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Integrated anaerobic-aerobic system for the biodegradation of highly chlorinated aromatic compounds

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New Jersey Institute of Technology, 1991

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INTEGRATED ANAEROBIC-AEROBIC SYSTEM FOR THE BIODEGRADATION OF HIGHLY CHLORINATED AROMATIC COMPOUNDS

by
Cheng-Ming Kung

A Dissertation
Submitted to the Faculty of the Graduate Division of the
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In Partial Fulfillment of the Requirements of the Degree of
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and Environmental Science
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APPROVAL PAGE

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ABSTRACT

Integrated Anaerobic-Aerobic System for the
Biodegradation of Highly Chlorinated Aromatic Compounds

by
Cheng-Ming Kung

A two-step process was developed for the complete mineralization of hazardous chlorinated aromatic compounds. The system consisted of an anaerobic reactor, in which reductive dehalogenation took place, coupled with an aerobic reactor in which the complete mineralization of the products of the anaerobic dehalogenation occurred.

A mixed anaerobic culture from a municipal sewage treatment plant was enriched and then immobilized on silica-based porous beads to treat 2,4,6-trichlorophenol (TCP), which is the model target compound in this work. In the anaerobic reactor, TCP was degraded to 2,4-dichlorophenol which was, in turn, reduced to 4-chlorophenol (4-CP). Stoichiometric amounts of 4-CP were recovered at the end of the anaerobic step. No other unknown compound was produced to any significant extent during the anaerobic process. As the anaerobic culture got acclimated to the TCP, the dehalogenation rate of TCP increased from 21.7 to 43.2 μM/day.

The effluent from the anaerobic reactor was subsequently treated in a suspended growth aerobic reactor to remove the 4-CP. The anaerobic effluent had to be buffered with a phosphate solution to adjust its pH to about 7 and maintain aerobic activity.

When the system was run in batch mode, 106.4 μM of TCP could be dechlorinated to 4-CP in three days by an acclimated anaerobic culture. 98 μM of the 4-CP produced were then mineralized in the aerobic reactor in less than three days.

When the system was run in continuous mode, 120 μM of TCP were entirely and continuously dechlorinated to 4-CP in the anaerobic reactor, and then completely mineralized in the aerobic reactor. The average residence times (which were not optimized) in the anaerobic and aerobic reactors were respectively 105 and 98 hours, although there are indications that these were longer than necessary.

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Configurations of Two Interconnected Chemostats." Biotech. Bioeng. 30 1006-1018.

- C. M. Kung. 1991. "Anaerobic-Aerobic Biodegradation of Highly Chlorinated Compounds." 2nd Annual Student Mini-Technical Conference, NJIT, April 5.

- P. M. Armenante, G. Lewandowski, C. M. Kung, and D. Kafkewitz. 1990. "Anaerobic-Aerobic Treatment of Hazardous Wastewater." AICHE Annual Meeting, Chicago, November 1990.

- B. C. Baltzis, and C. M. Kung. 1990. "Steady State Coexistence of Two Pure And Simple Competitors in a Distributed Medium." Conference on Mathematical Aspects of Microbial Ecology, East Lansing, MI. May 21-23.

- C. M. Kung. 1990. "Anaerobic Degradation of Highly Chlorinated Compounds." 1st Annual Student Mini-Technical Conference, NJIT, April 20.

- G. Lewandowski, B. C. Baltzis, C. M. Kung and M. E. Frank. 1988. "An Approach to Biocatalyst Modeling of Mixed Populations Using Pure Culture Kinetic Data." Engineering Foundation Conference on BIOTECHNOLOGY APPLICATIONS IN HAZARDOUS WASTE TREATMENT. Longboat Key, Fl. October 30 - November 4.

- C. M. Kung, P. T. Lagonikos, and B. C. Baltzis. 1987. "Bioreactors Configurations and Characteristics as Factors Determining the Ability to Maintain a Mixed Culture." NERM 17 ACS Meeting, Rochester, New York November 8-11.

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INTRODUCTION

Halogenated hydrocarbons are used in many aspects of our daily life. They have applications as solvents (e.g., chloroform, trichloroethylene, and chlorobenzene), as insulating liquids (polychlorinated biphenyls), as wood preservatives (pentachlorophenol, PCP), and as herbicides and fungicides.

Polychlorinated phenols, particularly PCP, are produced in the hundreds of thousands of tons each year. Wood preservatives constitute 80% of this production. A large amount of these phenols enter the environment (Dickson, 1980). Because of chemical and thermal stability, these compounds are persistent in nature. Half-lives of more than 15 years are not uncommon (Müller and Lingens, 1986).

The toxicity of the family of chlorophenols has been tested on mice and rats. Increased respiration, motor weakness, tremors, CNS depression, convulsions, dyspnea (difficulty in breathing), coma, and then death were observed. The order of acute oral toxicity of the compounds is PCP > tetrachlorophenols > monochlorophenols > trichlorophenols > dichlorophenols (Borzelleca, et al., 1984). The results indicate that the more chlorines are on the compound, the more toxic it is. However, monochlorophenol is more toxic than tri- and di-chlorophenol.

Another problem is that biodegradation systems for treating wastewater are inhibited by the presence of halogenated compounds. PCP has been shown to be strongly inhibitory to methanogenesis in both pure cultures (Guthrie, et al., 1984) and anaerobic sludges and sediments. Thus the presence of PCP or other halogenated hydrocarbons

in wastewater is a potentially serious problem if anaerobic digestion is to be successful (Mikesell and Boyd, 1986).

Table 1 shows the physical and chemical properties of phenol and chlorinated phenols.

Since nature itself produces a variety of halogenated compounds in large amounts, it would be surprising if mechanisms for their biodegradation did not exist. Although most microorganisms tend to be inhibited by these compounds, microorganisms do indeed exist which play a major role in the degradation of halocarbons.

Besides biological processes, there are other physicochemical processes capable of dealing with these toxic compounds. An advantage of biological processes is that they are usually less energy consuming and often less expensive than physicochemical ones. The most attractive feature of the biodegradation processes over others is that the compounds can be mineralized (to water, carbon dioxide, methane, and halides), rather than just being transformed.

Traditional biological systems use primarily aerobic rather than anaerobic processes, because aerobic organisms are generally more stable and have higher growth rates than anaerobic organisms. However, anaerobes have an extremely important property in waste treatment applications, namely their ability to reductively dehalogenate organic compounds. This property is largely unaffected by the number or position of the halogen atoms in the molecule (Bollag, 1974; Suflita et al., 1982). This is not the case for aerobes, which are much more sensitive to the number and position of halogens (Bollag, 1974; DiGeronimo et al. 1979; Okey and Borgan, 1965). On the other hand, dehalogenated compounds are more water soluble and more amenable to aerobic mineralization. Therefore, coupling the two processes would appear to have a number of inherent advantages over a conventional aerobic process.

The purpose of the present study was to precisely test that hypothesis in a practical engineered system, consisting of an anaerobic reactor where dechlorination of the

toxic halogenated compound took place, followed by an aerobic reactor (fed with the effluent of the anaerobic reactor) in which complete mineralization was obtained

LITERATURE REVIEW

2.1. CLASSIFICATION OF ANAEROBIC MICROORGANISMS

According to their trophic requirements anaerobic microorganisms may conveniently be divided into three broad categories (Barnes and Fitzgerald, 1987). The first comprises hydrolytic bacteria, commonly referred to as acidogens, because they initially ferment their substrate into short-chain organic acids and other small molecules. The second group is that of the heteroacetogens which produce acetic acid and hydrogen, and the third is that of the methanogens, which produce methane. This group may be further subdivided into hydrogen utilizers (lithotrophs) and acetic acid users (acetotrophs). Feedstocks containing oxidized sulphur and nitrogen may give rise to two additional groups of bacteria, the sulphate-reducers and the denitrifiers.

The methanogenic bacteria (Group III) are possibly responsible for the anaerobic degradation of halogenated toxic compounds.

The methanogens are the most culturally fastidious group in the symbiosis of anaerobic digestion. They require a broad spectrum of nutrients in order to grow, including carbon, phosphorus, nitrogen, sulphur, calcium, magnesium, potassium, sodium, organic nutrients such as amino-acids and vitamins and trace metals. In addition to iron, zinc and manganese, it was established that methanogens require trace amounts of cobalt, molybdenum and nickel (Schönheit, 1979). Biomass conformation plus adjustment of yield coefficient of carbon is 93C:5N:1P.

The majority of mesophilic methanogens will not grow at pH values below 5.5. Low pH values favour proton reduction to hydrogen, rather than hydrogen reduction to methane, and therefore methane production normally ceases. Empirical results have additionally shown that an upper pH limit of pH 8 is desirable.

2.2. ENRICHMENT SOURCES

Enrichment for microorganisms that are able to degrade target toxic compounds efficiently is the first step, and sometimes the most time consuming step for a researcher to develop a microbial degradation system. In order to cut the time spent in this phase to get powerful microorganisms, it is crucial to start sampling from the right sources.

In Berry's review (1987), listed enrichment sources include: soil (5 references), river or marine mud, sludge (7 references), sewage, subsurface, marine or lake sediment.

For chlorinated hydrocarbons, the most promising sources are soil or water samples chronically polluted with these compounds (Müller and Lingens, 1986).

The complete conversion of halobenzoic acids and halophenolic compounds to methane by lake sediment and sewage sludge microorganisms has been demonstrated (Sahm et al., 1986).

Hexachlorobenzene (Fathepure et al., 1988) and at least some polychlorinated biphenyls (PCB) (Tiedje et al., 1987) were dechlorinated by sewage sludge and river sediments, respectively.

Bacterial strains from sediments from the PCB-contaminated Hudson river (NY, USA) were able to degrade a majority of the congeners included in a commercial polychlorinated biphenyl oil (Bedard et al., 1986).

Chlorinated phenols and guaiacols can be degraded by mixed bacterial cultures obtained from areas polluted by bleach plant effluents which contain these compounds (Neilson et al., 1983).

Mixed bacterial cultures originating from soil contaminated by polychlorinated phenols, which are used as wood preservatives in sawmills, degrade PCP (Valo et al., 1985).

A long lag period is usually observed before the onset of anaerobic transformation, which is most likely due to low initial numbers of microorganisms present in aquifer solids and ground water (Edwards et al., 1990). The concentration of the toxic compounds can also affect the lag period.

Horowitz et al. (1983) found that before the degradation of halobenzoate occurred, the lag time in an unacclimated sediment ranged from 0.4 to 40 weeks. They concluded that the lag periods varied according to the type and position of the aryl halide. The substrate concentration also had an effect on the length of the lag period for 4-amino-3,5-dichlorobenzoate.

Anaerobically digested municipal sewage sludge which had been acclimated to monochlorophenol degradation for more than 2 years was shown to degrade PCP (Mikesell and Boyd, 1986).

2.3. EVIDENCE OF ANAEROBIC DEHALOGENATION

Horowitz and co-workers (1983) ran a series of experiments on halobenzoate to demonstrate that the dehalogenation reactions are brought about by living anaerobic bacteria and not by abiotic photochemical reactions of chemical reductants.

1. The reactions occur only in the dark and in the absence of oxygen. Sterilized sludge samples do not carry out any reaction even in the presence of the strong reductant titanium citrate.

2. Upon increasing the temperature above 39°C, dehalogenation reactions in freshwater sediments are lost, since sediment microbes are not adapted to these conditions. On the contrary, a chemical mechanism would be enhanced by such temperatures.

3. Usually, when haloaromatics are added to unadapted sediments, long lag periods with a low background of methanogenesis are followed by a phase of rapid degradation. This indicates a specific biological adaptation, as does the acclimation of sediments by prior exposure to the haloaromatic compounds.

2.4. ANAEROBIC TECHNIQUES

There are several derivative variations of the technique described by Hungate for the cultivation of anaerobic microorganisms (Bryant, 1972; Holdeman and Moore, 1972; Hungate, 1969; Macy et al., 1972). The fundamental unit of operation (Miller and Wolin, 1974) is a serum bottle closed with a butyl rubber stopper with a crimped metal seal. Media are prepared under nonsterile conditions, usually with an appropriate reducing agent. The media are gassed with O_2-free gas and added to gassed serum bottles. Stoppers are inserted as the gassing needles are withdrawn from the bottles. Metal seals are then crimped to seal the caps to the bottles, and the bottled media are autoclaved. All inoculations are carried out with a hypodermic syringe and needle.

2.5. COMETABOLISM

In many instances it appears that several distinct organisms may be responsible for the substrate degradation with one organism modifying the substrate in such a manner that the second and subsequent organisms can now use the products as substrates and effect further modifications. In such cases no organism in pure culture would use the substrate as sole carbon and energy source. Therefore it is necessary to add an alternative carbon source to provide energy for growth (Slater and Somerville, 1979).

This phenomenon is called cometabolism and is defined as the transformation of a non-growth substrate in the obligate presence of a growth substrate or another transformable compound (Dalton and Stirling, 1982). Cometabolism is important in the biodegradation of pesticides either aerobically or anaerobically.

Dietrich and Winter (1990) were able to match the stoichiometric relation of *n*-butyrate (co-substrate) oxidation to 2-chlorophenol or 2,6-dichlorophenol reductive dehalogenation, respectively.

In Slonim's work (1985) to degrade 4,6-dinitro-o-cresol (DNOC) in a continuous system, the performance of the anaerobic system was highly dependent on the influent concentration of the co-substrate sucrose. An influent sucrose to DNOC ratio of 2:1 or higher resulted in 95-100% removal (or conversion) of DNOC in the anaerobic process. However, when influent sucrose to DNOC ratio was less than 2:1, the anaerobic microorganisms failed to co-metabolize DNOC.

When Golovleva (1981) utilized a *Pseudomonas* species to degrade DDT, that did not serve as a source of carbon or energy, alternative sources of carbon and energy, therefore, needed to be supplied. In their experiments, lactic acid as carbon source, and nitrate as electron acceptor were added to the medium.

The aerobic transformation of trichloroethylene (TCE) is a cometabolic process (Edwards et al., 1990). TCE is not utilized by the bacteria, but only fortuitously transformed by virtue of their powerful methane monooxygenase.

On the other hand, although *Flavobacterium* cells (Topp et al., 1988) need readily metabolizable carbon to reduce the lag period to degrade PCP, it is not cometabolism because PCP could be used as sole carbon source for the cells.

2.6. MEDIUM

In the anaerobic degradation of aromatic toxic compounds, the availability of co-substrates and electron acceptors in the medium plays a crucial role in influencing microbial activity and diversity (Berry et al., 1987). Sometimes, an extra nutrient in the medium which is used as co-substrate or electron acceptor would enhance the degradation greatly. In many cases, semi-defined or undefined media are used in the biodegradation of toxic compounds, because of the difficulties in defining the medium.

In the paper by Long-de Vallère et al. (1989), 35 references were listed using defined media in experiments. However, only other 4 and 14 references using semi-defined or undefined media, respectively, were reported.

In Dolfing and Tiedje's work (1987), unknown supplements that were present in rumen fluid, were also required by the isolated strain DCB-1 in degrading 3-chlorobenzoate together with two other pure cultures.

Edwards et al. (1990) observed an increase of the anaerobic degradation rate of TCE by adding *p*-cresol into the medium.

No growth and no dehalogenation of 2-chlorophenol was obtained when yeast extract and peptone were omitted completely in the medium of Dietrich and Winter's work (1990).

Topp et al. (1988) suggest that available carbon in polluted environments could facilitate PCP removal by inoculated *Flavobacterium* cells by attenuating the toxicity of PCP and contributing to the production and maintenance of PCP-degrading biomass.

2.7. ADVANTAGES OF ANAEROBIC DEGRADATION

The choice between aerobic and anaerobic processes for wastewater treatment has tended to favor the former because the systems were considered to be more reliable, more stable, and better understood. However, these classical disadvantages of

anaerobic processes have been overcome recently because of the advance of the knowledge in the anaerobic cultures (Heijnen et al., 1989). The processes also offer several clear advantages (Forday and Greenfield, 1983; Long-de Vallère et al., 1989; Anderson et al., 1984) such as:

1. Anaerobic processes generate less sludge than aerobic processes. Aerobic processes are likely to yield between 0.5 and 1.5 kg of biomass (sludge) solids for each kg of BOD removed, while anaerobic processes are likely to yield only 0.1-0.2 kg for each kg of BOD removed.

2. Anaerobic processes generate methane which can be used as a fuel source.

3. Even without the use of methane as an energy source, the aeration energy requirements of aerobic processes exceed the mixing energy requirements of anaerobic processes.

4. Anaerobic microorganisms have the ability to lie dormant for several months and then be fully operational within 2-3 days (of great value when seasonal waste waters are to be treated).

5. Anaerobic system has the capability of operation on a stop/start basis.

Also, from a microbiological point of view, it may turn out that anaerobic techniques are better suited to remove halogenated aromatic compounds from industrial effluents than aerobic biological treatment because:

a. Haloaromatic compounds tend to polymerize when degraded by ordinary aerobic bacteria (Knackmuss, 1982). These polymerization products are rather resistant to further bacteria attack. However, under anaerobic conditions, oxidation, and hence polymerization, are not possible.

b. Theoretically, polyhalogenated aromatics should be more easily degraded by anaerobes than by aerobic bacteria since an increasing degree of halogenation of the aromatic ring decreases the electron density of the aromatic nucleus. Thus, electrophilic attack of oxygen on aromatic structures is more difficult. To the contrary, a

decreased electron density of the aromatic nuclues should enhance anaerobic enzymatic attack by a reductive (nucleophilic) mechanism (Klinman, 1972).

c. Since anaerobic enrichments have a high affinity for haloaromatics, anaerobic techniques seem to be well-suited to remove trace levels from industrial effluents.

The interest in anaerobic treatment has been accelerated by the more stringent requirements to pretreat industrial wastewaters prior to sewer discharge, the need to reduce the energy costs of treatment, particularly for high strength wastewaters, and the unsuitability of alternative treatment methods for some types of wastewaters (Barnes and Fitzgerald, 1987).

2.8. METHODS FOR THE DETERMINATION OF ANAEROBIC BIODEGRADATION

Some of the intermediates and products of their anaerobic transformation (such as vinyl chloride produced from the dehalogenation of chloroform), can be more hazardous than the parent compounds (Edwards et al., 1990). Thus, it is essential to trace the fate of target compound in waste water. Monitoring the disappearance of the target compounds is not usually enough.

When specific toxic compounds are to be treated, the concentration of the transformed compounds in the effluent could be directly determined by GC or HPLC. The degradation products could be determined by GC-MS, if complete mineralization has not occurred.

Calculations from mass balances of the conversion of organic carbon to carbon dioxide and methane allow predictions of the theoretical yields of gas expected if the compound are to be completely mineralized. Therefore, measurements of gas production allow easy assessment of the degree of degradation achieved (Long-de Vallère et al., 1989; Shelton and Tiedje 1984a).

Hakulinen and Salkinija-Salonen (1982) measured the amount of $^{14}CO_2$ to ascertain the complete mineralization of ^{14}C PCP.

2.9. ISOLATION OF PURE CULTURES

Pure bacterial cultures able to metabolize PCP under aerobic conditions have been isolated by a number of research groups. Among these are several strains of a *Flavobacterium* sp. (Saber and Crawford, 1985), a *Pseudomonas* (Watanabe, 1973), and a *Coryneform* bacterium (Chu and Kirsch, 1972). A strain of *Arthrobacter* which utilized PCP has been added to soil in an attempt to decontaminate polluted areas (Edgehill and Finn, 1983).

However, no anaerobic bacteria with the ability to degrade PCP have been isolated or identified (Mikesell and Boyd, 1986).

A single anaerobic microorganism can rarely take up a complex organic molecule and mineralize it to carbon dioxide. Rather, a succession of specialized organisms modify the molecule in turn, each deriving a small amount of carbon and/or energy from the reaction (Hamilton, 1979; Lovely and Klug, 1982; Sleat and Robinson, 1984). As a result, it is very difficult to isolate pure cultures for anaerobic degradation.

Dietrich and Winter's (1990) attempts to isolate an anaerobic 2-chlorophenol dechlorinating organism failed. The mixed culture was reduced to three morphologically distinctive microorganisms.

When Hakulinen et al., (1985) mixed the isolated cultures of *Pseudomonas aeruginosa* and *Klebsiella oxytoca*, the mixed cultures were able to utilize 2,4,6-trichlorophenol (TCP) as a sole carbon and energy source under anaerobic conditions. However, none of the isolates were able to degrade TCP under anaerobic conditions in pure culture.

Shelton and Tiedje (1984b) isolated three pure cultures that could grow on 3-chlorobenzoate as the sole carbon and energy source when they were mixed. These three cultures performed different reaction steps in mineralizing 3-chlorobenzoate.

In practice, mixed microbial cultures offer the best possibilities for efficient and robust treatments. In this respect, the results of laboratory based studies with pure cultures growing in defined media under optimal conditions cannot be immediately applied to complex in situ and downstream treatments where considerations of fluctuating physico-chemical conditions and interactions between microorganisms may be as important as considerations of biodegradative mechanisms (Müller and Lingens, 1986).

2.10. DEGRADATION PATHWAYS

Many chlorophenols have recently been shown to be anaerobically biodegradable. However, neither the biochemical pathway nor the organism or organisms responsible for degradation have been unambiguously identified (Hakulinen et al., 1985).

Proposed degradation pathways in strict anaerobic conditions involve reductive dehalogenation (Suflita et al., 1982) and the addition of hydroxyl group to the aromatic ring (Taylor et al., 1970). With respect to the microorganisms which carry out the anaerobic dechlorination reaction, it has been shown that some gram negative bacteria may be particularly important (Hakulinen et al., 1985).

Anaerobic (Murthy et al., 1979; Guthrie et al., 1984) and aerobic degradation (Murthy et al., 1979; Watanabe, 1977; Moos et al., 1983; Valo et al., 1985) of PCP by mixed microbial cultures has been reported. Murthy et al. (1979) found that CO_2 formation was severely reduced under anaerobic conditions but identified 2,3,5,6-tetrachlorophenol, 2,3,4,5-tetrachlorophenol, 2,3,6-trichlorophenol, and pentachloroanisole as degradation products. Ide et al. identified 2,3,4,6-tetrachlorophenol, 2,4,5-

trichlorophenol, 3,4- and 3,5-dichlorophenol, and 3-chlorophenol as degradation products.

These results strongly indicated that sequential reductive dechlorination reactions can be carried out by microorganisms degrading PCP under anaerobic conditions.

Suflita et al. (1982) demonstrated that in the dehalogenation of halogenated benzoate, the primary degradative event was the loss of the aryl halide without the alteration of the aromatic ring. Dehalogenation required strict anaerobic conditions and depended on the halogen and position, but not on the number of halogen substituents. These results suggest that reductive dehalogenation of aromatics could be important in the removal of some chlorinated xenobiotics from the environment.

In contrast, aerobic metabolism of aromatic compounds can be characterized by (i) direct replacement of the halogen by a hydroxyl group (Johnston et al., 1972; Klages and Ligens, 1979); (ii) the occasional nonenzymatic loss during NIH shifts (Guroff et al., 1967); and (iii) removal of the halogen from the alkyl moiety after cleavage of the ring, which is the most frequently cited case (Goldman et al., 1967; Hartmann et al., 1979; Horvath and Alexander, 1970; Reineke and Knackmuss, 1980; Spokes and Walker, 1974).

In the aerobic degradation of PCP, evidence (Suzuki, 1977; Rott et al., 1979) show that the process proceeds by replacement of a chlorine substituent by a hydroxyl group, although the reaction mechanism remains unclear.

The study of the degradation pathways could be applied in the synthesis of new chemicals. Compounds that are liable to contaminate the environment could be designed so as to combine efficacy with biodegradability. More direct applications may come from the use of isolated microorganisms of purified enzymes for the decontamination of pollutant spillages. Another application of the enzymes is to use them as catalysts for commercial chemical syntheses (Müller and Lingens, 1986).

2.11. BIODEGRADATION OF HALOGENATED AROMATICS

Microbial degradation, either aerobically or anaerobically, of halogenated compounds has been widely studied. As noted by Bollag (1974), *meta*-substituted aromatic compounds tend to be more recalcitrant. The persistence of the compounds against aerobic degradation is positively correlated with the number of halogens (DiGeronimo et al. 1979; Okey and Borgan, 1965). However, this is not apparent for anaerobic metabolism (Suflita et al., 1982).

When Mikesell and Boyd (1986) dechlorinated PCP, Cl substituents *ortho* to the phenolic OH group are removed more rapidly than Cl in the *meta* and *para* positions. Their data showed that the less extensively chlorinated phenols (tetrachlorophenols and trichlorophenols) were less subject to dechlorination reactions than PCP and thus tended to accumulate.

Dietrich and Winter's mixed culture (1990) could dehalogenate 2-chlorophenol, 2-bromophenol or completely dechlorinate 2,6-dichlorophenol, whereas from 2,4-dichlorophenol only the substituent in the *ortho*-position could be eliminated.

Hakulinen et al. (1985) added various concentrations of 8 polychlorinated phenols (2,3-, 2,4-, 2,5-, 3,4- and 3,5-dichlorophenols, 2,4,6- and 3,4,5-trichlorophenols, 2,3,4,5-tetrachlorophenol, and PCP) in their anaerobic system. The extent of dechlorination was variable for the different isomers, but ranged from no reaction of 3,4-dichlorophenol to as high as 90-99% removal of *ortho* chlorines from tri and tetra chlorophenols. There was no evidence of ring cleavage.

Tiedje and Boyd (Boyd et al., 1983; Boyd and Shelton, 1984) demonstrated that microbial populations from lake sediments and sewage sludge meditated the anaerobic degradation to methane of some 19 chloro-, bromo-, and iodo-benzoate and phenolic compounds. From these results, it appears that *meta* halogens are more susceptible to attack by anaerobic bacteria when compared to the *ortho* or *para* isomers. Suflita et al. (1982) reached a similar conclusion.

Several researchers (Suflita et al., 1982; Mikesell and Boyd, 1986; Boyd and Shelton, 1984; Boyd et al., 1983) found that bromo- and iodo- substituents are degraded after a shorter lag time than their chloro- or fluoro- counterparts, which suggests that the Br and I species are more readily dehalogenated.

It was found by Suflita et al. (1982) that complete dehalogenation was required before a substrate could be mineralized to CH_4 and CO_2. Horowitz et al. (1983) reached the same result in their experiments.

In the anaerobic degradation of PCBs both the number and position of the chlorine atoms determined the extent of biodegradability. For example, the more highly substituted PCBs were metabolized less extensively than the mono- and dichlorinated biphenyls. Furthermore, the isomers bearing chlorine substituents on only one ring were more easily metabolized than those with substituents on both rings, and isomers doubly substituted in the *o*-positions (e.g., 2,2'- or 2,6-substitution) were generally recalcitrant to microbial degradation.(Müller and Lingens, 1986)

Flavobacterium (Frick et al., 1988) was used to treat numerous types of pollutants in water, aerobically, including river water, lake water, and groundwater. PCP concentrations were reduced to undetectable levels from initial levels ranging from 10 ppb to 100ppm, usually within 48 hr after inoculation.

3,4,5-trichloroguaiacol, 3,4-dichlorophenol, 2,4,5-trichlorophenol, and PCP were tested in Larsson's experiment (1988). The resulting concentrations of the phenolic pollutants were 30 to 537 μg/liter. During 120 days 6-15% of above compounds were consumed in the humic water under aerobic condition.

2.12. AEROBIC MICROORGANISMS IN ANAEROBIC SYSTEMS

The microbial flora of an anaerobic reactor fed with chlorophenols may include bacteria that are classified as aerobes, such as *Pseudomonas aeruginosa* (Hakulinen et

al., 1985). Ferry and Wolfe (1976), Taylor et al. (1970), Toerien (1967) and McCarty (1962) have also isolated *Pseudomonas* species from anaerobic conditions. Taylor (1970) as well as Ferry and Wolfe (1976) isolated such bacteria from anaerobic cultures fed an aromatic carbon source.

In the Hakulinen's work (1985), pure and mixed cultures were tested for use of 2,4,6-trichlorophenol (TCP) as a sole carbon and energy source under anaerobic conditions. None of the isolates were able to degrade TCP under anaerobic conditions in pure culture. However, mixed cultures containing only *Pseudomonas aeruginosa* and *Klebsiella oxytoca* were able to degrade TCP. It seems that the presence of *K. oxytoca* enables the *Pseudomonas* strain to survive under anaerobic conditions.

2.13. IMMOBILIZATION

Immobilization of microorganisms is the first step toward the application of microbial degradation in the use of packed-bed or fluidized bed reactors. Immobilization has the advantage of keeping a high concentration of biomass in the reactor, preventing the washout of microorganisms, and protecting microorganisms from being exposed to high concentration of toxic compounds.

Applications of immobilization might be: 1) to develop a reactor for a microbial process involving organisms which grow too slowly for continuous suspended culture operation, 2) to isolate organisms which grow so slowly on inhibitory substrates that continuous suspended culture techniques become difficult, and 3) to design a high-rate water-treatment process in which cell retention is provided by a biofilm reactor (Longde Vallère et al., 1989).

Chou et al. (1979) showed that fixed-film or attached growth systems were greatly superior to suspended growth systems in the aspects of acclimation and stability

to compounds like monocyclic aromatics and organic acids commonly found in petrochemical wastewater.

Frick et al. (1988) ran series of experiments in the degradation of PCP, comparing the activity in aliquots of reactor liquid with equal volume samples of immobilizing saddles indicating that 30 to 50% of the activity was in the immobilized biofilm.

Caunt and Chase (1988) did a detailed study on the immobilization of *Alcaligenes denitrificans* in Celite particles (R-630, R-631, R-633, and R-635), made of calcined diatomaceous earths. Their results showed that incorporation of 0.25 M sodium chloride in the medium, a high initial cell concentration, and presaturation of the beads with substrate gave the best results.

2.14. TYPES OF BIOREACTOR

2.14.1. Packed-Bed Reactors

A packed-bed bioreactor contains solid supporting media on which a biofilm is grown. The supporting media retain large amount of biomass and protect the microorganisms against concentrated toxic compounds or washout. Although there are some restrictions (Mosey, 1977) on the use of this type of reactor, Anderson (1984) used anaerobic packed-bed reactors to treat industrial wastewater, because of the above mentioned advantages.

According to the direction of flow, the packed-bed reactors can be classified into two sub-groups:

1. Upflow Packed Bed Reactor

A plug flow pattern usually results within the reactor. However, rising bubbles caused by gas production may tend to stir up the flow pattern. In general, this reactor is operated without recycle. The reactor may need periodic backwashing to remove accumulated solids (Barnes and Fitzgerald, 1987).

Krumme and Boyd (1988) used the upflow configuration to successfully degrade a mixture of all three monochlorophenols and 3,4,5-trichlorophenol at efficiencies greater than 90%. The substrate loading rate was 20 ppm per day at a hydraulic retention time of 2 or 4 days. However, there was little biodegradation of either 2,4,6-trichlorophenol or PCP at the same operating conditions.

2. Downflow Packed Bed Reactor

By using media of high void volume and irrigating the wastewater over the media, clogging, as seen in upflow filters, may be overcome. These reactors can be operated with the liquid partially or completely filling the reactor. It was previously thought that biogas production was sufficient to improve reactor mixing (Kennedy and van den Berg, 1982; van den Berg, 1984). Further work now indicates that although biogas production improves mixing slightly by increasing the axial dispersion, the major improvement comes from recycling (Long-de Vallère et al., 1989).

2.14.2. Expanded & Fluidized Bed Reactors

The difference between expanded and fluidized beds is not clear cut. A popular definition is that expanded beds have an expansion of 10-20% of static height, while fluidized beds expand from 30% to 100% of static height (Callander and Barford, 1983). Factors that contribute to the effectiveness of these reactors include the following (Barnes and Fitzgerald, 1987; Hickey and Owens, 1981; Henze and Harroemoes et al., 1983; Heijnen, 1984):

1. Small media may be used, resulting in high specific surface area, and therefore large biomass volume for a relatively small reactor.

2. There is good mixing and mass transfer in the bed, so problems of channeling, plugging, and gas hold-up commonly encountered in packed beds are avoided.

3. Shear forces create a thin, dense biofilm comprising mainly nondiffusion-limited, active biomass.

4. Hydraulic retention times may be low, resulting in cost-effective installation and operation for an industrial application.

Their major disadvantage is the power required for the fluidization process.

In Hakulinen and Salkinoja-Salonen's work (1981), PCP was mineralized in an anaerobic fluidized bed reactor in their anaerobic-aerobic two-stage reactor setup.

2.15. ANAEROBIC-AEROBIC TREATMENT

The integration of these two processes has advantages in the following cases:

1. When aerobes are not able to effectively treat the compound, but are able to treat the metabolic products of anaerobic processes (Hakulinen and Salkinija-Salonen. 1982; Su, 1990).

2. When volatile compounds that can be stripped by aeration are present in the wastewater (Dienemann et al., 1990).

Hakulinen and Salkinoja-Salonen (1981) demonstrated that an anaerobic-aerobic fluidized bed process was able to reduce the concentration of organics and chlorinated phenolics in a pulp mill bleaching effluent. It was observed that the chlorophenols were degraded in the first anaerobic stage, followed by toxicity and biological oxygen demand (BOD) removal in the second-stage aerobic filter.

Su (1990) used an anaerobic-aerobic CSTR process for treating waste water containing high concentration of nitrite and nitro-aromatic compounds that are resistant to aerobic microbial degradation. Nitrite and nitro-aromatics were biotransformed to nitrogen and amino derivatives using ethanol and glucose as carbon sources, respectively, in the anaerobic reactor. The amino derivatives were then degraded in the aerobic reactor.

Ying et al., (1990) developed a two-stage anaerobic-aerobic biological activated carbon (BAC) process for treating high concentration wastewaters from phenolic resin

manufacturing. Most organic contaminants were removed in the first step anaerobic BAC bed: more than 90% of chemical oxygen demand (COD, from as high as 30,000 mg/l), and more than 99% of the major constituents - phenol (from 12000 mg/l), formaldehyde (from 3000 mg/l) and methanol (from 2500 mg/l). The second stage aerobic BAC treatment removed virtually all remaining phenol and more than 90% of the residual COD, which was due mostly to organic acids resulting from anaerobic degradation byproducts.

Dienemann et al. (1990) employed serial anaerobic/aerobic packed-bed bioreactors to biodegrade organic contaminants in leachate from a high priority Superfund site in the U.S. Classical, secondary aerobic treatment was not selected because volatilization of priority pollutants in the leachate would pose a significant secondary air pollution threat. The intention of the reactor configuration was that the larger part of the volatile species present would be biodegraded anaerobically, minimizing subsequent volatilization losses in the aerobic column.

Slonim et al., (1985) used an anaerobic recycle fluidized bed reactor as a pretreatment stage for 4,6-dinitro-o-cresol (DNOC), followed by an activated sludge reactor as the aerobic treatment stage to mineralize the compound.

It is evident that the integrated anaerobic-aerobic system offers an alternative for the biodegradation of industrial wastewater with efficiency and flexibility. The enriched microorganisms, the nutrients (co-substrate, or electron acceptor) and the reactor design of both reactors all play important roles in the performance of the system.

METHODS AND MATERIALS

3.1. HPLC ANALYSIS OF CHLOROPHENOLS

3.1.1. Procedure

Samples were stored in a freezer after they were taken. To monitor phenolic substrate transformation, samples were thawed and prepared for HPLC analysis by acidifying centrifuged supernatant, which was then mixed and filtered. The solution was sucked into a syringe, and then was forced to pass through a 13 mm $\times$ 0.20 μm filter paper. Sample volumes of 25 μl were injected into a Waters Component System, consisting of a Waters 600E System Controller, a Waters Model U6K universal liquid chromatograph injector (which was later replaced by a Waters 715 Ultra Wisp Sample Processor) and a Waters 484 Tunable Absorbance Detector. The column used was an Alltech Econosphere C8 5μ, 4.6 mm i.d. $\times$ 150 mm (Cat.# 70090). An Alltech Direct Connect™ Refillable Guard Column (Cat.# 28950) filled with Guard Column Refill Pell. C8 (Stock# 28971) was employed to protect the column. The system was calibrated using a solution of known concentrations of TCP, and its metabolites, 2,4-DCP, 4-CP and phenol.

3.1.2. Analysis Conditions

Mobile Phase A (1% acetic acid in methanol) : Mobile Phase B (1% acetic acid in Milli-Q Water), 50:50, were run isocratically. The flow rate was 1 ml/min. The UV detection was at 280 nm, 0.5 AUFS. The data were processed by PE Nelson chromatography software rev. 5.10 interfaced with 760 series Model 2600.

3.1.3. Calibration Curves

Calibration curves for phenol, 2-CP, 3-CP, 4-CP, 2,4-DCP, 2,6-DCP, 3,5-DCP and, TCP were prepared for the determination of the concentrations in parts per million as shown in Figures 1 through 8. The correlation coefficients of the regression were all satisfactorily close to unity. At the beginning of each HPLC run, standards of 20 ppm 4-CP, 2,4-DCP, and, TCP were injected to test the effectiveness of the calibration curves. A factor could be derived from the division of 20 ppm by the calculated concentration of the standard. If the calculated concentrations of the standards were off by less than 5 ppm, the real sample concentrations would be the calculated sample concentrations times the aforementioned factor. If they were off by more than 5 ppm, the calibration curves would be re-done.

After the project was done, it was found that the filter papers (Gelman Science, Prod.# 66600) used to filter the samples could absorb chlorophenols when the samples passed through. However, since the standard solutions for making the calibration curves were treated with the filter papers too, this absorbance effect could be cancelled when the concentrations of the samples were determined with the calibration curves.

3.2. CHLORIDE ION MEASUREMENT

Two methods could be used: direct measurement and known addition. The former was used here. Standard solutions whose concentrations ranged from 100 to 500 ppm Cl^- were prepared. 2 ml ISA per 100 ml standard or sample were added and

a calibration curve on the millivolt readings (linear axis) from Orion Model SA720 pH/ISE meter against concentration (log axis) was prepared.

3.3. PHOSPHATE BUFFER PREPARATION

Phosphate buffer was typically used to control the pH value of solutions in the range of 5.8 and 8.0 (Costilow, 1981). This was the range that most of the bacteria preferred. Another important reason for its extensive use in this project was that it did not constitute a carbon source for microorganisms. KH_2PO_4 (monobasic) and Na_2HPO_4 (dibasic) were used to prepare buffer solutions. The concentration of the stock phosphate buffer was 1 M at the beginning of the project, but it was later changed to 0.5 M because of the difficulty in preparing 1 M Na_2HPO_4 solution.

0.5 M of each solution was prepared first. Either one of the solutions could be used to titrate the other one, until the desired pH value was reached. A stock solution of pH 6.8 was used throughout the project.

3.4. CHEMICAL OXYGEN DEMAND MEASUREMENT

Since this measurement was done by Mr. Jou, a fellow student working on a related project at NJIT, only summary of the method is described here. Sample, blanks and standards in sealed tubes are heated in an oven or block digestor in the presence of dichromate at 150°C. After two hours, the tubes are removed from the oven or digestor, cooled and measured spectrophotometrically at 600 nm.

3.5. MEDIA PREPARATION

3.5.1. Undefined Medium

The media used for the anaerobic reaction were taken from the anaerobic digesters of two local sewage treatment plants located in Livingston and Elizabeth, New Jersey, respectively. Originally, the medium was taken from the Livingston sewage treatment plant. However, the anaerobic digester at the plant was later converted to an aerobic reactor at the beginning of 1990. Hence, a similar medium from the Joint Meeting company in Elizabeth was used thereafter.

The liquor taken from either digester was first autoclaved to kill all the microorganisms initially present, and then diluted 1:1 with deionized water. After settling overnight, the supernatant was removed, and centrifuged or filtered to remove any remaining solids. This solution was then used as a medium for the anaerobic reaction. Sometimes this solution was also used as a medium for the aerobic reactor.

Before use, resazurin dye was added to the medium as an oxygen indicator, and the solution was autoclaved again. TCP was injected after the medium cooled, which was then ready for use in the anaerobic reactor.

If there were any volatiles present in the original solution taken from the anaerobic digesters, they would be lost during autoclaving. COD from the inherent carbon cnotent of the sterilized medium was measured roughly an order of magnitude greater than the added TCP.

3.5.2. Defined Medium

At the beginning, the enrichment was performed in a defined medium, which consisted of (in every liter of the medium) sodium acetate (1 g), resazurin (1 ml), trace minerals (10 ml), mineral salts (25 ml), and ammonium chloride (1.25 g) were added in boiling water. When the water was cooled in ice and gassed under nitrogen,

$Fe(NH_4)_2(SO_4)_2 \cdot 6H_2O$ (0.018 g), and $NaHCO_3$ (7.5 g) were then added . Reducing agent (10 ml), and vitamin (5 ml) were added just before autoclaving. Sodium acetate was used as the carbon source.

The compositions of trace minerals solution (mg/liter) were Nitriloacetic acid (1500), $MgSO_4 \cdot 7H_2O$ (3000), $MnSO_4 \cdot H_2O$ (500), NaCl (1000), $FeSO_4 \cdot 7H_2O$ (100), $Co(NO_3)_2.6H_2O$ (100), $CaCl_2$, anhydrous (100), $ZnSO_4 \cdot 7H_2O$ (100), $CaSO_4 \cdot 5H_2O$ (10), $AlK_2(SO_4)_3$, anhydrous (10), Boric acid (10), and $Na_2MoO_4 \cdot 2H_2O$ (10).

The vitamins solution (mg/100 ml) contained: Biotin (4), *p*-Aminobenzoic acid (10), Folic acid (4), Pantothenic acid, calcium salt (10), Nicotinic acid (10), Vitamin B_{12} (0.2), Thiamine hydrochloride (10), Pryidoxine hydrochloride (20), Thioctic acid (10), and Riboflavin (1).

3.6. INOCULUM PREPARATION

The inoculum for the anaerobic batch reaction was those samples taken from the anaerobic digesters without any treatment. After the anaerobic microorganisms were successfully immobilized in the packed-bed reactor, the solution from the reactor, taken under nitrogen gassing, was the inoculum and medium for any later anaerobic batch reactions. This solution which contained 4-CP, the dechlorination product of TCP, was also used as inoculum and medium for the aerobic reaction when no external ATCC cultures were needed.

The ATCC cultures were grown in the undefined medium described in the previous section for three days. 5 ml of each culture was then inoculated to the aerobic reaction medium.

3.7. MATERIALS

(1) Serum bottles, 125 ml, from Fisher, Cat.# 06-406K

(2) Aluminum seal stoppers, from Bellco, Cat.# 2048-11800

(3) Aluminum seals, 20 mm, from Bellco, Cat.# 2048-11020

(4) Seal crimper, from Bellco, Cat.# 2048-10020

(5) Manville Celite catalyst carrier, R-635 silicon beads, a kind gift from Manville company. See Pak's work (1988) for the chemical and physical properties of the carrier.

(6) Resazurin, from Fisher, Cat.# EK113-7728. The stock solution was 0.1% by weight, and put in a serum bottle, crimped and autoclaved. The working concentration in the medium was 0.1% of the stock solution.

(7) Nitrogen gas, zero grade, from Liquid Carbonic

(8) Manosil silicone rubber tubing 3/16" i.d. × 1/16" wall, for the anaerobic reactor, Cat.# P8497-42

(9) Acetic acid glacial AR select from Baxter

(10) Methanol, for HPLC, GC, pesticide residue analysis and spectrophotometry, from Baxter

(11) Filter paper, 47 mm × 0.45 μm, for HPLC solvents, from Gelman Science, Prod.# 66608

(12) Filter paper, 13 mm × 0.20 μm, for HPLC samples, from Gelman Science, Prod.# 66600

(13) Plastic filter holder, 13 mm, for HPLC samples, from Gelman Science, Prod.# 4317

The following chemicals were from Aldrich Chemical Company:

(14) 2-Chlorophenol Cat.# 18577-9

(15) 3-Chlorophenol Cat.# C6280-8

(17) 2,6-Dichlorophenol Cat.# D7020-1

The following chemicals were from Sigma Chemical Company:

(16) 4-Chlorophenol Cat.# C4914

(18) 2,4-Dichlorophenol Cat.# D6023

(19) 2,4,6-Trichlorophenol Cat.# T-1266

(20) Pentachlorophenol Cat.# P-1045

The stock solutions of the chemicals from (14) to (20) were 2500 ppm in 0.1 N NaOH

(21) Bacto Thioglycollate Medium without Dextrose or Indicator, Cat.# 0432-02-6

This solution was used to differentiate the aerobes, facultative organisms and anaerobes. In the thioglycollate tubes, the aerobes would show growth on the top layer, while the anaerobes showed turbidity on the bottom. However, for facultative organisms, the growth was throughout the tube (Difco Manual, 10th. edition, 1984).

EXPERIMENTAL APPARATUS AND PROCEDURES

This project had three phases: the first phase was to build up an anaerobic system able to dechlorinate highly chlorinated phenols; the second phase was to build up an aerobic system able to mineralize the dechlorination product from the anaerobic system; the third phase was to integrate these two systems to perform the above jobs in a batch mode and eventually in a continuous mode.

Thus, in the first phase anaerobic samples were enriched by exposing them to various kind of chlorophenols. It was found that TCP could be dechlorinated to 4-CP successfully. TCP was then chosen as model compound in this study. The effect of phosphate buffer on the anaerobic culture was studied because in the aerobic system, the reaction solution had to be buffered in order to work properly. If the buffering decreased the activity of the anaerobic culture, the feed of the phosphate buffer had to bypass the anaerobic reactor in the anaerobic-aerobic system. The immobilization of the anaerobic culture was performed to prevent washout of the biomass.

In the second phase, an aerobic culture had to be enriched to mineralize 4-CP, the dechlorination product of TCP. Cultures from American Type Culture Collection (ATCC), a sample from the previous anaerobic system and a sample from the anaerobic

digester of a municipal treatment plant were used as sources of inocula. The optimum phosphate buffer concentration was determined for the aerobic system, as well as the effect of residual TCP on the aerobic degradation of 4-CP.

The ability of the sample from the anaerobic system to mineralize 4-CP aerobically led to an attempt to isolate facultative or aerobic organisms from the sample.

In the third phase, an anaerobic packed bed reactor was selected because of the advantages of cell immobilization. Channelling was a common problem in this type of reactor (Barnes and Fitzgerald, 1987), and efforts were made to eliminate it.

The following experiments on biodegradation of chlorophenols were conducted at 30°C in a constant temperature room which was a small, closed, walk-in laboratory whose temperature and humidity could be controlled from an outside panel.

4.1. ANAEROBIC REACTORS

4.1.1. Batch Reactor

Initially the anaerobic batch process was performed in serum bottles, sealed with a butyl rubber stopper and an aluminum crimp seal. The total volume of each bottle was 125 ml. The medium, chlorophenols, and resazurin were put into the bottle under nitrogen. The bottle was then sealed with a crimper. Samples were taken by inserting a syringe needle through the rubber stopper.

4.1.2. Recirculation Reactor System

The recirculation reactor system is shown in Figure 9. The reactor was made of Lucite with 2" diameter and 8" height. There was an inlet located 0.5" above the bottom, and an outlet 0.5" below the top. A screen supporting the immobilization beads was located above the inlet point. Silicon rubber sealant, purchased from R & S Auto Store, was used to seal the reactor from ambient air. A 250 ml storage flask, with a

working volume of approximately 100 ml, was used as a reservoir for the reactor. There was an opening on the top of the storage flask, connected to a flask full of water, for the release of gases produced by the microorganisms. Since this was a relatively small reactor, a small leak of air into either the reactor or the connecting tubing would have had an important impact on the anaerobiosis of the system.

The type of tubing had an important role in keeping the system anaerobic. Only rubber tubing could successfully prevent the permeation of oxygen into the medium. Other types of tubing like PVC or tygon were all subject to oxygen permeation. The fluid pump employed had to be compatible with the normally thick-walled rubber tubing.

4.2. AEROBIC BATCH REACTORS

The aerobic batch reaction was performed in a 250 ml fernbach flask with a white plastic cap. Air was supplied by shaking the flask in an incubator shaker. The working volume was 100 ml.

4.3. ANAEROBIC-AEROBIC REACTORS

This system consisted of an anaerobic reactor followed by an aerobic one as shown in Figure 10. Fluids were pumped via LKB peristaltic pumps.

The anaerobic reactor was a Lucite cylinder, 5" in diameter and 18" in height. Both ends of the reactor were sealed by rubber stoppers with glue and a silicon rubber sealant. The flow direction in the reactor was originally upward and then changed to downward in different runs. There was an I/O port on the reactor located approximately 2" above the bottom. Samples were taken from this port when the flow was downward. Because the I/O port of the lower end was not really at the bottom, about 500 ml of dead reactor volume remained when the reactor was run in the continuous mode.

There were three sampling ports 4" apart from each other along the reactor side. The highest one was 4" below the top. This port was used for sampling when the flow was upward in the reactor. The immobilization beads were stacked from the bottom of the reactor. There was no screen to support the beads. The other I/O port of the reactor was a glass tubing punching through the top rubber stopper. In order to release the gases produced by the anaerobes, there was an opening at the top rubber stopper. Tubing was connected to this opening, with the other end underneath water to provide a seal. Because of the dead volume, the working volume of the reactor was different between the batch mode and the continuous mode. With a void fraction of 0.4 (Pak, 1988), the working volume of the reactor in the batch mode was 2.3 liters, and 2.1 liters in the continuous mode. A T-connector was installed in the tubing leading from the reactor outlet to the aerobic reactor for the purpose of sampling.

The aerobic reactor was a glass cylinder, 4" in diameter and 16" in height. There was no packing, and the aerobic culture was simply suspended in the medium by air bubbling. The working volume was approximately 2.2 liters. Both ends of the cylinder were sealed with rubber stoppers. The flow direction in the reactor was upward. There were two inlets in the reactor, one for the liquid feed and the other for air. The aeration rate was 50 ml per minute. This aeration rate could keep the reaction medium at 60% of oxygen saturation. The solution inlet was a T-connector from two sources: the effluent from the anaerobic reactor, and a 0.5 M, pH 6.8 phosphate buffer solution. The flow rates of these two sources were in the ratio of 10:1, so that the final concentration of the phosphate buffer in the aerobic reactor was about 50 mM. A pH electrode was installed in the top of the reactor to monitor the pH of the solution. There was an opening in the top lid for sampling.

4.4. LKB 2132 MICRO PERPEX PUMP

The flow rate of the continuous anaerobic-aerobic system was in the range of 15 – 30 ml/hour because of the reactor volumes and the residence time required in the system. Typical residence times for each of the reactors were about 3 to 4 days, respectively. It was not easy to find pumps that were able to deliver the solution accurately and reliably at this low flow rate. In general, because the pump heads usually tend to "chew" the tubing, most of the peristaltic pumps commercially available are not reliable.

Micro Perpex was a two-channel peristaltic pump which produced a constant, low pulsation flow. Because of the reliability problem, the tubing for the pump should be inspected every 100 hours and changed after 500 hours, even if no physical damage could be seen. The pump can be used to pump liquids at flow rates of 0.5 to 500 ml/hour per channel, for temperatures from 0 to 40°C.

4.5. MISTRAL 3000i CENTRIFUGE – LARGE CAPACITY BENCH CENTRIFUGE

The centrifuge was used to separate the liquid from the biomass and other sediments for HPLC analysis. The condition was set at 5500 rpm and 20°C for 15 minutes. In the case of pretreatment of the anaerobic sludge, the setting was the same as above but for 4 minutes only.

Centrifuge Speed Range: 200 to 6000 rpm to an accuracy of 10 rpm of set speed.

Centrifuge Temperature Control: Control range is between 0 to 40°C with an accuracy of ± 2°C of the set temperature; however, the selectable range is from -19 to 40°C.

Rotor: 6-place universal angle with maximum speed of 6000 rpm. Cat.# 257-570.

4.6. NEW BRUNSWICK CONTROLLED ENVIRONMENT INCUBATOR SHAKERS

Two models of incubator shaker G-24 (16 sites) & G-25 (40 sites) were employed during the aerobic batch experiments. The operating condition was 30°C and 250 rpm.

Temperature Range: Approximately 5°C above ambient to 60°C

Stroke: Rotary motion with 1 inch circle

Speed Range: Rotary - 40 to 400 strokes/min.

Safety Thermostat: Secondary thermostat provides de-energizing of heater in case of Control Thermostat failure.

4.7. ORION MODEL SA720 pH/ISE METER

This meter has four modes of operation; i.e., rel mV/temp/pH/conc. Standard solutions of pH 7 & pH 10 were used for calibration purposes. The calibration could be done either automatically or manually. The calibration method adopted was "Manual Calibration with two Buffers." Please refer to the manual of the meter.

4.7.1. pH Electrode

An Orion Model 91-56 combination pH electrode with a BNC connector was used. The electrode is effective in the temperature range of 0 to 80°C. The filling solution was 4 M KCl saturated with AgCl (Orion Cat.# 900011). Between measurements and for short-term storage (up to one week) the electrode was stored in 200 ml pH 7 buffer with 1 g of KCl added. An Automatic Temperature Compensation probe (Cat.# 917001) was also employed together with the pH electrode. The temperature range of the ATC probe is 0 to 80°C with the accuracy of ± 0.1°C or 1%, whichever is greater. The applicable pH range for the ATC probe is 0 to 14.

4.7.2. Chloride Electrode

An Orion combination chloride electrode model 96-17B was used for the measurement of chloride concentrations in solution. Two kinds of filling solution, namely, Cat. No. 900017 and Cat. No. 900001, can be used with the electrode. The latter one was suitable for use in samples more concentrated than 10^{-2} M chloride. An ionic strength adjustor (ISA), i.e. 5 M $NaNO_3$, was required to provide a constant background ionic strength. The electrode may be stored upright in a standardizing solution. There is no need to drain the electrode for short periods of storage.

4.8. ANAEROBIC DEGRADATION

Although the media were taken from two sewage treatment plants, only the consortium from the Livingston plant was used as an anaerobic inoculum. The solution taken from the Joint Meeting Company was mostly used as medium.

4.8.1. Procedure Used to Determine Anaerobic Degradation of Various Chlorophenols in Batch Reactor

A number of monochlorophenols and dichlorophenols were individually tested for degradation by the anaerobic mixed culture in the original medium from the sewage plant. The experiments were performed over a two month period in the serum bottles, with chlorophenols and resazurin added.

4.8.2. Procedure Used to Determine Anaerobic Degradation of TCP in Batch Reactor Without Addition of Phosphate Buffer

Unautoclaved medium containing anaerobic mixed culture was injected into serum bottles about 90 - 95 % full. TCP and native organic compounds in the medium served as carbon sources.

4.8.3. Procedure Used to Determine Effect of Phosphate Buffer on the Anaerobic Degradation of TCP in Batch Reactor

An anaerobic mixed culture taken from the anaerobic reactor, where TCP dechlorination had been observed and 4-CP was already produced, was incorporated with different levels of 1 M phosphate buffer of pH 6.8 to make final phosphate concentrations of 0, 50, 100, and 150 mM. TCP was then added to each reactor.

4.8.4. Procedure Used to Determine Immobilization of the Anaerobic Culture

In the anaerobic reactor, R-635 beads were immersed in the unautoclaved medium which contained anaerobic culture together with 101 μM of TCP. The reactor was in batch mode to allow the microorganisms' growth. Another 40 μM of TCP was spiked into the system after 317 hours when the original input TCP had been consumed. To verify that the immobilization process was successful, the original medium was drained, leaving only the immobilized beads in the reactor. The autoclaved medium with 152 μM of TCP was then added to the reactor.

4.8.5. Procedure Used to Determine Anaerobic Degradation of TCP in the Recirculation Reactor

Autoclaved medium including TCP was added to the recirculation reactor packed with Manville R-635 beads, on which the anaerobic mixed culture was immobilized. TCP was spiked into the system in the second run of the experiment. At this time, 4-CP had already accumulated in the reactor from the first run.

4.9. AEROBIC DEGRADATION

4.9.1. Medium Preparation

All media used in these experiments were the effluents from the anaerobic reactor, unless otherwise specified. These media contained 4-CP from the previous anaerobic process.

4.9.2. Air Stripping of 4-CP

This experiment was performed in the aerobic reactor to determine the rate of removal of 4-CP by air stripping. Two liters of autoclaved fresh medium, buffered with 100 ml of 1 M, pH 6.8 phosphate, was put into the reactor. A 4-CP concentration of 169.4 μM was spiked into the reaction medium. The aeration rate was 50 ml per minute. The stripping test lasted for 4 days.

4.9.3. Procedure Used to Determine Aerobic Degradation of 4-CP Without Buffering the Medium

Twelve ATCC cultures (see Table 8) were grown individually in autoclaved supernatant from the sewage plant in shaker flasks for three days. The medium was the same as those for anaerobic microorganisms. Then, 140 μM of 4-CP was added to each flask to test for biodegradation. Two runs of this experiment were performed. However, in the second run only the four ATCC cultures that showed better degradation of 4-CP were used.

4.9.4. Procedure Used to Determine Aerobic Degradation of 4-CP with the Addition of Phosphate Buffer in the Middle of the Reaction

Eleven ATCC cultures (see Table 9) were individually inoculated into shaker flasks to repeat the above experiments. ATCC culture #14235 was not used. Forty-two hours later, the pH values increased to 9, and no significant degradation was ob-

served. At this time, 5 ml of each pure culture were again inoculated and 250 mM phosphate buffer was added to adjust the values of pH in the range of 7.8 to 8.2.

4.9.5. Procedure for Studying the Effect of Different Concentrations of Phosphate Buffer in the Medium on the Aerobic Degradation of 4-CP

ATCC cultures #93 (*Serratia Marcescens*), #17514 (*Pseudomonas Putida*), #17991 (*Serratia Marcescens*), #29195 (*Pseudomonas Glathei*), and #33668 (*Pseudomonas Pseudoflava*) were grown in nutrient broth for one day and separately inoculated into the shaker flasks, where different levels of 1 M phosphate buffer of pH 6.8 were added to adjust the pH value of each culture. The final concentrations of phosphate in the media were 50, 100 and 150 mM, respectively. Uninoculated solutions served as controls. Four controls with different levels of phosphate from 0 to 150 mM were examined.

4.9.6. Procedure for Studying the Effect of the Presence of TCP on the Degradation of 4-CP

An effluent of 200 ml from the anaerobic reactor was buffered with 0.5 M, pH 6.8 phosphate to reach a final concentration of 50 mM phosphate. The 4-CP produced from the anaerobic dechlorination of TCP was the only chlorophenol present in the effluent. The effluent was separated into two parts. TCP was added into one part, while the other part served as a control. These two solutions were then added to two batch aerobic reactors.

4.10. ISOLATION OF FACULTATIVE AND AEROBIC MICROORGANISMS IN THE ANAEROBIC CONSORTIA

About 100 ml of the effluent from the anaerobic reactor was transferred to a shaker flask. Only 4-CP was present in the solution, as a result of anaerobic dechlorination of TCP. After three days of aerobic degradation, the 4-CP was completely mineralized. A sample from the shaker flask was streaked on a nutrient agar plate. Three different types of colonies were observed. They were further streaked to make sure that the grown colonies were pure.

4.10.1. Oxygen Requirement Test for the Isolated Pure Cultures

Thioglycollate tubes were used to test the oxygen requirement of the cultures. The bacteria were taken by a flamed inoculating loop from the agar plate mentioned in the previous section, and were inoculated into the thioglycollate tube. The tubes were incubated at 30°C overnight. The categories of oxygen requirement could be determined from the way the bacteria grew in the tubes.

4.10.2. Anaerobic Dechlorination of TCP by the Isolated Pure Cultures

Fresh autoclaved medium with about 90 μM of TCP was prepared in four serum bottles. No phosphate buffer was added. The inocula were the bacteria grown in the thioglycollate tubes. The inoculum size was 5 ml. The fourth bottle was not inoculated.

4.10.3. Aerobic Degradation of 4-CP by the Isolated Pure Cultures

Fresh autoclaved medium with about 220 μM of 4-CP was prepared in four shaker flasks. The medium was buffered with 0.5 M phosphate buffer pH 6.8 to reach a final phosphate concentration of 50 mM. The inocula were the bacteria grown in the

thioglycollate tubes. The inoculum size was 5 ml. The fourth flask was not inoculated.

4.11. ANAEROBIC-AEROBIC DEGRADATION

The anaerobic-aerobic system consisted of two reactors in series. The first reactor was operated anaerobically and loaded with Manville R-635 beads. The effluent from this reactor plus 0.5 M pH 6.8 phosphate buffer were brought together into the bottom of the suspended growth aerobic reactor. Aeration was performed by bubbling house air from the bottom. The pH of the medium in the aerobic reactor was monitored.

4.11.1. Procedure Used to Determine Degradation of TCP in the Batch Mode

The first part of this experiment was performed in the anaerobic reactor. The fresh autoclaved medium with TCP and resazurin was put into the anaerobic reactor. The sampling was done every day to monitor the dechlorination process. When the TCP was completely converted to 4-CP, the medium was transferred to the aerobic reactor. 0.5 M phosphate buffer pH 6.8 was added in the ratio of 1:4 to the medium. Thus, the final concentration of 4-CP in the anaerobic reactor was different from the initial concentration of 4-CP in the aerobic reactor. The aeration rate was 50 ml per minute. The working volume for the anaerobic reactor was 2.3 liters, and the final volume for the aerobic one was 1.9 liters. Originally, the anaerobic reactor was operated under nitrogen to make sure no oxygen was present in the system, but the procedure was then found unnecessary.

4.11.2. Procedure Used to Determine Degradation of TCP in the Continuous Mode

The system was initially started in batch mode with TCP in the anaerobic reactor and 4-CP in the aerobic one at the same time. After the reactions were completed in both reactors, the continuous mode was started. The working volumes for the anaerobic and aerobic reactors were 2.1 and 2.15 liters, respectively. The input flow rate to the anaerobic reactor was 20 ml per hour. The input flow rate to the aerobic reactor was 20 ml per hour anaerobic effluent, plus 2 ml per hour 0.5 M, pH 6.8 phosphate. Hence, the residence times for the reactors were 105 and 97.7 hours, respectively.

Originally, the flow direction in the packed-bed anaerobic reactor was upward, but it was then changed to downward because of channelling. No nitrogen gassing was necessary in the anaerobic reactor, as determined by the color of the resazurin dye. The flow direction in the suspended growth aerobic reactor was always upward.

RESULTS AND DISCUSSIONS

5.1. ANAEROBIC DECHLORINATION PROCESS

The anaerobic culture was obtained from the anaerobic digester of the Livingston sewage treatment plant which treated solely domestic sewage. It was supposed to be composed of obligate anaerobes and facultative organisms, but aerobes were later found in the culture.

5.1.1. Anaerobic Medium

For the culture to work properly, it is important for the medium to have a low redox potential, not only a low oxygen level.

A reduction in the redox potential of the medium is usually achieved by applying reducing agents. Sodium thioglycollate (0.05%), Cysteine·HCl (0.025%), Dithiothreitol (0.05%), $Na_2S·9H_2O$ (0.025%), and Cysteine·HCl + $Na_2S·9H_2O$ (0.025% + 0.025%) are some of the most widely used reducing agents (Costilow, 1981).

When the anaerobic experiments were first started, a mixture of Cysteine·HCl and $Na_2S·9H_2O$, typically used for the most stringent anaerobes, was employed as the reducing agent. However, it was then found that the consortia adapted to the environment so well that the reducing agent was not needed in the medium.

The Chemical Oxygen Demand (COD) of the anaerobic medium before TCP was added was over 500 ppm, which suggested that there were a great deal of carbon sources in the medium. The COD's of the medium after anaerobic treatment and aerobic treatment were not measured.

5.1.2. Isolation of the Consortia

A significant amount of work was spent to enrich the anaerobic culture in the presence of chlorophenols. The anaerobic inoculum from the Livingston Sewage Treatment Plant was enriched to degrade chlorophenols.

At the beginning, the enrichment was performed in the defined medium, where sodium acetate was used as the main carbon source. No degradation of the chlorophenols was observed.

The medium from the sewage plant was then directly used as nutrient source for the enrichment after some pretreatment steps including sterilization (see section 3.5.1.).

Anaerobic samples from the Livingston plan were then exposed to 2,4-DCP, 2,6-DCP, 3,5-DCP, 2-CP, 3-CP, and 4-CP for a period of two months. At the end of this period, the cultures were analyzed to see if any degradation of the toxic compounds had occurred. Table 2 shows the results.

For the three types of monochlorophenol, the *para*-isomer was the most recalcitrant to anaerobic degradation. The *ortho*- and *meta*- isomers were degraded at approximately the same rates. Among the three dichlorophenols, the one with chlorines on the *meta* position was the most difficult to attack.

Several research groups (Hakulinen et al., 1985; Mikesell and Boyd, 1986; Dietrich and Winter, 1990) have reached similar results in the anaerobic degradation of chlorophenols. The chlorine atom in the *ortho* position is removed more rapidly than from *meta* and *para* positions.

For the removal of *meta* Cl, Suflita and Boyd (Suflita et al., 1982; Boyd et al., 1983; Boyd and Shelton, 1984) reported that it was most susceptible to attack by anaerobic consortia, when halogenated benzoates were treated.

For those chlorophenols which were attacked by the consortia, the more chlorines on the benzene ring, the faster the degradation rate proceeded. This is consistent with observations made by Mikesell and Boyd (1986).

5.1.3. Mass Balance for the Anaerobic Dechlorination of TCP by Suspended Microorganisms in the Batch System

At the beginning of these experiments, there was 4-CP and phenol in the reactor from previous runs. The TCP was first dechlorinated to 2,4-DCP, and then further into mostly 4-CP, with some phenol. Two runs of the experiment were performed in series and the degradation rates of TCP increased from 21.7 to 43.2 μM/day. It took more than five days for the consortia to degrade 2,4-DCP in the first run; on the other hand, in less than three days, 2,4-DCP was degraded completely in the second run. The speeding up of the dechlorination process implied that the bacteria became acclimated to the chlorophenols. Table 3 and Figures 11 and 12 show the experimental results. In the Table 3, the column of Sum was calculated by summing up the concentrations of TCP, 2,4-DCP, 4-CP and phenol. The column of % of Recovery was calculated by dividing the sums after the reactions were started by the sums at the beginning of the reactions. Thus, the % of recovery at the beginning of the reaction was set at 100. The initial concentrations of 4-CP and phenol were deducted from the measured concentrations to be shown in the Figure 12.

The degradation products were first identified by comparison with the retention times of the known standards in the HPLC. The results were further confirmed by comparing the UV spectra of the dechlorination products with those of known stan-

dards. Anaerobic dechlorination instead of dearomatization was expected from other researchers' works (Suflita et al., 1982; Horowitz et al. 1983).

The characteristic of an enzymatic reaction is that it is site-specific. Hence, no other degradation compounds, for example, 2,6-DCP or 2-CP, were found among the reaction products. By accounting for 2,4-DCP, 4-CP, and phenol the mass balances closed quite well, suggesting that there were no additional compounds produced during the degradation process.

In this work, the intermediate and the final product of the anaerobic dechlorination of TCP was determined although complete mineralization was not occurred. This enabled one the possibility to get the microorganisms already known to degrade the product for further treatment.

5.1.4. Effect of Phosphate Buffer on the Anaerobic Degradation of TCP by the Free Microorganisms in the Batch System

Anaerobic dechlorination in the presence of four concentrations of the phosphate buffer, namely, 0, 50, 100 and 150 mM, in the medium was also tested.

The starting pH of the reaction broth was between 7.4 and 9.0. Within 63 hours, all TCP was totally converted to 4-CP without adding any amount of phosphate. If there was any phosphate present, the dechlorination was slowed down dramatically. In the 50 mM phosphate solution, there was still some dichlorophenol to be dechlorinated after more than one week's reaction. The dechlorination of TCP did not take place in the 100 mM phosphate solution until one week later. No dechlorination took place at all when the phosphate concentration was 150 mM. Table 4 and Figures 13 to 16 show the results.

It was obvious that the activity of the anaerobic consortia was inhibited by the phosphate buffer. In all cases, the mass balances were close to 100%. Phenol was no longer present as a degradation product.

Later, it was shown that phosphate buffer solution was necessary for the subsequent aerobic mineralization of 4-CP. These results suggested that in the anaerobic-aerobic system the feed of buffer solution should feed directly to the aerobic reactor so that the anaerobic dechlorination of TCP would not be inhibited.

5.1.5. Immobilization of the Anaerobic Microorganisms in the Porous Silica Beads and TCP Dechlorination during Immobilization

Manville Celite catalyst carrier, R-635 silica beads were used as a support for the immobilization of the microorganisms. The beads were immersed in the reaction medium containing the anaerobic consortia and 101.3 μM of TCP. After one week, a biofilm of bacteria could be seen on the surface of the R-635 beads. The first sample was taken after about two weeks. TCP had been completely dechlorinated to 4-CP at this time. Thus, 40.5 μM of TCP was again spiked into the reactor to feed the microorganisms. The spiked TCP was dechlorinated to 4-CP within one day, and the mass balance was closed. Table 5 and Figure 17 show the results.

In order to ensure that the biofilm on the beads was the culture degrading TCP, another experiment was performed. The reaction broth of the immobilization process was drained out from the system. Then, fresh autoclaved medium with 150 μM of TCP was introduced into the reactor. This insured that only the microorganisms in the biofilm would be responsible for the dechlorination of TCP in the system. The TCP disappeared after two days, and 2,4-DCP appeared after one day and disappeared after four days. 4-CP was produced and remained in the reactor. Figure 18 shows the results.

From this experiment, we concluded that the immobilized bacteria were responsible for the dehalogenation reaction, and were able to complete the process at a faster rate than previously observed (comparing these results with those in Table 3, and Figure 11 & 12). The mass balance was satisfactorily closed in these experiments.

5.1.6. Mass Balance for the Anaerobic TCP Degradation Process in the Immobilized Recirculated Reactor System

In Run 6-1, the initial concentration of TCP was 111.4 μM. There were still 23 μM of TCP left in the system after six days. No 2,4-DCP was observed, although 4-CP was produced during the reaction period. In Run 6-2, 152 μM of TCP disappeared after two days. In the same experiment, 2,4-DCP was formed after one day and disappeared after another day. The entire reaction was completed in two days, resulting in a reaction product containing 4-CP only. The mass balance could be closed in both runs. Table 6 and Figures 19 and 20 show the results.

5.2. AEROBIC DEGRADATION PROCESS

Anaerobic dechlorination of TCP did not proceed beyond 4-CP. However, this degradation product was relatively easy for the aerobic culture to attack. One way to achieve this goal was to purchase pure cultures from ATCC, which were known to degrade phenol. Another possibility was to select for a 4-CP-degrading aerobic population from the mixed culture obtained from a wastewater treatment plant assuming that some indigenous microorganisms capable of degrading 4-CP under aerobic conditions could be obtained from the anaerobic culture.

The level of dissolved oxygen was not measured in the following experiments performed in shaker flasks.

5.2.1. Stripping of 4-CP

In the aerobic system, disappearance of 4-CP could be due to air stripping as well as biodegradation. Thus, it was necessary to study the effect of stripping of 4-CP in the aerobic reactor. Table 7 and Figure 21 show the results. In a period of 4 days, the concentration of 4-CP (164.9 μM) remained essentially the same. Hence, microbial activity would account for any disappearance of 4-CP during that time period.

5.2.2. Chloride Ion Measurement in the Aerobic Reactor

Because the medium was from a sewage treatment plant, it always contained a large amount of chloride ions. The working medium was found to have a chloride concentration of about 8.5 mM. However, from theoretical calculation, the chloride released from the mineralization of 4-CP usually ranged from 100 to 200 μM. With such a high amount of background chloride, the measurement of the increased chloride in the aerobic system was not practical. Therefore, no chloride concentration measurement were made in this work.

A synthetic medium without background chloride should be formulated for this purpose. This may not easy to find, since the co-substrates and electron acceptors added in the medium should match the characteristic need of the anaerobic and aerobic cultures. Rumen fluid (Dolfing and Tiedje, 1987), or yeast extract and peptone (Dietrich and Winter, 1990) could be a first trial in future work.

5.2.3. Measurement of Reaction Rates

Rates of reaction can only be compared qualitatively since biomass was not measured. The original idea of using the ATCC cultures was to test if any of them were capable of degrading 4-CP. Thus, the biomass was not measured.

5.2.4. Aerobic Degradation of 4-CP by the ATCC Cultures

Twelve ATCC cultures able to degrade phenol were tested for the aerobic degradation of 4-CP present in the treated anaerobic medium. The initial concentration was 140 μM and no phosphate buffer was added. After 78 hours, four of the ATCC cultures showed better ability to degrade 4-CP than the other eight. The experiment was repeated on these four cultures. This time the initial concentration of 4-CP was about 200 μM. Only a slight degradation of 4-CP was observed for all four cultures after 46 hours. Table 8 shows the results.

Similar experiments were repeated on eleven ATCC cultures since the above experiments did not give satisfactory results. All cultures started with 165.6 μM of 4-CP. After forty-two hours of reaction, it was found that the pH's of all the cultures rose to about 9. Consequently, each culture was re-inoculated to prevent the extinction of the bacteria, and phosphate buffer was added to bring down the values of pH in the range of 7.8 to 8.2. At 87 hours, all the cultures showed appreciable degradation. Five of the cultures, ATCC cultures #93 (*Serratia Marcescens*), #17514 (*Pseudomonas Putida*), #17991 (*Serratia Marcescens*), #29195 (*Pseudomonas Glathei*), and #33668 (*Pseudomonas Pseudoflava*) completely degraded 4-CP. Table 9 shows the results. This experiment demonstrated that the pH of the medium could rise to 9 and cause inhibition of 4-CP degradation.

The indigenous cells in the anaerobically treated medium were removed from the culture by centrifugation. The rest of the solution was put in a shaker flask. Without any further treatment, the pH value of the solution rose. This suggested that lysis of the strict anaerobes was not the cause of the rise in pH.

The stock solution of TCP was prepared in 0.1 N NaOH. Thus it was highly basic. When stocked TCP was added to the anaerobic reactor, the pH of the reaction medium usually ranged from 8.5 to 9.0 depending on the amount of TCP added. The pH was observed first dropping in the anaerobic reactor and then increasing back to approximately the initial pH in the aerobic reactor. Sodium hydroxide from the stock solution could be a possible cause of the pH rise.

The rise in pH could be reduced by adding phosphate buffer to the cultures. Three concentrations of phosphate buffer, i.e. 50, 100, and 150 mM, were tested on the aforementioned five ATCC cultures. Tables 10 to 14 and Figures 22 to 31 show the results. There were altogether fifteen runs of this set of experiments whose pH values ranged from 7.0 to 7.2 at the beginning of the reaction. The pH values went up to 7.4 – 8.7 in six hours and then remained constant.

ATCC cultures #93 and #17991 degraded 4-CP effectively in the 50 mM phosphate buffer in eighteen hours. When 100 or 150 mM phosphate buffers were used, there were still appreciable amounts of the compound left at the end of reactions.

On the contrary, ATCC culture #17514 in 50 mM phosphate could not degrade 4-CP as effectively as in the 100 or 150 mM phosphate buffers. An important observation was that this culture produced an unidentified degradation product while 4-CP was degraded. This product appeared in the chromatograph of HPLC and had a retention time of around three minutes. There was no further study on this compound.

ATCC culture #29195 degraded 4-CP completely in three concentrations of the phosphate buffer in eighteen hours. However, the degradation rate in the 100 mM phosphate buffer seemed to be the fastest among the three, since the concentration of 4-CP dropped to 4.7 μM in thirteen hours.

ATCC culture #33668 could not completely consume 4-CP in all three concentrations of the phosphate buffer within the reaction period. Even worse was that a degradation product was formed during the reaction. The retention time of this product was similar to the product formed by ATCC #17514 in the HPLC chromatograph.

In conclusion, ATCC cultures #93, #17991, and #29195 were better 4-CP degraders, since they could degrade 4-CP effectively without forming any degradation product.

It should be stressed that these experiments were conducted in a medium where 4-CP was not the only carbon source. However, COD, was not measured at the beginning of the experiments. The outcome might be different if the experiments were performed in a defined medium where 4-CP was the only carbon source.

5.2.5. Aerobic Degradation of 4-CP by the Indigenous Culture from the Immobilized Anaerobic Reactor

Experiments were made to test whether the anaerobic culture which degraded TCP could also degrade the dechlorinated product, 4-CP, under aerobic conditions. Table 15 and Figures 32 to 36 show the results. There was no inoculation of bacteria from outside in these experiments.

In Run 15-1 (see Figures 32 and 33), the effect of different concentrations of phosphate buffer was examined. Four concentrations of phosphate buffer were tested, namely, 0, 50, 100 and 150 mM. The 4-CP in the medium was from the dechlorination of TCP in the anaerobic reactor. The starting concentrations of 4-CP in different phosphate concentrations were different because of the dilution of the phosphate buffer. The control run without any phosphate added showed no degradation of 4-CP at all, and the pH went up to 8.8. In the 50 and 100 mM phosphate solutions, the indigenous cultures degraded 4-CP in a period of eighteen hours. For an even higher concentration of phosphate, i.e. 150 mM, the activity of the culture was inhibited, and the degradation was slowed down appreciably. In all cases, a rise in pH was observed.

The ATCC culture #29195 (see Table 13 and Figure 28) degraded 4-CP to 21.0, 4.7 and 50.5 μM in 50, 100 and 150 mM phosphate buffered solutions (respectively), in thirteen hours, as compared to 77.8, 84.8 and 89.4 μM by the indigenous culture. The ATCC culture #17514 (see Table 11 and Figure 24) degraded 4-CP to 44.3 and 59.9 μM in 100 and 150 mM phosphate buffered solutions (respectively), also, in thirteen hours. ATCC culture #93 (see Table 10 and Figure 22) degraded 4-CP to 28 μM in 50 mM phosphate buffered solution in thirteen hours. In all other cases (see Tables 10-15 and Figures 22, 24, 26, 28, 30 and 32) the indigenous culture was a better degrader of 4-CP. However, since the amount of biomass was not measured, the above comparisons may not reflect the real specific degradation rates.

In Run 15-2 (see Figures 34 and 35), three concentrations of phosphate, namely, 0, 50, and 100 mM, were tested. Similar results were obtained. About 200 μM of 4-CP were completely degraded in 66 hours with 50 or 100 mM phosphate buffer. However, a lag phase of one day was observed.

In Run 15-3 (see Figure 36), the phosphate concentration was only 50 mM. 57.7 μM of 4-CP were degraded in two days. No lag phase was observed in this run.

The indigenous aerobic culture from the immobilized anaerobic reactor could degrade 4-CP aerobically when buffered with the phosphate solution. The lag phase in Run 15-2 might be caused by the high starting 4-CP concentration (inhibition).

After the anaerobic treatment of TCP in the medium, the HPLC chromatographs still showed some peaks which derive from the original sterilized medium. These peaks were present at the beginning of the anaerobic process. After aerobic treatment, these peaks did not appear in the HPLC chromatographs.

The ability of the indigenous culture from the anaerobic reactor to aerobically degrade the product (4-CP) of TCP dechlorination is an advantage of the anaerobic-aerobic system. First, there is no need to inoculate an aerobic culture from outside. Secondly, since the aerobic culture is continuously fed from the anaerobic reactor, the possibility of washing out the culture from the aerobic reactor is minimized when the reactors are run in continuous mode. Therefore, there is no need to immobilize the culture in the aerobic reactor, and as a result the volume of the suspended growth aerobic reactor can be smaller than a comparable packed-bed reactor, because of the elimination of the bead volume.

5.2.6. Aerobic Degradation of 4-CP by the Anaerobic Culture From a Different Municipal Treatment Plant

An anaerobic culture from a different municipal treatment plant (Joint Meeting Company, Elizabeth, NJ) was tested for the aerobic degradation of 4-CP. The medium

was again the treated anaerobic medium. Three concentrations of phosphate buffer, namely, 50, 100 and, 150 mM, were tested. Their pH values and 4-CP concentrations were monitored. Table 16 and, Figures 37 and 38 show the results.

The pH values went up from 7.0 − 7.2 to 7.4 − 8.5 in six hours and remained quite steady afterwards. When the medium was not buffered, there was no degradation of 4-CP. If the medium was buffered by 50, or 100 mM phosphate, 4-CP could be completely degraded in eighteen hours. However, when the medium was buffered by 150 mM phosphate, the final concentration of 4-CP was still half of the initial concentration after 18 hours.

The degradation rate of 4-CP by the culture from the Joint Meeting Company was comparable to that by the indigenous culture except in the 50 mM phosphate solution (see Tables 15 and 16, and Figures 32 and 37). In the 50mM phosphate solution, there were still 28 μM of 4-CP left in the medium as compared to complete consumption of 4-CP in the indigenous culture.

5.2.7. The Effect of TCP on the Aerobic Degradation of 4-CP

The indigenous culture from the anaerobic reactor and ATCC culture #29195 were tested for this effect in the shaker flasks. ATCC culture #29195 was chosen because it was the best 4-CP degrader among other ATCC cultures tested (see section 5.2.4.). In both cultures, the media were buffered with 50 mM phosphate to facilitate the degradation process. The 4-CP in the media was the dechlorination product from the anaerobic reactor. The medium in each culture was divided into two parts. One of them was spiked with TCP. Tables 17 and 18, and Figures 39 to 42 show the results.

Although TCP could be degraded aerobically by the indigenous culture, the rate was slower than anaerobic degradation (compared to Run 3-2, Figure 12; Run 4-1, Figure 13; Run 5-2, Figure 18; and Run 6-2, Figure 20).

The initial concentrations of 4-CP were 61.4 and 66.9 μM in the media without and with TCP, respectively, for the test on the indigenous culture from the anaerobic reactor. The initial TCP concentration was 94.7 μM. In both media 4-CP was almost completely degraded in one day. The presence of TCP did not influence the degradation rate of 4-CP.

A noteworthy observation was that TCP could be degraded by the indigenous culture aerobically. However, the reaction pattern was different, in the sense that TCP was not dechlorinated to 4-CP in this case. Instead, TCP was mineralized, since no other peaks were found in the HPLC chromatographs. This result indicated that the presence of TCP in the aerobic reactor would not prevent 4-CP degradation.

For the test of ATCC culture #29195, the starting concentrations of 4-CP were 172.6 and 170.3 μM in the media without and with TCP, respectively. The starting TCP concentration was 52.2 μM. In both runs, the concentration of 4-CP dropped to about 18 μM within one day. However, only a little TCP was degraded in the reaction period of two days.

In a comparison of the performance of the two cultures, the indigenous one was more desirable for its ability to degrade TCP aerobically. Thus, even in the event that any residual TCP remained in the solution after the anaerobic treatment step, it would be subject to degradation in the aerobic reactor (albeit at a slower rate).

5.3. FACULTATIVE AND AEROBIC MICROORGANISMS ISOLATED FROM THE ANAEROBIC CONSORTIA

Three kinds of bacteria were isolated from nutrient agar plates. When these were inoculated into the thioglycollate tubes, bacterium #1 showed growth throughout the tube; bacteria #2 and #3 showed growth on the surface layers only. This indicated that bacterium #1 was a facultative organism, while bacteria #2 and #3 were aerobes.

A drop of the solution from the anaerobic reactor was inoculated into this thioglycollate tube, and there was growth throughout the tube.

It is surprising that aerobic microorganisms were surviving in the anaerobic system. However, this observation has been made by other researchers. McCarty (1962), Toerien (1967), Taylor et al. (1970), Ferry and Wolfe (1976), and Hakulinen et al. (1985) have isolated *pseudomonas* species from anaerobic conditions. Hakulinen et al. (1985) found that the survival of *pseudomonas* under anaerobic conditions might be due to the presence of another anaerobic microorganism, *Klebsiella oxytoca.*

Due to experimental limitations, this work was not performed under anaerobic conditions, which meant that there might be undetected obligate anaerobes in the anaerobic reactor.

5.3.1. Anaerobic Degradation of TCP by the Isolated Pure Cultures

This experiment was performed in serum bottles without immobilization of the cells. Four bottles were used: bacteria #1 to #3 plus a control in which no bacteria were inoculated. The starting concentrations of TCP ranged from 87.1 μM to 122.1 μM. Table 19 and Figure 43 show the results. After about six days, none of the TCP in the reactors was degraded.

Hence, the dechlorination of TCP to 4-CP was not likely to be done by any of these three bacteria alone. The obligate anaerobes existing in the anaerobic reactor were likely to be responsible.

5.3.2. Aerobic Degradation of 4-CP by the Isolated Pure Cultures

This experiment was conducted in shaker flasks. A control was included in the experiment. The initial concentrations of 4-CP ranged from 210.0 to 254.3 μM, as effluent from the packed-bed anaerobic reactor. Table 20 and Figure 44 show the re-

sults. The 4-CP in the control reactor and the facultative anaerobe reactor was not degraded at all after six days. Bacterium #2 consumed 225.5 μM of 4-CP in four days. And bacterium #3 degraded 210.0 μM of 4-CP in between four and six days. It was likely that these two were responsible for the aerobic degradation of 4-CP in the anaerobic-aerobic system. Since the amount of biomass inoculated was not controlled, it was hard to compare the reaction rates between these pure cultures and the previously used mixed culture.

5.4. ANAEROBIC-AEROBIC SYSTEM IN BATCH MODE

The initial concentration of TCP was 106.4 μM. The TCP was not attacked for two days. It was then dechlorinated completely after another three days. After two days, 2,4-DCP was produced and was subsequently converted to 4-CP in another three days. 104.2 μM of 4-CP appeared in the anaerobic medium in five days, and remained almost constant for another day. The pH value of the solution started at 8.6 and remained almost constant when the TCP was not dechlorinated. At the end of the second day, the pH began to drop from 8.5 to 8.3 in a period of four days.

At beginning of the seventh day, the anaerobic medium was transferred to the aerobic reactor to degrade the 4-CP generated anaerobically. In the aerobic reactor, the initial concentration of 4-CP was 75.1 μM, which was different from the final concentration of 4-CP (98.0 μM) in the anaerobic reactor. This resulted from the addition of phosphate buffer to the reactor. 4-CP was completely degraded in less than three days. The corresponding pH value increased from 7.2 to 8.0 in six days and stayed at 8.0. Table 21 and Figures 45 and 46 show the results.

Before this experiment was performed, the anaerobic reactor was not in use for approximately a month. Thus, the immobilized microorganisms were not fed TCP during this period. The microorganisms were just immersed in the sterile medium

containing some 4-CP from previous runs. The lag phase in the dechlorination of TCP was probably due to this reason. If this lag period was deducted, the dechlorination rate was comparable to previous experimental results (see Run 3-2).

The fast recovery of the anaerobic consortia to dechlorinate TCP suggested that the consortia was very stable. Hence, the consortia needed little maintenance to keep it active. This is consistent with the claim made by Anderson et al. (1984).

The pH varied in both the anaerobic and aerobic reactors in a manner similar to previous experiments (i.e., pH dropped in the anaerobic reaction and rose in the aerobic one). Volatile fatty acids produced from the sterile sewage under anaerobic processes, and HCl produced from the reductive dechlorination of TCP, were expected to lower the pH (see also Dietrich and Winter, 1990).

The total reaction took 9 days. However, if the lag period of two days was deducted and the anaerobic solution was transferred right after the complete disappearance of the TCP, the complete mineralization took 6 days only, divided roughly evenly between the anaerobic and aerobic reactors. This degradation rate was comparable to those of previous experiments (see Run 3-2 and Run 17-1) for 106.4 μM of TCP.

5.5. ANAEROBIC-AEROBIC SYSTEM IN CONTINUOUS MODE

5.5.1. Upflow Anaerobic Reactor

Two runs were performed. Table 22 and Figures 47 to 50 show the results. In both runs, TCP in the anaerobic reactor was dechlorinated as expected, and 4-CP was successfully degraded during batch mode. However, when the continuous mode was started up, the concentrations of 4-CP and TCP gradually increased in the aerobic reactor (although only 4-CP was detected in the samples taken from the anaerobic reactor). The input feed concentration of TCP was about 100 μM.

A stripe of particularly dark color was observed above the feed inlet port in the anaerobic reactor. This stripe was due to the biomass growing on the input nutrients. Channelling has been a common drawback of upflow packed bed reactors (Barnes and Fitzgerald, 1987). In both runs the appearance of TCP in the aerobic reactor without being detected in the anaerobic reactor was a result of the channelling. The sampling port in the anaerobic reactor was not directly above the inlet. Therefore, the collected samples contained no TCP but 4-CP from previous experiments.

It was not understood why the aerobic system could not degrade TCP and 4-CP, since from the results of previous experiments, the aerobic culture was expected to be able to degrade these two compounds together.

In Run 17-2, the culture degraded 66.9 μM of 4-CP in about two days; 94.7 μM of TCP was degraded to 12.2 μM in six days. The residence time of the aerobic reactor was 97.7 hours. Short residence time might be a reason for the TCP accumulation in the reactor.

5.5.2. Downflow Anaerobic Reactor

Because of the channelling problem in the previous experiment, the flow direction in the anaerobic reactor was changed from upward to downward. The flow direction in the aerobic reactor remained upward.

The problem of channelling was then eliminated with this change. In the upflow reactor, the influent feed hit the immobilizing beads as soon as it entered the reactor which might cause channelling. On the other hand, in the downflow reactor, the feed mixed with the medium before it contacted the beads since there was about one inch high of the medium above the beads in the reactor.

In batch mode, the anaerobic reactor started with 231.9 μM of TCP, all of which disappeared within 3 days. In a period of 6 days, 2,4-DCP was produced and consumed and 4-CP accumulated from 51.3 to 289.3 μM. In the aerobic reactor,

205.3 μM of 4-CP disappeared in 2 days. Thus continuous mode was ready to start after 6 days' batch reaction.

Table 23 and Figures 51 & 52 show the results in both batch and continuous reactions.

In continuous mode, the input concentrations of TCP ranged from 113.4 to 130.6 μM because of the need to change storage tanks. Samples from the anaerobic reactor were taken from the outlet port. No TCP or 2,4-DCP was detected in the samples. The concentration of 4-CP at the anaerobic reactor outlet was about 230 μM for 7 days. It then dropped to 80.1 μM and rose to a steady concentration about 105 μM. This concentration of 4-CP approximately matched the input concentration of TCP from 81 (105/130.6) to 93% (105/113.4). 4-CP concentration of 230 μM was due to the dechlorination of original TCP in batch mode.

In the aerobic reactor, no 4-CP was detected in the effluent samples.

The system was operated for four weeks. Hence the integrated anaerobic-aerobic system successfully mineralized input TCP in a two-step process in which TCP was dechlorinated to 4-CP in the anaerobic reactor, and 4-CP was mineralized in the aerobic reactor.

During the process, it was observed that the feed solution in the storage tank was contaminated with external bacteria, since the original pink color of the resazurin dye disappeared, indicating oxygen consumption. Bacterial colonies were observed when a sample from the feed tank was streaked on nutrient agar. Frick et al. (1988) had the same problem in the aerobic degradation of PCP. Their system failed because of the contamination. Fortunately, in our system bacterial contamination of the feed tank did not appear to alter the results of the experiments.

Hakulinen and Salkinija-Salonen (1982) successfully used an anaerobic fluidized bed reactor and an aerobic trickling filter to treat PCP. In the outlet of the aerobic re-

actor, only carbon dioxide was detected. However, they did not mention the fate of PCP in the anaerobic reactor, nor the degradation rate.

CONCLUSIONS

6.1. Anaerobic System

- An enriched anaerobic consortium from the anaerobic digestor of the Livingston Sewage Treatment Plant was able to dechlorinate 2,4,6-trichlorophenol, 2,4-dichlorophenol, and 2,6-dichlorophenol.
- The effluent from the same anaerobic digester was directly used as the medium for the anaerobic consortia, after autoclaving and clarification. No reducing agent was added to the above medium.
- The anaerobic consortium was able to partially degrade 2-chlorophenol, and 3-chlorophenol in a period of two months.
- The anaerobic consortium was not able to attack 3,5-dichlorophenol and 4-chlorophenol in a period of two months.
- For those chlorophenols which were attacked by the anaerobic consortium, the more chlorines on the benzene ring, the faster the degradation rate proceeded.
- 2,4,6-trichlorophenol was first dechlorinated to 2,4-dichlorophenol and then to 4-chlorophenol by the consortium.
- The mass balance for the dechlorination of 2,4,6-trichlorophenol was satisfactorily closed. All 2,4,6-trichlorophenol was converted to 4-chlorophenol in the anaerobic process.
- After acclimation of the consortium, the dechlorination rate of 2,4,6-trichlorophenol increased from 21.7 to 43.2 μM/day.

- The consortium worked best without adding phosphate buffer. When a phosphate buffer was added, the more concentrated the phosphate buffer was, the slower the dechlorination rate of 2,4,6-trichlorophenol. The starting pH was 9.0 and ended up with 8.0.
- The anaerobic microorganisms were successfully immobilized on the Manville R-635 silica beads.
- After immobilization, the consortium was able to dechlorinate 150 μM of 2,4,6-trichlorophenol in four days.
- The consortium was able to dechlorinate 2,4,6-trichlorophenol in the immobilized recirculation reactor with stoichiometric recovery of 4-chlorophenol.
- The consortium was very stable, even after it had not been exposed to 2,4,6-trichlorophenol for one month. Its activity could be revived in a few days.

6.2. Aerobic System

- The indigenous culture from the immobilized anaerobic reactor was able to degrade 4-chlorophenol in the buffered medium.
- There was no degradation of 4-chlorophenol when the medium was not buffered with phosphate, since the pH would rise from 8.2 to 9 in six hours.
- ATCC cultures #93 (*Serratia Marcescens*), #17514 (*Pseudomonas Putida*), #17991 (*Serratia Marcescens*), #29195 (*Pseudomonas Glathei*), and #33668 (*Pseudomonas Pseudoflava*), and the anaerobic culture from another treatment plant (Joint Meeting company) were able to degrade 4-chlorophenol in the buffered medium.
- In all cases, the medium used was the anaerobically treated solution.
- 4-chlorophenol was the main compound treated in this system.

- There was 10% of disappearance from 164.9 μM of 4-chlorophenol due to air stripping in a period of four days.
- The best phosphate concentration range for the indigenous culture to degrade 4-chlorophenol was 50 to 100 mM.
- No degradation product was formed when 4-chlorophenol was degraded by the indigenous culture.
- When the indigenous culture was used, immobilization of the aerobic culture was not necessary, since it was coming from the anaerobic reactor.
- The indigenous culture was able to mineralize 4-chlorophenol and 2,4,6-trichlorophenol together.
- ATCC culture #29195 was able to mineralize 4-chlorophenol in the presence of 2,4,6-trichlorophenol, which had remained unattacked.
- The indigenous culture contained at least one type of facultative and two types of aerobes. The facultative did not degrade 2,4,6-trichlorophenol anaerobically or 4-chlorophenol aerobically. The aerobes were able to degrade 4-chlorophenol aerobically but not 2,4,6-trichlorophenol anaerobically.

6.3. Anaerobic-Aerobic System

- An integrated anaerobic-aerobic continuous system successfully mineralized 2,4,6-trichlorophenol at an inlet concentration of 120 μM in a process in which 2,4,6-trichlorophenol was dechlorinated to 4-chlorophenol in the anaerobic reactor, and 4-chlorophenol was mineralized in the aerobic reactor. The residence times of each reactor were 105 and 98 hours, respectively.
- The integrated anaerobic-aerobic batch system dechlorinated 106.4 μM of 2,4,6-trichlorophenol in the anaerobic batch reactor in five days (including a lag period

of two days). The resulting 4-chlorophenol was mineralized in the aerobic batch reactor in less than three days.

- In continuous mode, there was a problem of channelling which failed to complete the degradation of 2,4,6-trichlorophenol when the reaction solution flowed upward in the anaerobic reactor.
- No channelling was observed when the reaction solution flowed downward in the anaerobic reactor.
- The anaerobic system regained its activity in two days after lying dormant for a month.

REFERENCES

Anderson, G. K., K. J. McKeown, and T. Donnelly. 1984. "The Application of Anaerobic Packed-Bed Reactros to Industrial Waste-Water Treatment." Wat. Pollut. Control **83**:491-498.

Barnes, D. and P. A. Fitzgerald. 1987. "Anaerobic Wastewater Treatment Precesses." in C. F. Forster and D. A. Wase (eds.): Environmental Biotechnology. Chichester, UK: Ellis Horwood. 57-113.

Bedard, D. L., R. Unterman, L. H. Bopp, M. J. Brennan, M. L. Haberl, and C. Johnson. 1986. "Rapid Assay for Screening and Characterizing Microorganisms for the Ability to Degrade Polychlorinated Biphenyls." Appl. Environ. Microbiol. **51**:761-768.

Berry. D. F., A. J. Francis, and J. M. Bollag. 1987. "Microbial Metabolism of Homocyclic and Heterocyclic Aromatic Compounds under Anaerobic Conditions." **51**:43-59.

Bollag, J. M. 1974. Adv. Appl. Micorbiol. **18**:75.

Borzelleca, J. F., L. W. Condle, and J. R. Hayes. 1984. "Toxicological Evaluation of Selected Chlorinated Phenols." in Jolley et al. (eds.): Water Chlorination: Chem., Environ. Impact Health Eff. Proc. Conf., 5th (Pub. 1985), Chelsea, Mich. 331-343.

Boyd, S. A., D. R. Shelton, D. Berry, and J. M. Tiedje. 1983. "Anaerobic Biodegradation of Phenolic Compounds in Digested Sludge." Appl. Environ. Microbiol. **46**:50-54.

Boyd, S. A., and D. R. Shelton. 1984. "Anaerobic Biodegradation of Chlorophenols in Fresh and Acclimated Sludge." Appl. Environ. Microbiol. **47**:272-277.

Bryant, M. P. 1972. "Commentary on the Hungate Technique for Culture of Anaerobic Bacteria." Amer. J. Clin. Nutr. **25**:1324-1328.

Callander, I. J. and J. P. Barford. 1983. "Recent Advances in Anaerobic Digestion Technology." Process Biochem. **18(4)**:24.

Caunt, P. and H. A. Chase. 1988. "Biodegradation by Bacteria Immobilized on Celite Particles." Bio/Technology **6**:721-725.

Chou, L. W., P. E. Speece, and R. H. Siddiqi. 1979. "Acclimation and Degradation of Petrochemical Wastewater Components by Methane Fermentation." Biotechnol. Bioeng. Symp. **8**:391.

Chu, J. P., and E. J. Kirsch. 1972. "Metabolism of Pentachlorophenol by an Axenic Bacterial Culture." App. Microbiol. **23**:1033-1035.

Costilow, R. N., 1981. "Biophysical Factors in Growth." in Gerhardt et al. (eds.) Manual of Methods for General Bacteriology, American Society for Microbiology, Washington DC, 66-78.

Dalton, H., and D. I. Stirling. 1982. "Co-metabolism." Phi. Trans. R. Soc. Lond. B **297**:481-496.

Dickson, D. 1980. "Pentachlorophenol Dioxins Found to Pose Health Risks." Nature (London). **283**:418.

Dienemann, E. A., D. S. Kosson and R. C. Ahlert. 1990. "Evaluation of Serial Anaerobic/Aerobic Packed Bed Bioreactors for Treatment of a Superfund Leachate." J. Hazardous Materials **23**:21-42.

Dietrich G. and J. Winter. 1990. "Anaerobic Degradation of Chlorophenol by An Enrichment Culture." Appl. Microbiol. Biotechnol. **34**:253-258.

DiGeronimo, M. J., M. Nikaido and M. Alexander. 1979. "Utilization of Chlorobenzoates by Microbial Populations in Sewage." Appl. Environ. Microbiol. **37**:619-625.

Dolfing, J. and J. M. Tiedje. 1987. "Growth Yield Increase Linked to Reductive Dechlorination in a Defined 3-chlorobenzoate Degrading Methanogenic Coculture." Arch. Microbiol. **149**:102-105.

Edgehill, R. U. and R. K. Finn. 1983. "Microbial Treatment of Soil to Remove Pentachlorophenol." Appl. Environ. Microbiol. **45**:1122-1125.

Edwards, E., E. M. Godsy, S. Henry, K. P. Mayer, and D. Grbic-Galic. 1990. "Transformation of Aromatic Hydrocarbons and Halogenated Solvents by Subsurface Microorganisms." Presented Before the Division of Environmental Chemistry, American Chemical Society. Boston, Massachusetts. April 22-27.

Fathepure, B. Z., J. M. Tiedje, and S. A. Boyd. 1988. "Reductive Dechlorination of Hexachlorobenzene to Tri- and Dichlorobenzenes in Anaerobic Sewage Sludge." Appl. Environ. Microbiol. **54**:327-330.

Ferry, J. G. and R. S. Wolfe. 1976. "Anaerobic Degradation of Benzoate to Methane by a Microbial Consortium." Arch. Microbiol. **107**:33-40.

Frick, T. D., R. L. Crawforld, M. Martinson, T. Chresand, and G. Bateson. 1988. "Microbiological Cleanup of Groundwater Contaminated by Pentachlorophenol." Basic Life Sci. **45**(Environ. Biotechnol.):173-191.

Forday, W. and P. Greenfield. 1983. "Anaerobic Digestion." Effl. Water Tr. J. **23**:405-413.

Goldman, P., G. W. A. Milne and M. T. Pignataro. 1967. "Fluorine-Containing Metabolites Formed from 2-Fluorobenzoic Acid by *Pseudomonas* Species." Arch. Biotech. Biophys. **118**:178-184.

Golovleva, L. A. and G. K. Skryabin. 1981. in T. Leisinger, A. M. Cook, R. Harper, J. Nuesch (eds.): Microbial Degradation of Xenobiotic and Recalcitrant Compounds. Academic Press, London. 287.

Guroff, G., J. W. Daly, D. M. Jerina, J. Renson, B. Witkop, and S. Udenfriend. 1967. "Hydroxylation - Induced Migration: The NIH Shift." Science **157**:1524-1530.

Guthrie, M. A., E. J. Kirsch, R. F. Wukasch, and C. P. L. Grady, Jr. 1984. "Pentachlorophenol Biodegradation II, Anaerobic." Water Res. **18**:451-461.

Hakulinen, R., and M. Salkinija-Salonen. 1981. "An Anaerobic Fluidized-Bed Reactor for the Treatment of Industrial Wastewater Containing Chlorophenols." in P. F. Cooper and B. Atkinson (eds.): Biological Fluidized Bed Treatment of Water and Wastewater. Chichester, UK: Ellis Horwood. p.374.

Hakulinen, R., and M. Salkinija-Salonen. 1982. "Treatment of Pulp and Paper Industry Wastewaters in an Anaerobic Fluidised Bed Reactor." Process Biochemistry, 18-22.

Hakulinen, R., S. Woods, J. Ferguson and M. Benjamin. 1985. "The Role of Facultative anaerobic Microorganisms in anaerobic Biodegradation of Chlorophenols." **17**:289-301.

Hamilton, W. A. 1979. "Microbial Energetics and Metabolism." in J. M. Lynch and N. J. Poole, (eds.): Microbial Ecology: A Conceptual Approach, Blackwell Scientific Publications. Oxford.

Hartmann, J., W. Reineke, H.-J. Knackmuss. 1979. "Metabolism of 3-Chloro-, 4-Chloro- and 3,5-Dichlorobenzoate by a *Pseudomonad.*" Appl. Environ. Microbiol. **37**:421-428.

Heijnen, J. J. 1984. "Biological Industrial Waste-Water Treatment Minimizing Biomass Production and Maximizing Biomass Concentration." Doctoral Dissertation. Gist-Brocades, Delft, Holland.

Heijnen, J. J., A. Mulder, W. Enger, and F. Hoeks. 1989. "Review on the Application of Anaerobic Fluidized Bed Reactors in Wastewater Treatment." The Chemical Engineering Journal **41**:B37-B50.

Henze, M. and P. Harroemoes. 1983. "Anaerobic Treatment of Wastewater in Fixed Film Reactors -- a Literature Review." Water Sci. Technol. **15**:1.

Hickey, R. F. and R. W. Owens. 1981. "Methane Generation from High-Strength Industrial Wastes with the Anaerobic Biological Fluidized Bed." Biotechnol. Bioeng. Symp. **11**:399.

Holdeman, L. V., and W. E. C. Moore. 1972. Anaerobe Laboratory Manual. Virginia Polytechnic Institute and State University, Blacksburg.

Horowitz, A., J. M. Suflita, and J. M. Tiedje. 1983. "Reductive Dehalogenations of Halobenzoates by Anaerobic Lake Sediment Microorganisms." Appl. Environ. Microbiol. **45**:1459-1465.

Horvath R. S. and M. Alexander. 1970. "Cometabolism of m-Chlorobenzoate by an *Arthrobacter*." Appl. Microbiol. **20**:254-258.

Hungate, R. E. 1969. "A Roll Tube Method for Cultivation of Strict Anaerobes." 117-132. In J. R. Norris and D. W. Ribbons (ed.): Methods in Microbiology Vol. 3B, Academic Press Inc. New York.

Johnston, H. W., G. G. Briggs, and M. Alexander. 1972. "Metabolism of 3-Chlorobenzoic Acid by a *Pseudomonad*." Soil Biol. Biochem. **4**:187-190.

Kennedy, K. J. and L. van den Berg. 1982. "Anaerobic Digestion of Piggery Waste Using a Stationary Fixed Film Reactor." Agric. Wastes **4**:151.

Klages, U. and F. Ligens. 1979. "Degradation of 4-Chlorobenzoic Acid by a *Nocardia* Species." FEMS Microbiol. Lett. **6**:201-203.

Klinman, J. P., 1972. "The Mechanism of Enzyme Catalyzed Reduced Nicotinamide Adenine Dinucleotide-Dependent Reductions." J. Biol. Chem. **247**:7977-7987.

Knackmuss, H. J., 1982. "Xenobiotic Degradation in Industrial Sewage: Haloaromatics as Target Substrates." Biochem. Soc. Symp. **48**:173-190.

Krumme, M. L. and S. A. Boyd. 1988. "Reductive Dechlorination of Chlorinated Phenols in Anaerobic Upflow Bioreactors." Wat. Res. **22**:171-177.

Larsson, P., L. Okla, and L. Tranvik. 1988. "Microbial Degradation of Xenobiotic, Aromatic Pollutants in Humic Water." Appl. Environ. Microbiol. **54**:1864-1867.

Long-de Vallère, C. L., S. Petrozzi, D. Zürrer, U. Baier, and I. J. Dunn. 1989. "Methods of Anaerobic Degradation of Toxic Compounds in Chemical and Industrial Wastewaters." Biological Waste Treatment 35-72.

Lovely, D. R., and M. J.Klug. 1982. "Intermediary Metabolism of Organic Matter in the Sediments of a Eutrophic Lake." Appl. Environ. Microbiol. **43**:552-560.

Macy, J. M., J. E. Snellen, and R. E. Hungate. 1972. "Use of Syringe Methods for Anaerobiosis." Amer. J. Clin. Nutr. **25**:1318-1323.

McCarty, P. L., J. S. Jeris, P. McKinney, R. E. Reed, and C. A. Wath. 1962. "Microbiology of Anaerobic Digestion." Report R62-29, Segwick Laboratories of Sanitary Science. Massachusetts Institute of Technology, Cambridge, MA.

Mikesell, M. D., and S. A. Boyd. 1986. "Complete Reductive Dechlorination and Mineralization of Pentachlorophenol by Anaerobic Microorganisms." Appl. Environ. Microbiol. **52**:861-865.

Miller, T. L., and M. J. Wolin. 1974. "A Serum Bottle Modification of the Hungate Technique for Cultivating Obligate Anaerobes." Applied Microbiology **27**:985-987.

Moos, L. P., E. J, Kirsch, R. F. Wukasch, and C. P. L. Grady, Jr. 1983. "Pentachlorophenol Biodegradation -I, Aerobic." Water Res. **17**:1575-1584.

Mosey, F. E. 1977. "Anaerobic Filtration: A Biological Treatment Process for Warm Trade Waste Waters: An Account of the Development of the Process and Its Possible Future Applications Illustrated by Laboratory Studies of the Anaerobic Filtration of a Concentrated Carbohydrate Waste." Tech. Rep. TR – Water Res. Cent (Medmenham Engl.), TR48.

Müller, R. and F. Lingens. 1986. "Microbial Degradation of Halogenated Hydrocarbons: A Biological Solution to Pollution Problems?." Angew. Chem. Int. Ed. Engl. **25**:779-789.

Murthy, N. B. K., D. D. Kaufman, and G. F. Fries. 1979. "Degradation of Pentachlorophenol (PCP) in Aerobic and Anaerobic Soil." J. Environ. Sci. Health Part B14 1-14.

Neilson, A. H., A.-S. Allard, P. A. Hynning, M. Remberger, and L. Landner. 1983. "Bacterial Methylation of Chlorinated Phenols and Guaiacols: Formation and Veratroles form Guaiacols and High-Molecular-Weight Chlorinated Lignin." Appl. Environ. Micorbiol. **45**:774-783.

Okey R. W. and R. H. Borgan. 1965. "Apparent Involment of Electronic Mechanisms in Limiting the Microbial Metabolism of Pesticides." J. Water Poll. Cont. Fed. **37**:692-712.

Pak, D. 1988. "Reactor Design for Hazardous Waste Treatment Using a White Rot Fungus." Doctoral Dissertation. NJIT, Newark, NJ.

Reineke W. and H. J. Knackmuss. 1980. "Hybrid Pathway for Chlorobenzoate Metabolism in *Pseudomonas* sp. B13 Derivatives." J. Bacteriol. **142**:467-473.

Rott, B., S. Nitz, and F. Korte. 1979. "Microbial Decomposition of Sodium Pentachlorophenolate" J. Agric. Food Chem. **27**:306-310.

Saber, D. L. and R. L. Crawford. 1985. "Isolation and Characterization of *Flavobacterium* Strains That Degrade Pentachlorophenol." Appl. Environ. Microbiol. **50**:1512-1518.

Sahm, H., M. Brunner, and S. M. Schoberth. 1986. "Anaerobic Degradation of Halogenated Aromatic Compounds." Microb. Ecol. **12**:147-153.

Schönheit, P.,J. Moll, and R. K. Thauer. 1979. "Nickel Cobalt and Molybdenum Requirement for Growth of *Methanobacterium Thermoautotrophicum*." Arch. Microbiol. **123**:105-107.

Shelton, D. R., and J. M. Tiedje. 1984a. " General Method for Determining Anaerobic Biodegradation Potential." Appl. Environ. Microbiol. **47**:850.

Shelton, D. R., and J. M. Tiedje. 1984b. " Isolation and Partial Characterization of Bacteria in An Anaerobic Consortium That Mineralized 3-chlorobenzoic Acid." Appl. Environ. Microbiol. **48**:840-848.

Slater J. H and H. J. Somerville. 1979. "Microbial Technology: Current State, Future Prospects." Microbial Aspects of Waste Treatment with Particular Attention to the Degradation of Organic Compounds. Symp. Soc. Gen. Microbiol. **29**:221-261.

Sleat, R. and J. P Robinson. 1984. "The Bacteriology of Anaerobic Degradation of Aromatic Compounds." J. Appl. Bacteriol. **57**:381-394.

Slonim, Z., L. T. Lien, W. W. Eckenfelder, and J. A. Roth. 1985. "Anaerobic-Aerobic Treatment Process for the Removal of Priority Pollutants." EPA Project Summary EPA/600/S2-85/077.

Spokes J. R. and N. Walker. 1974. "Chlorophenol and Chlorobenzoic Acid Cometabolism by Different Genera of Soil Bacteria." Arch. Microbiol. **96**:125-134.

Su, T. M. 1990. "Anoxic/Oxic Two-Stage Biological Treatment of Nitrite and Nitroaromatic Wastes." Presented at the Symposium of Biotechnology for Hazardous Waste Treatment. Chinese American Academic & Professional Society (CAAPS), Annual Convention. Albany, New York.

Suflita, J. M., A. Horowitz, D. R. Shelton, and J. M. Tiedje. 1982. "Dehalogenation: A Novel Pathway for the Anaerobic Biodegradation of Haloaromatic Compounds." Science **218**:1115-1117.

Suzuki, T. 1977. J. Environ. Sci. Health Part B12 113.

Taylor, B. F., W. L. Campbell, and Chinoy. 1970. "Anaerobic Degradation of the Benzene Nucleus by a Facultatively Anaerobic Microorganisms." J. Bacteriology **102**(2):430-437.

Tiedje, J. M., S. A. Boyd, and J.F. Quensen III. 1987. "Reductive Dechlorination of PCBs in Anaerobic Microbial Communities." in H. L. Finkbeiner and S. B. Hamilton, (eds.): Research and Development Program for the Destruction of PCBs, General Electric Company Corporate Research and Development. Sixth Progress Report. Schenectady, New York. pp.29-33.

Toerien, D. F. 1967. "Enrichment Culture Studies on the Aerobic and Facultative Anaerobic Bacteria Found in Anaerobic Digesters." Water Research **1**:147-155.

Topp, E., R. L. Crawford, and R. S. Hanson. 1988. "Influence of Readily Metabolizable Carbon on Pentachlorophenol Metabolism by a Pentachlorophenol-Degrading *Flavobacterium* sp.." Appl. Environ. Microbiol. **54**:2452-2459.

Valo, R., J. Apajalathi, and M. Salkinoja-Salonen. 1985. "Studies on the Physiology of Microbial Degradation of Pentachlorophenol." Appl. Microbiol. Biotechnol. **21**:313-319.

van den Berg, L. 1984. "Developments in Methanogensis from Industrial Waste Water." Can. J. Microbiol. **30**:975.

Watanabe, I. 1973. "Isolation of Pentachlorophenol Decomposing Bacteria from Soil." Soil Sci. Plant Nutr. **19**:109-116.

Watanabe, I. 1977. "Pentachlorophenol Decomposing and PCP-Tolerant Bacteria in Field Soil Treated with PCP." Soil Biol. Biochem. 9:99-103.

Ying, W. C., J. G. Goeddertz, W. Lin and A.S. Weber. 1990. "Biological Activated Carbon Process for Treating High Concentration Phenolic Wastewaters." Presented at the Symposium of Biotechnology for Hazardous Waste Treatment. Chinese American Academic & Professional Society (CAAPS), Annual Convention. Albany, New York.

Table 1[a] Physical and Chemical Properties of Phenol and Chlorinated Phenols

	Molecular Weightt	Physical State	Melting Point °C	Boiling Point °C	Solubility[b]			pK_a
					Water	Alcohol	Ether	
					ppm			
Phenol	94.11	Crystals	41	182	8.2 at 15°	S	VS	9.89
2-CP[c]	128.56	Liquid		175-176	2.85	S	S-alk	8.65
3-CP	128.56	Needles	32-33	214	2.6	S	S	8-12
4-CP	128.56	Needles	41-43	217	2.7	VS	VS	9.37
2,3-DCP[c]	163.01	Needles	45	209-210	0.45	VS	VS	
2,4-DCP	163.01	Hexagonal needles	45	206-208	SS	VS	VS	7.85
2,5-DCP	163.01	Prisms	59	211	SS	VS	VS	7.50
2,6-DCP	163.01	Needles	68-69	219-220		VS	VS	6.91
3,4-DCP	163.01	Needles	68	253.5				8.58
3,5-DCP	163.01	Prisms	68	233	SS	VS		
2,3,5-TCP[c]	197.46	Needles	55	249-250	SS	S	S	
2,4,5-TCP	197.46	Needles	61-63	252	I	S	S	7.07
2,3,6-TCP	197.46	Needles	58		S	V	V	5.98
2,4,6-TCP	197.46	Needles	68-69	246	0.09	VS	VS	6.62
3,4,5-TCP	197.46	Needles	101	271-277		S		7.83
2,3,4,5-TeCP[c]	231.90	Needles	69-70	164	SS	VS	VS	
2,3,4,6-TeCP	231.90	Needles	70	150				5.46
2,3,5,6-TeCP	231.90	Leaflets	115					
PCP[c]	266.35	Solid	188-189	310				5.00

[a]Adapted from Borzelleca, et al., 1984.

[b]S: soluble; SS: slightly soluble; VS: very soluble; I: insoluble.

[c]CP: Chlorophenol; DCP: Dichlorophenol; TCP: Trichlorophenol; TeCP: Tetrachlorophenol; PCP: Pentachlorophenol

Table 2. Anaerobic Degradation of Tested Chlorophenols in the Batch System

The reaction time was two months.

Compounds	Initial Concentration, μM	Final Concentration, μM
3,5-DCP	71.8	71.2
2,6-DCP	55.2	0.0
2,4-DCP	54.0	0.0
4-CP	77.0	73.1
3-CP	112.0	56.0
2-CP	119.0	46.7

Table 3. Mass Balance for the Anaerobic Dechlorination of TCP by the Freely Suspended Microorganisms in the Batch System

Run 3-1

Time, day	TCP, μM	DCP, μM	4-CP, μM	Phenol, μM	Sum, μM	% Recvy
0	108.7	0.0	196.0	8.4	313.1	100
2	59.3	21.9	226.0	8.7	315.9	101
5	0.0	21.0	285.5	11.9	318.4	102
7	0.0	0.0	309.5	21.1	330.6	106
9	0.0	0.0	315.5	26.6	342.1	109
11	0.0	0.0	319.4	26.8	346.2	111

Run 3-2

Time, hours	TCP, μM	DCP, μM	4-CP, μM	Phenol, μM	Sum, μM	%, Recvy
0	94.9	0.0	263.1	19.1	377.1	100
12	67.3	10.3	263.1	17.4	358.1	95
24	53.4	16.3	288.9	17.5	376.1	100
36	30.3	22.5	305.9	19.8	378.5	100
49	6.7	26.9	330.4	20.9	384.9	102
59	0.0	14.4	344.3	22.0	380.7	101
72	0.0	0.0	361.8	27.3	389.1	103
83	0.0	0.0	358.6	30.0	388.6	103
96	0.0	0.0	346.2	32.3	378.5	100

Table 4. Effect of Phosphate Buffer on the Anaerobic Dechlorination of TCP by the Freely Suspended Microorganisms in the Batch System

Run 4-1 No phosphate buffer

Time, hours	TCP, μM	DCP, μM	4-CP, μM	Sum, μM	%, Recvy
0	101.7	0.0	128.3	230.0	100
30	0.0	46.0	182.3	228.3	99
63	0.0	0.0	214.6	214.6	93

Run 4-2 50mM phosphate buffer

Time, hours	TCP, μM	DCP, μM	4-CP, μM	Sum, μM	%, Recvy
0	94.7	0.0	118.2	231.6	100
30	106.4	0.0	115.9	222.3	96
63	98.3	17.2	116.6	232.1	100
75	69.4	20.9	116.6	206.9	89
172	0.0	16.6	205.3	221.9	96

Run 4-3 100mM phosphate buffer

Time, hours	TCP, μM	DCP, μM	4-CP, μM	Sum, μM	%, Recvy
0	75.5	0.0	112.8	188.3	100
30	65.9	0.0	101.1	167.0	89
63	66.4	0.0	105.8	172.2	91
75	64.3	0.0	106.5	170.8	91
172	65.3	16.6	116.6	198.5	105

Run 4-4 150mM phosphate buffer

Time, hours	TCP, μM	DCP, μM	4-CP, μM	Sum, μM	%, Recvy
0	51.2	0.0	100.3	151.5	100
30	39.0	0.0	91.0	130.0	86
63	54.2	0.0	93.3	147.5	97
75	84.1	0.0	94.1	178.2	118
172	57.2	0.0	80.1	137.3	91

Table 5. Anaerobic Dechlorination of TCP during the Immobilization of the Anaerobic Microorganisms in the Manvil R-635 Beads

Run 5-1 Dechlorination of TCP During the Immobilization Phase

Time, hours	TCP, μM	DCP, μM	4-CP, μM	Sum, μM	% Recvy
0	101.3	0.0	0.0	101.3	100
51	0.0	23.9	80.1	104.0	103
316	0.0	0.0	120.5	120.5	119
*317	40.5	0.0	118.2	158.7	100
336	0.0	0.0	136.1	136.1	86
362	0.0	0.0	141.5	141.5	89
407	0.0	0.0	152.4	152.4	86
431	0.0	0.0	146.2	146.2	92

*New spike

Run 5-2 Anaerobic Dechlorination of TCP in the Autoclaved Fresh Medium After Immobilization

Time, hours	TCP, μM	DCP, μM	4-CP, μM	Sum, μM	% Recvy
0	152.0	0.0	0.0	152.0	100
24	49.6	20.2	77.8	147.6	97
48	0.5	51.5	93.3	145.3	96
72	0.0	12.3	145.4	157.7	104
96	0.0	0.0	154.0	154.0	101

Table 6. Mass Balance of the Anaerobic Dechlorination of TCP in the Immobilized Recirculation Reactor System

Run 6-1

Time, hours	TCP, μM	4-CP, μM	Sum, μM	%, Recvy
0	111.4	0.0	111.4	100
24	44.1	58.3	102.4	92
68	35.5	71.5	107.0	96
99	21.8	66.9	88.7	80
124	36.0	77.0	113.0	101
147	23.3	84.8	108.1	97

Run 6-2

Time, hours	TCP, μM	DCP, μM	4-CP, μM	Sum, μM	%, Recvy
0	152.0	0.0	112.8	264.8	100
24	70.4	30.1	131.4	231.9	88
50	0.0	0.0	236.4	236.4	89
95	0.0	0.0	277.6	277.6	105
119	0.0	0.0	297.0	297.0	112
143	0.0	0.0	275.3	275.3	104

Table 7. Stripping Test of 4-CP in the Autoclaved Fresh Medium

Time, hours	4-CP, μM
0	164.9
12	171.1
22	167.2
36	169.5
47	152.4
61	155.5
73	143.9
84	157.9
96	148.5

Table 8. Aerobic Degradation of 4-CP by ATCC Cultures Without Buffering

Run 8-1 Initial concentration of 4-CP = 140.0μM

Reaction time = 78 hours

ATCC Culture #	Final Concentration, μM
93 *Serratia Marcescens*	8.6
9446 *Pseudomonas Chlororaphis*	115.1
10145 *Pseudomonas Aeruginosa*	93.3
13270 *Pseudomonas Maltophilia*	150.1
14235 *Pseudomonas Resinovorans*	117.4
17514 *Pseudomonas Putida*	91.0
17991 *Serratia Marcescens*	136.1
19706 *Pseudomonas Indigofera*	118.2
23328 *Pseudomonas Pictorum*	130.0
29195 *Pseudomonas Glathei*	84.0
31800 *Pseudomonas Putida*	138.4
33668 *Pseudomonas Pseudoflava*	152.4

Run 8-2

ATCC Culture #	@0 hr, μM	@46 hr, μM
93 *Serratia Marcescens*	210.0	178.8
10145 *Pseudomonas Aeruginosa*	202.0	182.7
17514 *Pseudomonas Putida*	211.5	171.0
29195 *Pseudomonas Glathei*	201.4	168.7

Table 9. Aerobic Degradation of 4-CP by ATCC Cultures with the Addition of Phosphate Buffer in the Middle of the Reaction

All cultures start with 165.6μM of 4-CP

ATCC Culture #	@42 hr, μM	@87 hr, μM
93 *Serratia Marcescens*	144.6	0.0
9446 *Pseudomonas Chlororaphis*	164.8	18.7
10145 *Pseudomonas Aeruginosa*	127.5	75.4
13270 *Pseudomonas Maltophilia*	143.9	23.3
17514 *Pseudomonas Putida*	136.1	0.0
17991 *Serratia Marcescens*	106.5	0.0
19706 *Pseudomonas Indigofera*	121.3	17.9
23328 *Pseudomonas Pictorum*	151.6	21.0
29195 *Pseudomonas Glathei*	147.0	0.0
31800 *Pseudomonas Putida*	157.9	18.7
33668 *Pseudomonas Pseudoflava*	152.4	0.0

Table 10. Aerobic Degradation of 4-CP in the Anaerobic Treated Medium by ATCC culture #93 *Serratia Marcescens*

4-CP Concentration in μM during the Reaction

Time, hours	50mM Phosphate	100mM Phosphate	150mM Phosphate
0	113.5	133.7	115.1
6	101.9	140.7	126.0
13	28.0	119.8	123.6
18	0.0	54.4	94.9

pH Variation during the Reaction

Time, hours	50mM Phosphate	100mM Phosphate	150mM Phosphate
0	7.22	7.09	7.04
6	8.31	7.83	7.43
13	8.56	7.85	7.45
18	8.59	7.86	7.45

Table 11. Aerobic Degradation of 4-CP in the Anaerobic Treated Medium by ATCC Culture #17514 *Pseudomonas Putida*

4-CP Concentration in μM during the Reaction

Time, hours	50mM Phosphate	100mM Phosphate	150mM Phosphate
0	154.0	140.0	135.3
6	145.4	131.4	123.6
13	102.6	44.3	59.9
18	21.0	3.9	3.9

pH Variation during the Reaction

Time, hours	50mM Phosphate	100mM Phosphate	150mM Phosphate
0	7.16	7.05	7.03
6	8.49	7.83	7.43
13	8.57	7.78	7.44
18	8.36	7.72	7.44

A degradation product was fromed while 4-CP was degraded.

Table 12. Aerobic Degradation of 4-CP in the Anaerobic Treated Medium by ATCC Culture #17991 *Serratia Marcescens*

4-CP Concentration in μM during the Reaction

Time, hours	50mM Phosphate	100mM Phosphate	150mM Phosphate
0	131.4	143.1	133.7
6	123.6	126.0	136.1
13	80.9	120.5	
18	0.0	59.1	82.4

pH Variation during the Reaction

Time, hours	50mM Phosphate	100mM Phosphate	150mM Phosphate
0	7.20	7.08	7.01
6	8.50	7.87	7.41
13	8.45	7.83	7.45
18	8.48	7.86	7.45

Table 13 Aerobic Degradation of 4-CP in the Anaerobic Treated Medium by ATCC Culture #29195 *Pseudomonas Glathei*

4-CP Concentration in μM during the Reaction

Time, hours	50mM Phosphate	100mM Phosphate	150mM Phosphate
0	107.3	110.4	107.3
6	100.3	98.8	91.0
13	21.0	4.7	50.5
18	3.1	3.9	3.1

pH Variation during the Reaction

Time, hours	50mM Phosphate	100mM Phosphate	150mM Phosphate
0	7.19	7.07	7.03
6	8.51	7.65	7.36
13	8.35	7.65	7.36
18	8.41	7.64	7.43

Table 14. Aerobic Degradation of 4-CP in the Anaerobic Treated Medium by ATCC Culture #33668 *Pseudomonas Pseudoflava*

4-CP Concentration in μM during the Reaction

Time, hours	50mM Phosphate	100mM Phosphate	150mM Phosphate
0	150.1	140.0	136.1
6	141.5	137.6	130.6
13	115.9	113.5	119.8
18	64.5	42.8	80.1

pH Variation during the Reaction

Time, hours	50mM Phosphate	100mM Phosphate	150mM Phosphate
0	7.18	7.07	7.04
6	8.54	7.71	7.39
13	8.72	7.74	7.43
18	8.37	7.76	7.43

A degradation product was fromed while 4-CP was degraded.

Table 15. Aerobic Degradation of 4-CP by the Indigenous Culture from the Anaerobic Reactor

Run 15-1

4-CP Concentration in μM during the Reaction

Time, hours	0mM Phosphate	50mM Phosphate	100mM Phosphate	150mM Phosphate
0	138.4	120.5	113.5	108.1
6	168.7	114.3	108.9	105.0
13	140.0	77.8	84.8	89.4
18	159.4	0.0	0.0	63.8

pH Variation during the Reaction

Time, hours	0mM Phosphate	50mM Phosphate	100mM Phosphate	150mM Phosphate
0	8.19	7.15	7.06	7.02
6	8.67	8.27	7.65	7.35
13	8.71	8.46	7.65	7.35
18	8.76	8.48	7.61	7.36

Run 15-2

4-CP Concentration in μM during the Reaction

Time, hours	0mM Phosphate	50mM Phosphate	100mM Phosphate
0	226.3	202.2	198.3
5	220.8	203.7	188.2
16	206.8	196.7	188.2
26	202.2	202.0	187.4
66	211.5	0.0	0.0

pH Variation during the Reaction

Time, hours	0mM Phosphate	50mM Phosphate	100mM Phosphate
0	7.84	7.19	7.08
5	8.63	8.35	7.54
16	8.98	8.64	7.70
26	9.06	8.76	7.68
66	9.05	8.76	7.69

Run 15-3 4-CP Concentration In the 50mM Phosphate Buffered Culture

@0 hr, μM	@24 hr, μM	@50 hr, μM
57.5	35.8	0.0

Table 16. Aerobic Degradation of 4-CP in the Anaerobic Treated Medium by the Anaerobic Culture from the Joint Meeting Company

4-CP Concentration in μM during the Reaction

Time, hours	50mM Phosphate	100mM Phosphate	150mM Phosphate
0	112.0	104.2	97.2
6	107.3	100.3	89.4
13	78.5	76.2	78.5
18	28.0	3.1	56.0

pH Variation during the Reaction

Time, hours	50mM Phosphate	100mM Phosphate	150mM Phosphate
0	7.16	7.06	7.02
6	8.52	7.73	7.38
13	8.62	7.73	7.38
18	8.62	7.70	7.37

Table 17. The Effect of TCP on the Aerobic Degradation of 4-CP by the Indigenous Culture from the Anaerobic Reactor

Run 17-1 No TCP added

Hours	0	25	59	73	96	120
4CP,μM	61.4	3.1	0.0	0.0	0.0	0.0

Run 17-2 With TCP added

Hours	0	25	59	73	96	120
4-CP,μM	66.9	2.3	0.0	0.0	0.0	0.0
TCP,μM	94.7	60.8	54.2	68.9	50.2	12.2

Table 18. The Effect of TCP on the Aerobic Degradation of 4-CP by ATCC Culture #29195 *Pseudomonas Glathei*

Run 18-1 No TCP added

Hours	0	23	47
4-CP, μM	172.6	18.7	19.4

Run 18-2 With TCP added

Hours	0	23	47
4-CP, μM	170.3	10.9	17.8
TCP, μM	52.2	54.7	48.6

Table 19. Anaerobic Degradation of TCP by the Pure Cultures Isolated from the Anaerobic Reactor

Run 19-1 The control, autoclaved medium without inoculation of bacteria

Hours	0	23	48	71	93	142
TCP,μM	122.1	120.5	117.3	119.6	116.2	115.5

Run 19-2 Bacteria #1, facultative

Hours	0	23	48	71	93	142
TCP,μM	87.1	85.1	95.7	86.3	93.3	71.4

Run 19-3 Bacteria #2, aerobe

Hours	0	23	48	71	93	142
TCP,μM	91.7	95.4	102.9	90.3	85.5	97.3

Run 19-4 Bacteria #3, aerobe

Hours	0	23	48	71	93	142
TCP,μM	92.4	112.4	95.7	107.3	93.6	90.7

Table 20. Aerobic Degradation of 4-CP by the Pure Cultures Isolated from the Anaerobic Reactor

Run 20-1 The control, autoclaved medium without innoculation of bacteria

Hours	0	23	48	71	93	142
4CP,μM	254.3	249.6	227.7	230.8	245.5	250.8

Run 20-2 Bacteria #1, facultative

Hours	0	23	48	71	93	142
4CP,μM	225.5	207.6	213.8	196.7	179.6	206.8

Run 20-3 Bacteria #2, aerobe

Hours	0	23	48	71	93	142
4CP,μM	225.5	210.0	154.0	56.0	0.0	0.0

Run 20-4 Bacteria #3, aerobe

Hours	0	23	48	71	93	142
4CP,μM	210.0	219.3	153.2	80.9	14.0	0.0

Table 21. Anaerobic-Aerobic Degradation of TCP in Batch Mode

First stage – Anaerobic reactor

Time, hours	TCP, μM	DCP, μM	4-CP, μM	Sum, μM	pH
0	106.4	0.0	0.0	106.4	8.56
23	102.3	0.0	0.0	102.3	8.58
46	103.9	0.0	0.0	103.9	8.50
73	84.8	27.0	0.0	111.8	8.44
119	0.0	0.0	104.2	104.2	8.25
143	0.0	0.0	98.0	98.0	8.27

Second stage – Aerobic reactor

Time, hours	4-CP, μM	pH
0	75.1	7.15
25	52.9	7.42
49	42.0	7.84
69	0.0	7.93
117	0.0	7.97
145	0.0	8.02
172	0.0	8.02

Table 22. Anaerobic-Aerobic Degradation of TCP in the Continuous Upflow Reactors

Run 22-1

First stage – Anaerobic reactor

Time, hours	TCP, μM	DCP, μM	4-CP, μM	Sum, μM
0	30.4	0.0	31.9	62.3
22	21.3	0.0	44.3	65.6
*63	14.7	4.9	52.1	71.7
112	48.1	11.7	49.0	108.8
135	0.0	30.7	80.1	110.8
160	0.0	6.1	91.8	97.9
184	0.0	0.0	103.7	103.7
212	0.0	0.0	84.0	84.0
233	0.0	0.0	104.2	104.2
262	0.0	0.0	105.0	105.0
281	0.0	0.0	118.2	118.2
305	0.0	0.0	105.0	105.0

Second stage – Aerobic reactor

Time, hours	TCP, μM	4-CP, μM	Sum, μM
0	0.0	59.1	59.1
22	0.0	0.0	0.0
*63	0.0	0.0	0.0
112	5.6	0.0	5.6
135	27.9	4.7	32.6
160	57.8	14.0	71.8
184	62.8	18.7	81.5
212	44.1	25.7	69.8
233	30.4	35.8	66.2
262	22.8	38.9	61.7
281	22.8	44.3	67.1
305	16.7	48.2	64.9

*From 0 to 63 hours, the reactors were in batch mode

Run 22-2

First stage – Anaerobic reactor

Time, hours	TCP, μM	DCP, μM	4-CP, μM	Sum, μM
0	239.1	0.0	0.0	239.1
22	238.1	6.7	10.1	254.9
*70	0.0	25.2	94.9	120.1
89	0.0	0.0	114.3	114.3
113	0.0	6.7	107.3	114.0
165	0.0	6.7	58.3	65.0
185	0.0	0.0	62.2	62.2
210	0.0	0.0	61.4	61.4
233	0.0	0.0	87.9	87.9

Second stage – Aerobic reactor

Time, hours	TCP, μM	4-CP, μM	Sum, μM
0	0.0	63.8	63.8
22	0.0	21.8	21.8
*70	0.0	0.0	0.0
89	1.0	6.2	7.2
113	12.2	9.3	21.5
165	37.0	16.3	53.3
185	52.7	18.7	71.4
210	52.7	28.8	81.5
233	61.3	38.9	100.2

*From 0 to 70 hours, the reactors were in batch mode

Table 23. Anaerobic-Aerobic Degradation of TCP in the Continuous Anaerobic Downflow Reactor, and Aeroic Upflow Reactor

First stage – Anaerobic reactor

Time, hours	TCP, μM	DCP, μM	4-CP, μM	Sum, μM
0	231.9	0.0	51.3	283.2
26	251.9	9.2	132.2	293.3
49	111.4	50.9	134.5	296.8
73	0.0	68.7	180.4	249.1
96	0.0	12.3	275.3	287.6
*146	0.0	0.0	289.3	289.3
170	0.0	0.0	199.1	199.1
199	0.0	0.0	230.2	230.2
224	0.0	0.0	229.4	229.4
267	0.0	0.0	234.1	234.1
290	0.0	0.0	244.9	244.9
314	0.0	4.3	223.2	227.5
337	0.0	3.1	150.0	153.1
367	0.0	1.2	80.1	81.3
414	0.0	0.0	87.9	87.9
437	0.0	0.0	83.2	83.2
458	0.0	0.0	95.6	95.6
486	0.0	0.0	104.2	104.2
508	0.0	0.0	110.4	110.4
527	0.0	0.0	108.9	108.9
556	0.0	0.0	109.6	109.6
581	0.0	0.0	103.4	103.4
610	0.0	0.0	101.9	101.9
633	0.0	0.0	108.9	108.9
654	0.0	0.0	90.2	90.2

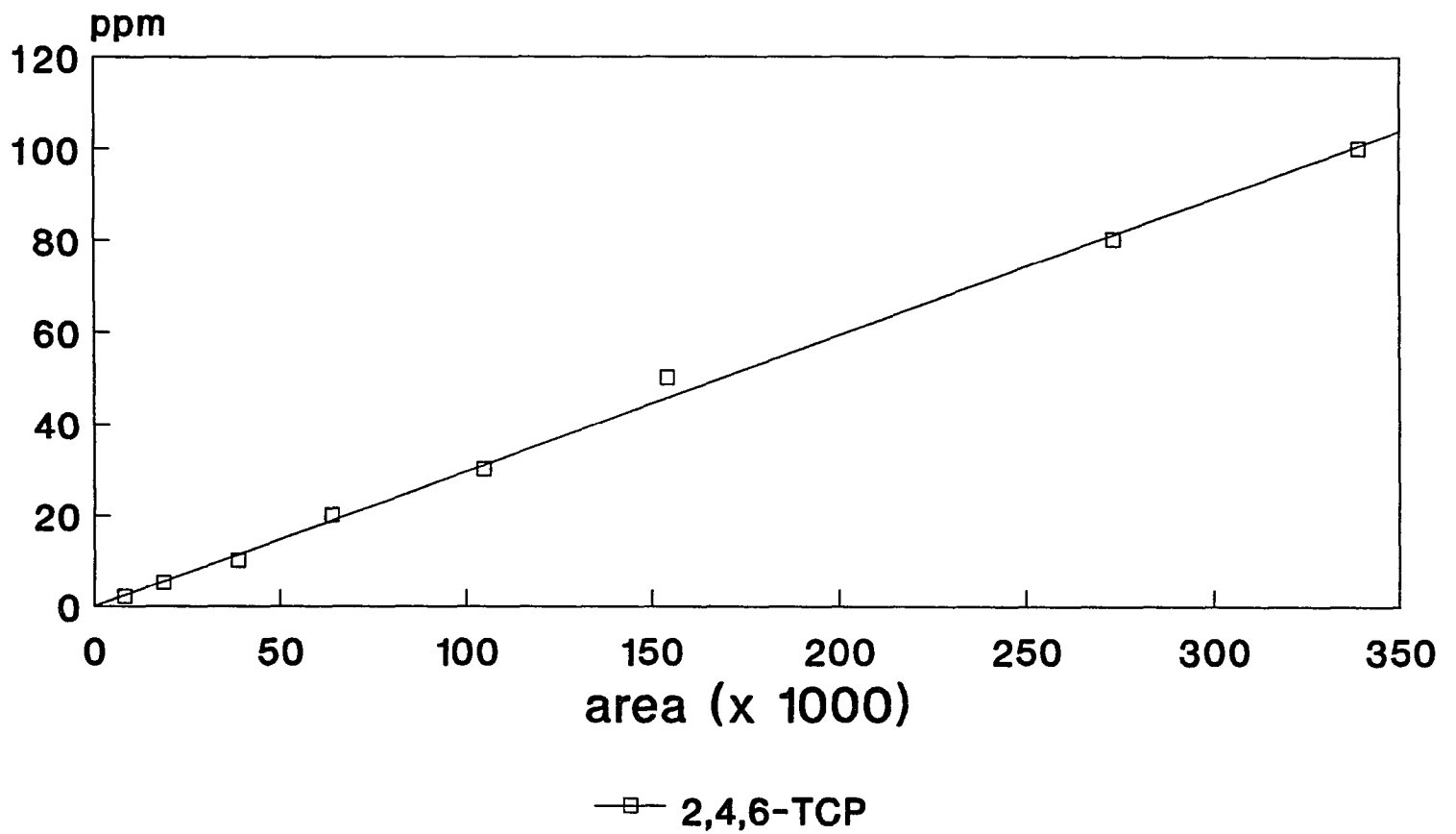
*From 0 to 146 hours, the reactor was in batch mode

Second stage – Aerobic reactor

Time, hours	4-CP, μM
0	205.3
26	202.2
49	144.6
73	0.0
96	0.0
*146	0.0
170	0.0
199	0.0
224	0.0
267	0.0
290	0.0
314	0.0
337	0.0
367	0.0
414	0.0
437	0.0
458	0.0
486	0.0
508	0.0
527	0.0
556	0.0
581	0.0
610	0.0
633	0.0
654	0.0

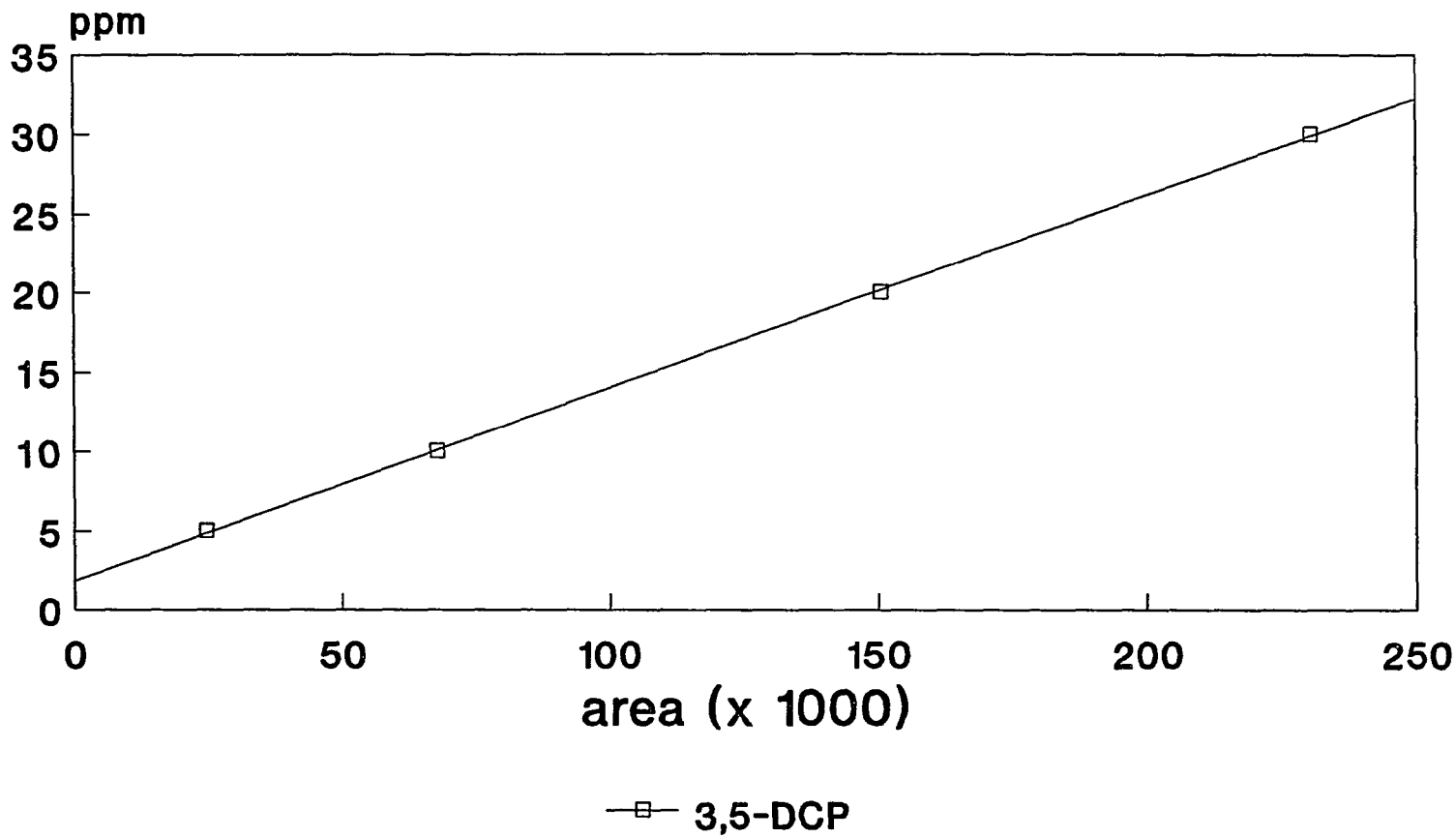
*From 0 to 146 hours, the reactor was in batch mode

Figure 1. Calibration Curve of 2,4,6-TCP by HPLC



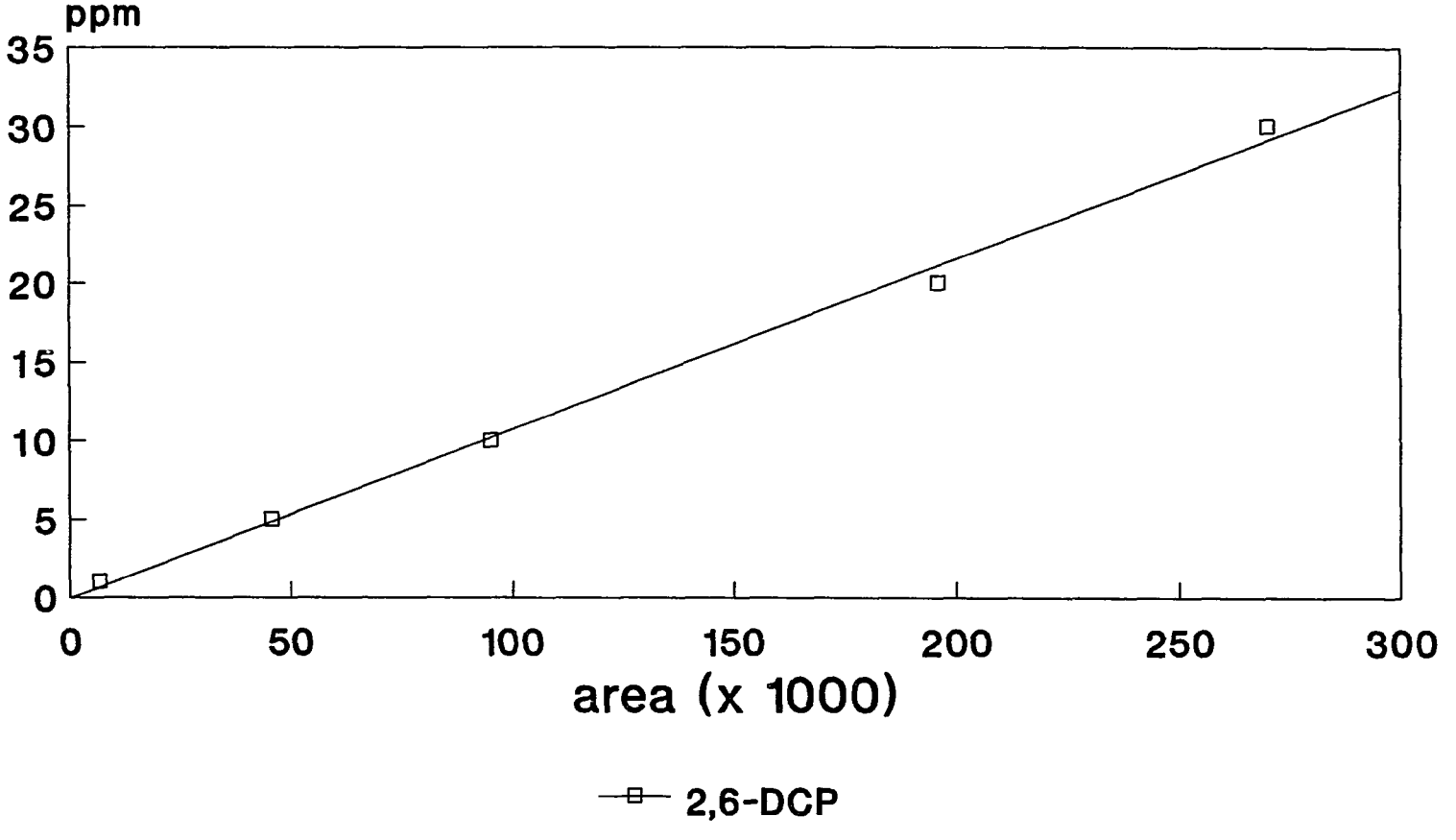
ppm = 2.98E-4 * area - 0.095
Correlation Coefficient = 0.9973

Figure 2. Calibration Curve of 3,5-DCP by HPLC



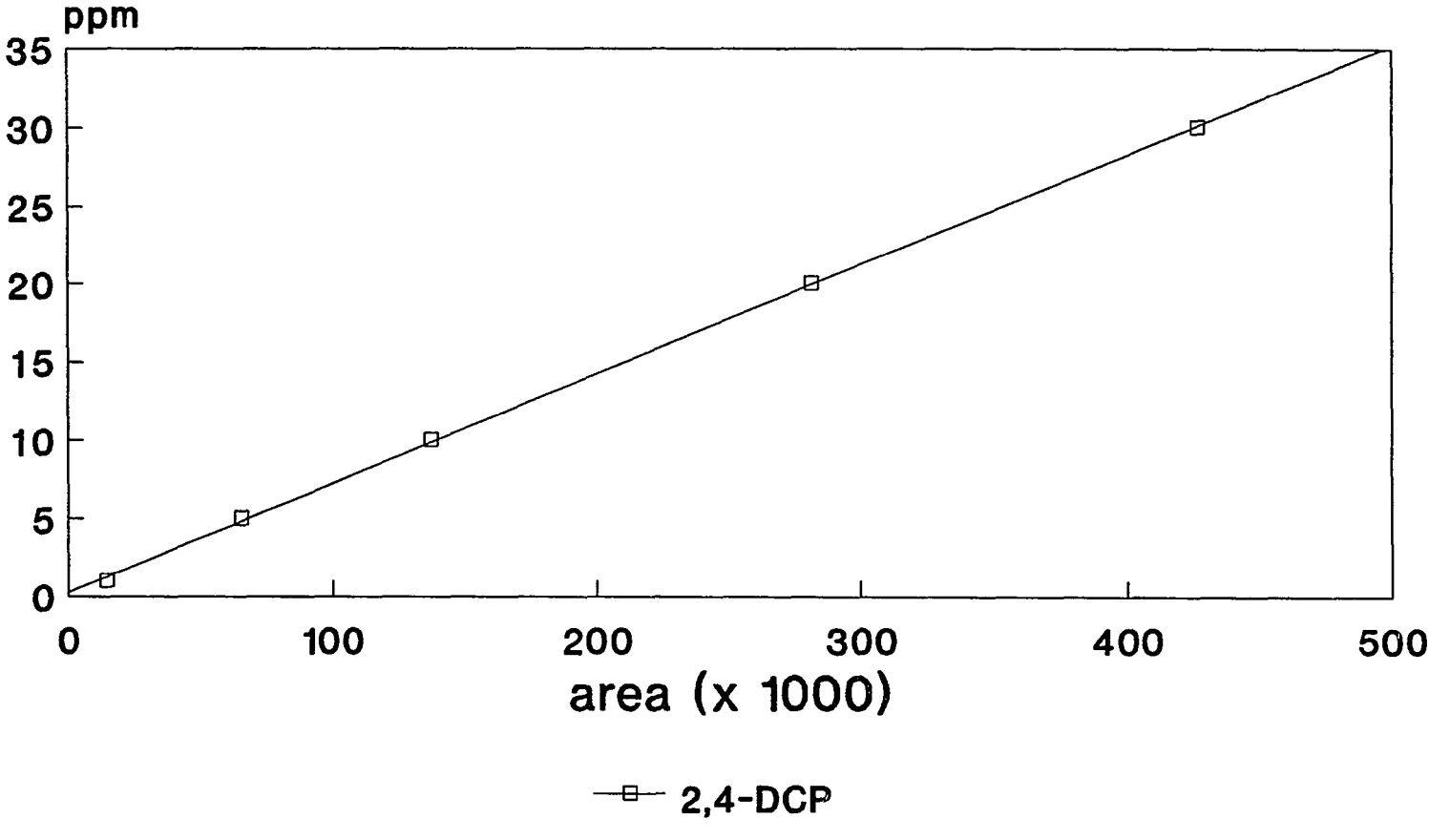
ppm = 1.22E-4 * area + 1.87
Correlation Coefficient = 0.9998

Figure 3. Calibration Curve of 2,6-DCP by HPLC



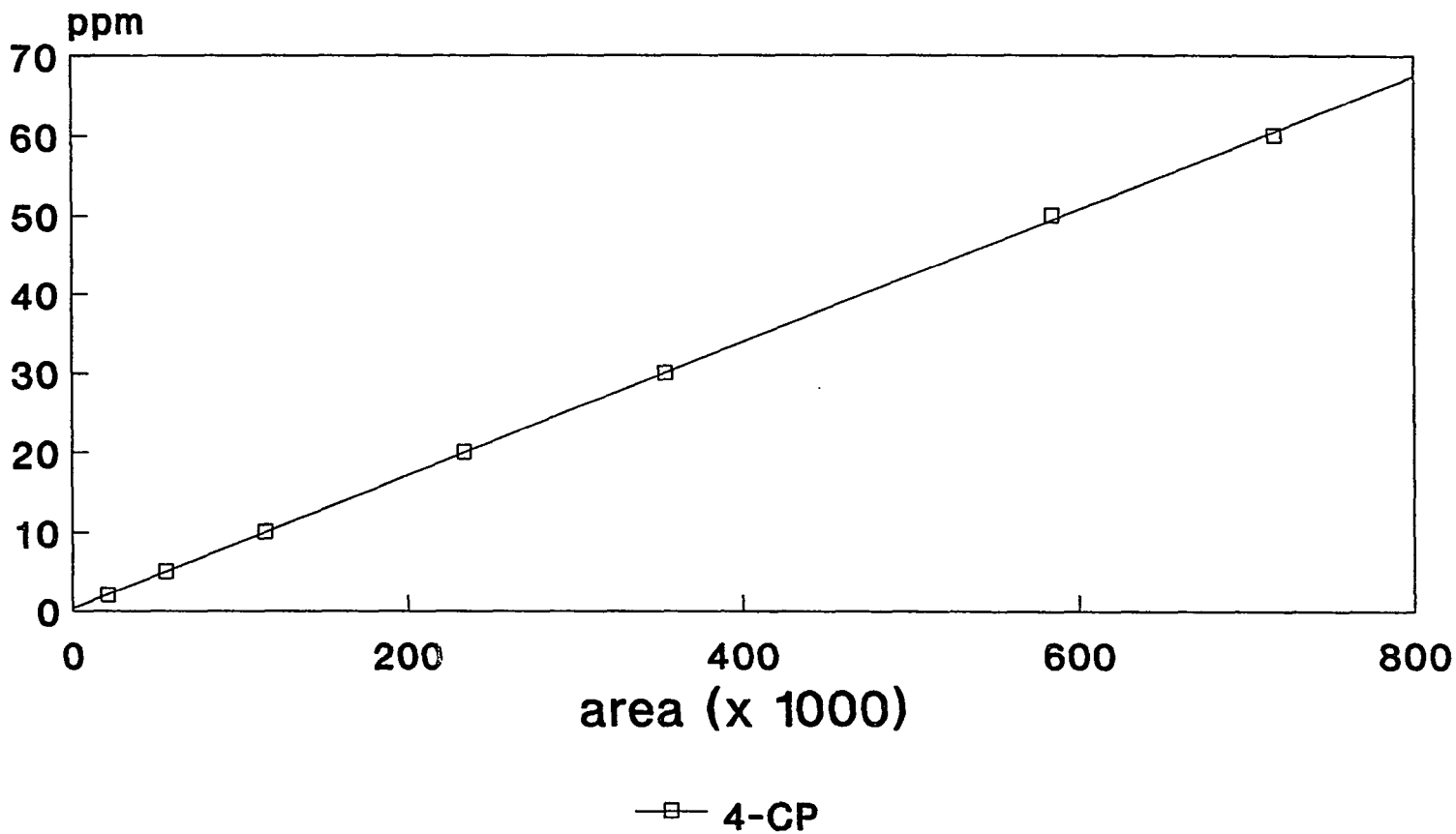
ppm = 1.09E-4 * area - 0.11
Correlation Coefficient = 0.9959

Figure 4. Calibration Curve of 2,4-DCP by HPLC



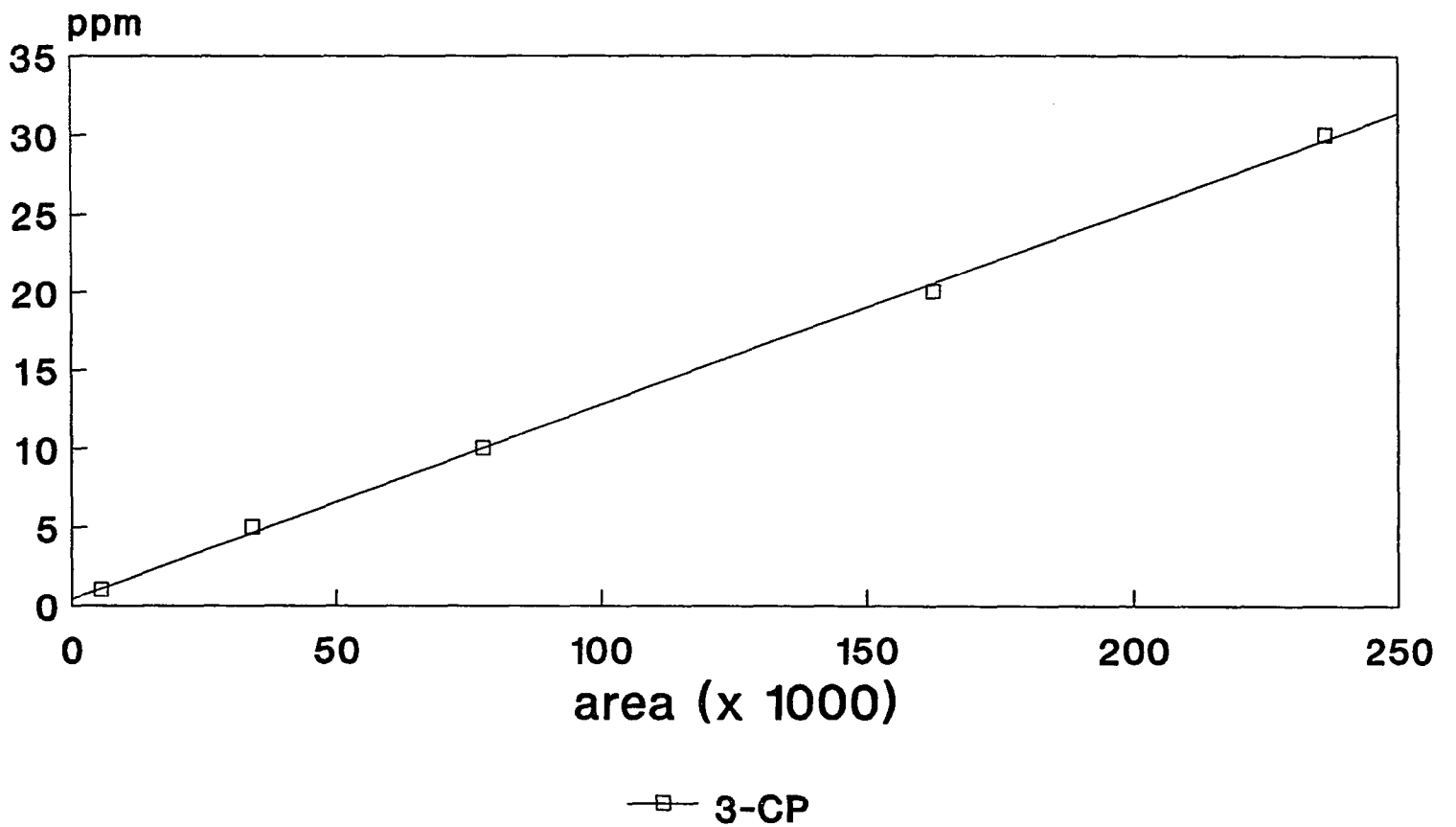
ppm = 7.00E-5 * area + 0.26
Correlation Coefficient = 0.9998

Figure 5. Calibration Curve of 4-CP by HPLC



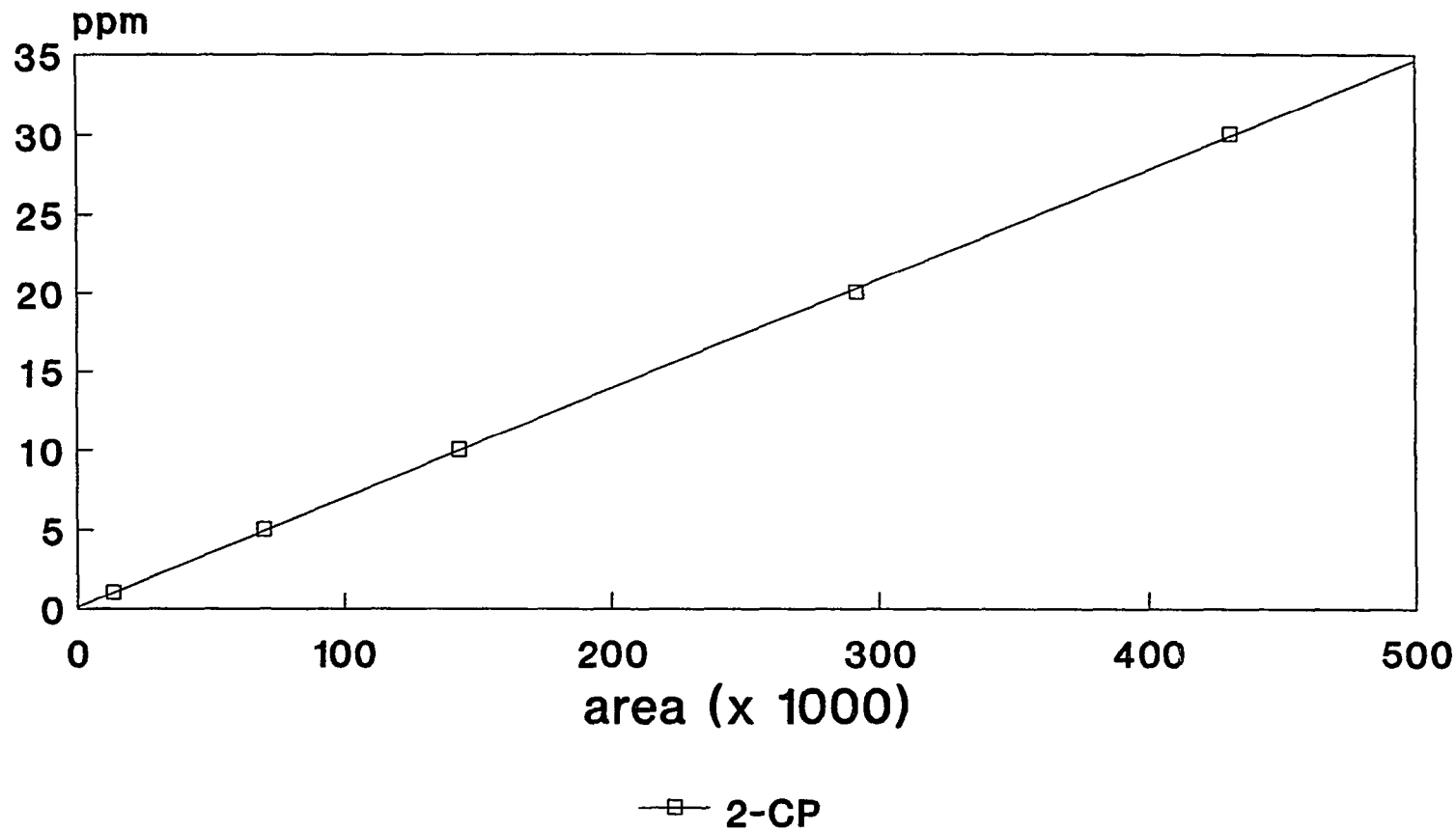
ppm = 8.42E-5 * area + 0.25
Correlation Coefficient = 0.9998

Figure 6. Calibration Curve of 3-CP by HPLC



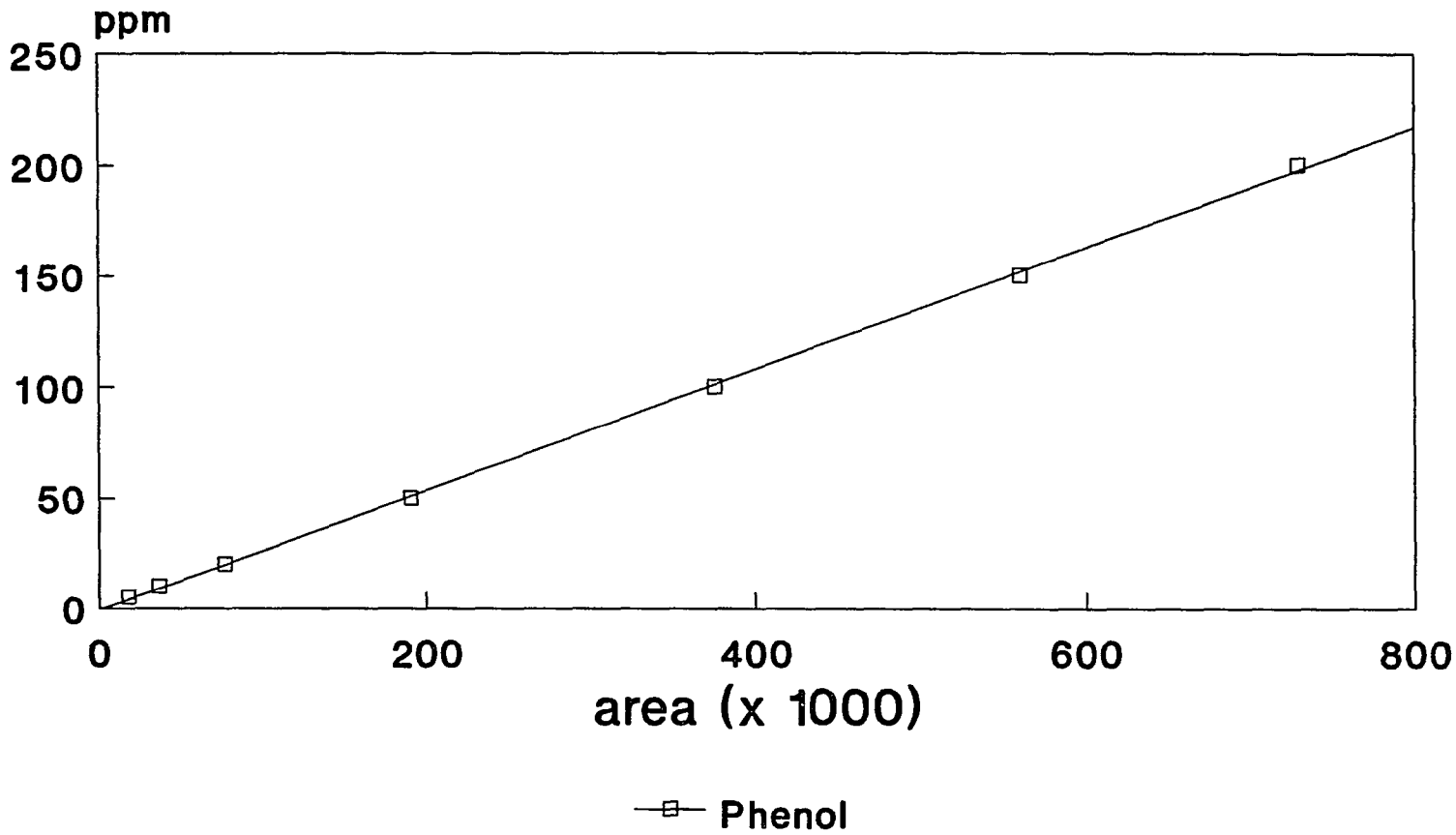
ppm = 1.24E-4 * area + 0.41
Correlation Coefficient = 0.9990

Figure 7. Calibration Curve of 2-CP by HPLC



ppm = 6.92E-5 * area + 0.092
Correlation Coefficient = 0.9998

Figure 8. Calibration Curve of Phenol by HPLC



ppm = 2.72E-4 * area - 0.62
Correlation Coefficient = 0.9996

Figure 9, Diagram of Anaerobic Recirculation Reactor

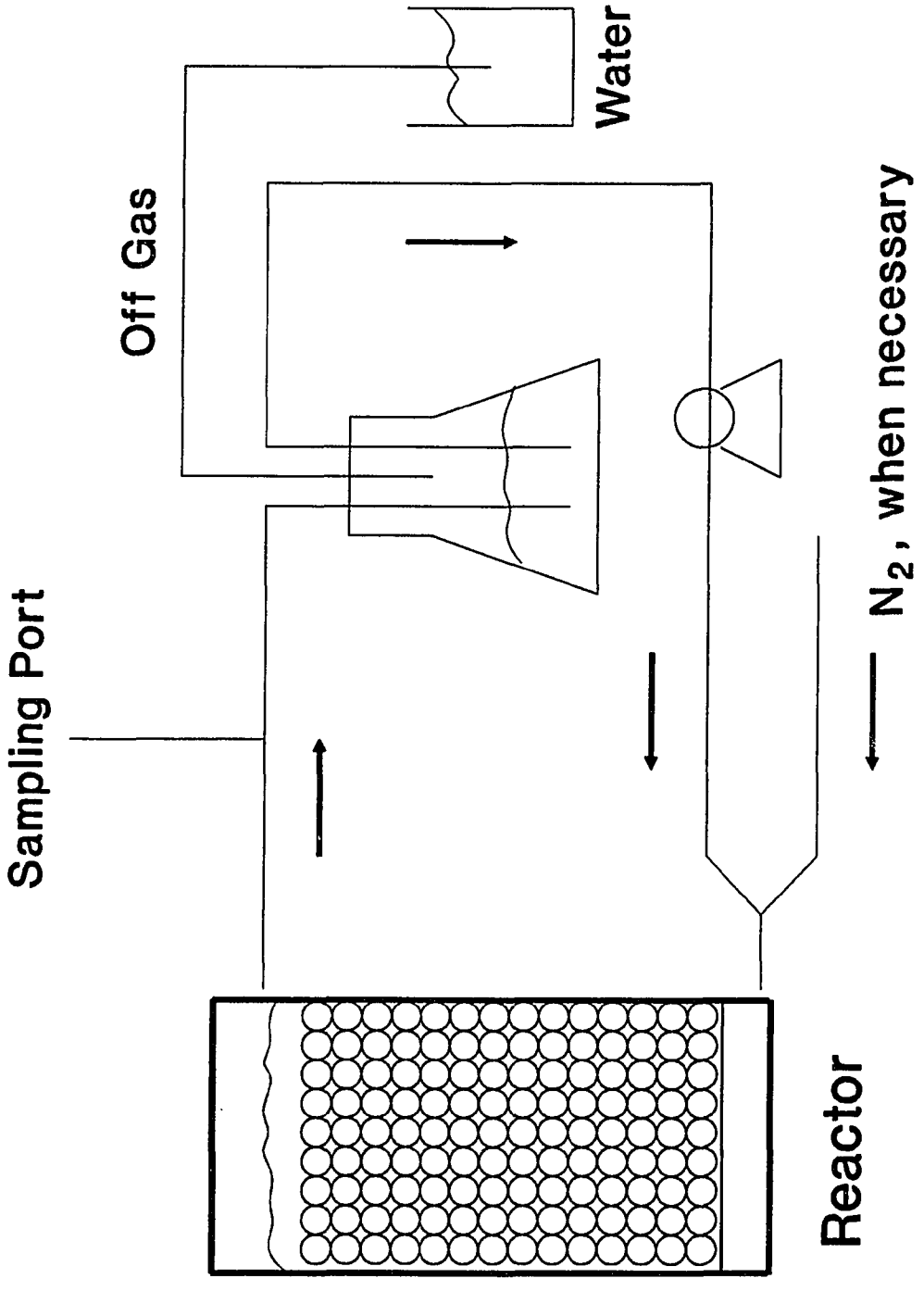


Figure 10. Diagram of Anaerobic-Aerobic Reactor System

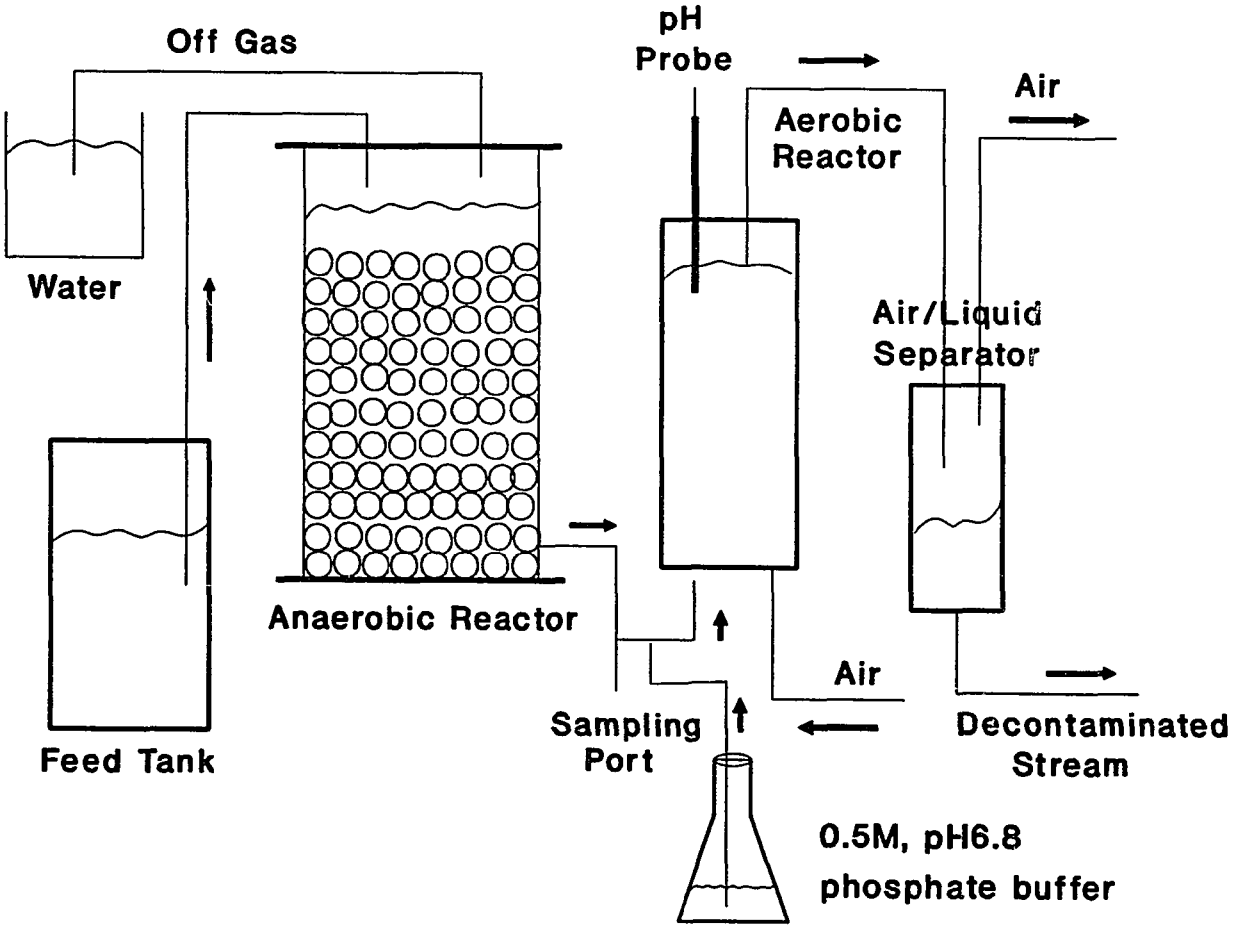


Figure 11. Anaerobic Degradation of 2,4,6-TCP in Batch System, Run 3-1

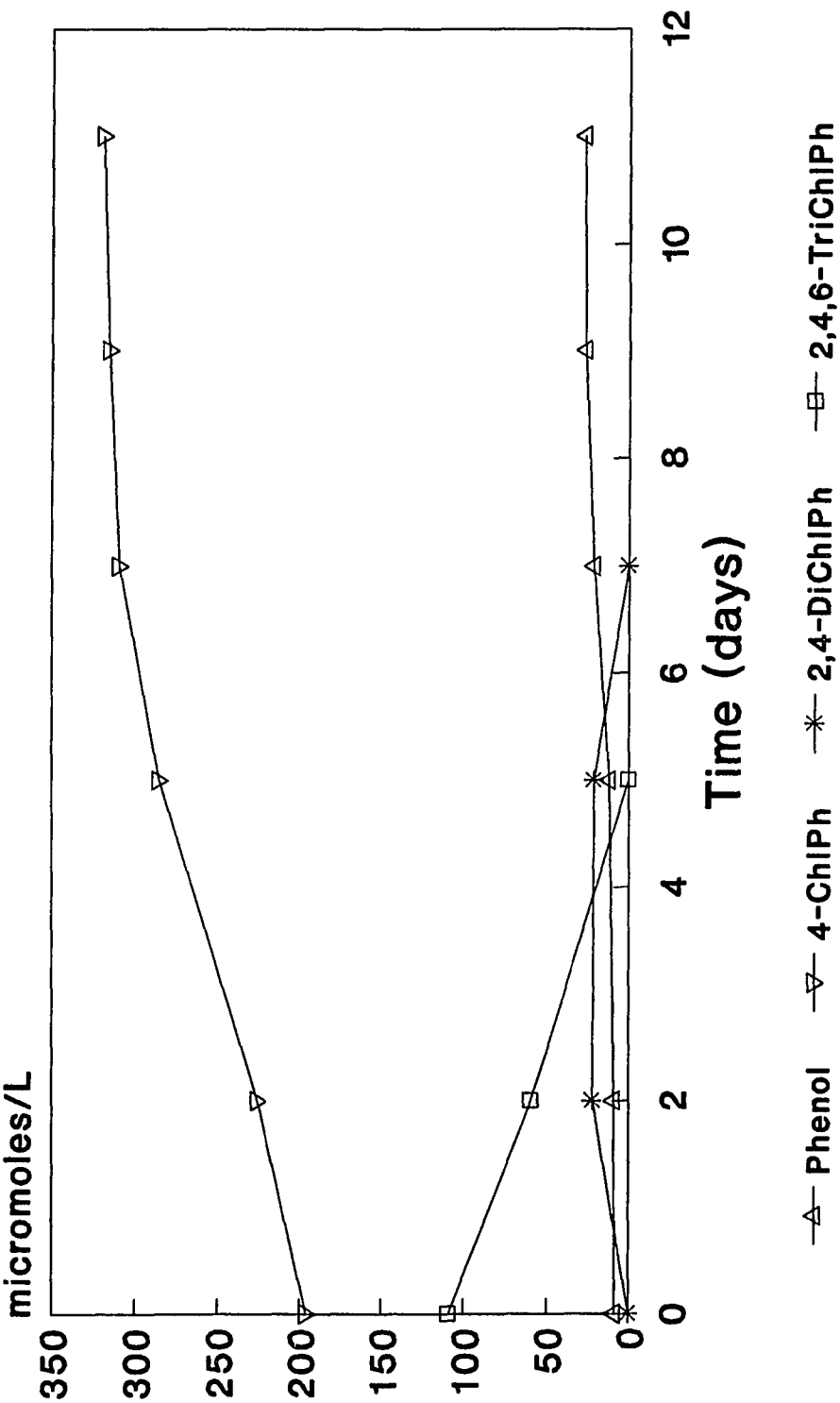


Figure 12. Anaerobic Degradation of 2,4,6-TCP in Batch System, Run 3-2

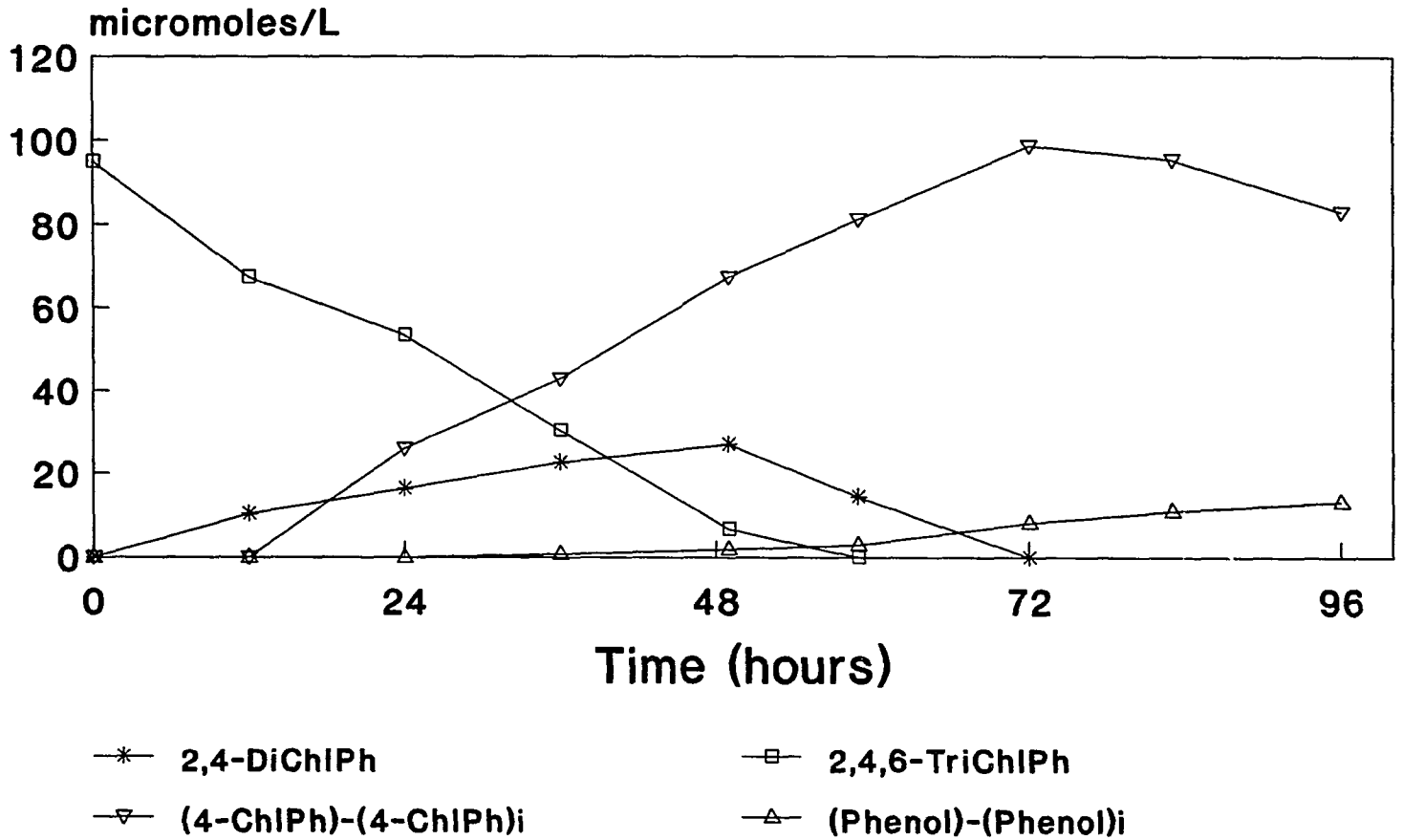


Figure 13. Effect of Phosphate Buffer on The Anaerobic Degradation of 2,4,6-TCP

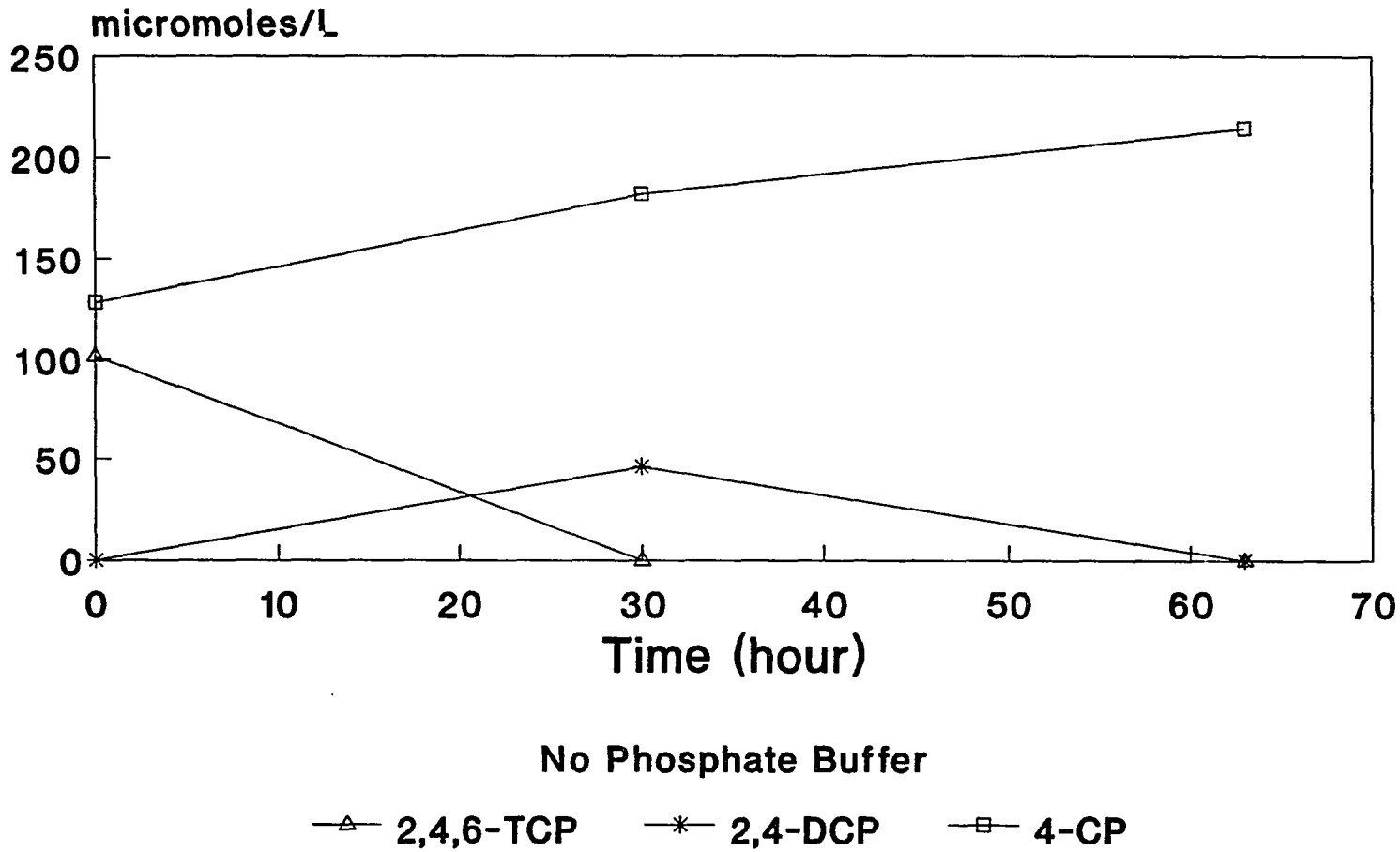


Figure 14. Effect of Phosphate Buffer on The Anaerobic Degradation of 2,4,6-TCP

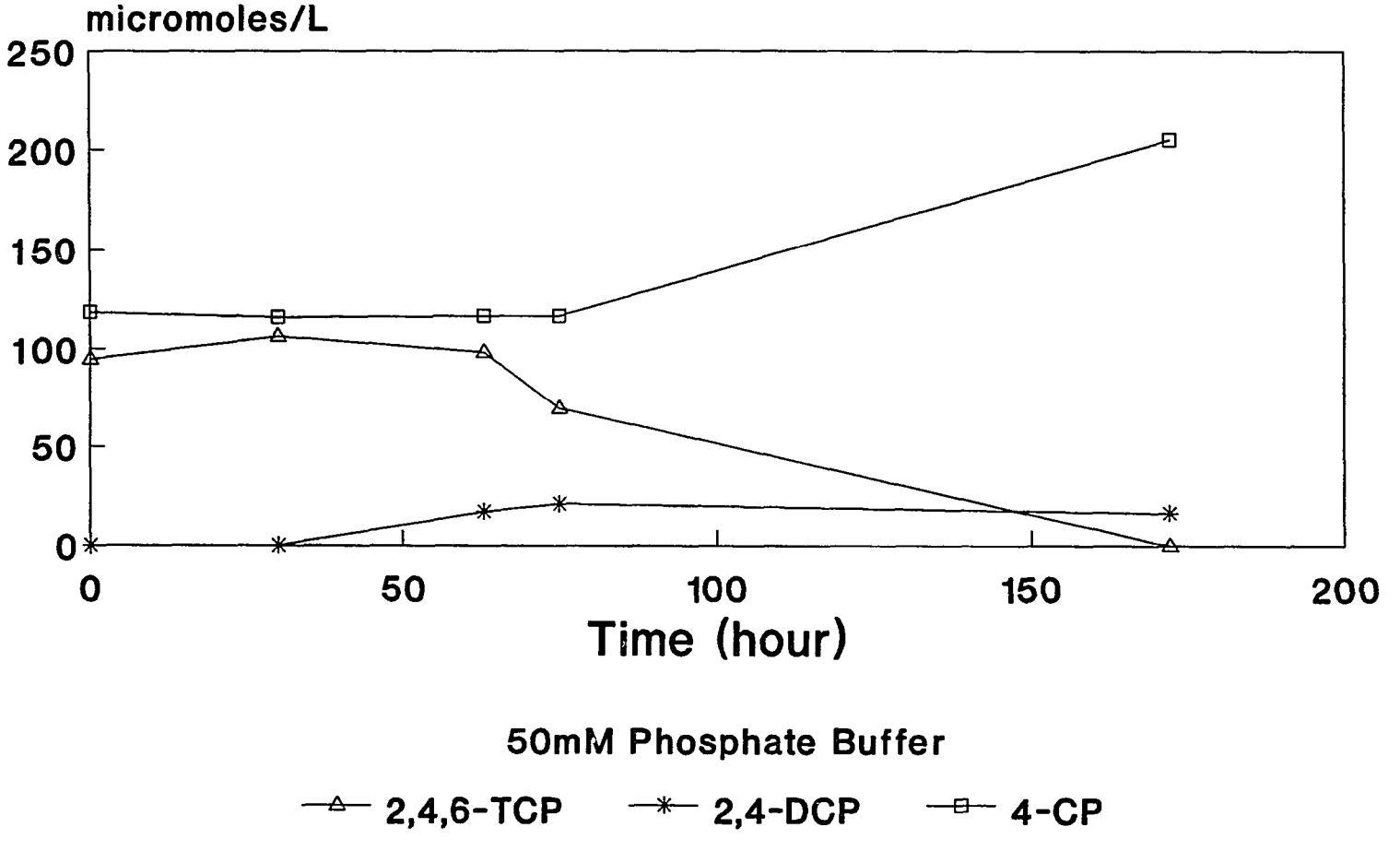


Figure 15. Effect of Phosphate Buffer on The Anaerobic Degradation of 2,4,6-TCP

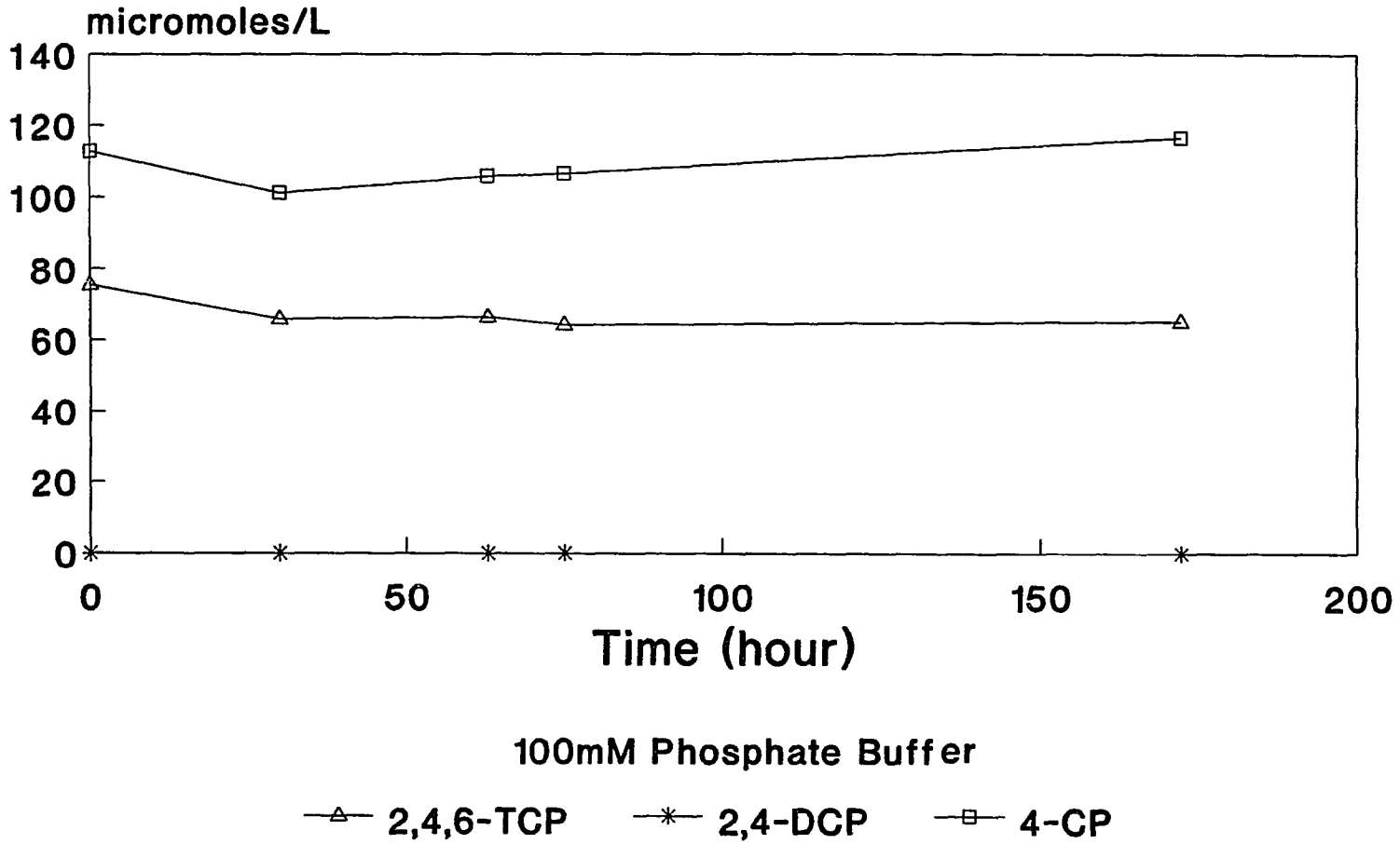


Figure 16. Effect of Phosphate Buffer on The Anaerobic Degradation of 2,4,6-TCP

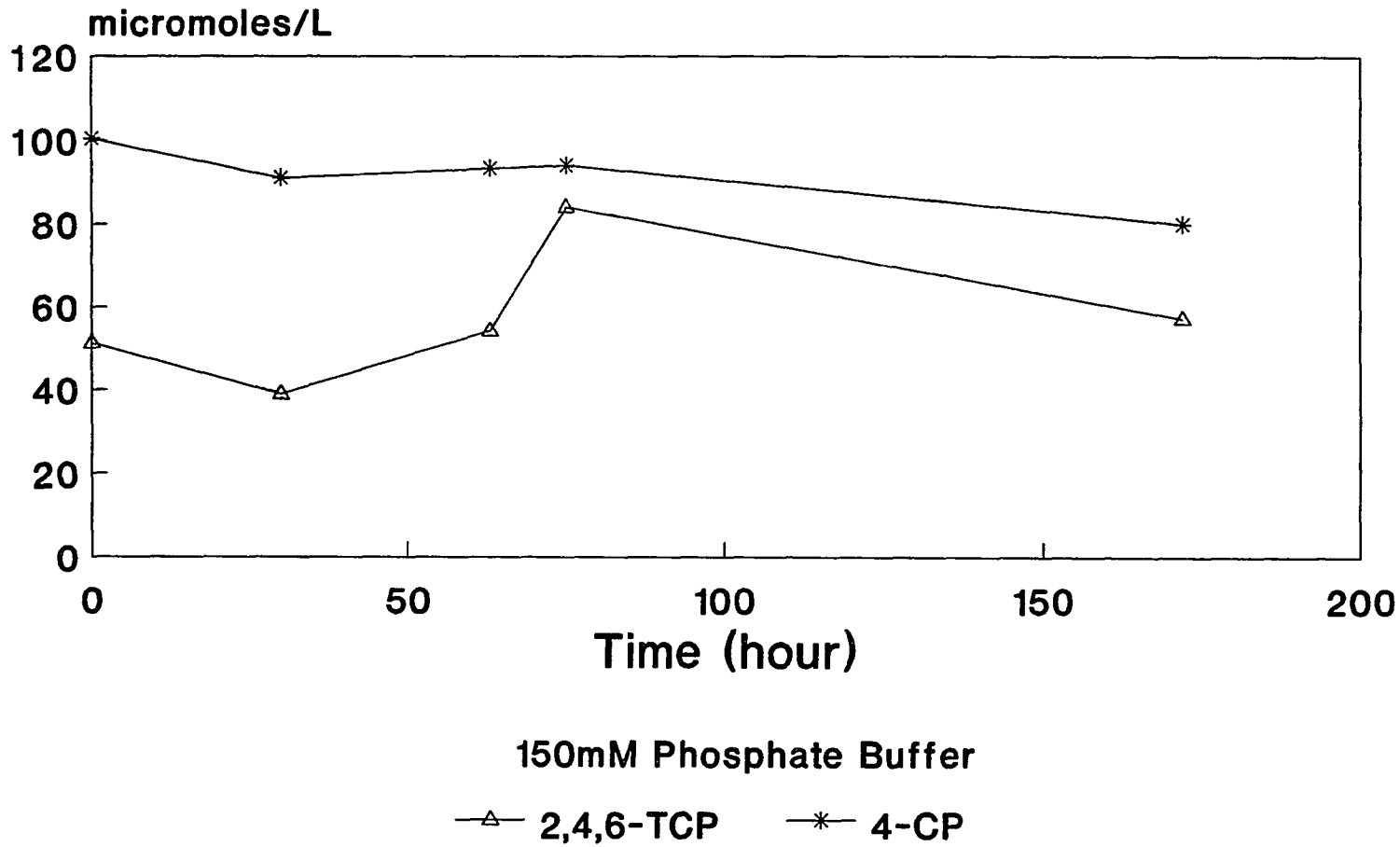


Figure 17. Anaerobic Degradation of 2,4,6-TCP in the Immobilization Phase

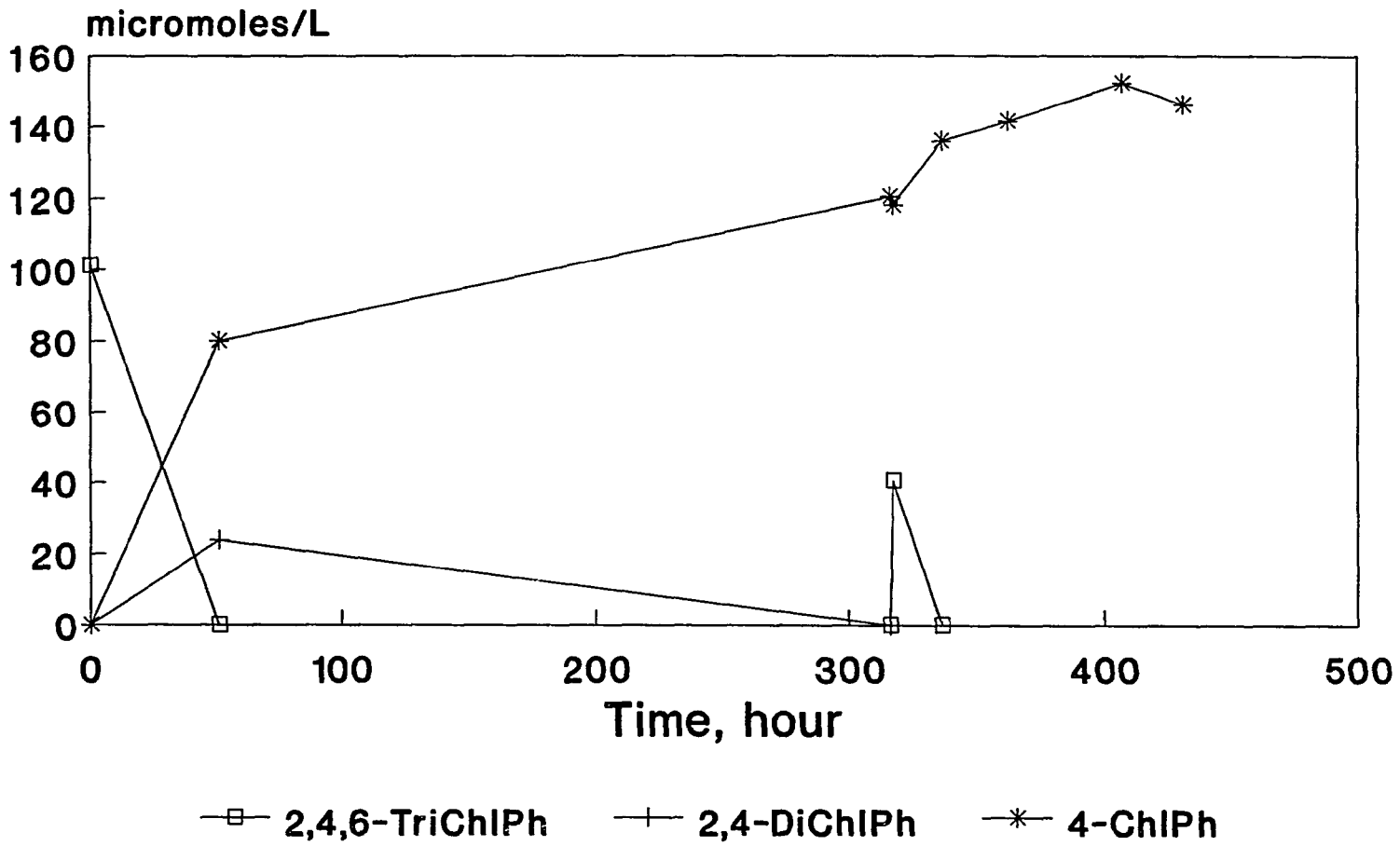


Figure 18. Anaerobic Degradation of 2,4,6-TCP After Immobilization

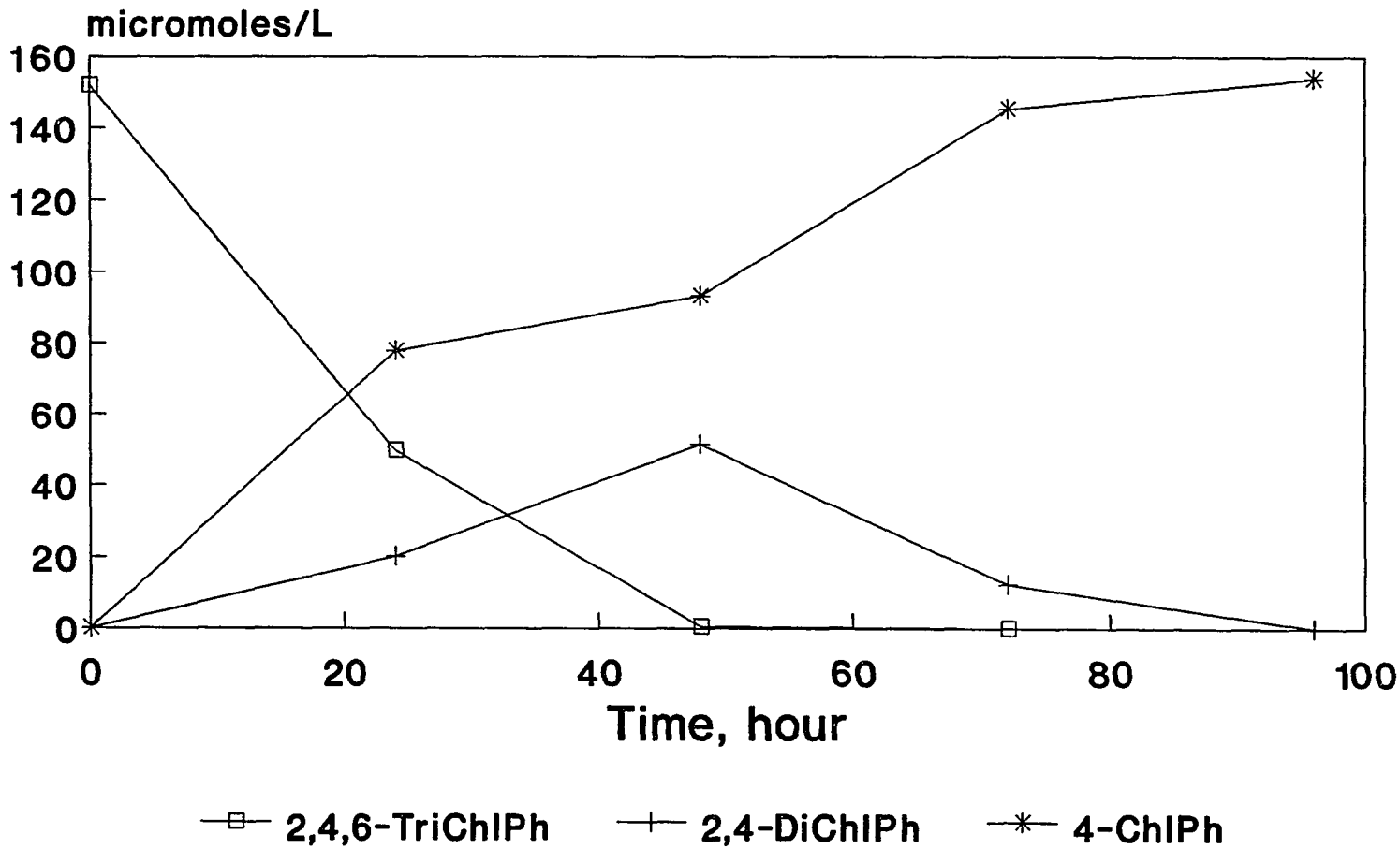


Figure 19. 2,4,6-TCP Degradation in the Immobilized Recirculation Reactor Run 6-1

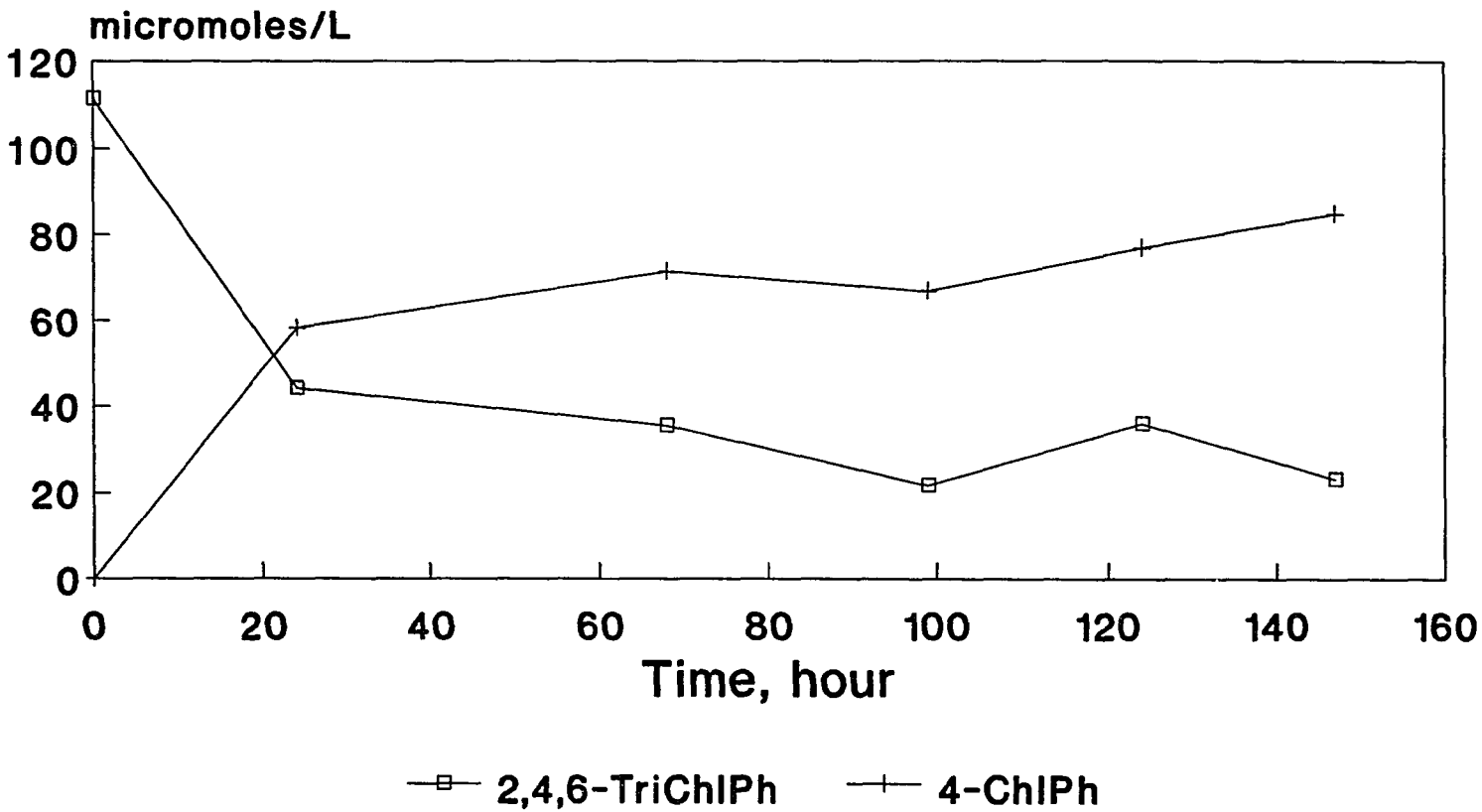


Figure 20. 2,4,6-TCP Degradation in the Immobilized Recirculation Reactor Run 6-2

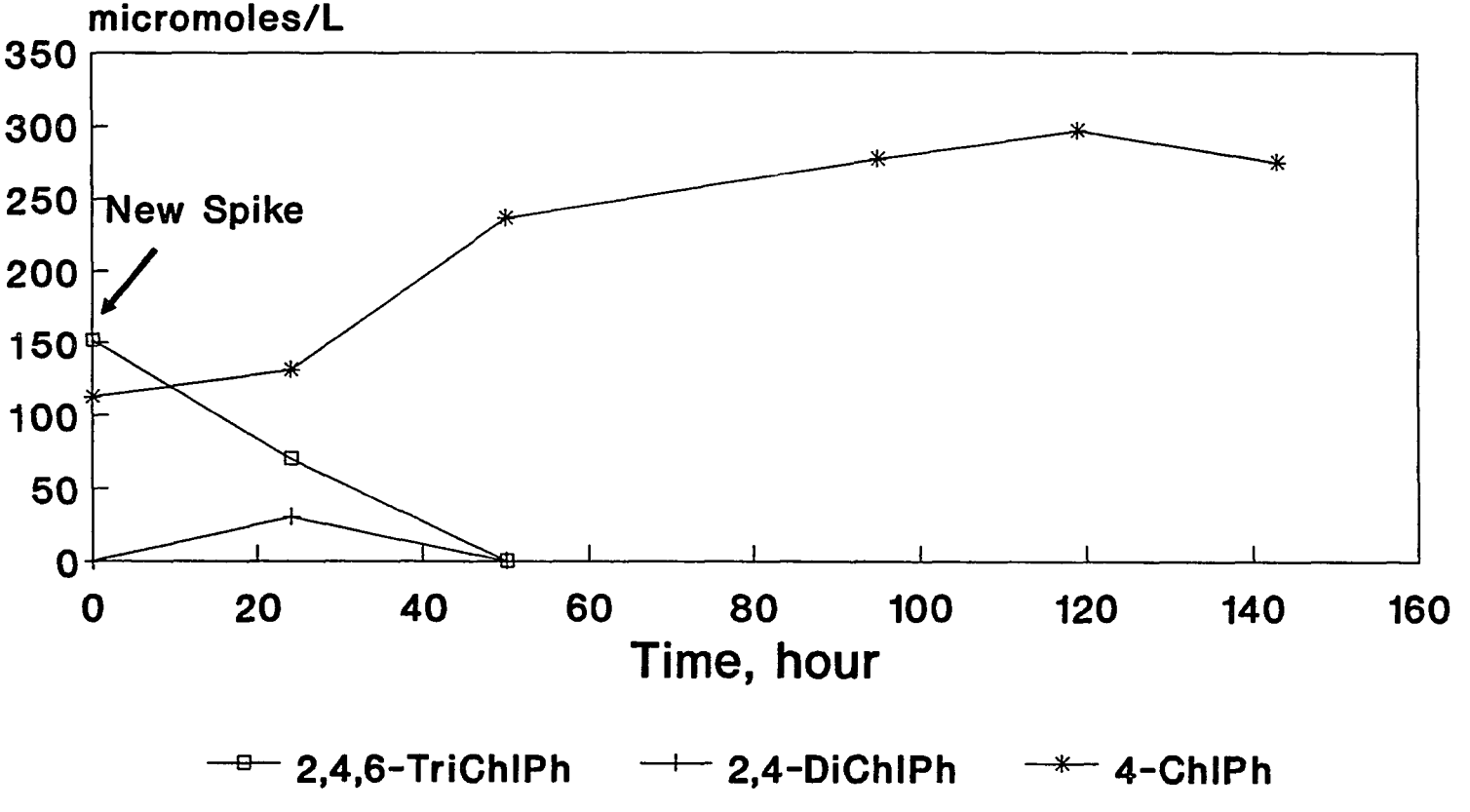


Figure 21. Stripping of 4-CP in the Aerated Aerobic Reactor

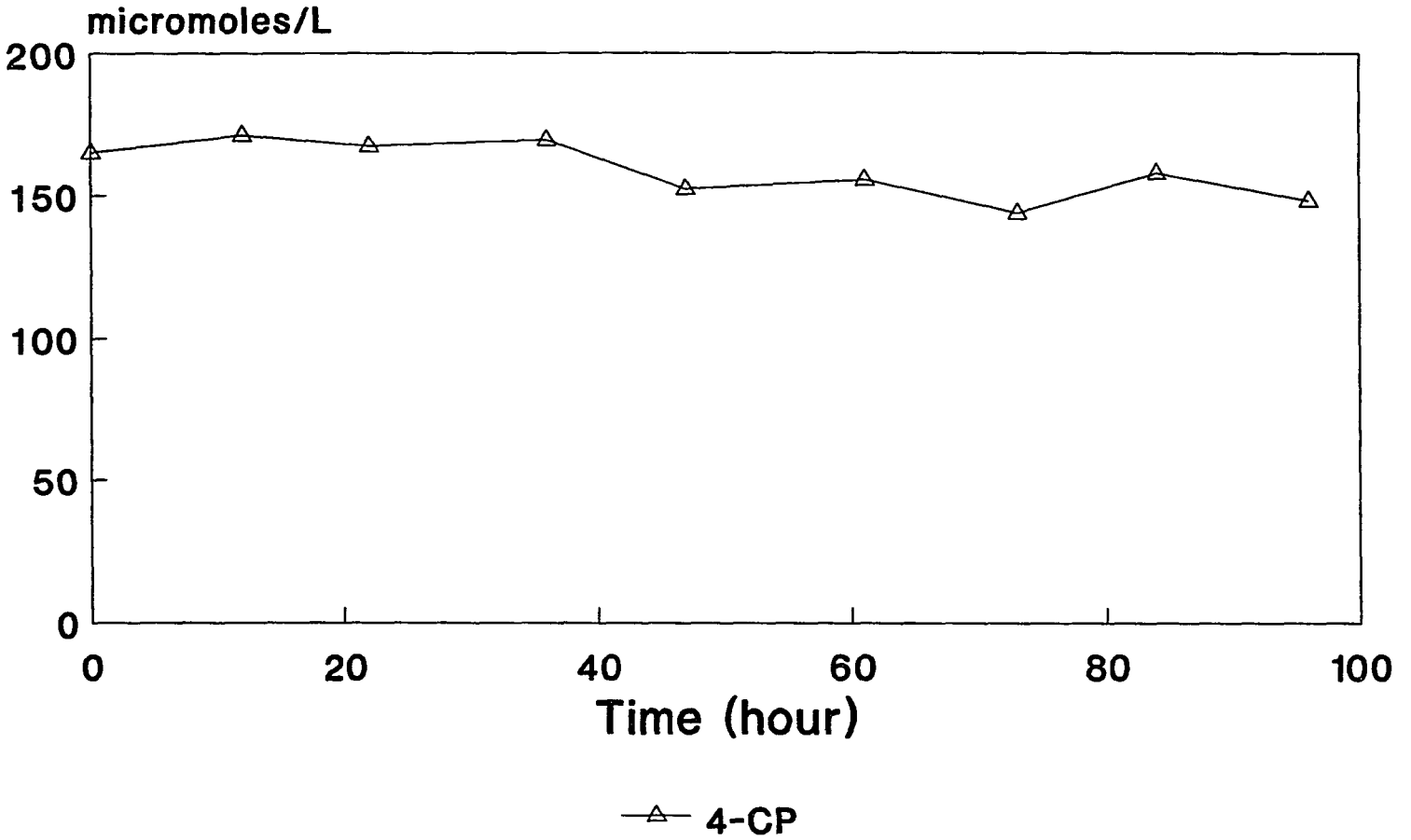


Figure 22. Aerobic Degradation of 4-CP in the Effluents from the Anaerobic Reactor by ATCC #93

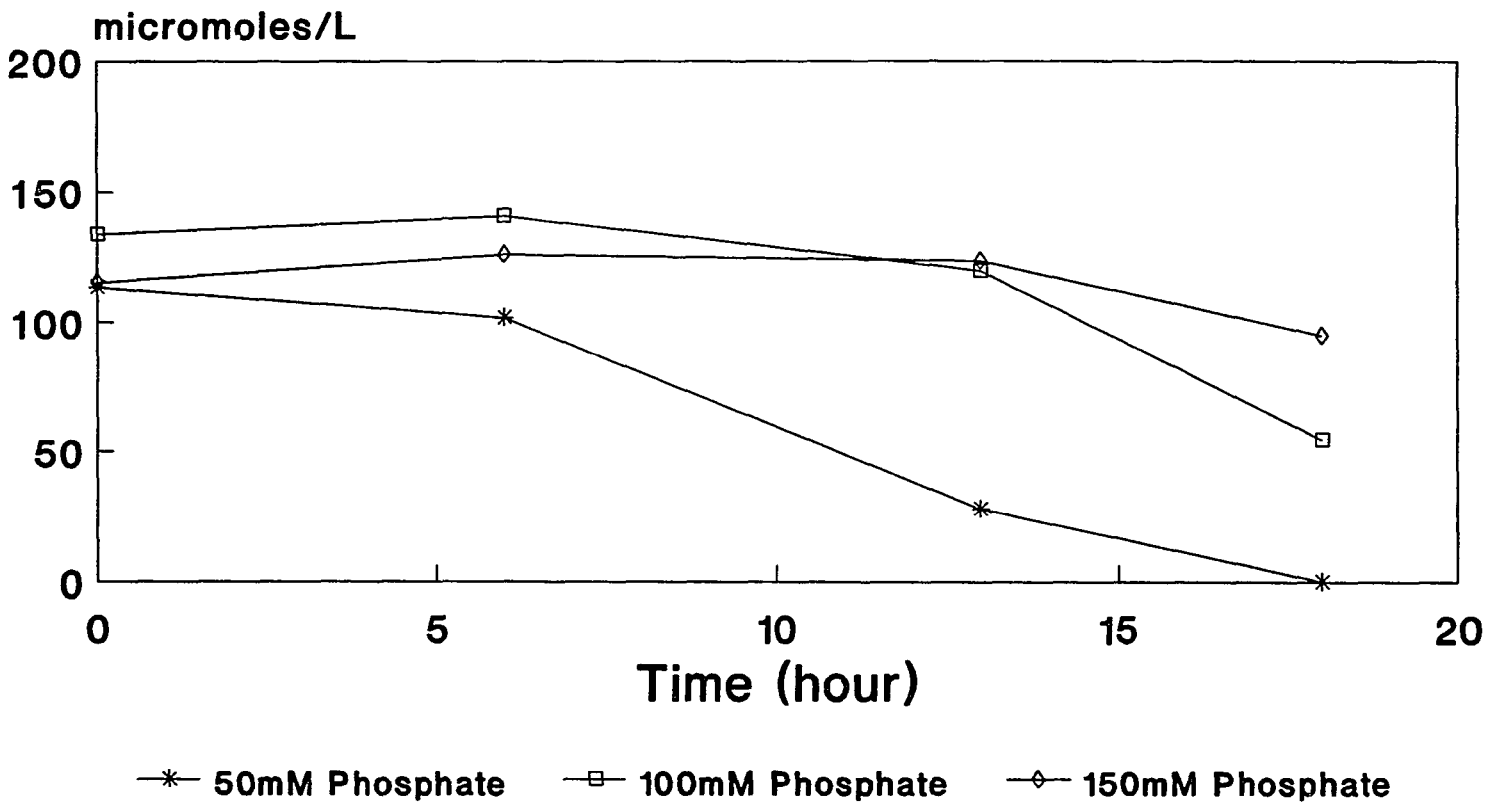


Figure 23. pH Change During the Aerobic Degradation of 4-CP by ATCC #93

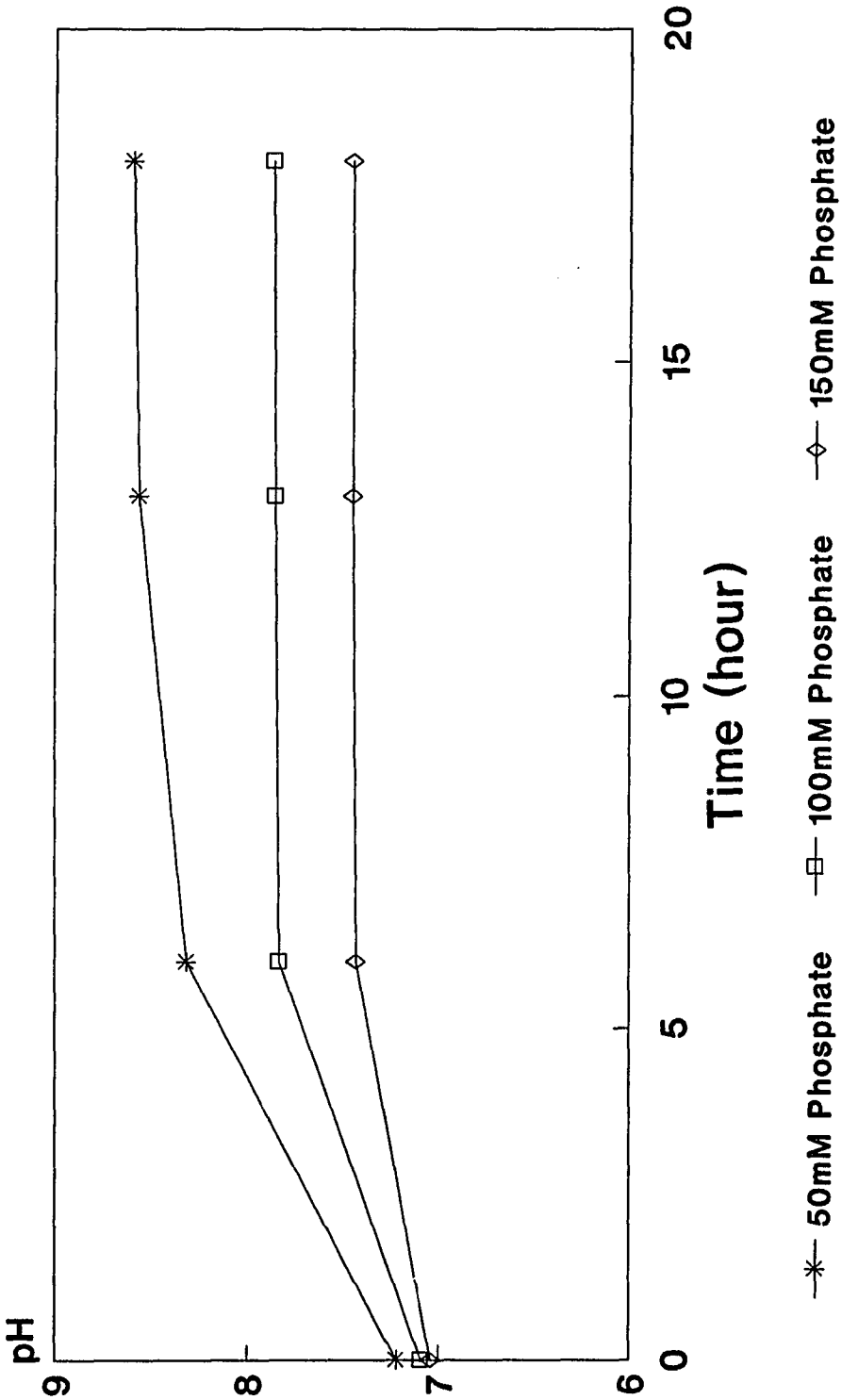


Figure 24. Aerobic Degradation of 4-CP in the Effluent from the Anaerobic Reactor by ATCC #17514

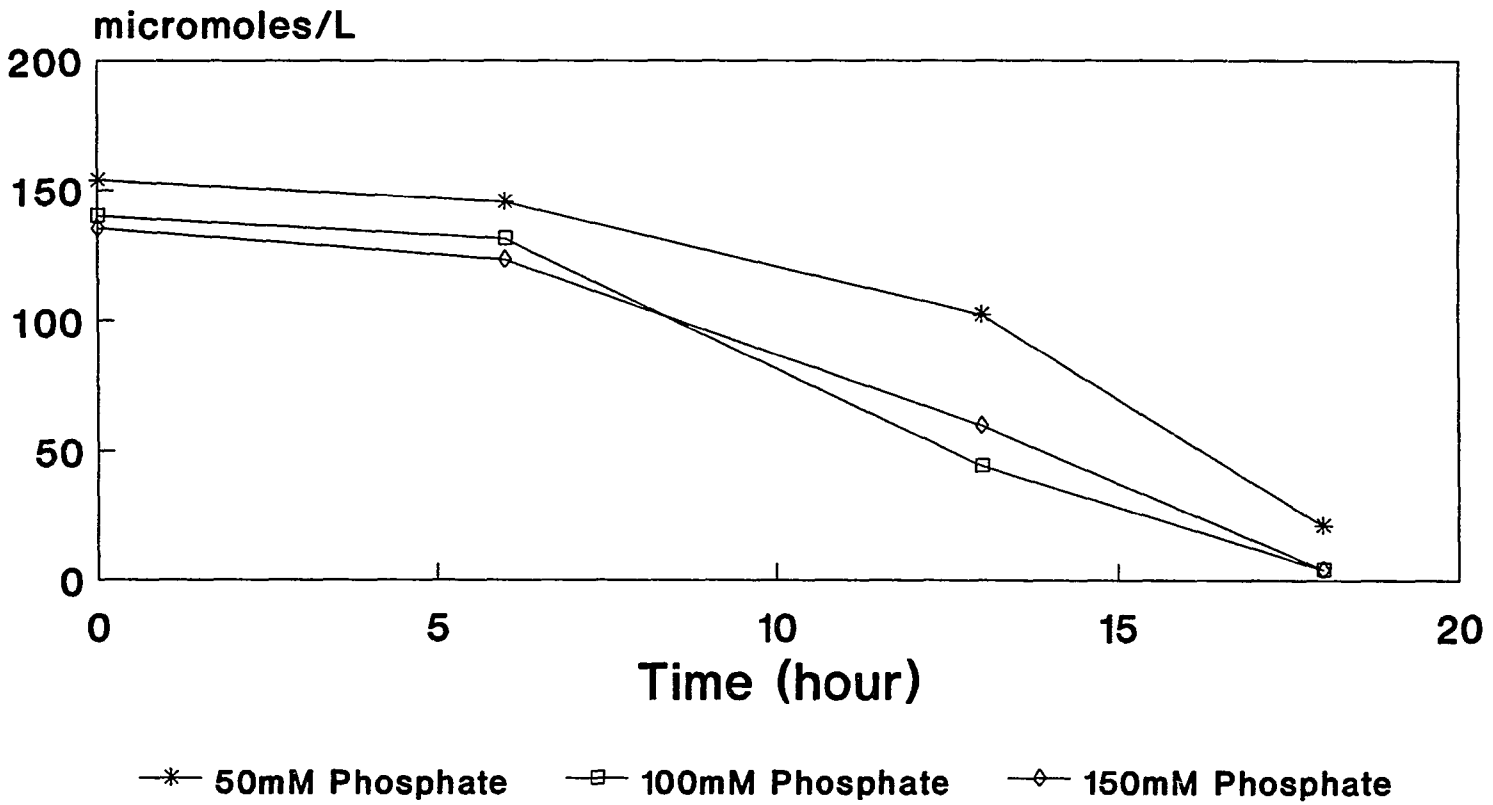


Figure 25. pH Change During the Aerobic Degradation of 4-CP by ATCC #17514

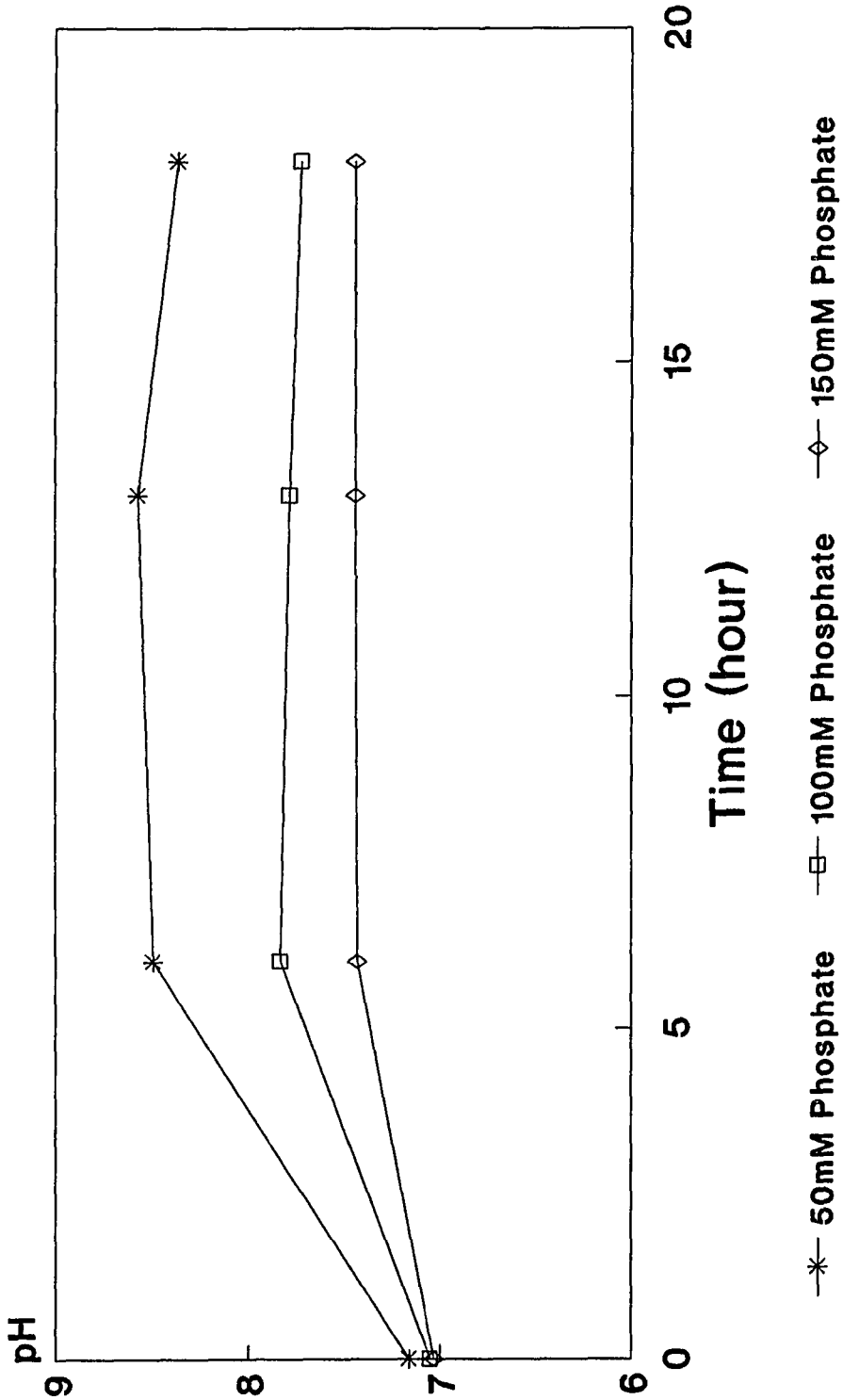


Figure 26. Aerobic Degradation of 4-CP in the Effluents from the Anaerobic Reactor by ATCC #17991

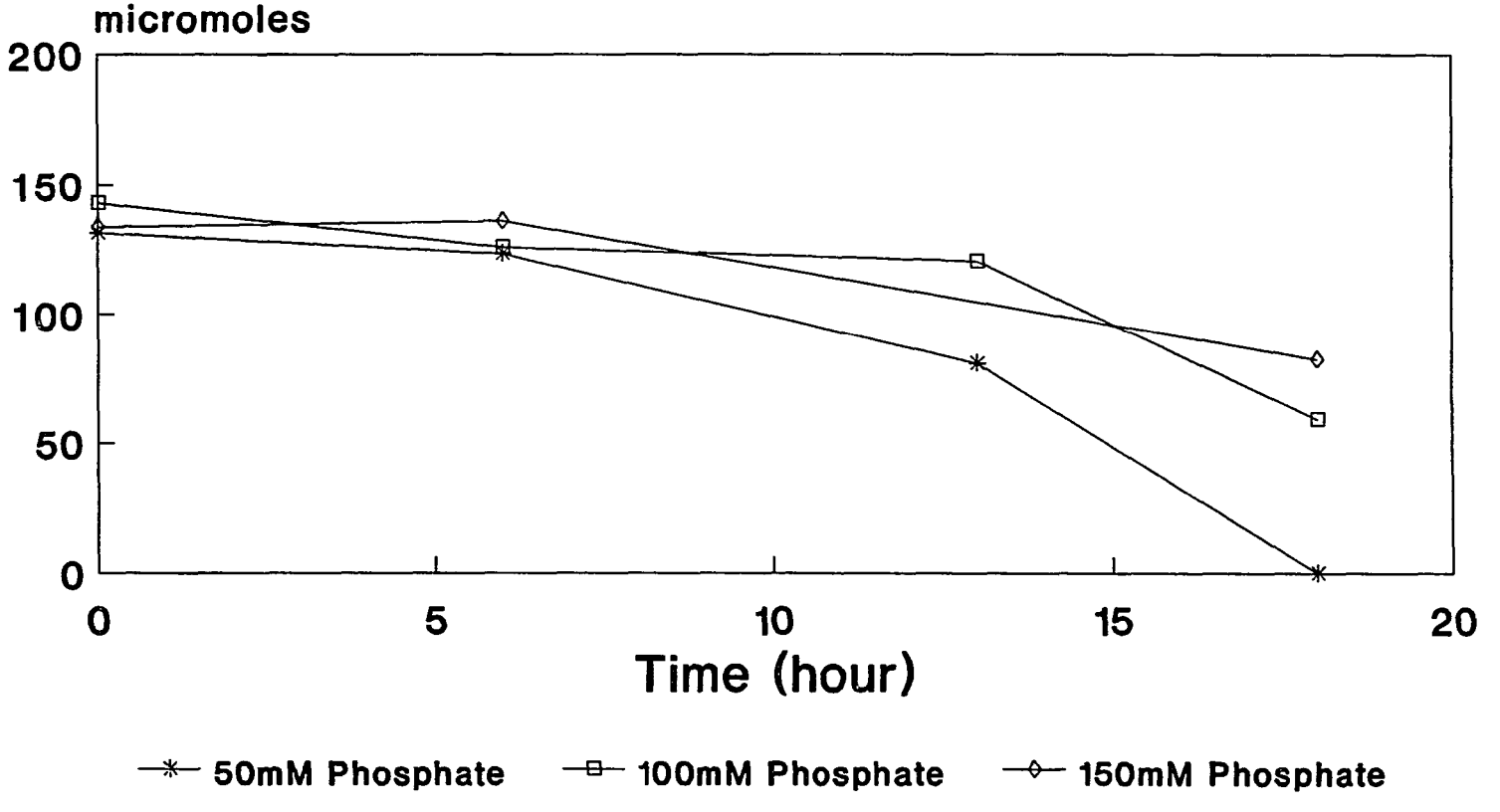


Figure 27. pH Change During the Aerobic Degradation of 4-CP by ATCC #17991

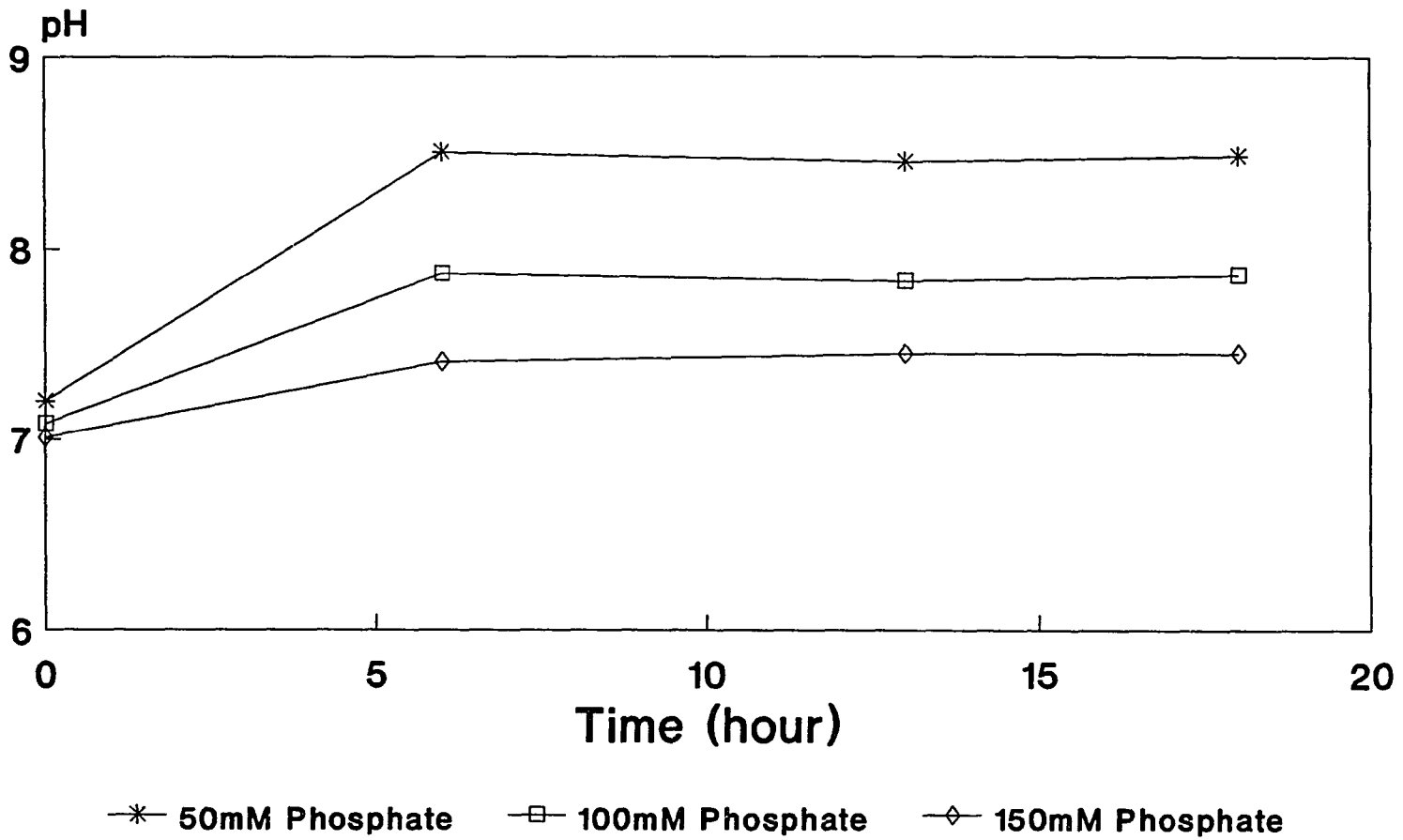


Figure 28. Aerobic Degradation of 4-CP in the Effluents from the Anaerobic Reactor by ATCC #29195

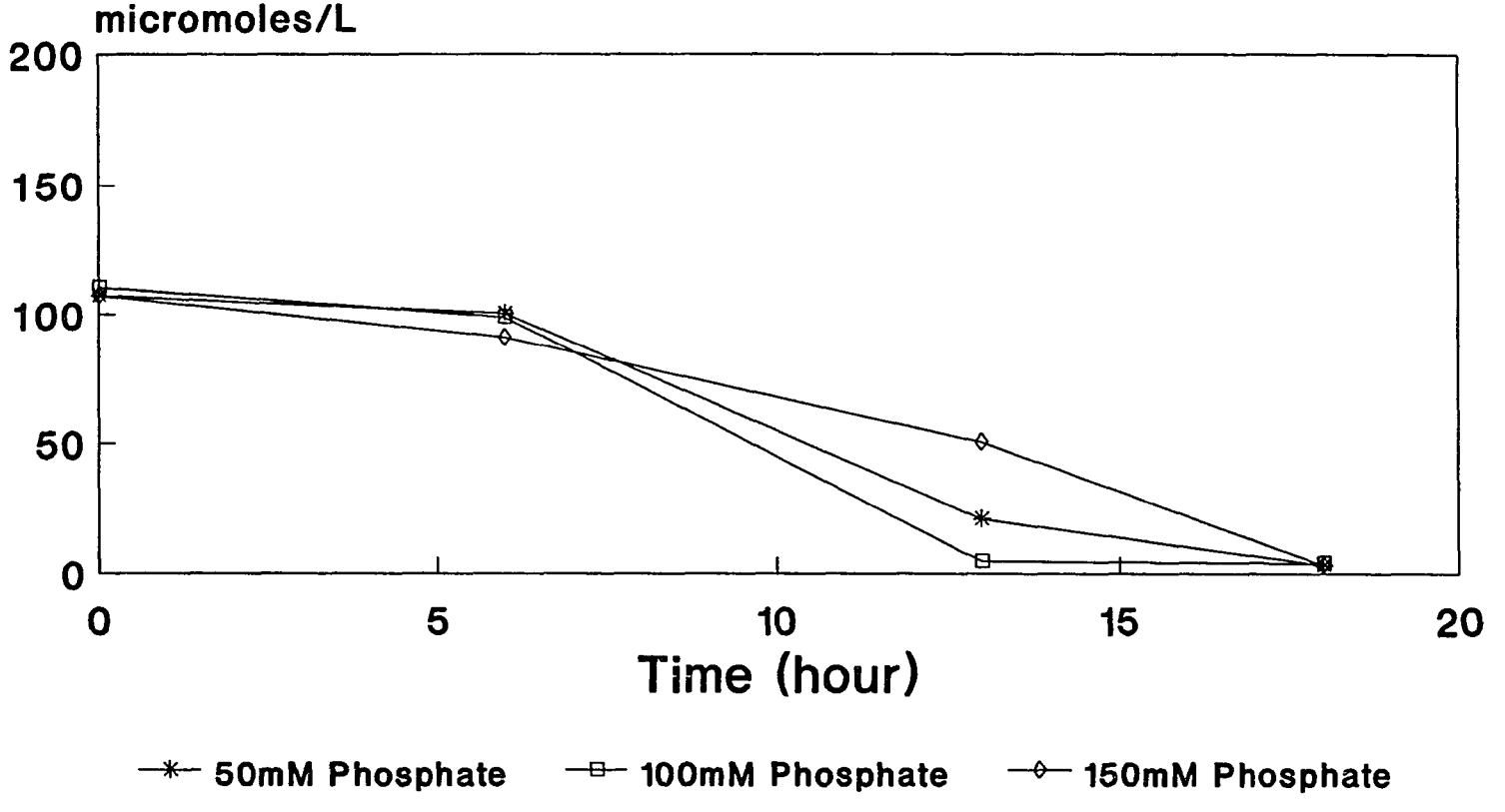


Figure 29. pH Change During the Aerobic Degradation of 4-CP by ATCC #29195

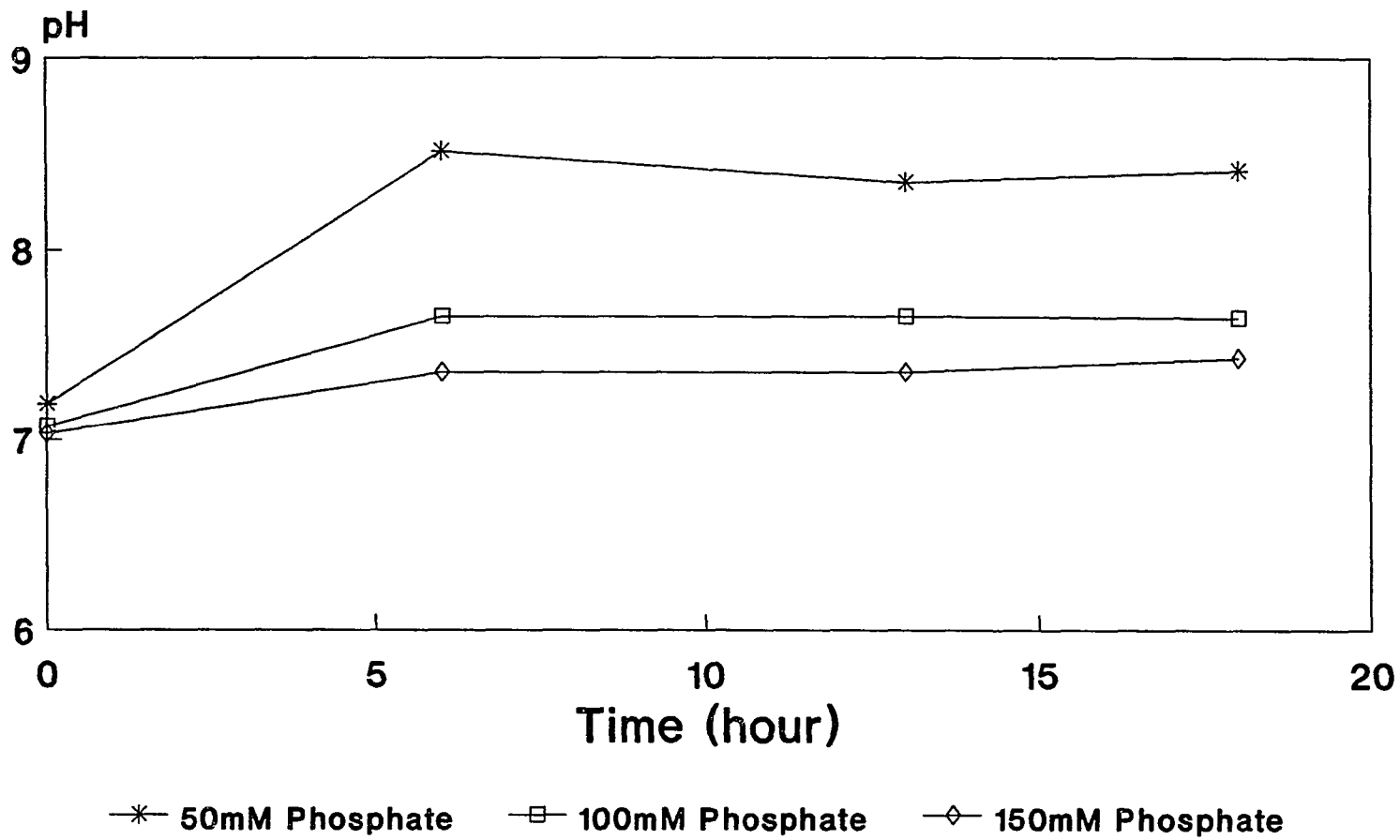


Figure 30. Aerobic Degradation of 4-CP in the Effluents from the Anaerobic Reactor by ATCC #33668

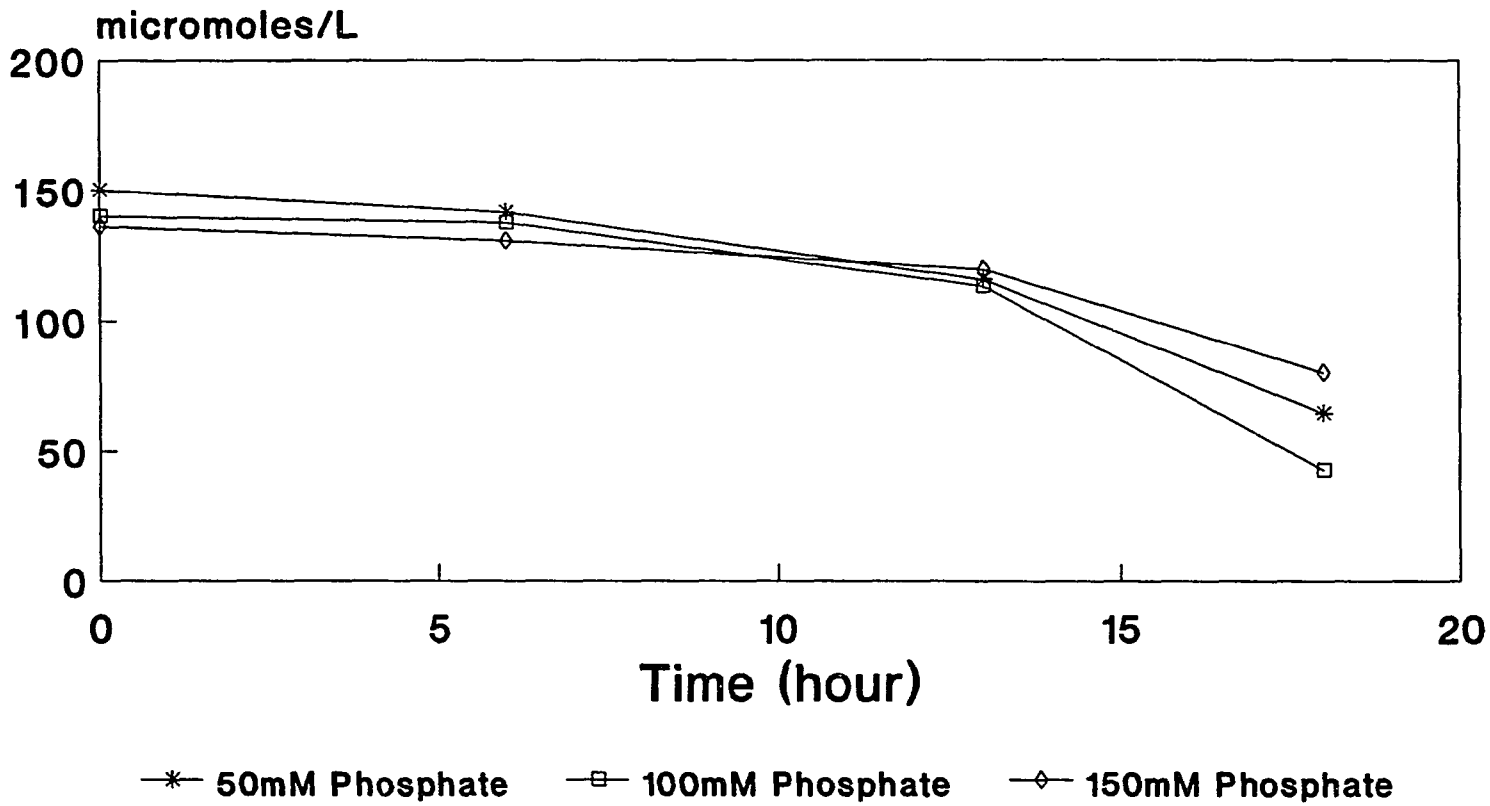


Figure 31. pH Change During the Aerobic Degradation of 4-CP by ATCC #33668

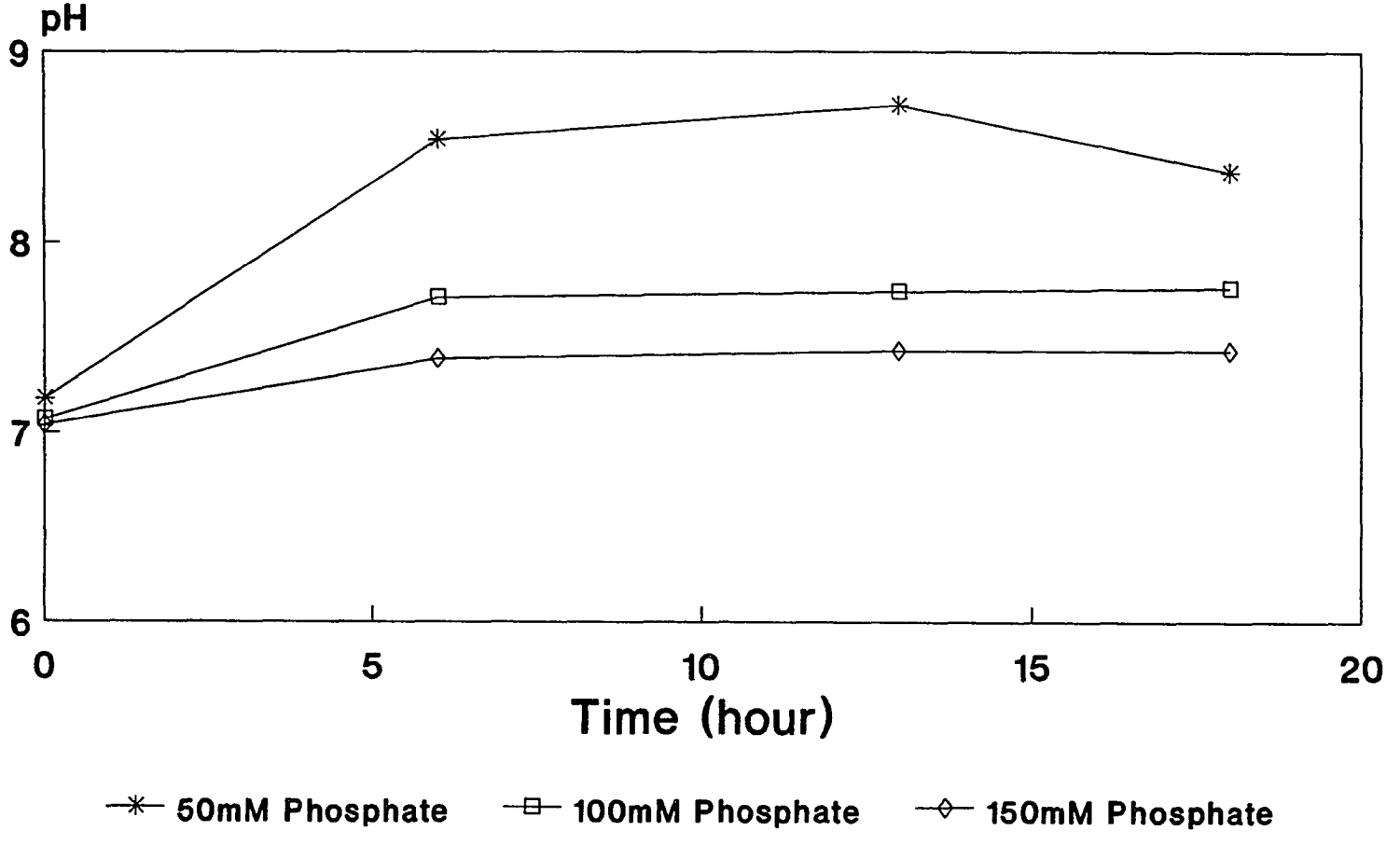


Figure 32. Aerobic Degradation of 4-CP In the Effluents from the Anaerobic Reactor by the Indigenous Culture, Run 15-1

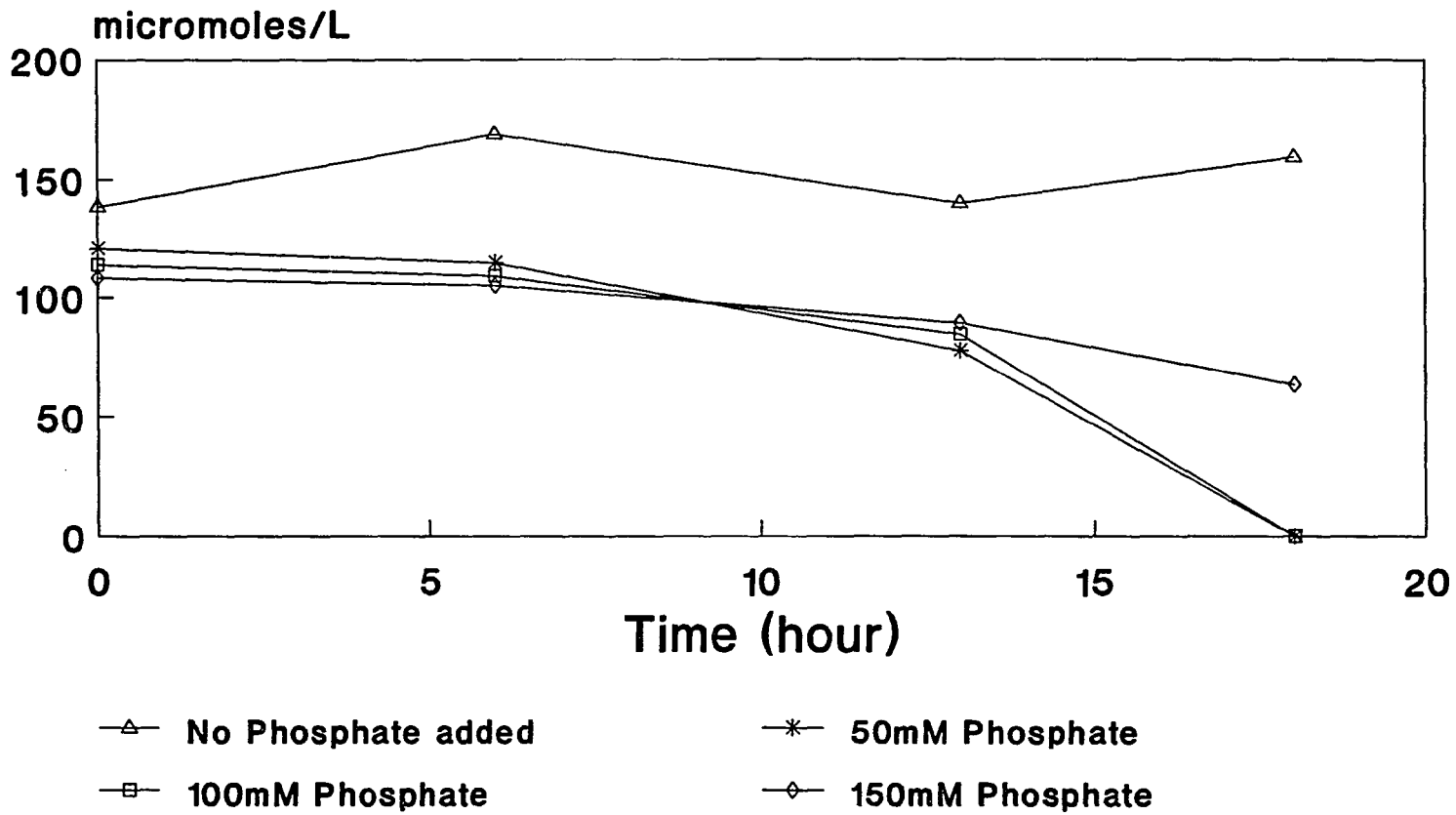


Figure 33. pH Change During the Aerobic Degradation of 4-CP by the Indigenous Culture, Run 15-1

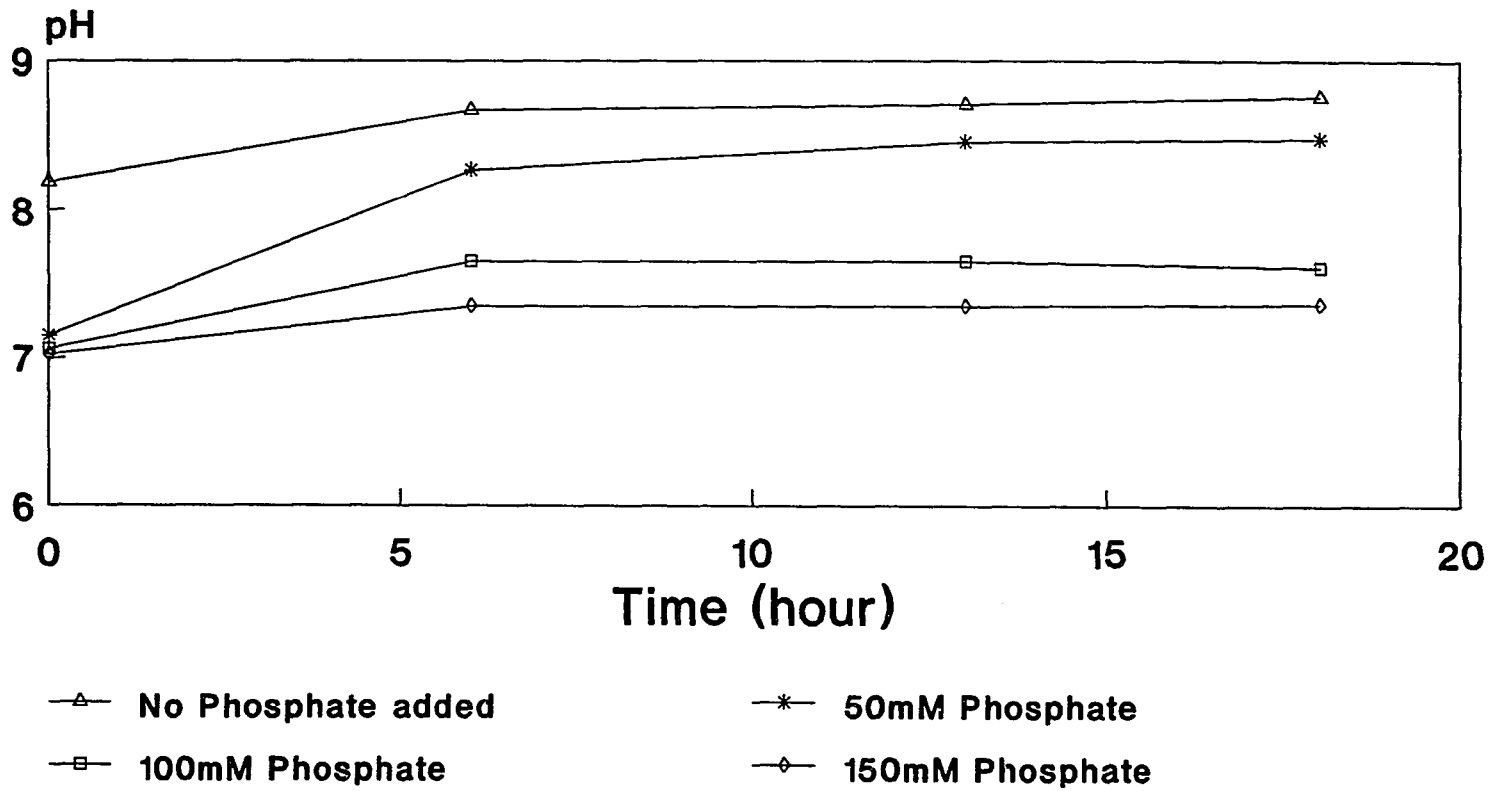


Figure 34. Aerobic Degradation of 4-CP In the Effluents from the Anaerobic Reactor by the Indigenous Culture, Run 15-2

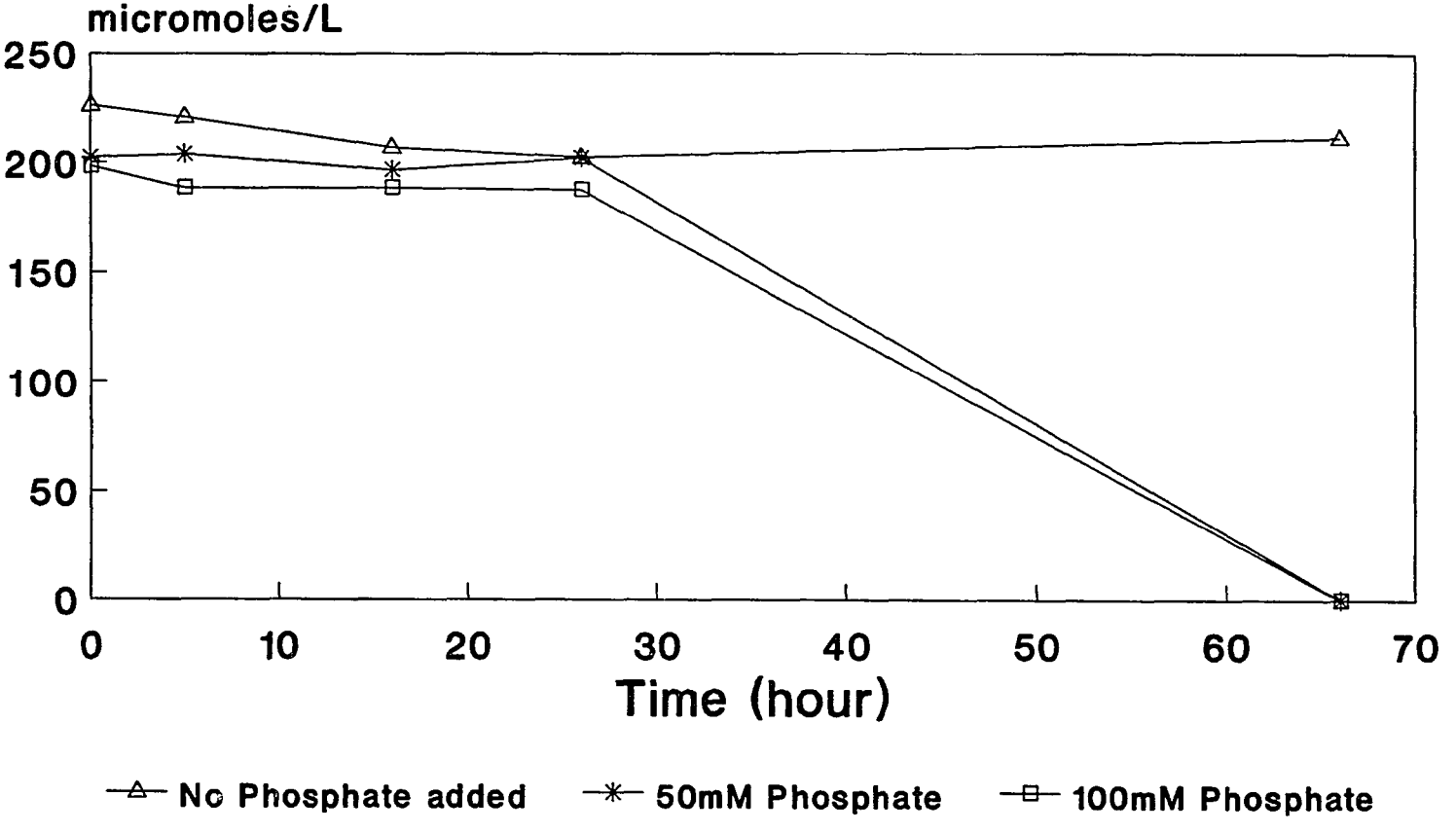


Figure 35. pH Change During the Aerobic Degradation of 4-CP by the Indigenous Culture, Run 15-2

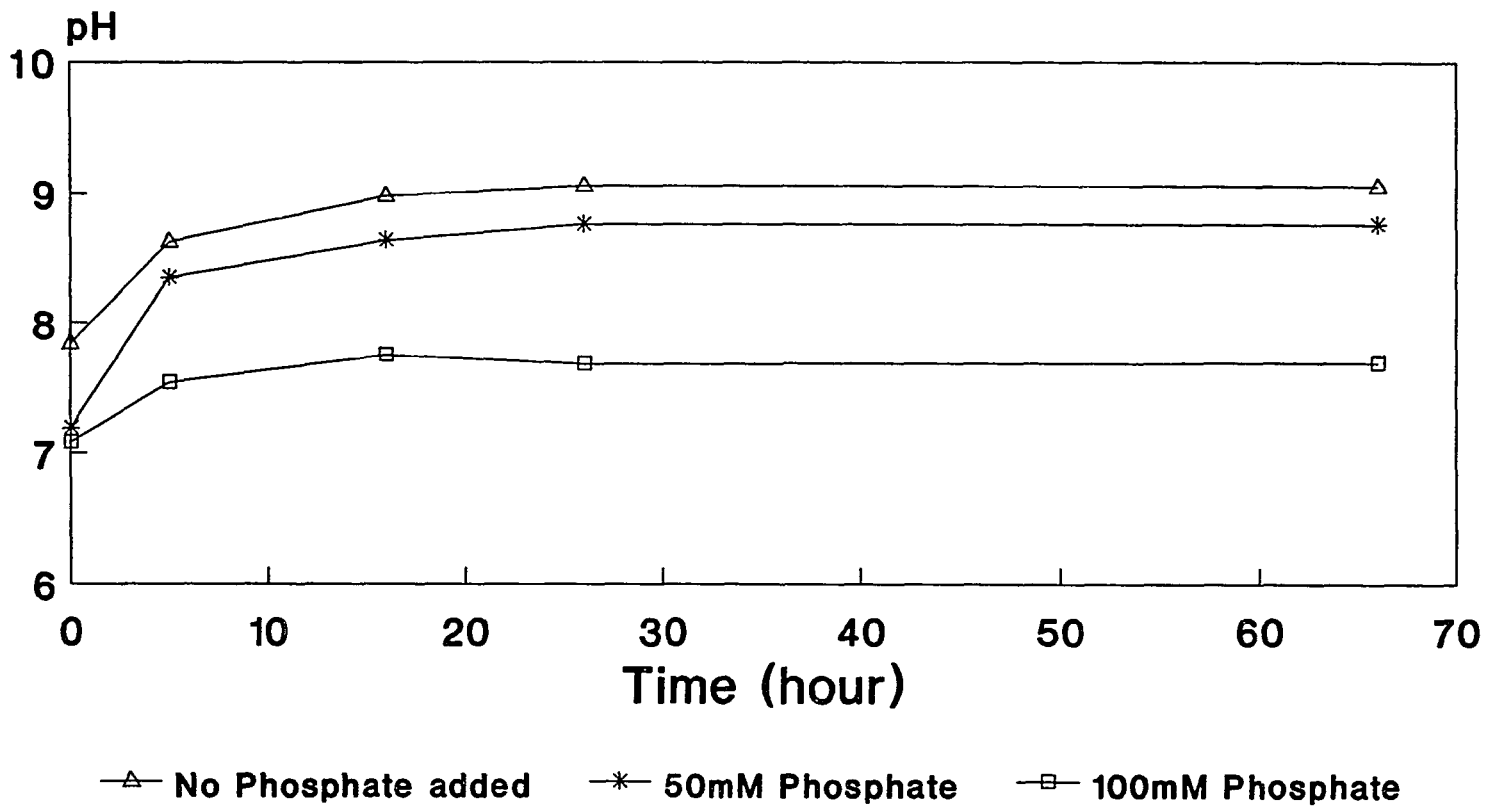


Figure 36. Aerobic Degradation of 4-CP In the Effluents from the Anaerobic Reactor by the Indigenous Culture, Run 15-3

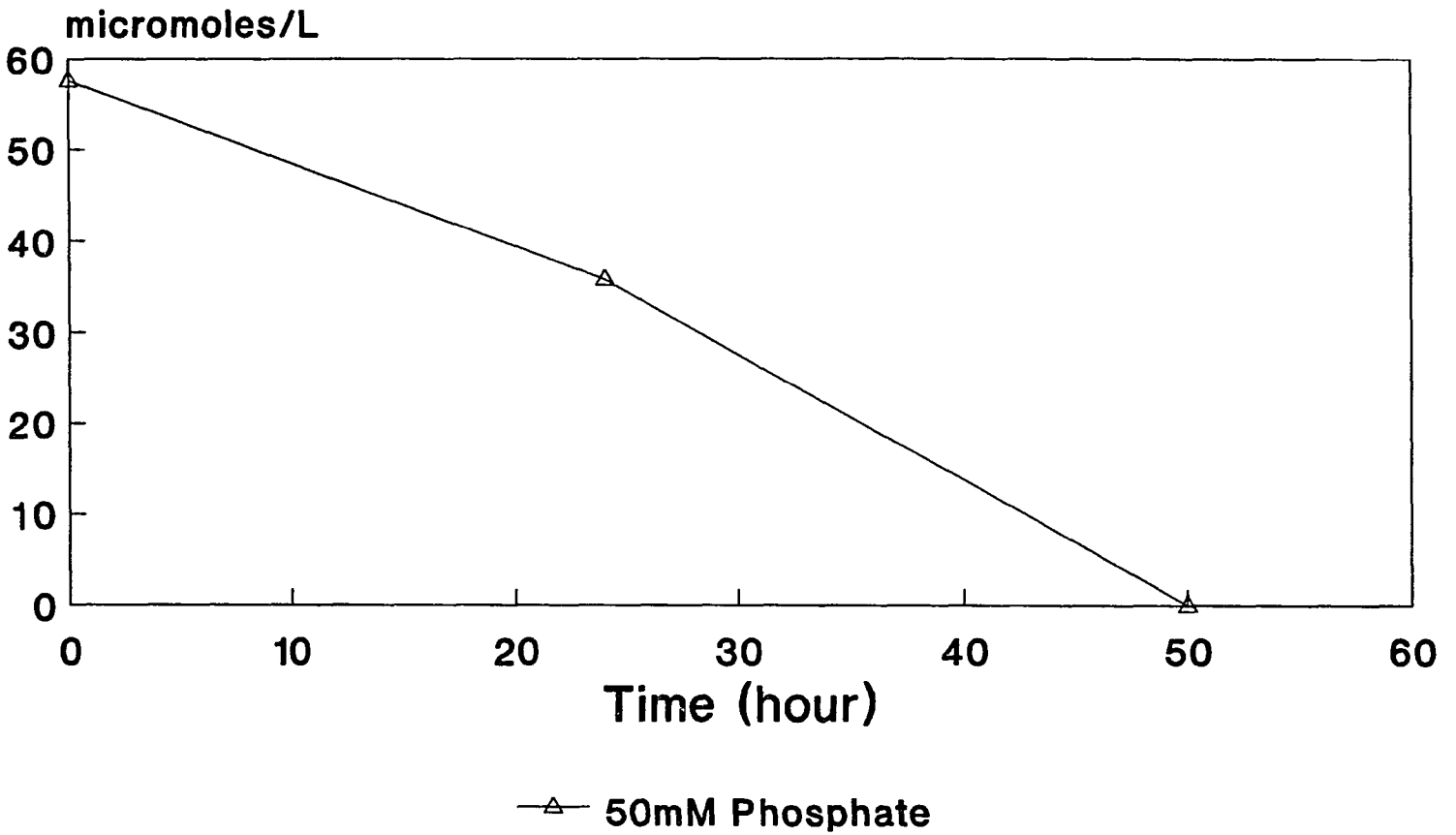


Figure 37. Aerobic Degradation of 4-CP in the Effluents from the Anaerobic Reactor By J.M. Anaerobic Sludge

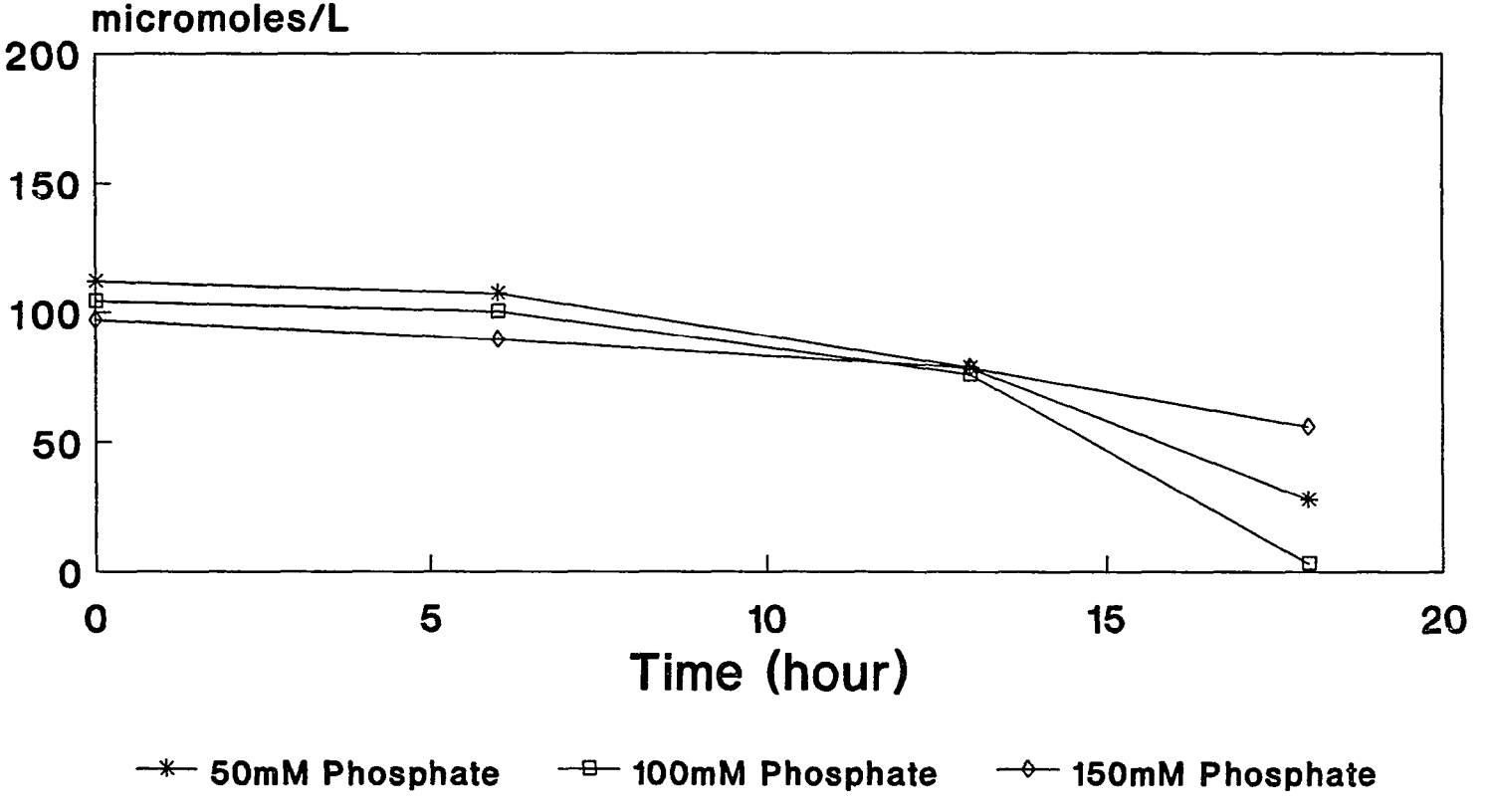


Figure 38. pH Change During the Aerobic Degradation of 4-CP by J.M. Anaerobic Sludge

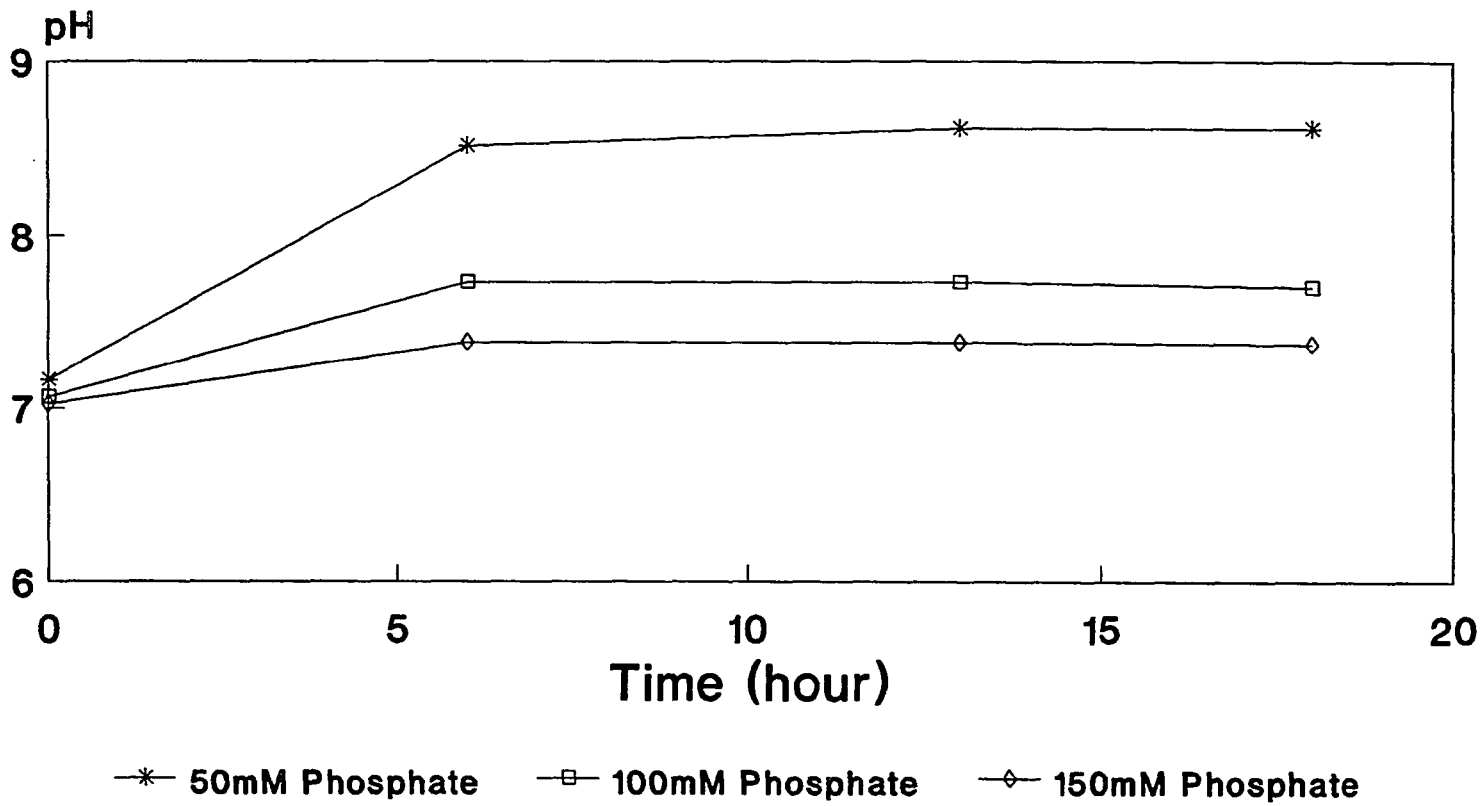


Figure 39. Aerobic Degradation of 4-CP By the Indigenous Culture without TCP

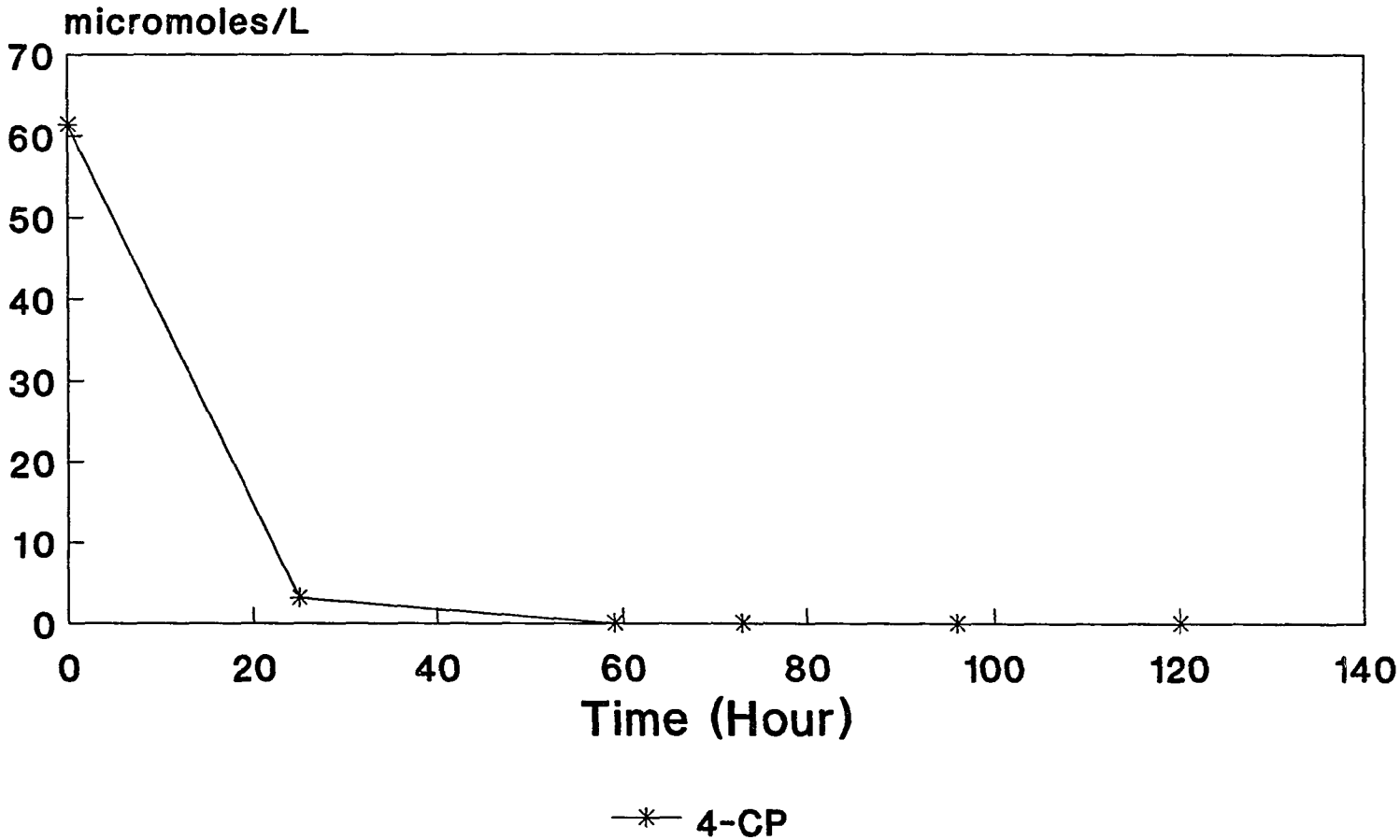


Figure 40. Aerobic Degradation of 4-CP By the Indigenous Culture with TCP

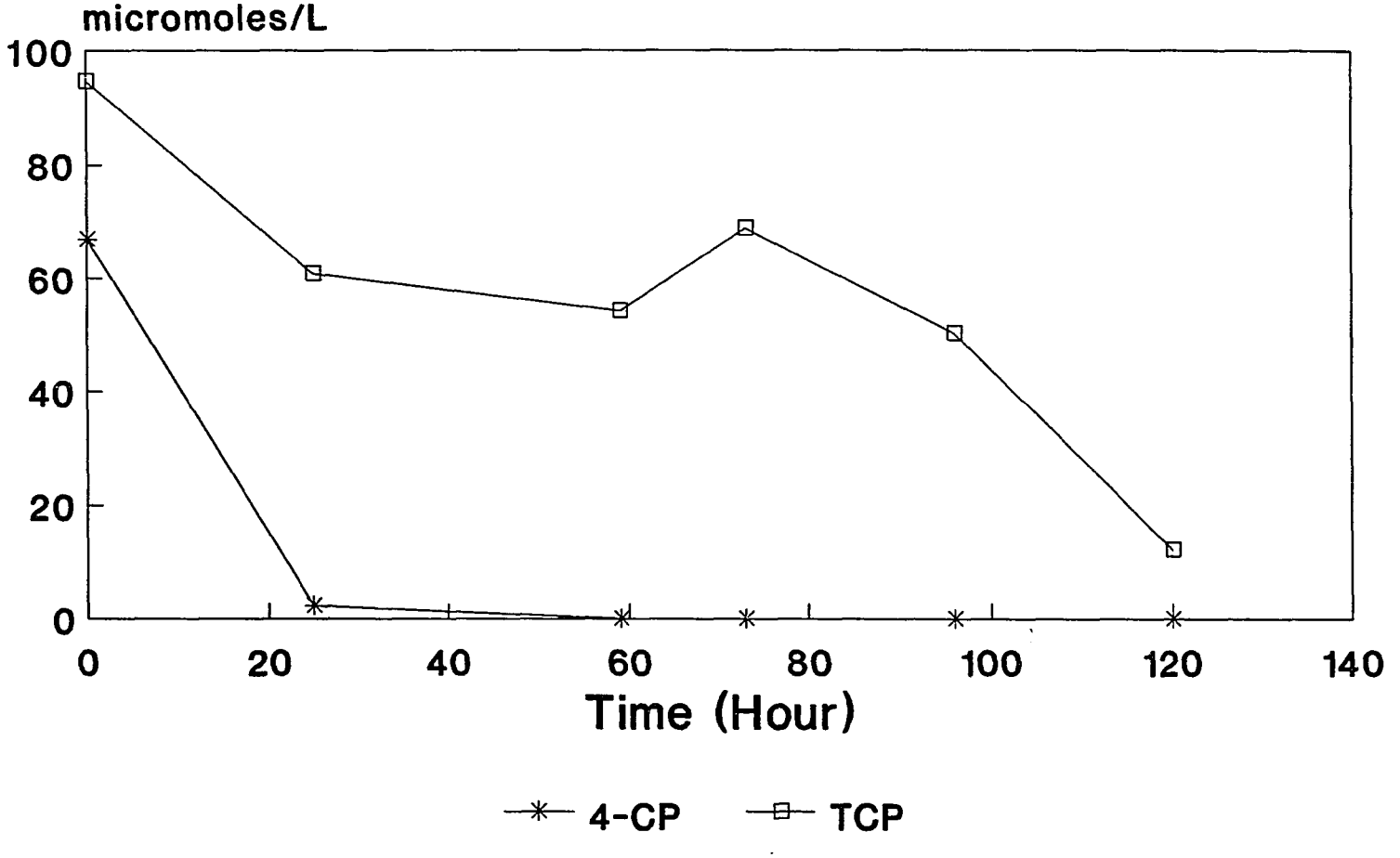


Figure 41. Aerobic Degradation of 4-CP by ATCC #29195 without TCP

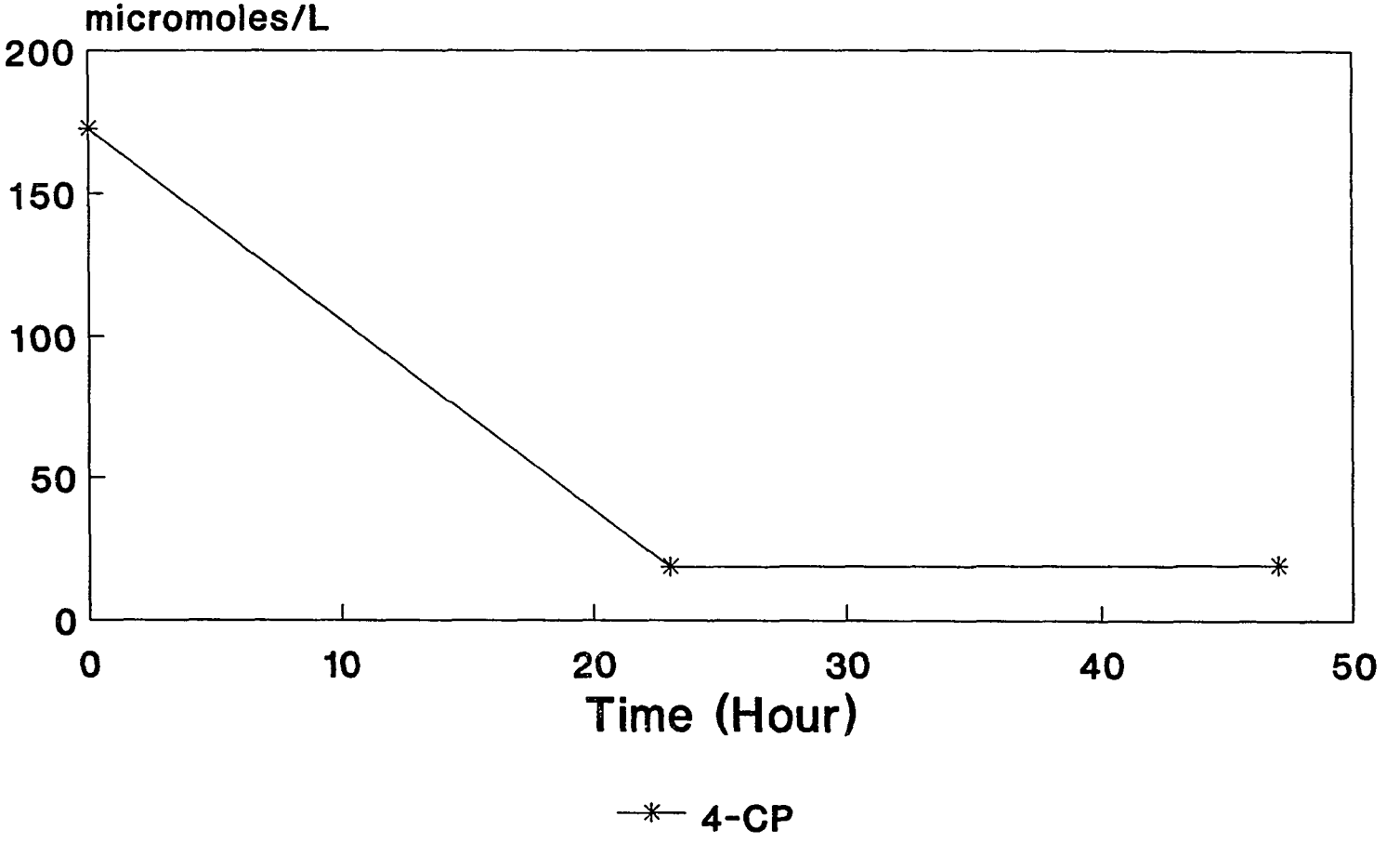


Figure 42. Aerobic Degradation of 4-CP by ATCC #29195 with TCP

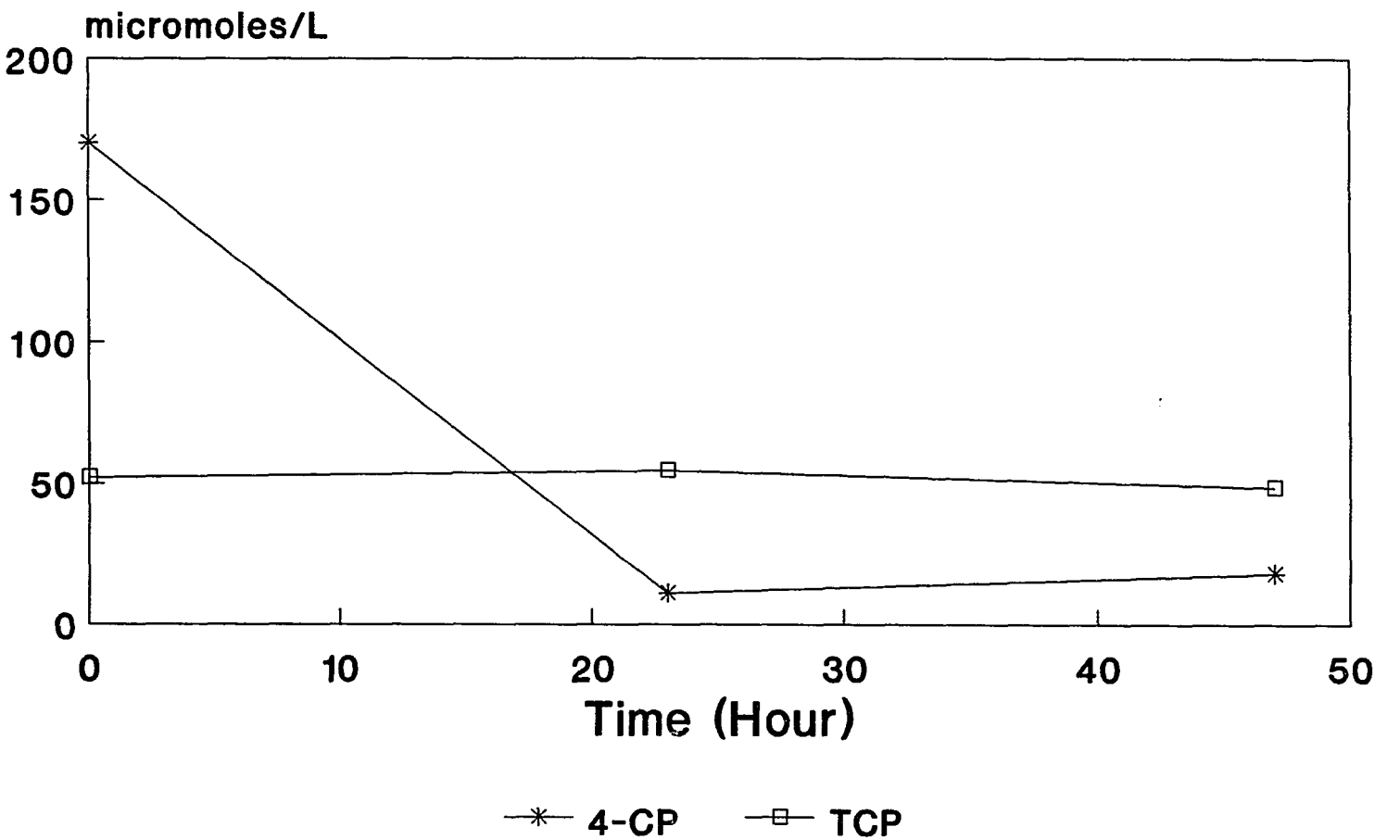


Figure 43. Anaerobic Degradation of TCP by the Pure Cultures Isolated From the Anaerobic Reactor

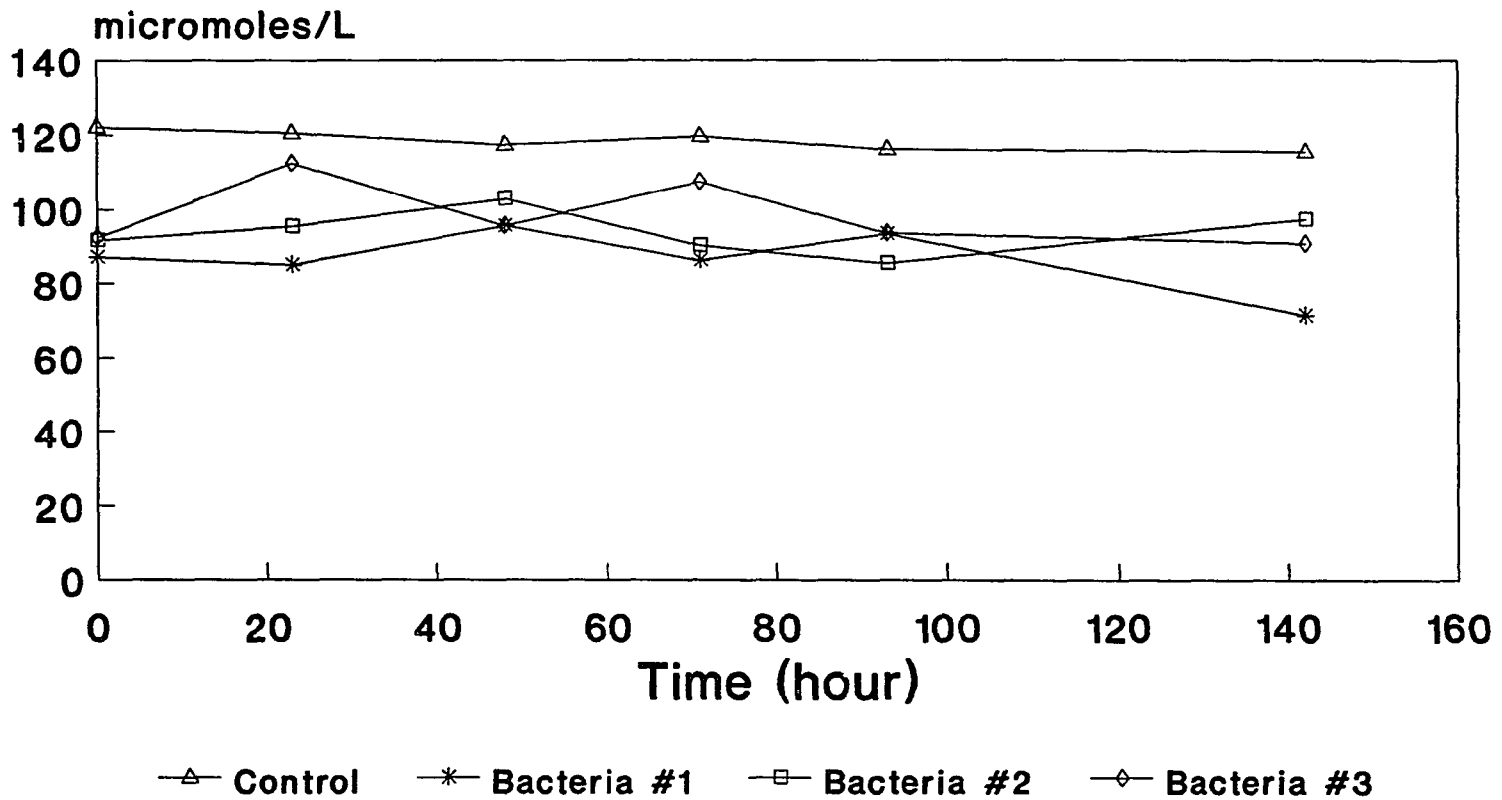


Figure 44. Aerobic Degradation of 4-CP by the Pure Cultures Isolated From the Anaerobic Reactor

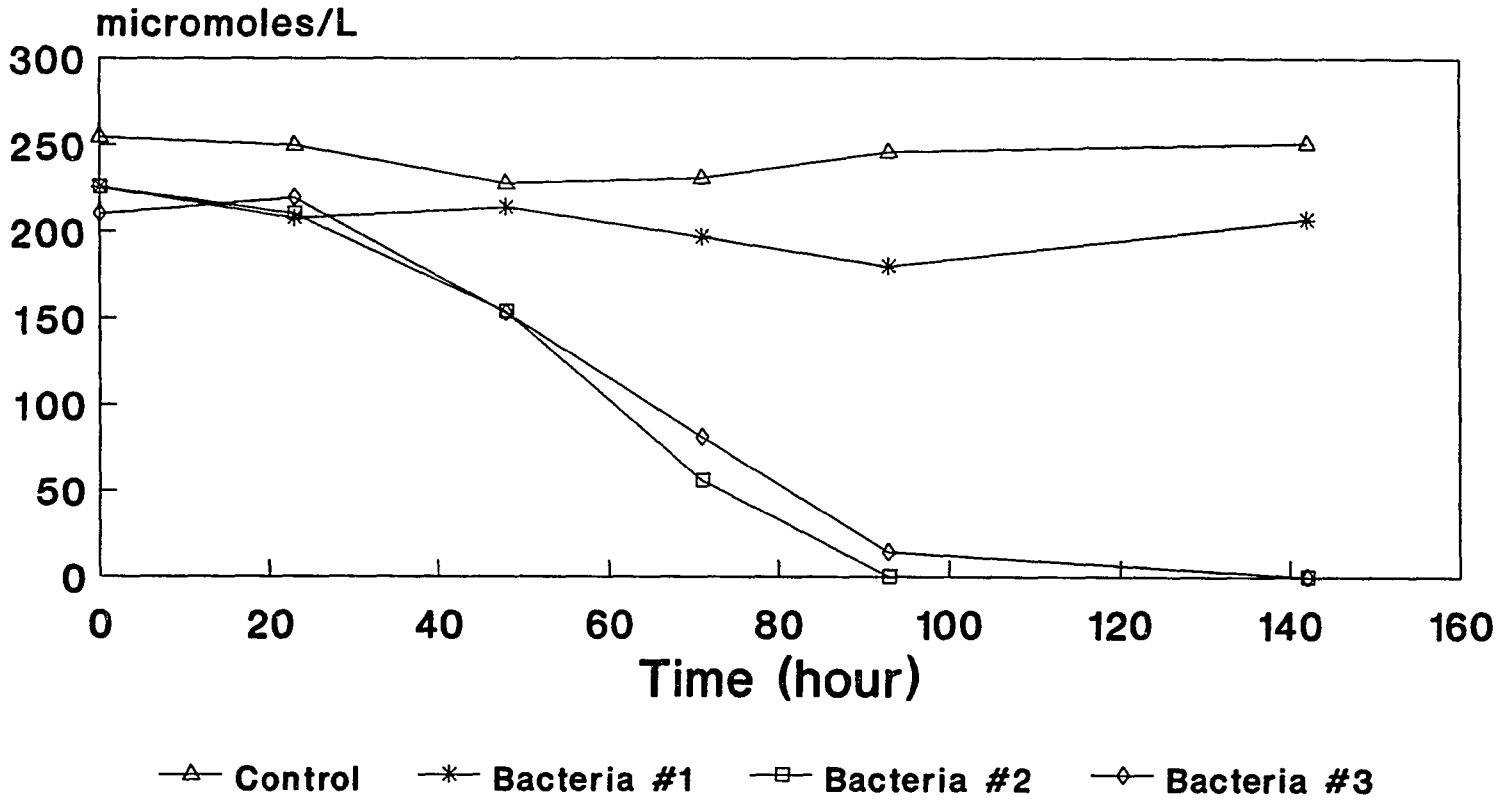


Figure 45. Anaerobic-Aerobic Degradation Of TCP in Batch Mode

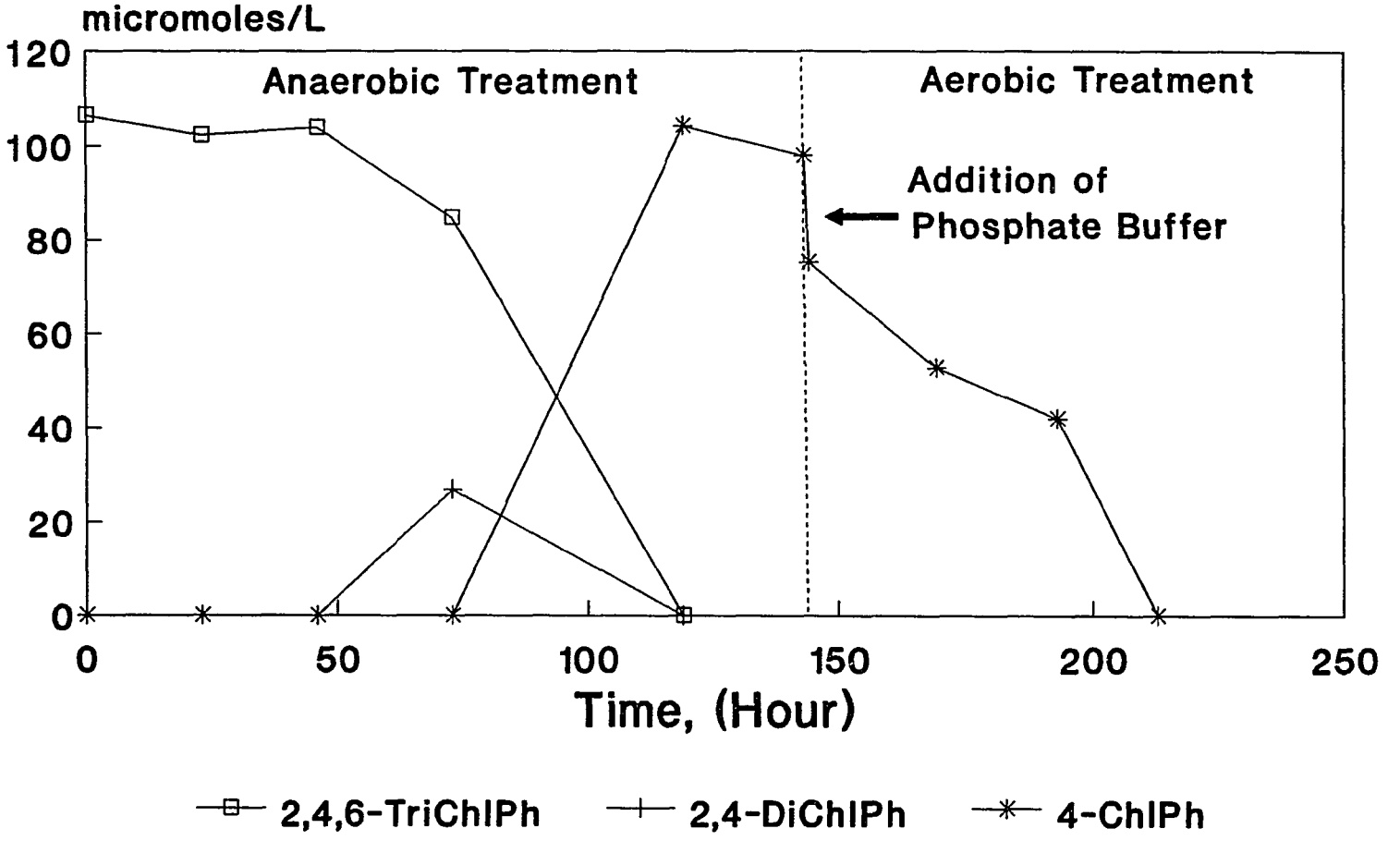


Figure 46. pH Variation in the Anaerobic-Aerobic Degradation Of TCP in Batch Mode

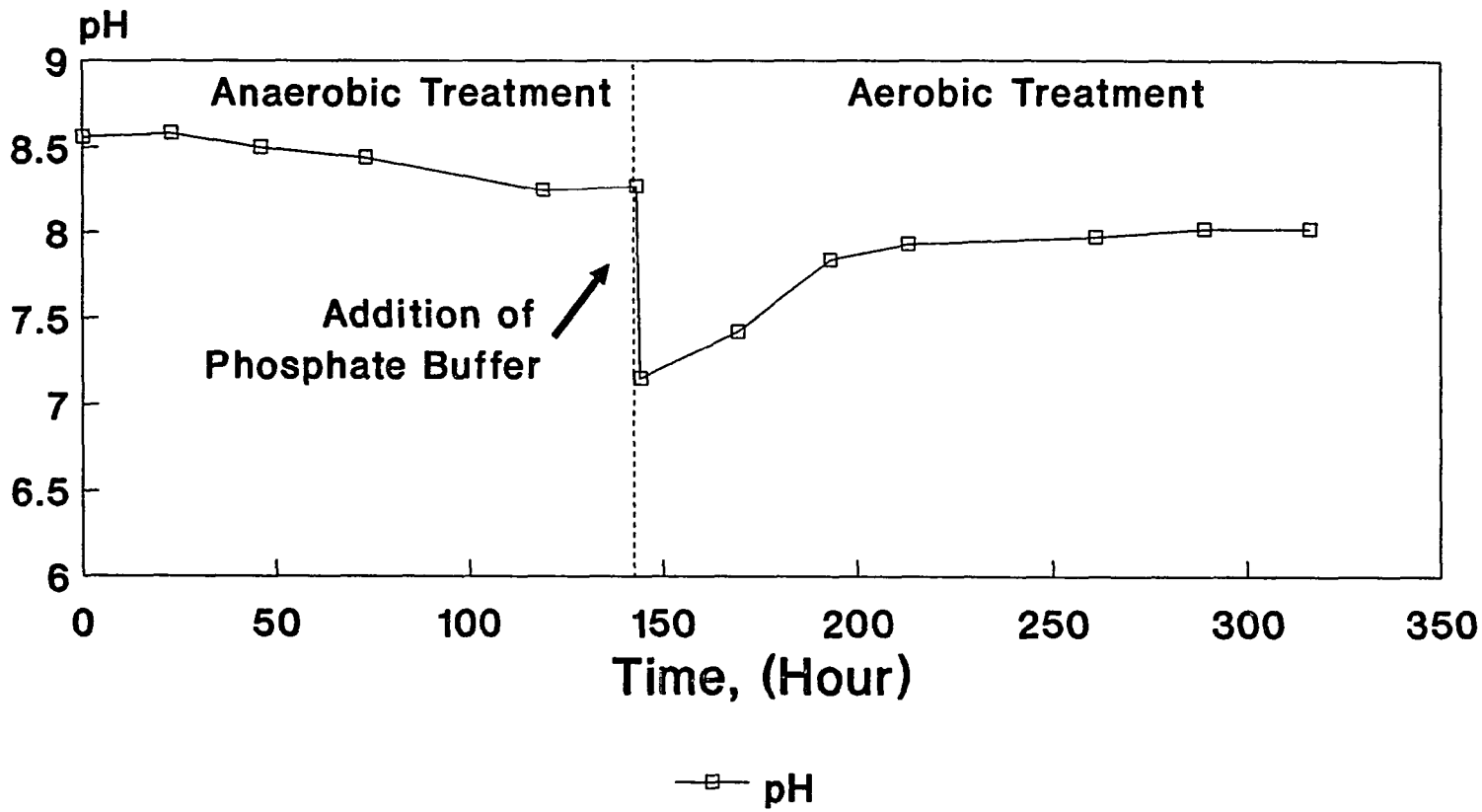


Figure 47. Anaerobic-Aerobic System: Degradation of TCP in Continuous Upflow Anaerobic Reactor, Run 22-1

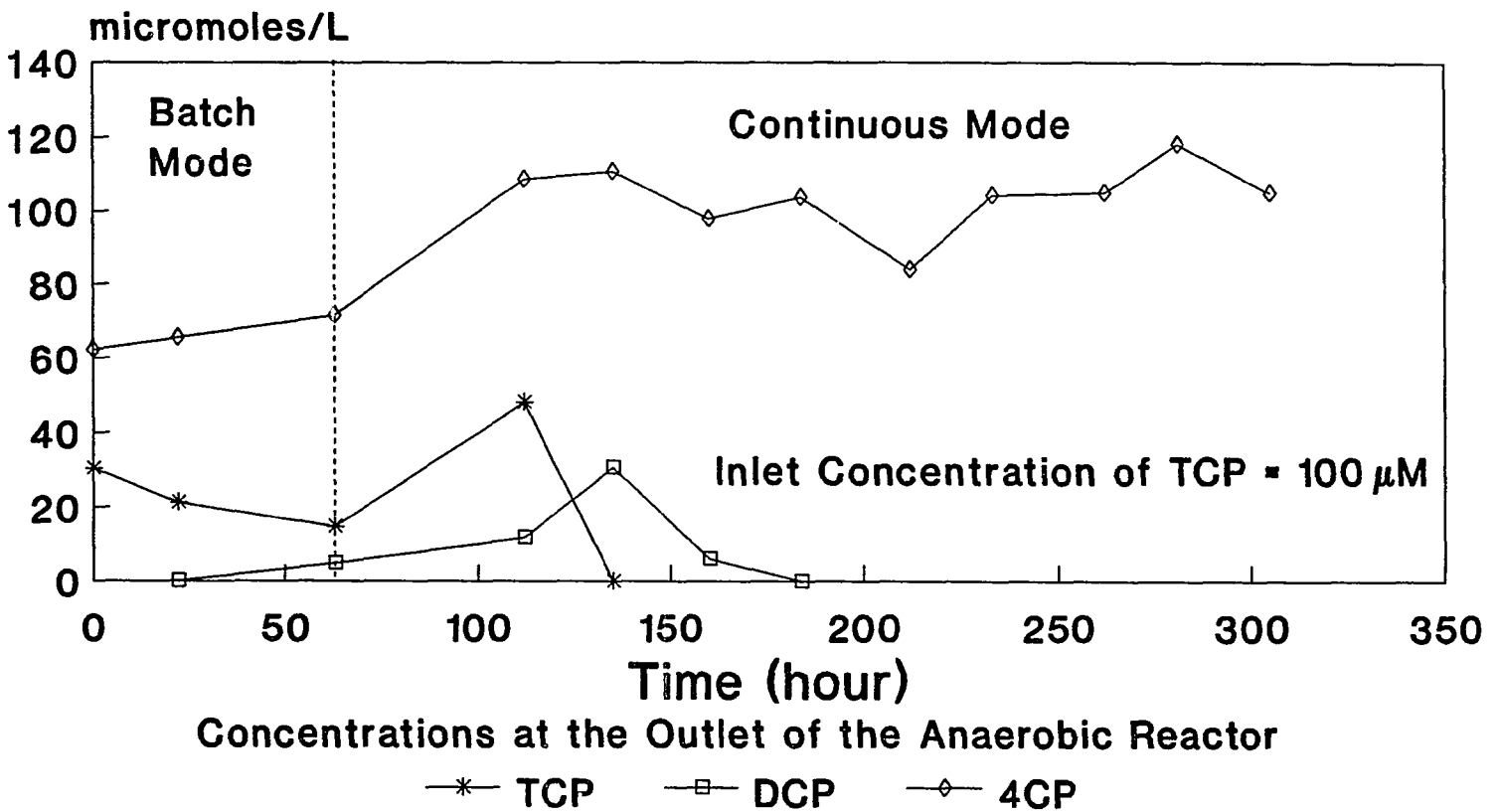


Figure 48. Anaerobic-Aerobic System: Mineralization of 4CP in the Aerobic Reactor, Run 22-1

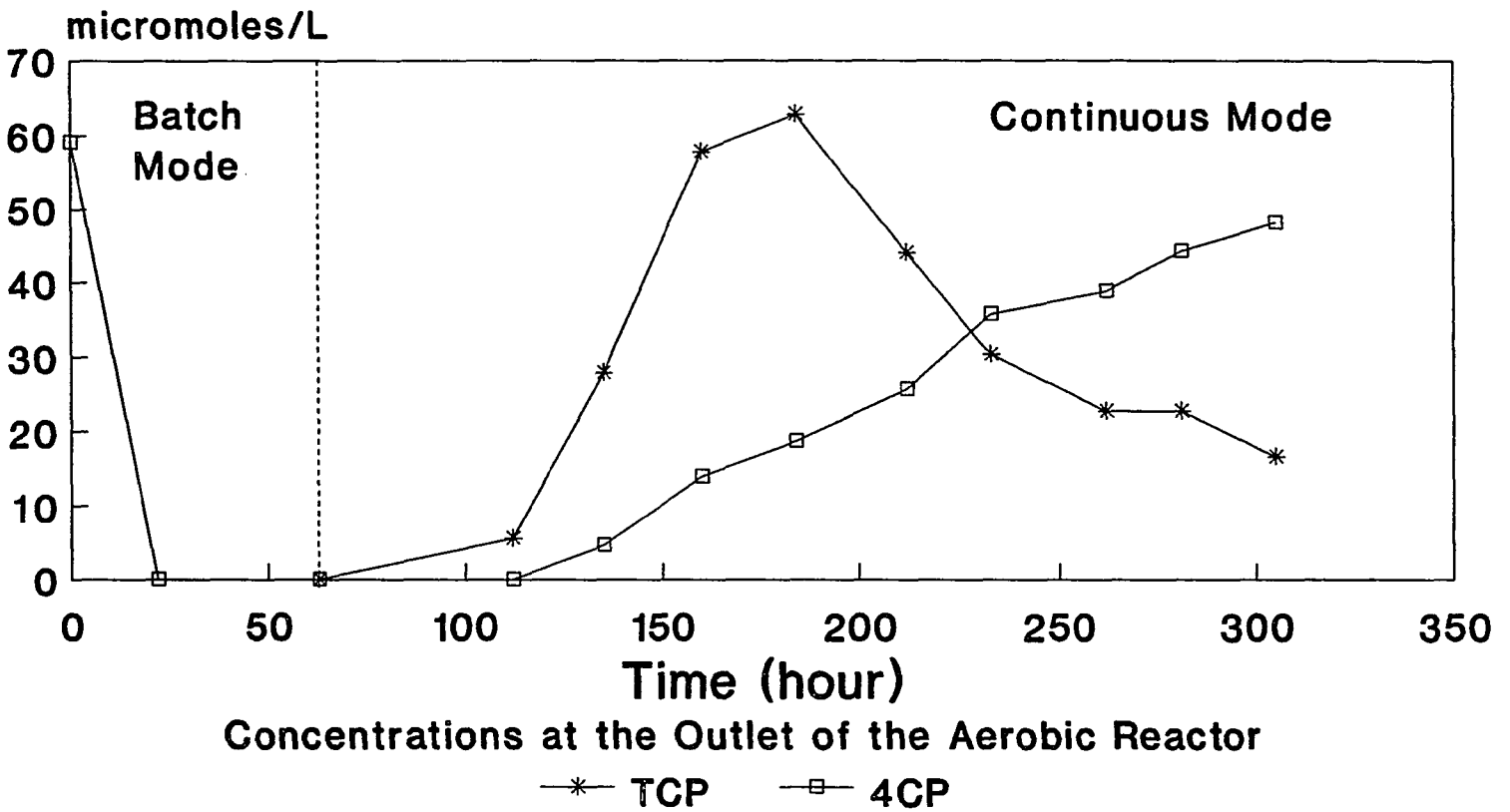


Figure 49. Anaerobic-Aerobic System: Degradation of TCP in Continuous Upflow Anaerobic Reactor, Run 22-2

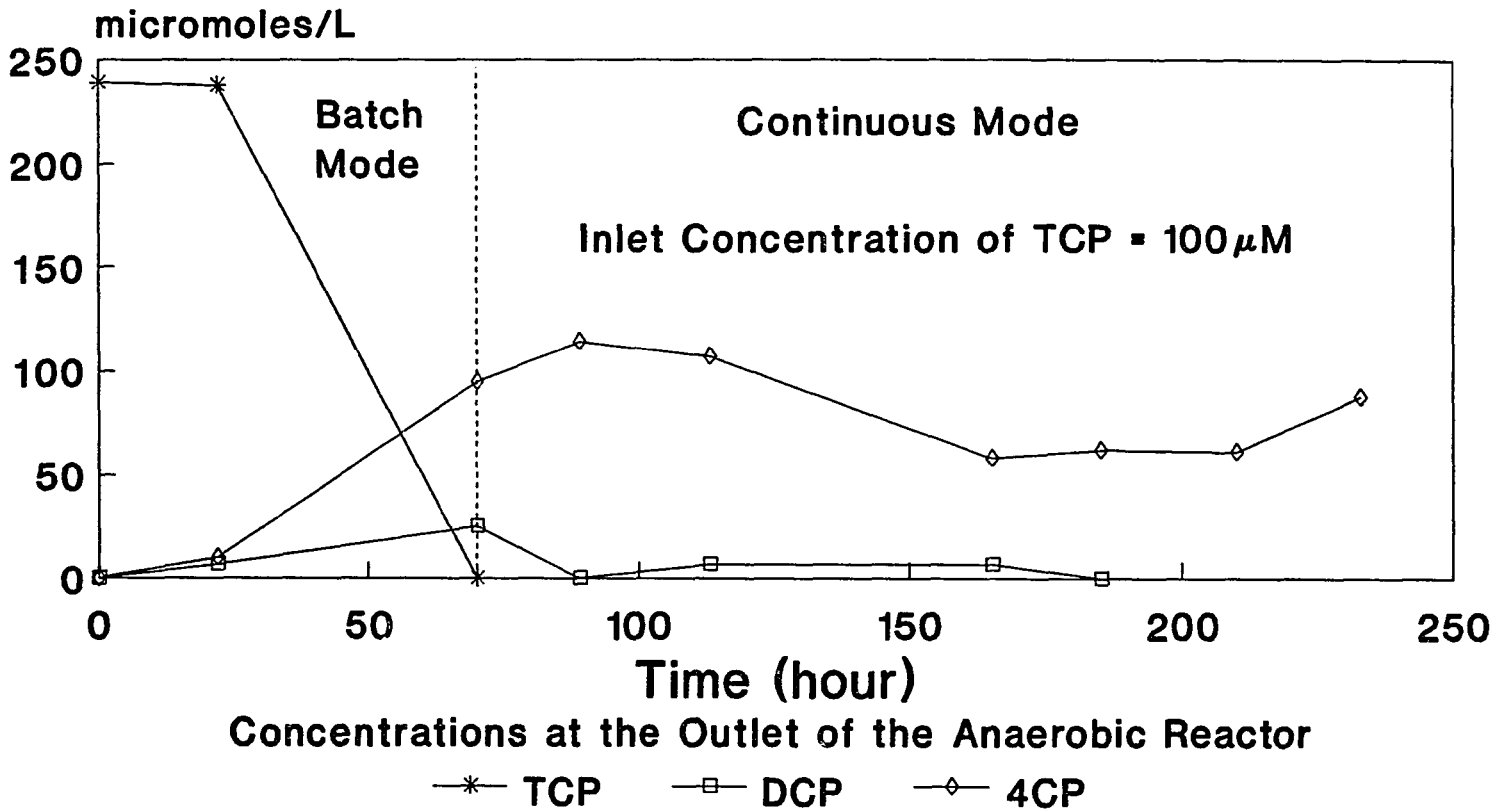


Figure 50. Anaerobic-Aerobic System: Mineralization of 4CP in the Aerobic Reactor, Run 22-2

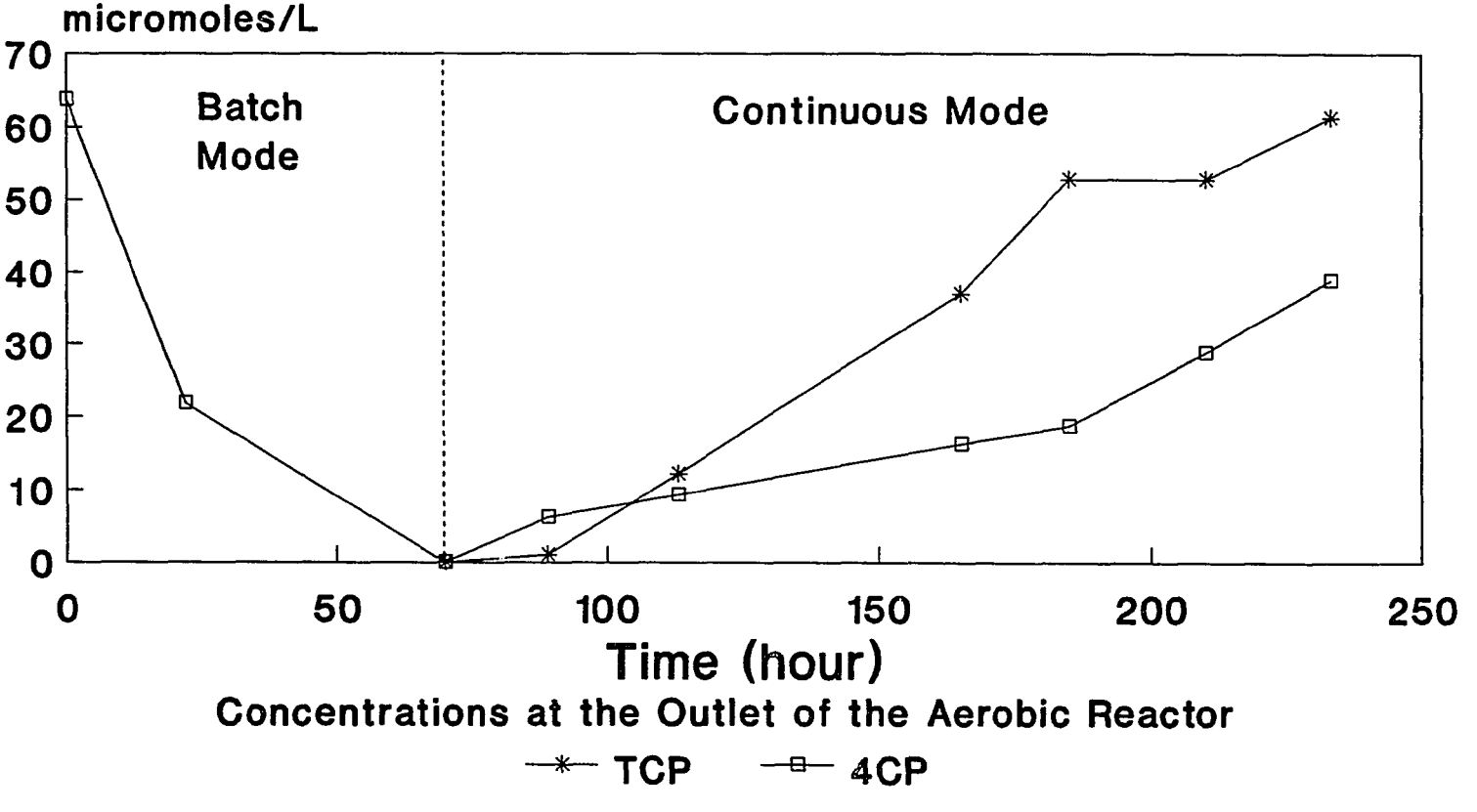


Figure 51. Anaerobic-Aerobic System: Degradation of TCP in Continuous Downflow Anaerobic Reactor

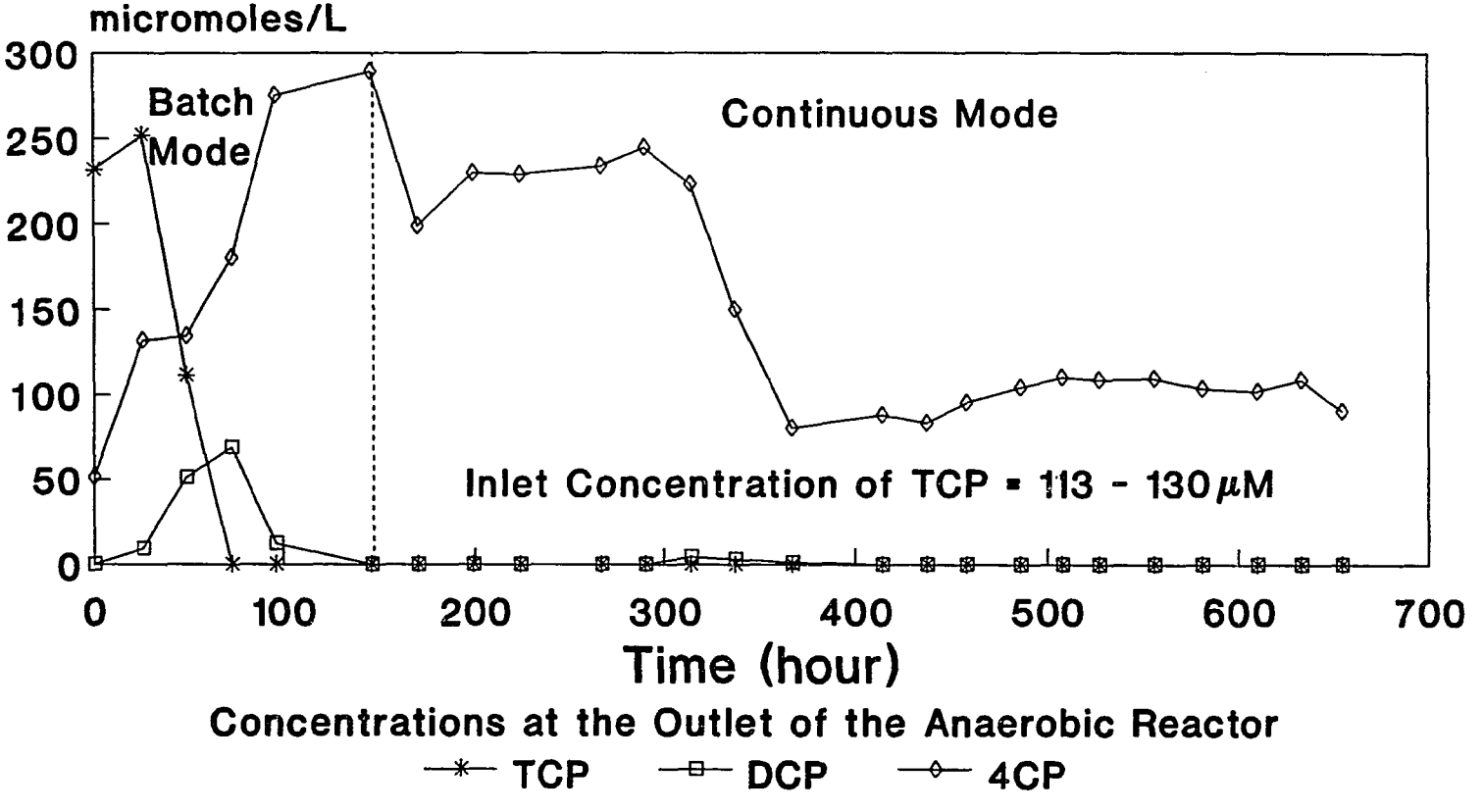


Figure 52. Anaerobic-Aerobic System: Mineralization of 4CP in the Aerobic Reactor