Pseudomonas sp. in the treatments and the controls for the first 7 days (Figure 23a). The Student t-test showed that at all other times Pseudomonas sp. growth in the treatment experiments were highly significantly different ($p\leq 0.05$) from that of the controls. In addition, analysis of covariance on colony forming units

Fig. 23a--Log mean value of CFUs mL^{-1} and mean ATP concentration of Pseudomonas sp. grown in the basal medium containing anthracene as the sole source of carbon (treatment). Control = isolate and basal medium, without anthracene. Bars show the two standard deviation on both sides of the ATP means.

Fig. 23b--Log value of CFUs mL^{-1} of Pseudomonas sp. grown in the basal medium containing anthracene as the sole source of carbon (treatment). Control = isolate and basal medium, without anthracene.


a
Log CFUs mL⁻¹
ATP CONCENTRATION (nM)
Treatment
Control
CFU
ATP
INCUBATION TIME (DAYS)

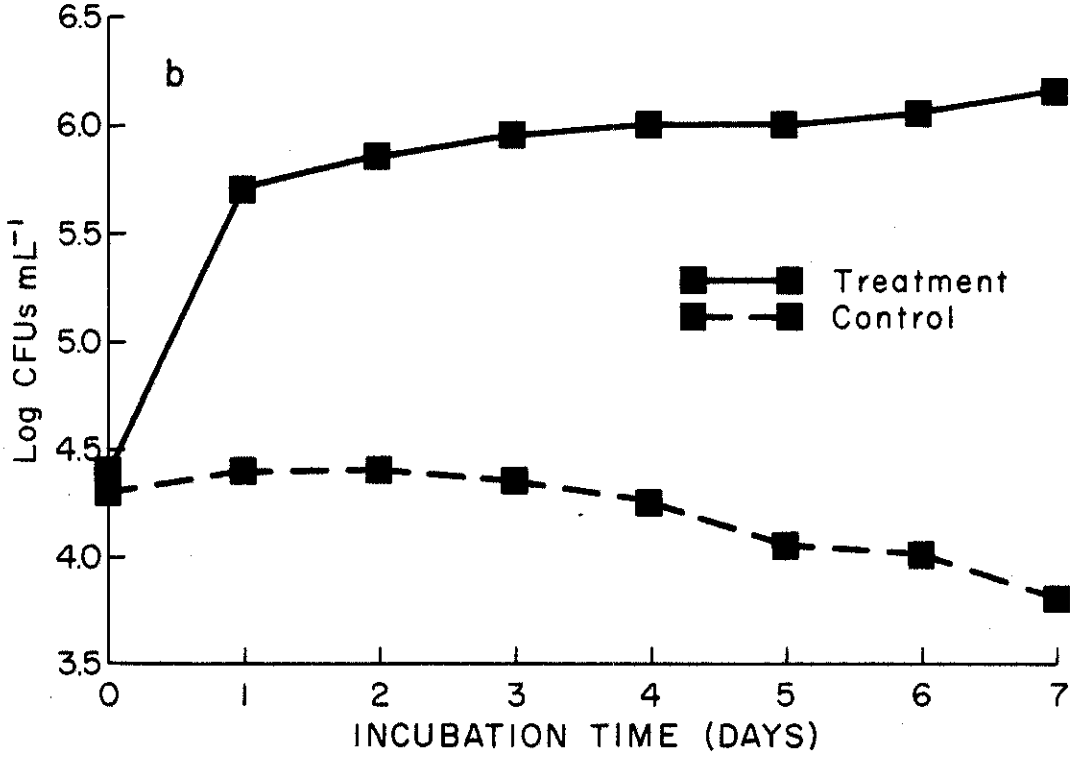
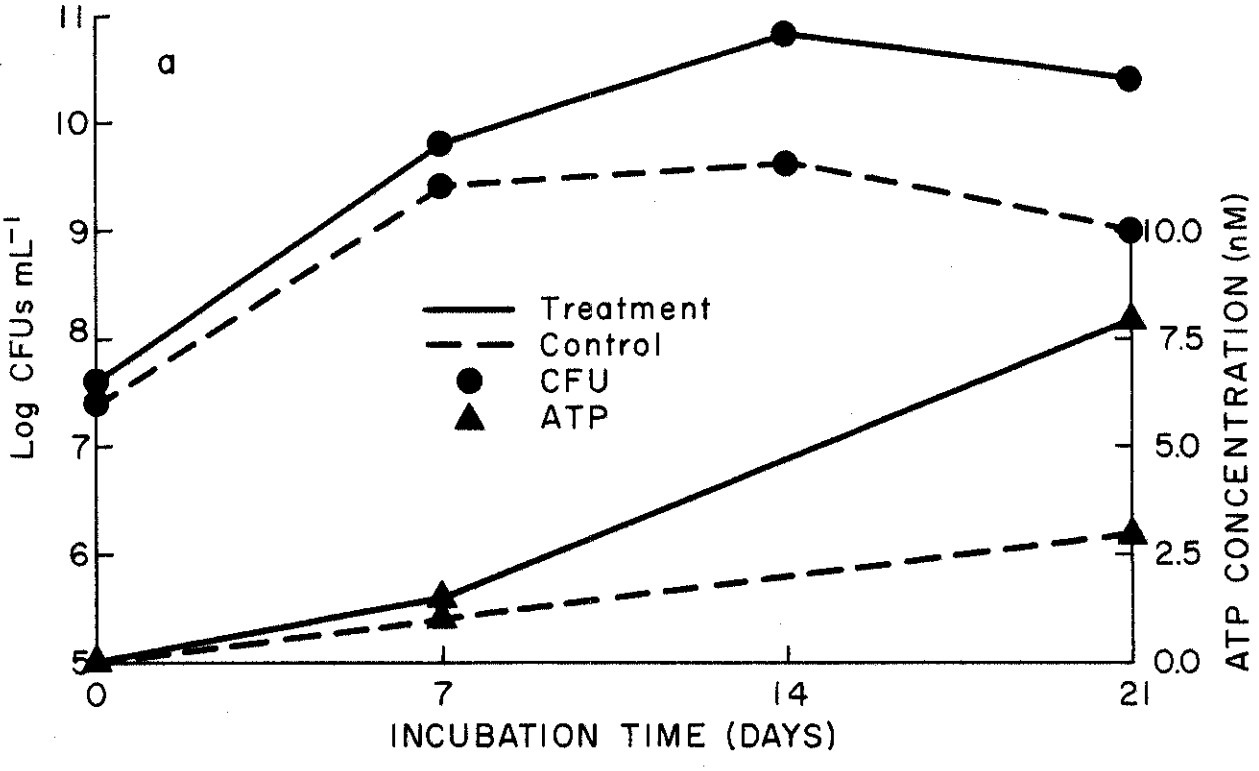
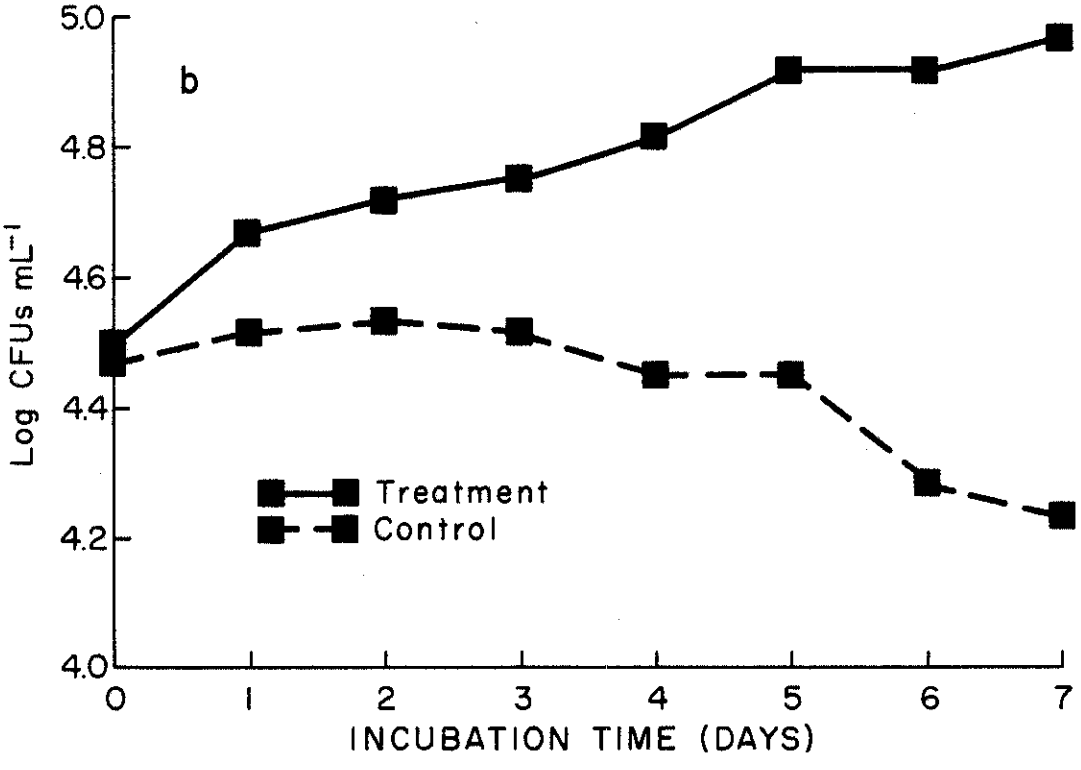

b
Log CFUs mL⁻¹
Treatment
Control
INCUBATION TIME (DAYS)

Fig. 24a--Log mean value of CFUs mL^{-1} and mean ATP concentration of Corynebacterium sp. grown in the basal medium containing anthracene as the sole source of carbon (treatment). Control = isolate and basal medium, without anthracene.

Fig. 24b--Log value of CFUs mL^{-1} of Corynebacterium sp. grown in the basal medium containing anthracene as the sole source of carbon (treatment). Control = isolate and basal medium without anthracene.


a
Log CFUs mL⁻¹
ATP CONCENTRATION (nM)
Treatment
Control
CFU
ATP
INCUBATION TIME (DAYS)


b
Log CFUs mL⁻¹
Treatment
Control
INCUBATION TIME (DAYS)

between the treatment and the control in short-term incubation yielded significant difference at the p=0.05 level (Table XVII, Figure 23b). The results from long-term incubation in the Trinity River water and BM experiments, and also from short-term incubation suggest that anthracene can be used by Pseudomonas sp. as the sole carbon source.

The results of the Corynebacterium sp. growth in the BM containing anthracene as the sole source of carbon are presented in Table XVI. As can be seen from Table XVI and Figure 24a the colony forming units and ATP concentration of Corynebacterium sp. until day 7 increased at relatively the same rate in both the treatments and in the controls and then number of viable cells in the treatments were significantly increased. The Student t-test also indicated a significant difference ($p \leq 0.05$) between the treatment and the control at days 14 and 21 of incubation. In addition, subsequent analysis of covariance of the short term studies showed similar results (Table XVIII and Figure 24b). The results from both long-term studies (Trinity River water and BM) and short term incubation studies indicated that Corynebacterium sp. can utilize anthracene as a sole carbon source for growth. However, the long-time survival or growth in the control flasks (BM only) might be attributed to some endogenous nutrient within the bacteria or possibility of contaminated media.

TABLE XVIII

CFUS AND ANALYSIS OF COVARIANCE (ANCOV) RESULTS FOR *CORYNEBACTERIUM* SP. ISOLATED FROM TRINITY RIVER WATER, GROWN IN BASAL MEDIUM SATURATED WITH ANTHRACENE

Time (Days)	Replicate	CFUs mL^{-1} Treatment Actual Value	CFUs mL^{-1} Control Actual Value	ANCOV Analysis
0	1	3.0×10^4	3.0×10^4	
	2	3.20×10^4	3.0×10^4	S
	3	3.0×10^4	3.0×10^4	
1	1	4.50×10^4	3.60×10^4	
	2	4.80×10^4	3.0×10^4	
	3	4.60×10^4	3.0×10^4	
2	1	5.10×10^4	3.0×10^4	
	2	5.0×10^4	3.60×10^4	
	3	5.0×10^4	3.40×10^4	
3	1	5.80×10^4	3.40×10^4	
	2	5.60×10^4	3.20×10^4	
	3	5.60×10^4	3.20×10^4	
4	1	6.60×10^4	3.0×10^4	
	2	6.40×10^4	3.0×10^4	
	3	6.50×10^4	2.80×10^4	
5	1	8.20×10^4	3.0×10^4	
	2	8.0×10^4	2.80×10^4	
	3	8.40×10^4	2.80×10^4	
6	1	8.80×10^4	2.0×10^4	
	2	8.50×10^4	1.90×10^4	
	3	8.0×10^4	1.80×10^4	
7	1	9.30×10^4	1.80×10^4	
	2	9.50×10^4	1.60×10^4	
	3	9.20×10^4	1.90×10^4	

S = Significant (Analysis of Corvariance) $p < 0.050$

Biotransformation of Pentachlorophenol by Pseudomonas sp. and Micrococcus sp. in Trinity River Water.--The results of the bacterial growth in the Trinity River water with PCP are presented in Table XIX. From this table can be seen that the numbers of viable cells (Pseudomonas sp.) decrease in treatments and controls following inoculation to the medium. The results of the Student t-test on CFUs and ATP concentration also show that there was no significant difference ($p \leq 0.05$) between the treatments and the controls (Table XIX). The results of experiments where Micrococcus sp. was incubated in Trinity River water containing PCP are illustrated in Table XIX and Figure 25. These results indicate that CFUs and ATP concentrations of the Micrococcus sp. in the treatments were significantly ($p \leq 0.05$) greater than the controls at various interval times except at initial time.

Biotransformation of Pentachlorophenol by Pseudomonas sp. and Micrococcus sp. in the Basal Medium.--Table XIX shows the results of PCP biotransformation in BM with Pseudomonas sp. Analysis of these data showed that there are no significant differences between the treatments and the controls for CFUs and ATP concentrations. These results indicate that Pseudomonas sp. is unable to utilize PCP as a primary or secondary substrate for their growth under these experimental conditions. Micrococcus sp. growth in the BM

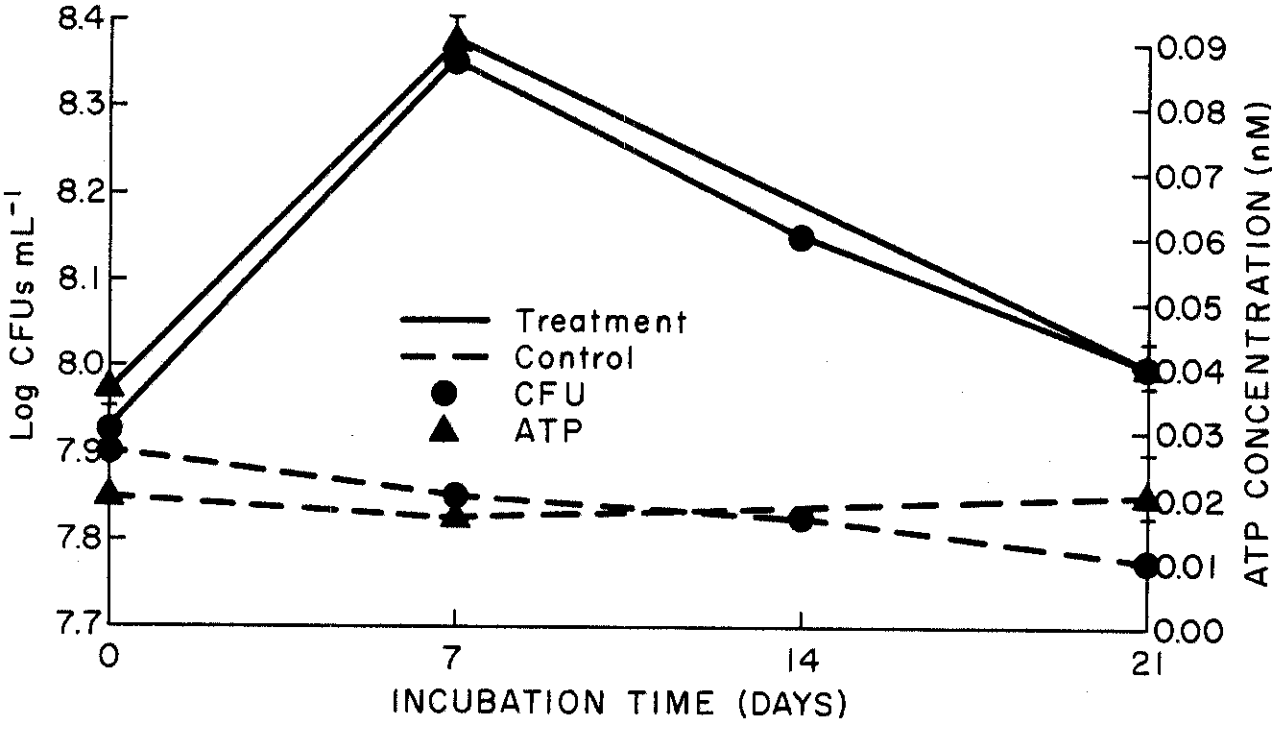
TABLE XIX

MEAN VALUES AND STATISTICAL COMPARISONS OF CFUS AND ATP CONCENTRATIONS BETWEEN TREATMENT AND CONTROL SAMPLES FOR *PSEUDOMONAS* SP. AND *MICROCOCCUS* SP. ISOLATED FROM TRINITY RIVER WATER, GROWN IN MEDIUM CONTAINING 1500 $\mu g L^{-1}$ OF PENTACHLOROPHENOL (PCP)

Medium	Isolates	Days	ATP (nM)		CFUs mL^{-1}		Student t-test	ANCOV Analysis
			Treatment $\bar{x}$	Control $\bar{x}$	Treatment $\bar{x}$	Control $\bar{x}$		
Trinity River water	*Pseudomonas* sp.	0	1.40×10^{-2}	1.33×10^{-2}	3.23×10^{7}	3.25×10^{7}	NS	-*
		7	7.9×10^{-3}	7.4×10^{-3}	2.16×10^{7}	2.1×10^{7}	NS	
		14	NO	NO	9.93×10^{6}	1.53×10^{7}	NS	
		21	7.5×10^{-2}	2.57×10^{-3}	2.20×10^{6}	6.4×10^{6}	NS	
BM	*Pseudomonas* sp.	0	1.30×10^{-2}	1.17×10^{-2}	3.04×10^{8}	3.03×10^{7}	NS	-*
		7	7.8×10^{-3}	1.12×10^{-2}	2.39×10^{7}	2.39×10^{7}	NS	
		14	NO	NO	1.42×10^{7}	1.37×10^{7}	NS	
		21	1.43×10^{-3}	1.67×10^{-3}	3.13×10^{6}	3.16×10^{6}	NS	
Trinity River water	*Micrococcus* sp.	0	3.53×10^{-2}	1.97×10^{-2}	8.46×10^{7}	7.9×10^{7}	NS	-*
		7	9.0×10^{-2}	1.77×10^{-2}	2.24×10^{8}	7.06×10^{7}	S	
		14	NO	NO	1.42×10^{8}	6.83×10^{7}	S	
		21	4.03×10^{-2}	2.10×10^{-2}	9.86×10^{7}	5.96×10^{7}	S	
BM	*Micrococcus* sp.	0	2.93×10^{-2}	3.33×10^{-2}	7.26×10^{7}	7.23×10^{7}	NS	S
		7	6.30×10^{-2}	3.40×10^{-2}	1.99×10^{8}	7.03×10^{7}	S	
		14	NO	NO	1.42×10^{8}	6.83×10^{7}	S	
		21	2.10×10^{-2}	2.10×10^{-2}	6.10×10^{7}	5.96×10^{7}	NS	

*Data was not analyzed due to the result of the Student t-test.
NS = Not significant (Student t-test, ANCOV analysis), $p \geq 0.05$.
S = Significant, $p < 0.05$.
NO = ATP was not measured at this time.

Fig. 25--Log mean value of CFUs mL^{-1} and mean ATP concentration of Micrococcus sp. grown in the Trinity River water containing pentachlorophenol (PCP) as additional carbon source (treatment). Control = isolate and water, without PCP. Bars show the two standard deviation on both sides of the ATP means.


8.4
8.3
8.2
8.1
8.0
7.9
7.8
7.7
Log CFUs mL⁻¹
0.09
0.08
0.07
0.06
0.05
0.04
0.03
0.02
0.01
0.00
ATP CONCENTRATION (nM)
Treatment
Control
CFU
ATP
0
7
14
21
INCUBATION TIME (DAYS)

containing PCP is also presented in Table XIX and Figure 26a. A significant difference existed between the treatments and the controls at days 7 and 14 as measured by CFUs and ATP concentration. However, in contrast, results for experiments with Trinity River water showed no increase in viable cell counts in cultures with PCP (treatments) at day 21. The ability of Micrococcus sp. to degrade PCP was further demonstrated in the short-term incubation studies (0-7 days) (Table XX and Figure 26b). Analysis of Covariance showed a significant difference ($p<0.05$) between the treatments and controls for CFUs mL^{-1} at 7 days. This result represents an appreciable enhancement of the CFUs and ATP level as compared to the controls. Comparisons of bacterial growth in experiments with low initial bacterial counts (~ 10 CFUs mL^{-1}) in BM containing anthracene or PCP as the sole source of carbon are shown in Figure 27.

Summary of Results of the Biotransformation Tests in River Waters and Basal Medium

The results obtained for the three river waters and the BM with anthracene or PCP indicate several points:

1. Closely related bacteria were shown to be capable of anthracene or PCP biotransformation in both the water samples and basal medium.
2. Biotransformation of anthracene and PCP were not correlated with the initial size of bacterial inoculum in five out of six experiments. These results

TABLE XX

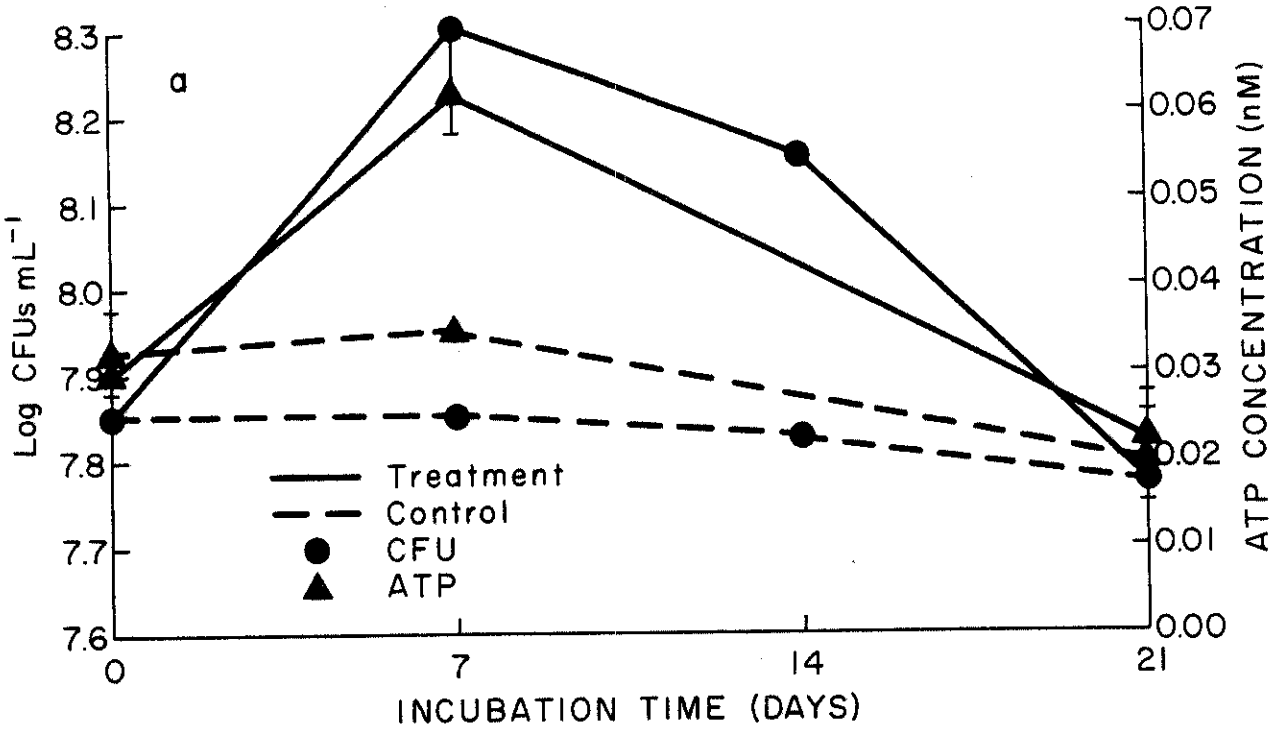
CFUS AND ANALYSIS OF COVARIANCE (ANCOV) RESULTS FOR MICROCOCCUS SP. ISOLATED FROM TRINITY RIVER WATER, GROWN IN BASAL MEDIUM CONTAINING 1500 μg^{-1} OF PENTACHLOROPHENOL (PCP)

Time (Days)	Replicate	CFUs mL^{-1} Treatment Actual Value	CFUs mL^{-1} Control Actual Value	ANCOV Analysis
0	1	2.50×10^4	2.30×10^4	
	2	2.40×10^4	2.40×10^4	S
	3	2.45×10^4	2.40×10^4	
1	1	5.20×10^4	2.80×10^4	
	2	5.0×10^4	2.90×10^4	
	3	4.90×10^4	3.0×10^4	
2	1	1.20×10^5	3.20×10^4	
	2	1.50×10^5	3.40×10^4	
	3	1.30×10^5	3.50×10^4	
3	1	2.60×10^5	3.20×10^4	
	2	2.40×10^5	3.0×10^4	
	3	2.30×10^5	2.90×10^4	
4	1	2.80×10^5	1.0×10^4	
	2	2.90×10^5	1.20×10^4	
	3	2.90×10^5	1.40×10^3	
5	1	3.40×10^5	1.0×10^4	
	2	3.30×10^5	1.0×10^4	
	3	3.50×10^5	9.0×10^3	
6	1	5.20×10^5	1.0×10^3	
	2	5.40×10^5	1.20×10^3	
	3	5.0×10^5	1.10×10^3	
7	1	5.90×10^5	9.0×10^2	
	2	6.0×10^5	8.90×10^2	
	3	5.80×10^5	9.0×10^2	

S = Significant (Analysis of Covariance) $p = < 0.050$

Fig. 26a--Log mean value of CFUs mL^{-1} and mean ATP concentration of Micrococcus sp. grown in the basal medium containing pentachlorophenol (PCP) as the sole source of carbon (treatment). Control = isolate and basal medium, without PCP.

Fig. 26b--Log value of CFUs mL^{-1} of Micrococcus sp. grown in the basal medium containing pentachlorophenol (PCP) as the sole source of carbon (treatment). Control = isolate and basal medium, without PCP.


a
8.3
8.2
8.1
8.0
7.9
7.8
7.7
7.6
Log CFUs mL⁻¹
0.07
0.06
0.05
0.04
0.03
0.02
0.01
0.00
ATP CONCENTRATION (nM)
Treatment
Control
CFU
ATP
0
7
14
21
INCUBATION TIME (DAYS)

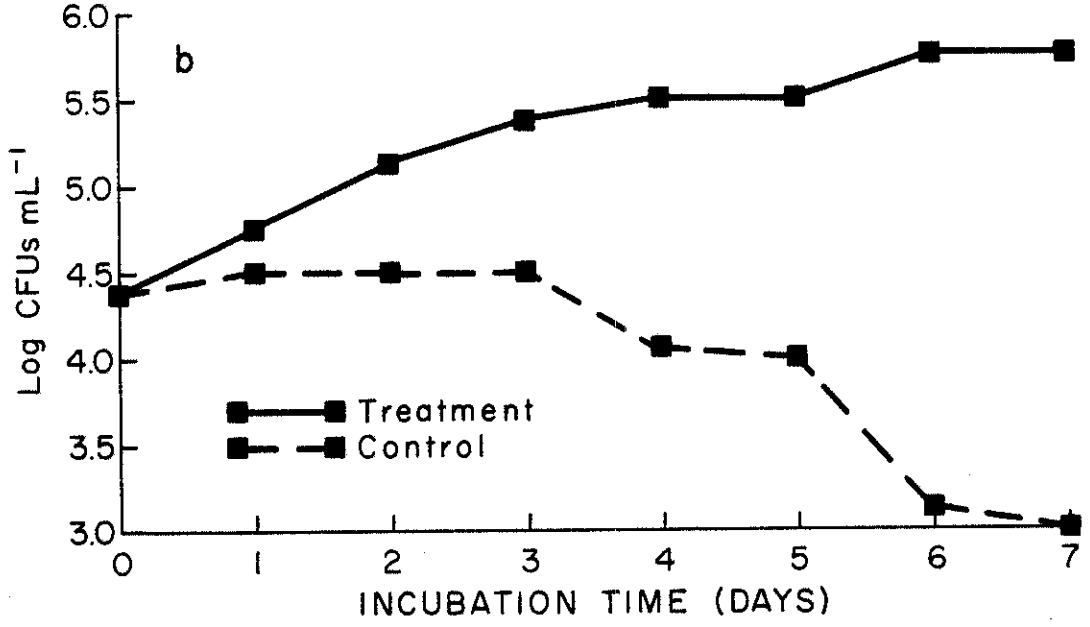
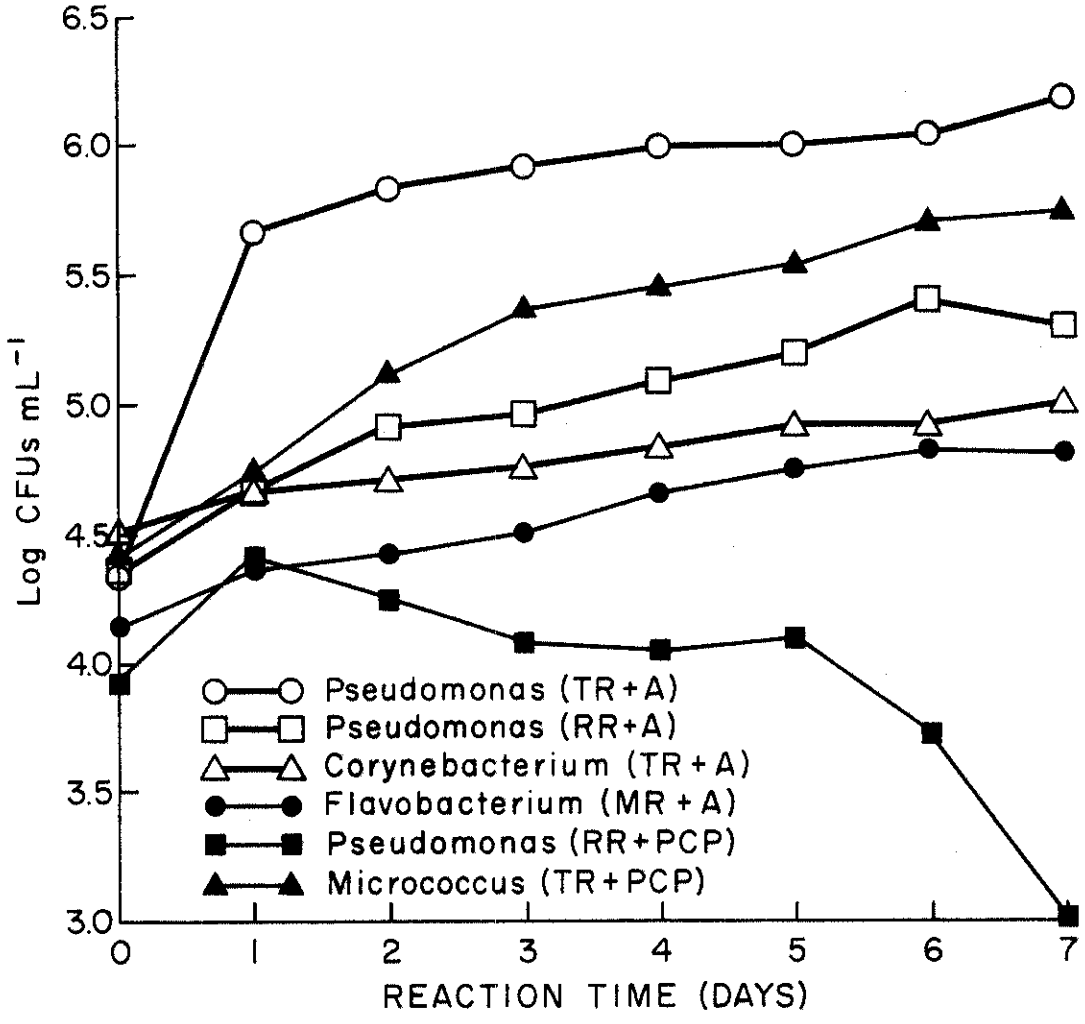

b
6.0
5.5
5.0
4.5
4.0
3.5
3.0
Log CFUs mL⁻¹
Treatment
Control
0
1
2
3
4
5
6
7
INCUBATION TIME (DAYS)

Fig. 27--Comparison of CFUs mL^{-1} of bacteria grown in the basal medium containing anthracene (A) or pentachlorophenol (PCP) as the sole source of carbon. Sources of water = Red River water (RR), Trinity River water (TR), and Mississippi River water (MR).


6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
Log CFUs mL⁻¹
0
1
2
3
4
5
6
7
REACTION TIME (DAYS)
Pseudomonas (TR+A)
Pseudomonas (RR+A)
Corynebacterium (TR+A)
Flavobacterium (MR+A)
Pseudomonas (RR+PCP)
Micrococcus (TR+PCP)

were obtained from long-term and short-term studies with different initial levels of inoculum. Out of these five studies, one experimental result conflicts with this observation. Results of Pseudomonas sp. inoculated in the Red River water containing PCP showed that the size of the initial inoculum had a discernable effect on the biotransformation of PCP. Based on cell yield, PCP proved to be a resistant substrate for the growth of this Pseudomonas sp. strain with a low initial inoculum. This result agrees with the results of Kirsch and Etzel (1973). Kirsch and Etzel reported that the absolute rate of PCP oxidation is related to a large degree on the cell concentration.

3. Four of the six bacterial isolates were able to metabolize anthracene. The most dominant and active utilizer of anthracene was a Pseudomonas sp. Other bacterial types include genera of Corynebacterium and sp. and Flavobacterium (Figure 27). Isolates of two genera, Acinetobacter and Alcaligenes, showed little (Acinetobacter) or no activity (Alcaligene). The reason Flavobacterium sp. growth was suppressed after 7 days incubation might be explained by one of two reasons. Either one of the classes of essential nutrients has been exhausted, or toxic products have accumulated. It is known

from the literature that anthracene can be metabolized in soil by Pseudomonas sp. (U.S. EPA, 1979a). Degradation of anthracene was reported by mixed cultures of P. aeruginosa with Flavobacterium sp., (Rogoff and Wender, 1957), but, so far, no conclusive reports are available for Flavobacterium sp. and Corynebacterium sp.

4. Of the six isolates tested for biotransformation of PCP, Micrococcus sp. (or a closely related genus) was the only isolate capable of growing on and using PCP at both high and low initial inoculum size in the experimental water (Trinity River water) and in BM as a sole source of carbon or carbon source. One species of Pseudomonas also showed, with a high initial inoculum, the ability to metabolize PCP in the Red River water and BM. However, no other species of Pseudomonas or similar genera were found to be able to use PCP for their growth. The absence of PCP degradation by other Pseudomonas sp. may therefore be a reflection of the habitats and experimental condition, or else the strains could be differentiated from one another and from Watanabe's isolated strain (1973). Other isolates, Pseudomonas sp., Bacillus sp., and Corynebacterium sp. were shown to have no ability to biotransform PCP. However, several bacteria

have been reported to utilize PCP as a sole source of carbon and energy: a Corynebacterium sp. isolated by Chu and Kirsch (1972), a Pseudomonas sp. isolated by Watanabe (1973), and the genus Arthrobacter KC3 by Gary and Finn (1982). It should be noted that none of the many PCP isolates tested so far were Micrococcus sp. or related to this genera as has been found in this study.

5. Certain bacteria which fail to grown on PCP or anthracene were still numerically dominant in the batch culture several days after transfer into PCP or anthracene medium (Acinetobacter sp. and Pseudomonas sp.), even though the only carbon source was either one of these chemicals when grown in basal salts medium. This suggests that these compounds at the levels used were not toxic to these organisms.

Effect of Glucose Additions on the Biotransformation of Anthracene and Pentachlorophenol in the River Waters

This aspect of my research was conducted to obtain additional information on the significance of biotransformation of anthracene and PCP by bacteria isolated from the three river waters. Glucose was used as an additional carbon source to determine whether or not chemical biotransformation was influenced by conditions that support cell proliferation. Biotransformation of anthracene and PCP in

glucose enriched media was determined by following the disappearance of the original chemical compounds (anthracene and PCP) through time of incubation. The disappearance of the original chemical concentration due to microbial action in experimental media as determined by analytical techniques previously described are expressed in terms of the concentration of chemical at various time intervals (zero, 7, 14, and 21 days) in each of the 100-ml water samples, plus the chemical with and without glucose (1 gL^{-1}). The ability of the isolates to biotransform anthracene or PCP via co-metabolism was tested statistically using analysis of covariance (ANCOV) for the comparison of slopes within controls (water + chemical; water + chemical + glucose) and treatment (water + chemical + glucose, and isolate). If the ANCOV indicated that there is a significant difference in slopes between two controls (water + chemical; water + chemical + glucose - 1 gL^{-1}) ($p=0.05$), then a Student-Newman-Keuls (SNK) multiple range test was conducted to show whether either of the control slopes were significantly different from the treatment.

The results from the biotransformation test incorporating sterile river water and additional glucose with and without bacterial isolates are summarized in table form. These tables show the actual concentrations of chemical compounds in the replicate treatment flasks (river water, chemical, glucose, and isolate) and in the sterile control flasks (river waters, chemical with and without glucose).

Also shown in these tables are results of Analysis of Covariance between slopes of treatments and controls. Selected graphical depictions of the disappearance of the compounds are presented for each experiment. These figures demonstrate the disappearance of the chemical compounds in the treatment flasks and in the sterile control flasks. The points depict the actual values of replicate flasks versus time. As can be seen in these figures, because of close relative values for each replicate, one point was depicted for each set of replicates. Based on statistical analysis the following results were obtained for each of the three water systems and chemicals.

Biotransformation of Anthracene by Alcaligenes sp. and Pseudomonas sp. in Glucose Enriched Red River Water.--ANCOV analysis for Red River water inoculated with Pseudomonas sp. or Alcaligenes sp. and anthracene demonstrated that there was a significant difference ($p \leq 0.05$) in slopes based on data obtained from the Red River water and anthracene with Pseudomonas sp. Subsequent analysis using SNK was not conducted since control slopes were not significantly different (Table XXI, Figure 28). Chemical data in Table XXI also show a 44% and 94% loss of total anthracene in the Red River water with glucose inoculated with Pseudomonas sp. after 14 and 21 days, respectively. Thus, 50% of anthracene disappeared between 14-21 days. This observation supports the

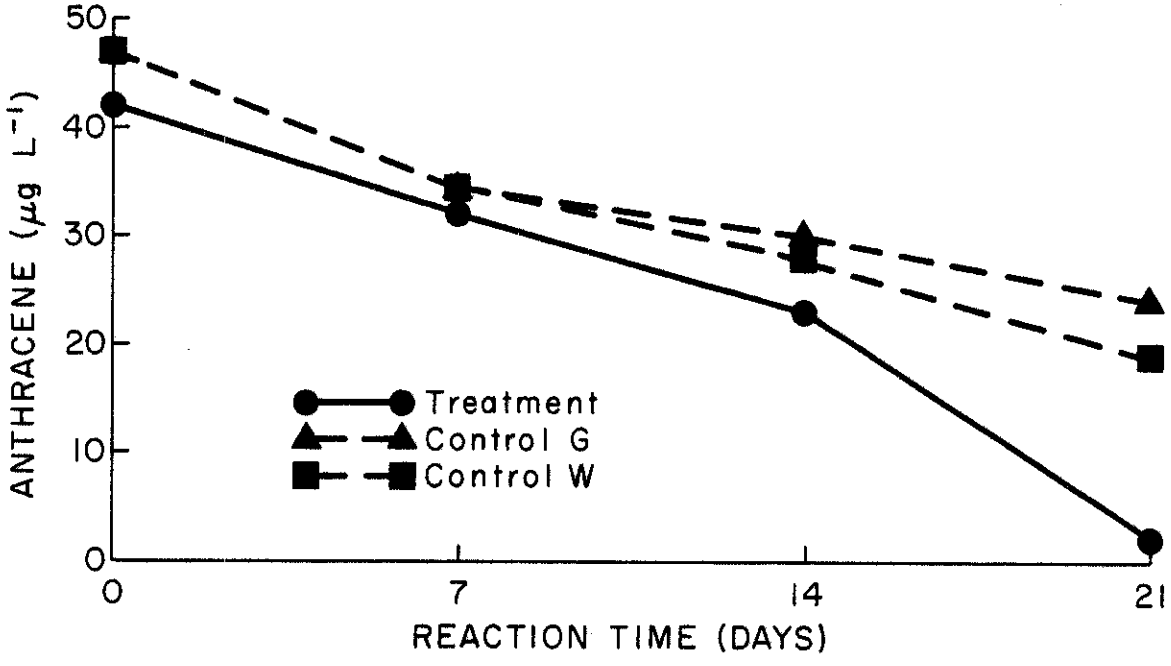
TABLE XXI

BIOTRANSFORMATION OF ANTHRACENE BY ALCALIGENES SP. AND PSEUDOMONAS SP. IN GLUCOSE ENRICHED RED RIVER WATER. RESULTS OF THE ANALYSIS OF COVARIANCE (ANCOV) BETWEEN TREATMENT AND CONTROLS. ORGANSISMS ISOLATED FROM RED RIVER WATER

Isolate	Days	Replicate	Concentration of Anthracene (μgL^{-1}) Actual Treatment*	Actual Control**	Actual Control***	ANCOV Analysis
	0	1	25.79	32.30	45.53	
		2	27.12	33.68	46.84	
		3	21.71	37.64	46.84	
	7	1	23.16	28.42	35.0	
		2	20.50	28.42	33.68	
		3	20.53	28.42	36.32	
Alcaligenes sp.	14	1	20.20	23.90	30.80	NS
		2	19.50	24.60	32.40	
		3	19.90	24.50	31.10	
	21	1	19.00	22.79	30.00	
		2	17.40	23.50	30.00	
		3	17.85	23.0	30.00	
	0	1	44.34	47.0	47.0	
		2	39.00	45.40	46.0	
		3	41.00	44.39	46.0	
	7	1	32.00	30.0	34.0	
		2	31.32	30.32	33.50	
		3	30.00	30.00	33.0	
Pseudomonas sp.	14	1	23.30	27.89	28.50	S
		2	22.63	28.50	29.0	
		3	22.63	28.95	29.0	
	21	1	1.20	18.30	23.0	
		2	1.20	17.90	24.0	
		3	1.20	20.20	23.5	

*Red River water + Anthracene + Glucose + Isolate
**Red River water + Anthracene + Glucose
***Red River water + Anthracene
NS = Not significant (ANCOV Analysis) $p \geq 0.05$.

Fig. 28--Biotransformation of anthracene by Pseudomonas sp. in glucose enriched Red River water (treatment). Controls = water, anthracene, and glucose without isolate (G); water and anthracene without isolate and no glucose (W).


50
40
30
20
10
0
ANTHRACENE (μg L⁻¹)
0
7
14
21
REACTION TIME (DAYS)
Treatment
Control G
Control W

idea that the Pseudomonas sp. may attack anthracene as a secondary substrate. Table XXI also shows the result of ANCOV analysis between slopes of the treatment and the controls for the Red River water plus anthracene with Alcaligenes sp. There was no significant differences ($p \geq 0.05$) shown by the ANCOV test for this bacterium.

Biotransformation of Pentachlorophenol by Pseudomonas sp. and Bacillus sp. in Glucose Enriched Red River Water.--Results of statistical tests conducted on data from the Red River water and PCP with Pseudomonas sp. or Bacillus sp. show that there was a significant difference for Bacillus sp. at the 0.05 level. Chemical data in Table XXII reveal that the disappearance of PCP in a medium inoculated with Bacillus sp. was 59% during the first 7 days (0-7 days), 23% during the second 7 days (7-14 day) and 27% during the third 7 days (14-21 days). This result suggests that the greatest disappearance of PCP with Bacillus sp. appears between zero and 7 days incubation time (Figure 29). For the control, the disappearance of PCP was 9.8% between 0-7 days and 55% between 7-14 days, and 24% for 14-21 days (Table XXII). No significant difference ($p \geq 0.05$) between slopes for Red River water and PCP inoculated with Pseudomonas sp. was found by ANCOV analysis (Table XXIII, Figure 30).

TABLE XXII

BIOTRANSFORMATION OF PENTACHLOROPHENOL (PCP) BY BACILLUS SP. IN GLUCOSE ENRICHED RED RIVER WATER. RESULTS OF THE ANALYSIS OF COVARIANCE (ANCOV) BETWEEN TREATMENT AND CONTROLS. ORGANISM ISOLATED FROM RED RIVER WATER

Days	Replicate	Concentration of PCP (μgL^{-1})			ANCOV Analysis
		Actual Treatment*	Actual Control**	Actual Control***	
0	1	1498.00	1500.00	1502.20	
	2	1455.00	1488.85	1500.00	
	3	1485.00	1482.85	1499.70	S
	4	1455.00	1488.50	1500.00	
	5	1492.00	1502.30	1498.30	
7	1	596.70	1397.40	1490.90	
	2	598.10	1368.40	1475.00	
	3	627.00	1391.70	1482.00	
	4	601.00	1314.50	1492.00	
	5	605.00	1322.10	1480.00	
14	1	583.75	566.70	633.20	
	2	572.75	561.00	629.00	
	3	565.00	682.30	602.00	
	4	560.50	587.00	670.00	
	5	540.50	645.80	698.00	
21	1	367.90	404.30	583.75	
	2	392.30	433.80	572.75	
	3	283.90	422.85	594.83	
	4	299.50	450.00	538.80	
	5	344.00	485.00	540.40	

*Red River water + Anthracene + Glucose + Bacillus sp.

**Red River Water + Anthracene + Glucose

***Red River Water + Anthracene

S = Significant (ANCOV Analysis) $p < 0.05$.

Fig. 29--Biotransformation of pentachlorophenol (PCP) by Bacillus sp. in glucose enriched Red River water (treatment). Controls = water, PCP, and glucose without isolate (G); water and PCP without isolate and no glucose (W).

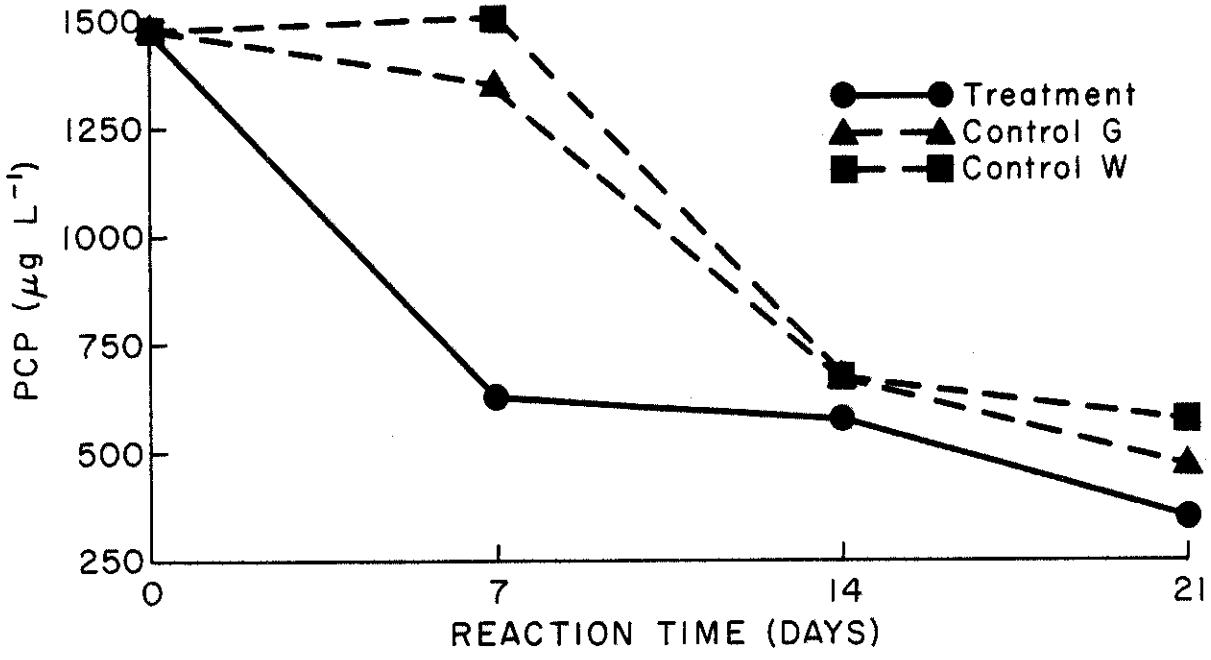

1500
1250
1000
750
500
250
PCP (μg L⁻¹)
0
7
14
21
REACTION TIME (DAYS)
Treatment
Control G
Control W

TABLE XXIII

BIOTRANSFORMATION OF PENTACHLOROPHENOL (PCP) BY PSEUDOMONAS SP. IN GLUCOSE ENRICHED RED RIVER WATER. RESULTS OF THE ANALYSIS OF COVARIANCE (ANCOV) BETWEEN TREATMENT AND CONTROLS. ORGANISM ISOLATED FROM RED RIVER WATER

Days	Replicate	Concentration of PCP (μgL^{-1})			ANCOV Analysis
		Actual Treatment*	Actual Control**	Actual Control***	
0	1	1486.75	1500.00	1502.20	
	2	1490.90	1488.85	1500.00	
	3	1498.60	1482.85	1499.70	NS
	4	1489.60	1488.50	1500.60	
	5	1500.00	1502.30	1498.30	
7	1	1197.60	1397.40	1490.90	
	2	1264.00	1368.40	1475.00	
	3	1189.10	1391.70	1482.00	
	4	1223.50	1314.50	1492.00	
	5	1250.00	1322.10	1480.00	
14	1	560.00	566.70	633.20	
	2	567.80	561.00	629.00	
	3	584.00	682.30	602.00	
	4	575.00	587.60	670.00	
	5	588.00	645.00	698.00	
21	1	491.00	404.30	583.75	
	2	460.00	433.80	572.75	
	3	484.00	422.85	594.83	
	4	450.00	450.00	538.80	
	5	485.00	485.00	540.40	

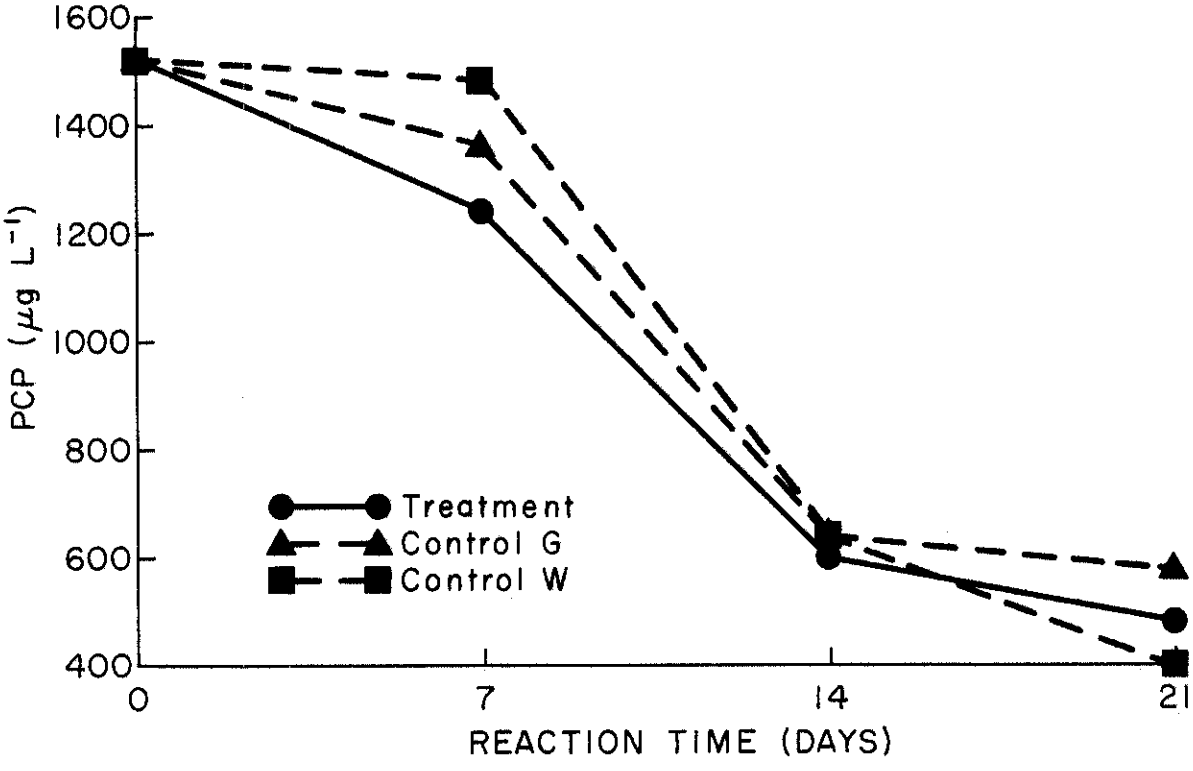
*Red River water + Anthracene + Glucose + Pseudomonas sp.

**Red River water + Anthracene + Glucose

***Red River water + Anthracene

NS + Not Significant (ANCOV Analysis), $p \geq 0.05$.

Fig. 30--Biotransformation of pentachlorophenol (PCP) by Pseudomonas sp. in glucose enriched Red River water (treatment). Controls = water, PCP, and glucose without isolate (G); water and PCP without isolate and no glucose (W).


1600
1400
1200
1000
800
600
400
PCP (μg L⁻¹)
0
7
14
21
REACTION TIME (DAYS)
Treatment
Control G
Control W

Biotransformation of Anthracene by Acinetobacter sp. and Flavobacterium sp. in Glucose Enriched Mississippi River Water.--The statistical test performed on data from the Mississippi River water plus anthracene with and without glucose inoculated with Acinetobacter sp. and Flavobacterium sp. were not significant at the $p=0.05$ level (Tables XXIV and XXV, Figure 31). However, as shown in Figure 31, disappearance of anthracene in control flasks was greater than in treatment flasks. This suggests that the biotransformation of anthracene in the Mississippi River water is not related to microbial action. It does not appear that the addition of glucose affected biotransformation of anthracene in the Mississippi River water inoculated with Flavobacterium sp. under these experimental conditions.

Biotransformation of Pentachlorophenol by Pseudomonas sp. and Corynebacterium sp. in Glucose Enriched Mississippi River Water.--Results of the biotransformation studies on the disappearance of PCP in the Mississippi River water inoculated with Pseudomonas sp. are in Table XXVI. Analysis of Covariance showed that the biotransformation of PCP in the treatment flasks was not significantly different ($p \geq 0.05$) from the control flasks. This indicated that this Pseudomonas sp. even in glucose enriched media was not able to biotransform PCP under these experimental studies. However, additional research with a focus on the different

TABLE XXIV

BIOTRANSFORMATION OF ANTHRACENE BY ACINETOBACTER SP. IN GLUCOSE ENRICHED MISSISSIPPI RIVER WATER. RESULTS OF THE ANALYSIS OF COVARIANCE (ANCOV) BETWEEN TREATMENT AND CONTROLS. ORGANISM ISOLATED FROM MISSISSIPPI RIVER WATER

Days	Replicate	Concentration of PCP (μgL^{-1})			ANCOV Analysis
		Actual Treatment*	Actual Control**	Actual Control***	
0	1	67.00	68.30	66.0	
	2	67.00	74.40	73.0	
	3	64.00	71.02	67.0	NS
	4	68.00	70.70	66.0	
	5	68.00	72.00	66.0	
7	1	63.00	37.00	39.00	
	2	65.00	38.00	39.00	
	3	66.00	38.00	41.50	
	4	65.00	24.00	39.00	
	5	61.00	37.00	39.00	
14	1	58.50	29.00	27.00	
	2	67.00	29.00	24.00	
	3	66.00	27.00	24.40	
	4	58.00	27.00	27.00	
	5	66.00	29.00	27.00	
21	1	47.60	24.50	24.40	
	2	57.00	24.50	20.00	
	3	58.50	25.60	20.00	
	4	58.53	25.60	24.40	
	5	48.78	25.60	22.00	

*Mississippi River water + Anthracene + Glucose + Acinetobacter Sp.

**Mississippi River + Anthracene + Glucose

***Mississippi River + Anthracene

NS = Not Significant (ANCOV Analysis), $p \geq 0.05$

TABLE XXV

BIOTRANSFORMATION OF ANTHRACENE BY FLAVOBACTERIUM SP. IN GLUCOSE ENRICHED MISSISSIPPI RIVER WATER. RESULTS OF THE ANALYSIS OF COVARIANCE (ANCOV) BETWEEN TREATMENT AND CONTROLS. ORGANISM ISOLATED FROM MISSISSIPPI RIVER WATER

Days	Replicate	Concentration of Anthracene (μgL^{-1}) Actual Treatment*	Actual Control**	Actual Control***	ANCOV Analysis
0	1	68.30	74.40	66.00	
	2	67.10	74.40	73.00	
	3	72.00	71.92	67.00	NS
	4	67.00	70.70	66.00	
	5	68.10	72.00	66.00	
7	1	39.10	37.00	39.00	
	2	41.50	38.00	39.00	
	3	39.00	38.00	41.50	
	4	39.00	24.00	39.00	
	5	40.00	37.00	39.00	
14	1	26.83	29.00	27.00	
	2	29.00	29.00	24.00	
	3	34.00	27.00	24.40	
	4	39.00	27.00	27.00	
	5	37.00	29.00	27.00	
21	1	29.00	24.50	24.40	
	2	29.00	24.50	20.00	
	3	33.00	25.60	20.00	
	4	33.00	25.60	24.40	
	5	29.00	25.60	22.00	

*Mississippi River water + Anthracene + Glucose + Flavobacterium sp.

**Mississippi River water + Anthracene + Glucose
***Mississippi River water + Anthracene
NS = Not significant (ANCOV Analysis), $p \geq 0.05$.

Fig. 31--Biotransformation of anthracene by Flavobacterium sp. in glucose enriched Mississippi River water (treatment). Controls = water, anthracene, and glucose without isolate (G); water and PCP without isolate and no glucose (W).

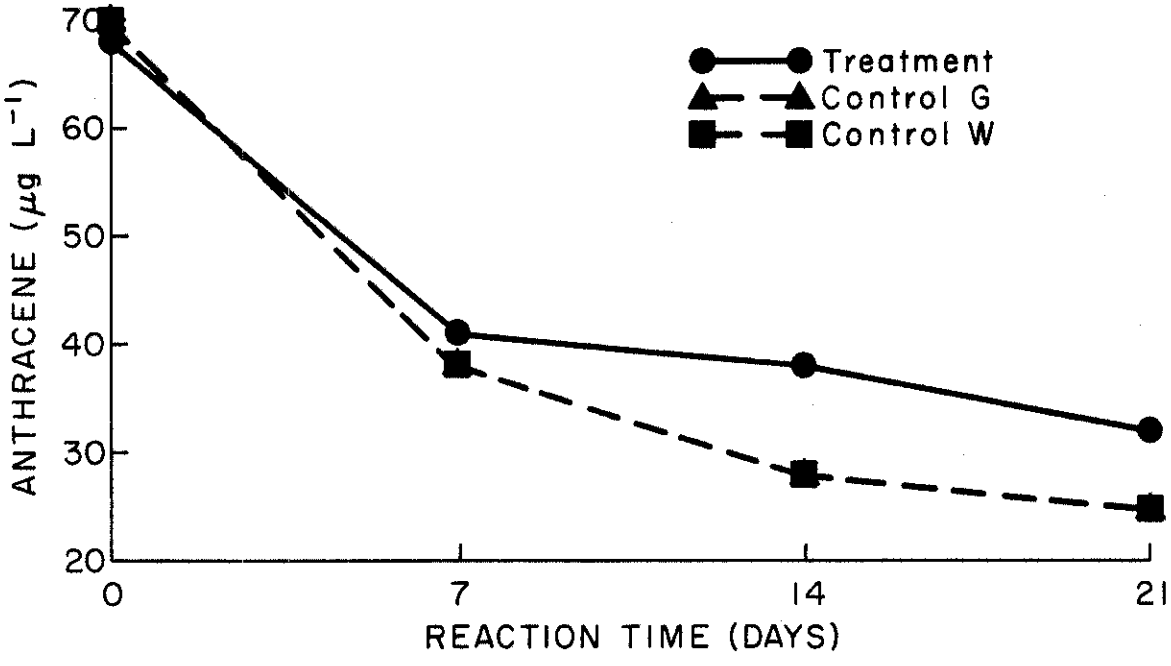

Treatment
Control G
Control W
ANTHRACENE (μg L⁻¹)
70
60
50
40
30
20
0
7
14
21
REACTION TIME (DAYS)

TABLE XXVI

BIOTRANSFORMATION OF PENTACHLOROPHENOL (PCP) BY PSEUDOMONAS SP. IN GLUCOSE ENRICHED MISSISSIPPI RIVER WATER. RESULTS OF THE ANALYSIS OF COVARIANCE (ANCOV) BETWEEN TREATMENT AND CONTROLS. ORGANISM ISOLATED FROM MISSISSIPPI RIVER WATER

Days	Replicate	Concentration of PCP (μgL^{-1}) Actual Treatment*	Actual Control**	Actual Control***	ANCOV Analysis
0	1	1498.00	1500.0	1500.00	
	2	1498.00	1498.0	1508.00	
	3	1500.00	1485.0	1499.00	NS
	4	1501.00	1490.0	1502.00	
	5	1498.00	1498.0	1498.00	
7	1	1060.00	1060.6	1060.60	
	2	1065.00	1085.8	1085.80	
	3	1045.30	1045.4	1045.30	
	4	1038.00	1000.0	1000.00	
	5	1054.00	1092.0	1042.00	
14	1	901.201	602.0	668.60	
	2	924.50	667.7	645.80	
	3	951.00	638.5	665.80	
	4	935.00	638.0	662.75	
	5	959	607.7	663.15	
21	1	584.73	588.8	581.00	
	2	532.90	575.5	550.00	
	3	529.75	570.5	595.00	
	4	559.90	577.0	544.00	
	5	570.90	580.0	545.20	

*Mississippi River water + Anthracene + Glucose + Pseudomonas sp.

**Mississippi River water + Anthracene + Glucose
***Mississippi River water + Anthracene
NS = Not significant (ANCOV Analysis), $p \geq 0.05$.

levels of glucose and concentrations of PCP are needed to establish definitely whether or not Pseudomonas sp. can biotransform PCP. Statistical ANCOV Analysis conducted on data from the biotransformation experiment with Corynebacterium sp. using Mississippi River water and addition of glucose and PCP show that there was a significant difference between slopes of treatment and controls (Table XXVII, Figure 32). This difference indicates that Corynebacterium sp. can biotransform PCP in a nutrient rich medium. Based on the chemical data, the greatest disappearance of PCP in treatment media was 56% during the first 7 days of incubation, 11% between 7-14 days, and 21% between 14-21 days. The disappearance from control was 27% for 0-7 days, 40% between 7-14 days, and 8.3% between 14-21 days.

Biotransformation of Anthracene by Corynebacterium sp. and Pseudomonas sp. in Glucose Enriched Trinity River Water.--Disappearance of anthracene concentration in the Trinity River water with Corynebacterium sp. show parallel results for treatment and controls. No significant difference ($p \geq 0.05$) between slopes was shown by ANCOV analysis (Table XXVIII). Significant differences ($p \leq 0.05$) by ANCOV analysis was not found for the Trinity River water and anthracene inoculated with Pseudomonas sp. (Figure 33). However, chemical data in Table XXVIII show that as much as 40% of the initial added anthracene disappeared after 14

TABLE XXVII

BIOTRANSFORMATION OF PENTACHLOROPHENOL (PCP) BY CORYNEBACTERIUM SP. IN GLUCOSE ENRICHED MISSISSIPPI RIVER WATER. RESULTS OF THE ANALYSIS OF COVARIANCE (ANCOV) BETWEEN TREATMENT AND CONTROLS. ORGANISM ISOLATED FROM MISSISSIPPI RIVER WATER

Days	Replicate	Concentration of PCP (μgL^{-1}) Actual Treatment*	Actual Control**	Actual Control***	ANCOV Analysis
0	1	1400.00	1500.00	1500.00	
	2	1489.00	1498.00	1508.00	
	3	1495.00	1498.00	1499.00	
	4	1495.00	1485.00	1502.00	
	5	1488.00	1490.00	1498.00	
7	1	636.21	1060.60	1060.60	
	2	643.46	1085.80	1085.80	
	3	640.00	1045.30	1045.30	
	4	653.00	1000.00	1000.00	
	5	648.00	1092.00	1092.00	
14	1	480.75	602.0	668.60	
	2	471.00	667.70	645.80	
	3	487.00	638.50	665.80	
	4	475.00	638.00	662.75	
	5	476.00	607.70	663.15	
21	1	340.00	588.80	581.00	
	2	326.00	575.50	550.00	
	3	324.00	570.50	595.00	
	4	339.60	577.00	544.20	
	5	340.50	580.00	545.20	

*Mississippi River water + Anthracene + Glucose + Corynebacterium sp.
**Mississippi River water + Anthracene + Glucose
***Mississippi River water + Anthracene
S = Significant (ANCOV Analysis), $p \geq 0.05$.

Fig. 32--Biotransformation of pentachlorophenol (PCP) by Corynebacterium sp. in glucose enriched Mississippi River water (treatment). Controls = water, PCP, and glucose without isolate (G); water, PCP without isolate and no glucose (W).

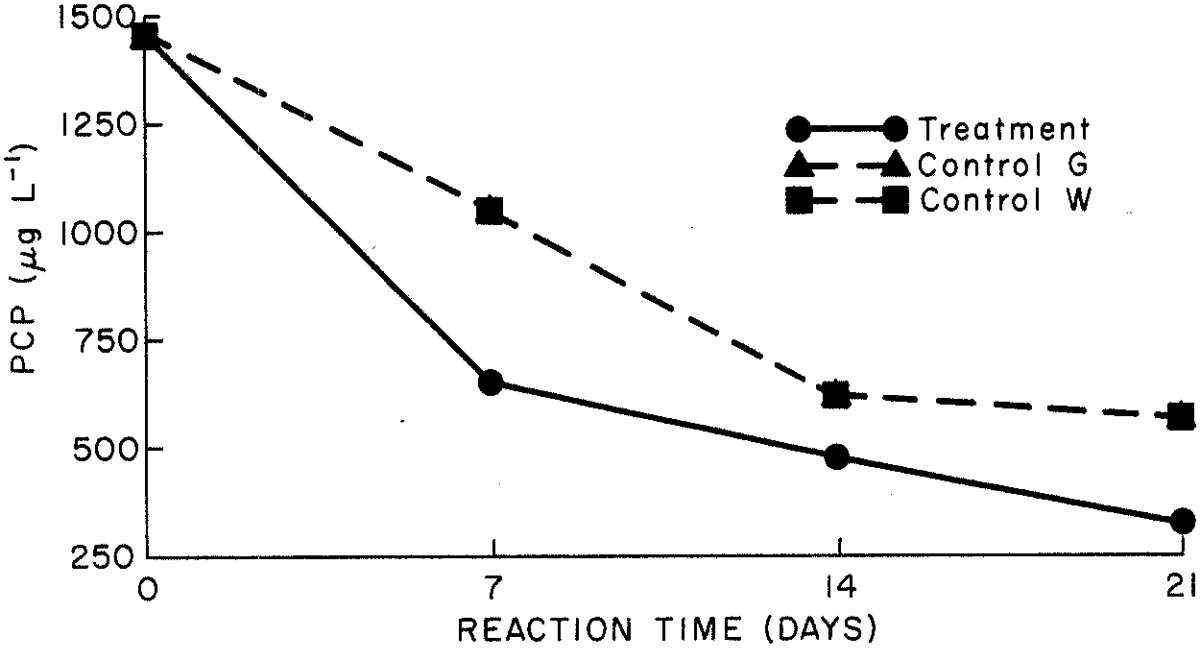

1500
1250
1000
750
500
250
PCP (μg L⁻¹)
0
7
14
21
REACTION TIME (DAYS)
Treatment
Control G
Control W

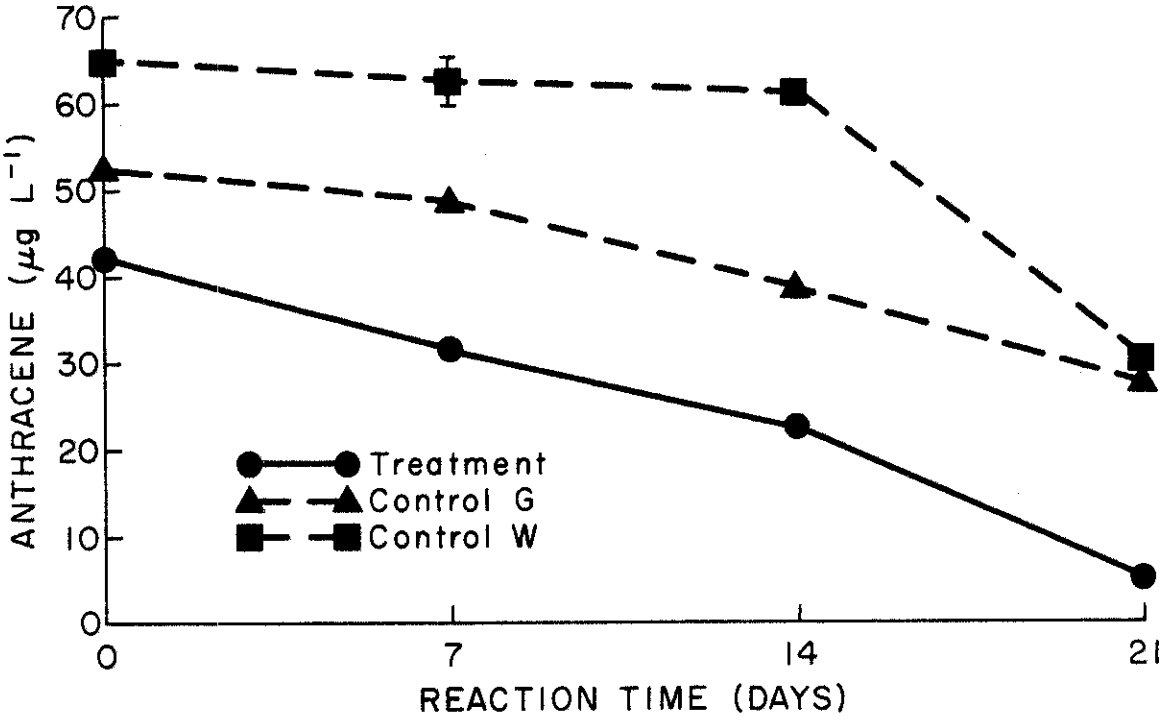
TABLE XXVIII

BIOTRANSFORMATION OF ANTHRACENE BY PSEUDOMONAS SP. AND CORYNEBACTERIUM SP. IN GLUCOSE ENRICHED TRINITY RIVER WATER. RESULTS OF THE ANALYSIS OF COVARIANCE (ANCOV) BETWEEN TREATMENT AND CONTROLS. ORGANISM ISOLATED FROM TRINITY RIVER WATER

Isolate	Days	Replicate	Concentration of Anthracene (μgL^{-1}) Actual Treatment*	Actual Control**	Actual Control***	ANCOV Analysis
Pseudomonas sp.	0	1	41.60	56.00	64.00	NS
		2	41.60	51.00	66.00	
		3	39.00	55.00	69.00	
	7	1	32.20	50.74	61.00	
		2	29.00	49.50	63.00	
		3	31.00	48.21	64.63	
	14	1	23.94	39.00	62.00	
		2	23.15	38.00	61.00	
		3	25.00	39.00	61.00	
	21	1	4.90	26.40	28.39	
		2	5.50	26.40	29.00	
		3	4.60	27.70	27.00	
Coryne-bacterium sp.	0	1	42.90	56.00	64.00	NS
		2	42.90	51.00	66.00	
		3	40.30	55.00	69.00	
	7	1	41.50	50.74	61.00	
		2	40.70	49.50	63.00	
		3	40.00	48.21	64.63	
	14	1	40.20	39.00	62.00	
		2	39.47	38.00	61.00	
		3	39.80	39.00	61.00	
	21	1	30.00	26.40	28.39	
		2	29.30	26.40	29.00	
		3	32.00	27.70	27.00	

*Trinity River water + Anthracene + Glucose + Isolate
**Trinity River water + Anthracene + Glucose
***Trinity River water + Anthracene.
NS = Not signifiant (ANCOV) Analysis) $p \geq 0.05$.

Fig. 33--Biotransformation of anthracene by Pseudomonas sp. in glucose enriched Trinity River water (treatment). Controls = water, PCP, and glucose without isolate (G); water and PCP without isolate and no glucose (W).


70
60
50
40
30
20
10
0
ANTHRACENE (μg L⁻¹)
Treatment
Control G
Control W
0
7
14
21
REACTION TIME (DAYS)

days of incubation, and 87% after 21 days. The disappearance of anthracene during these time periods for controls were 30% and 50%, respectively (Table XXVIII, Figure 33). This result suggests that disappearance of anthracene in the treatment between 14-21 days was greater than control, which was similar to results of my study with Red River water and anthracene inoculated with Pseudomonas sp. (Table XXI, Figure 28). However, this magnitude difference was not statistically significant ($p \geq 0.05$).

Biotransformation of Pentachlorophenol by Pseudomonas sp. and Micrococcus sp. in Glucose Enriched Trinity River Water.--The data from the biotransformation studies of PCP in the Trinity River water systems inoculated with Pseudomonas sp. are presented in Table XXIX. Results of Analysis of Covariance in Table XXIX shows no significant difference between slopes of controls and treatment ($p \geq 0.05$). This result which is consistent with the results from the other two waters confirms that Pseudomonas sp. is unable to biotransform PCP in all three water systems. These results also indicate that the source of river water and additional 1 g/L glucose did not affect biotransformation of PCP by Pseudomonas sp. under these specific laboratory conditions.

Table XXX and Figure 34 show the results of biotransformation of PCP in glucose enriched Trinity River water by

TABLE XXIX

BIOTRANSFORMATION OF PENTACHLOROPHENOL (PCP) BY PSEUDOMONAS SP. IN GLUCOSE ENRICHED TRINITY RIVER WATER. RESULTS OF ANALYSIS OF COVARIANCE (ANCOV) BETWEEN TREATMENT AND CONTROLS. ORGANISM ISOLATED FROM TRINITY RIVER WATER

Days	Replicate	Concentration of PCP (μgL^{-1}) Actual Treatment*	Actual Control**	Actual Control***	ANCOV Analysis
0	1	1500.00	1500.00	1500.00	
	2	1510.00	1510.00	1505.00	
	3	1545.00	1505.00	1508.00	NS
	4	1548.00	1495.00	1510.00	
	5	1500.00	1488.00	1495.00	
7	1	1351.00	1360.00	1411.00	
	2	1348.00	1370.00	1413.00	
	3	1352.00	1365.00	1415.00	
	4	1349.00	1358.00	1410.00	
	5	1353.00	1360.00	1412.00	
14	1	1100.00	1267.00	1360.00	
	2	1115.00	1280.00	1364.00	
	3	1115.00	1260.00	1368.00	
	4	1120.00	1275.00	1358.00	
	5	1126.00	1258.00	1362.00	
21	1	850.00	900.00	1070.00	
	2	885.00	895.00	1072.00	
	3	850.00	880.00	1064.00	
	4	848.00	887.00	1090.00	
	5	860.00	889.00	1078.00	

*Trinity River water + Anthracene + Glucose + Pseudomonas sp.
**Trinity River water + Anthracene + Glucose
***Trinity River water + Anthracene
NS = Not significant (ANCOV Analysis), $p \geq 0.05$.

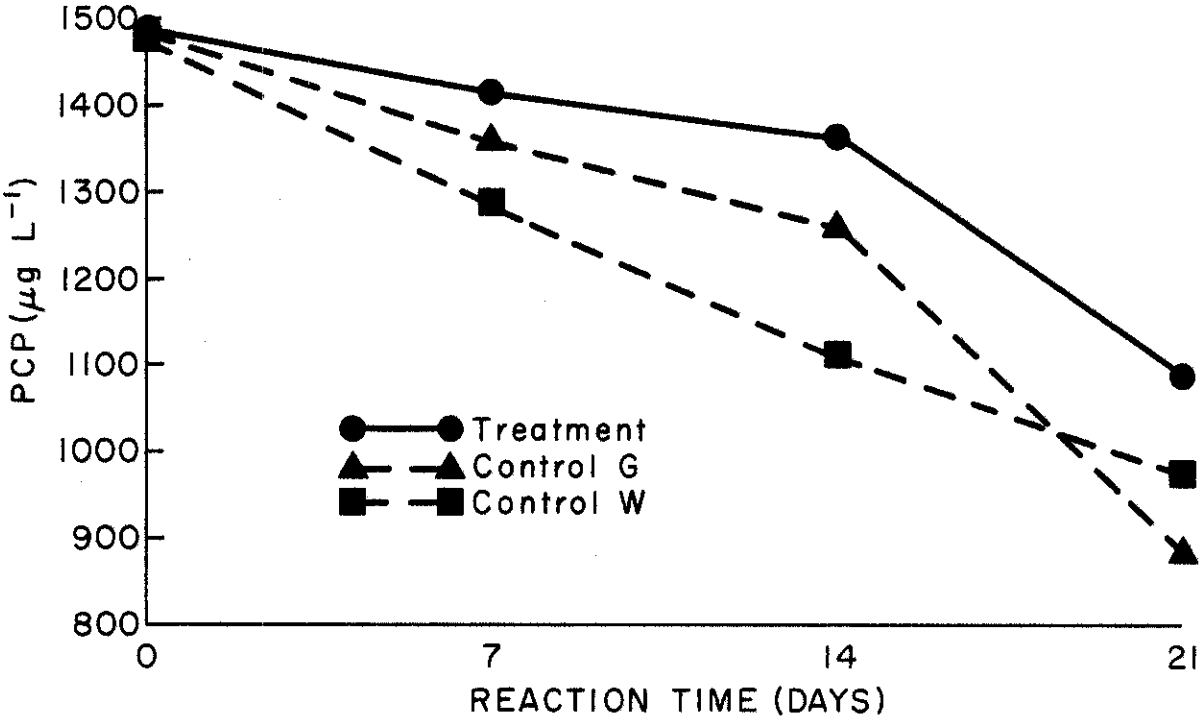
TABLE XXX

BIOTRANSFORMATION OF PENTACHLOROPHENOL (PCP) BY MICROCOCCUS SP. IN GLUCOSE ENRICHED TRINITY RIVER WATER. RESULTS OF ANALYSIS OF COVARIANCE (ANCOV) BETWEEN TREATMENT AND CONTROLS. ORGANISM ISOLATED FROM TRINITY RIVER WATER

Days	Replicate	Concentration of PCP (μgL^{-1}) Actual Treatment*	Actual Control**	Actual Control***	ANCOV Analysis
0	1	1509.00	1500.00	1500.00	
	2	1510.00	1510.00	1505.00	
	3	1489.00	1505.00	1508.00	NS
	4	1488.00	1495.00	1510.00	
	5	1485.00	1488.00	1495.00	
7	1	1280.00	1360.00	1411.00	
	2	1292.00	1370.00	1413.00	
	3	1286.00	1365.00	1415.00	
	4	1283.00	1358.00	1410.00	
	5	1280.00	1360.00	1412.00	
14	1	1100.00	1267.00	1360.00	
	2	1113.00	1280.00	1364.00	
	3	1118.00	1260.00	1368.00	
	4	1115.00	1275.00	1358.00	
	5	1113.00	1258.00	1362.00	
21	1	981.00	900.00	1070.00	
	2	960.00	895.00	1072.00	
	3	967.00	880.00	1064.00	
	4	999.00	887.00	1090.00	
	5	985.00	889.00	1078.00	

*Trinity River water + PCP + Glucose + Micrococcus sp.
**Trinity River water + PCP + Glucose
***Trinity River water + PCP
NS = Not significant (ANCOV Analysis), $p \geq 0.05$.

Fig. 34--Biotransformation of pentachlorophenol (PCP) by Micrococcus sp. in glucose enriched Trinity River water (treatment). Controls = water, PCP, and glucose without isolate (G); water and PCP without isolate and no glucose (W).


1500
1400
1300
1200
1100
1000
900
800
PCP (μg L⁻¹)
0
7
14
21
REACTION TIME (DAYS)
Treatment
Control G
Control W

Micrococcus sp. Statistical Analysis of Covariance showed biotransformation of PCP in the treatment is significantly different from the biotransformation of the chemical in the controls. However, as can be seen in Figure 34, this difference is not contributed to the Micrococcus sp. action. The reason for the disappearance PCP in the sterile controls is not fully understood. The amount of PCP lost in the controls is shown for visual comparisons to the amount lost by biotransformation. This result was similar to the result obtained from biotransformation of anthracene by Flavobacterium sp. in glucose enriched Mississippi River water. The results of the statistical analysis of all studies are summarized in Tables XXXI through XXXIII.

Summary of Results of the Biotransformation Tests with the Addition of Glucose

The results from these experiments demonstrate:

1. Pseudomonas sp., one of the most dominant bacterium isolated from anthracene rate kinetic experiment using Red River water and Trinity River water, was shown to have the ability to biotransform anthracene in each of these two water systems by addition of glucose. The other four dominant bacteria which were obtained from anthracene rate kinetic experiments and employed for this study failed to biotransform anthracene under these experimental conditions.

TABLE XXXI

SUMMARY OF STATISTICAL ANALYSIS OF BIOTRANSFORMATION RESULTS OF ANTHRACENE OR PENTACHLOROPHENOL BY ORGANISMS ISOLATED FROM RED RED RIVER WATER

			Student t-test				ANCOV Analysis	
			CFUs mL^{-1}		ATP (nM)		CFUs mL^{-1}	Concentration of Compound Remaining
Bacteria	Chemical	Time	Red River Water	BM	Red River Water	BM	BM (0-7 Days)	Red River Water (0-21 Days)
Pseudomonas sp.	Anthracene	0	NS	NS	NS	NS	S	S
		7	S	S	NO	NO		
		14	S	S	NO	NO		
		21	NS	NS	NS	NS		
Alcaligenes sp.	Anthracene	0	NS	NS	NS	NS	-*	NS
		7	NS	NS	NO	NO		
		14	NS	NS	NO	NO		
		21	NS	NS	NS	NS		
Pseudomonas sp.	PCP	0	NS	NS	NS	NS	NS	NS
		7	S	S	S	S		
		14	NS	NS	NO	NO		
		21	NS	NS	NS	NS		
Bacillus sp.	PCP	0	NS	NS	NS	NS	-*	S
		7	NS	NS	NO	NO		
		14	NS	NS	NS	NS		
		21	NS	NS	NS	NS		

*Test not performed due to the result of the Student t-test.
NS = Not significant (Student t-test, ANCOV Analysis), $p \geq 0.05$.
S = Significant, $p < 0.05$.
NO = ATP was not measured at this time.

TABLE XXXII

SUMMARY OF STATISTICAL ANALYSIS OF BIOTRANSFORMATION RESULTS OF ANTHRACENE OR PENTACHLOROPHENOL BY ORGANISMS ISOLATED FROM MISSISSIPPI RIVER WATER

			Student t-test				ANCOV Analysis	
			CFUs mL^{-1}		ATP (nM)		CFUs mL^{-1}	Concentration of Compound Remaining
Bacteria	Chemical	Time	Mississippi River Water	BM	Mississippi River Water	BM	BM (0-7 Days)	Mississippi River Water (0-21 Days)
Acinetobacter sp.	Anthracene	0	NS	NS	NS	NS	-*	NS
		7	NS	NS	NS	NS		
		14	NS	NS	NO	NO		
		21	NS	NS	NS	NS		
Flavobacterium sp.	Anthracene	0	NS	NS	NS	NS	S	NS
		7	S	S	S	S		
		14	NS	NS	NO	NO		
		21	NS	NS	NS	NS		
Pseudomonas sp.	PCP	0	NS	NS	NS	NS	-*	NS
		7	NS	NS	NS	NS		
		14	NS	NS	NO	NO		
		21	NS	NS	NS	NS		
Corynebacterium sp.	PCP	0	NS	NS	NS	NS	-*	S
		7	NS	NS	NS	NS		
		14	NS	NS	NO	NO		
		21	NS	NS	NS	NS		

*Test not performed due to the result of the Student t-test.
NS = Not significant (Student t-test, ANCOV Analysis), $p \geq 0.05$.
S = Significant, $p < 0.05$.
NO = ATP was not measured at this time.

TABLE XXXIII

SUMMARY OF STATISTICAL ANALYSIS OF BIOTRANSFORMATION RESULTS OF ANTHRACENE OR PENTACHLOROPHENOL BY ORGANISMS ISOLATED FROM TRINITY RIVER WATER

			Student t-test				ANCOV Analysis	
			CFUs mL^{-1}		ATP (nM)		CFUs mL^{-1}	Concentration of Compound Remaining
Bacteria	Chemical	Time	Trinity River Water	BM	Trinity River Water	BM	BM (0-7 Days)	Trinity River Water (0-21 Days)
Pseudomonas sp.	Anthracene	0	NS	NS	NS	NS	S	NS
		7	NS	S	S	S		
		14	S	S	NO	NO		
		21	S	S	S	S		
Coryne-bacterium sp.	Anthracene	0	NS	NS	NS	NS	S	NS
		7	NS	NS	NS	NS		
		14	S	S	NO	NO		
		21	S	S	S	S		
Pseudomonas sp.	PCP	0	NS	NS	NS	NS	-*	NS
		7	NS	NS	NS	NS		
		14	NS	NS	NO	NO		
		21	NS	NS	NS	NS		
Micrococcus sp.	PCP	0	NS	NS	NS	NS	S	S
		7	S	S	S	S		
		14	S	S	NO	NO		
		21	S	NS	S	NS		

*Test not performed due to the result of the Student t-test.
NS = Not significant (Student t-test, ANCOV Analysis), $p \geq 0.05$.
S = Significant, $p < 0.05$.
NO = ATP was not measured at this time.

2. Unlike Watanabe's (1973) and Karns et al. (1983) findings, no Pseudomonas isolates in this study were capable of biotransforming PCP under these conditions. Similar results have been reported by Gary and Finn (1982). Four out of six isolates tested were unable to biotransform PCP in the three sterile river waters enriched with glucose including Pseudomonas sp. and Micrococcus sp., even though these isolates were capable of utilizing PCP in the river waters without glucose enrichment. This phenomenon, bacteria that are able to biotransform anthracene or PCP in river water but not under nutrient rich conditions, support the idea that growth conditions are not necessarily favorable to biotransformation.
3. The disappearance of PCP from the Mississippi River water in the presence of Corynebacterium sp. indicates that this bacterium is capable of attacking PCP under nutrient rich conditions. A similar result had been reported by Chu and Kirsch (1972) and Gary and Finn (1982). This biological attack which results in disappearance of the halogenated phenol is indicated by the significant difference of percent lost chemical in the first 7 days of incubation. These data tend to be conservative estimates of the potential ability of Corynebacter-

ium sp. to transform PCP via co-metabolism.

4. The greatest disappearance of PCP from the Red River water inoculated with Bacillus sp. was shown between 0-7 days. This result suggests that under these conditions PCP may undergo biotransformation by Bacillus sp. via co-metabolism.
5. Bacillus sp. and Corynebacterium sp., were found to be the only dominant bacteria isolated from the PCP rate kinetic experiments to have the ability of biotransforming PCP under these nutrient rich conditions.

These results were obtained with a high initial population of bacteria that was incubated long enough for growth to have occurred at the expense of PCP or anthracene. These findings are thus consistent with the view that PCP is biotransformed by Bacillus sp. or Corynebacterium sp., may be co-metabolized in glucose enriched water systems and not by growth-linked processes. These two bacteria did not grow in pure culture when PCP was present as the only carbon source (in long-term incubation). This result also agrees with results of Moose et al. (1983). Moose reported that addition of dog food in media containing PCP increased the maximum specific PCP removal rate by an order of magnitude. Karns et al. (1983) also reported that resting cell suspensions of succinate-grown P. cepacia AC1100 are capable of rapidly and completely dechlorinating PCP even though this

strain is unable to grow with PCP as a sole source of carbon and energy.

Ide et al. (1972) and Kuwatsuka and Igarashi (1975) found that the degradation products of PCP in soil consist of dechlorinated compounds. Moreover, Suzuki and Nose (1971a and b) found that a species of a bacterium considered to be Pseudomonas sp. or a closely related bacterium isolated from soil transformed PCP into its methyl ether and into tetrachlorohydroquinone dimethyl ether in the culture medium. Also the methylation of PCP was reported with six species of Trichoderma (Cserjesi, 1972; Cserjesi and Johnson, 1972) and with a Bacillus sp. species from soil (Suzuki and Nose, 1971a and b).

Horvath and Alexander (1970) reported that co-metabolism is apparently common in the microbial transformation of halogenated aromatic molecules (i.e., 2,3,6-trichlorobenzoate by resting cells of benzoate-grown Brevibacterium, m-chloro benzoate by resting cell suspensions of benzoate-grown Arthrobacter sp., and 3,5-dichlorocatechol by resting cells of a benzoate-grown culture of Achromobacter sp.) and it should thus be possible to obtain the accumulation of a variety of halometabolities. It has also been observed that bacteria able to grow on certain halogenated compounds metabolize the corresponding unsubstituted molecules; in these instances, unsubstituted products corresponding to the halogenated intermediates may accumulate (Loos et al., 1967).

CHAPTER IV

CONCLUSION

The objectives of this research were to address some questions basic to biotransformation tests. Included in these stated objectives were:

1. To isolate and identify the five most dominant bacteria (to the genus level) present in tests assessing the influence of three different aquatic water/sediment systems on biotransformation rates of anthracene and pentachlorophenol (PCP).
2. To determine the ability of the two most dominant bacteria isolated from each aquatic system to grow in basal medium with anthracene and PCP as a sole source of carbon and energy, and in experimental water with the chemicals as an additional carbon source.
3. To determine the ability of the two most dominant bacteria isolated from each aquatic system to biotransform anthracene or PCP in the experimental waters via co-metabolism.

The stated objectives of this research were accomplished and all hypotheses were evaluated with some degree of success during this study. The conclusions which can be

drawn from the results of this research are summarized as follows:

1. The dominant microorganisms isolated from kinetic biotransformation rate studies of PCP and anthracene were all found to be typical aquatic species.
2. The most commonly isolated organisms which could be potentially useful in the biological treatment for industrial wastewater containing anthracene are represented by the genera of Pseudomonas, Corynebacterium and Flavobacterium sp. These were found to utilize anthracene as their carbon source. Likewise, Micrococcus sp. demonstrated the capability to utilize PCP in Trinity River water and BM. The two other bacteria including representatives of the genera of Bacillus from the Red River water and Corynebacterium from the Mississippi River water also demonstrated the capacity to biotransform PCP. It is not clear why certain organisms are able to biotransform anthracene or PCP under some conditions and not others. Possibilities include a co-substrate may be present in some experimental waters and missing in others or toxic products may be formed and prohibit utilization of the target chemical under certain circumstances. Also loss of chemical for unexplained reasons, from sterile controls, may

prevent the identification of organisms capable of biotransforming anthracene or PCP.

3. For all organisms except one out of six which are able to biotransform anthracene or PCP, the size of the initial inoculum had no discernable effect on these results. However, the lag time observed with PCP and anthracene was usually related to the biomass concentration at low cellular levels, which is similar to other experimental results (Andrews, 1968), but conflict with other growth models which predict that the lag time of PCP can be extended by increasing the size of the initial inoculum (Pamment and Hall, 1978). These results also seem to add to the controversy that currently exists in the literature as to whether chemicals biotransform by first or second order kinetic rate models. Paris et al. (1981) and others contend that the rate of a chemicals biotransformation is dependent upon the microbial biomass, i.e., second order, whereas Leslie et al. (1984) and other researchers show a first order kinetic relationship. However, the data from my research indicate that initial size of inoculation for five of the six organisms which are capable of biotransforming anthracene or PCP did not have an effect on biotransformation but it could have an effect on the

rate of biotransformation which was not the focus of this research. It is obvious more research is needed in this area.

4. Data were shown in which Pseudomonas sp. exposed only to the anthracene, degradation of the chemical seems to be more rapid (CFU mL^{-1} and ATP concentration at time intervals) than when supplied with organic nutrient such as glucose plus anthracene (lost chemical at time intervals). This suggests that anthracene is probably not a primary substrate for Pseudomonas sp. that do not compete favorably with more easily degradeable materials. In fact, based on the results obtained from this research it would not be at all surprising to show that PCP or anthracene become a secondary substrate to attack by actively growing cells which use some other compound as a primary energy source and nutrient.
5. The addition of glucose to the experimental systems was designed to indicate whether isolated bacteria can co-metabolize anthracene or PCP. The idea of co-metabolism suggests that in some experiments the target chemical is biotransformed though not a substrate, while the glucose (or other nutrient) is the primary substrate. The addition of 1 g/L of glucose in the Red River

water did not significantly affect on biotransformation of anthracene under these experimental conditions.

6. The addition of 1 g/L glucose in experimental waters significantly stimulated the biotransformation of PCP in the Red River water by Bacillus sp. and in the Mississippi River water by Corynebacterium sp. Also in the Trinity River water biotransformation of anthracene by Corynebacterium sp. and PCP by Pseudomonas sp. and Micrococcus sp. were significantly altered after addition of glucose.
7. Based on the results, it is obvious that all microorganisms do not have equal ability to biotransform all chemicals equally. This is shown by the fact that only a very limited number of isolated bacteria have been found to possess the capacity to biotransform either PCP or anthracene, and these bacteria have been studied only aerobically. However, The source of water and the type of microbe both are important factors influencing the biotransformation of anthracene or PCP.
8. Changes in pH have been observed from cultures in which bacteria were able to biotransform PCP (from 7.4 to 6.7). These changes in pH may affect PCP toxicity as has been shown for fish (Crandall and

Goodnight, 1959) and fungi (Cserjesi, 1972). Therefore, this result indicates that the pH should be maintained at the highest level possible commensurate with adequate bacterial growth for laboratory studies.

9. Anthracene and PCP are molecules (Alexander, 1965b; Vele-Muzquiz and Kasper, 1973; Gary and Finn, 1982) capable of being biotransformed by only a limited number of bacteria (Chu and Kirsch, 1972; Watanabe, 1973). PCP is related to those chemicals classified by Alexander (1973) as "chemicals that are suitable substrates for populations in axenic culture or in one or more microbial habitats but which are occasionally quite persistent." The results of my studies support the fact that PCP, a commercial biocide, is amenable to biological decomposition under specific laboratory conditions. However, the resistance of PCP and anthracene to microbial degradation would then be due to such factors as lack of microorganisms in the habitat capable of decomposing them; too high or too low a PCP or anthracene concentration, too low a pH, or other environmental conditions.

The overall goals of this research were met with varying degrees of success. Dominant organisms were isolated from each of the river systems studied and were all found

to be common aquatic microorganisms. It was found that several of these organisms can biotransform anthracene or PCP under these experimental conditions. This capability to use these chemicals was determined by different measurement procedures. One method by which utilization was measured by following loss of parent compound and comparing the loss between treatment and control systems. Another method used was to follow microbial growth as measured as CFU mL^{-1} and ATP concentration through time. The growth was then compared between treatment (organism + medium + chemical) and control (organism + medium) systems. Significant growth in test systems as compared to control systems ($p = 0.05$) was considered evidence of the ability of the organism to utilize the chemical under study.

The addition of glucose to the experimental systems was designed to gain some insight as to whether isolated bacteria can co-metabolize the target chemical (anthracene or PCP). The idea of co-metabolism suggests that the target chemical is biotransformed, though not a substrate, while the glucose (or other nutrient) is the primary substrate. The addition of glucose to the Red River water did not have any effect on the biotransformation of anthracene for any organisms tested. However, glucose addition did have effects in the biotransformation of PCP and anthracene in some experiments involving the Trinity and Mississippi River waters by certain microorganisms.

The initial microbial biomass in only one case had an effect on the biotransformation of the target chemicals. This is consistent with the views by many researchers. However, more research is needed in this area. It also became obvious during the course of this research that all microorganisms do not have equal ability to biotransform all chemicals equally. This is shown by the fact that only a very limited genera of microorganisms isolated were able to biotransform either anthracene or PCP.

The results of this research yielded biotransformation data that could be used to predict the fate of PCP or anthracene in a contaminated water. These findings may have useful implications for the biological treatment of wastewaters contaminated with these compounds thereby minimizing the environmental impact while maintaining the social benefits of these chemicals. However, incorporation of all these points from this study hopefully improve the efficiency of PCP and anthracene biotransformation in such treatment systems.

Some suggestions I would offer when conducting further research of this type are:

1. Measure the loss of parent compound in addition to microbial growth to determine the extent of utilization of the target chemical. In this case a simple comparison between treatments containing the chemical and controls without chemical were

deemed sufficient. However, this approach gives no insights to the metabolism, by-products, or rate of utilization of the compound. Let me here emphasize, however, these questions were not goals of my research.

2. Use a mass balance approach to determine the fate of the target chemical.
3. The use of one more control in the glucose experiments. The control should be organism + medium + chemical. In my case this control was considered useful but unnecessary for two reasons.
   a) The objective in this case was to measure biotransformation by growth (CFU and ATP) and loss of parent compound in glucose enriched river waters. The controls used were adequate for this objective.
   b) Economic and time constraints restricted the amount of additional research that actually could be conducted.

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