ACCLIMATION OF ACTIVATED SLUDGE TO PENTACHLOROPHENOL

by

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INTRODUCTION

Toxic pollutants in the aquatic environment are currently receiving attention from environmental interest groups, regulatory agencies, and industry. Legislation has initiated the regulatory process which will bring the discharge of toxic pollutants into U.S. waters under strict control in the near future. The Federal Water Pollution Control Act Amendments of 1972 (FWPCA) (1) and the subsequent Clean Water Act Amendments of 1977 (2) laid the groundwork for identification and regulation of toxic chemicals. However, it took the landmark legal decision known as the Consent Decree (3) - in which several environmentally concerned plaintiffs sued the U.S. Environmental Protection Agency (EPA) for failure to implement portions of the FWPCA - to expedite action to control toxic pollutants. As a result of the Consent Decree, the EPA published a list of 65 individual or groups of toxic chemicals, and this list was later unofficially expanded to the 129 so-called "priority pollutants." The EPA was instructed to develop water quality criteria for the listed compounds reflecting "the recommended maximum permissible concentrations consistent with the protection of aquatic organisms, human health, and recreational activities." These criteria are to be used in developing enforceable standards for the protection of our water resources.

Pentachlorophenol (also called PCP or penta), a priority pollutant, is a potent, broad-spectrum biocide whose major use is

in the preservation of wood and wood products. The value of PCP as a preservative is due to its relative resistance to biological and chemical degradation. Therefore, when this substance finds its way into the environment it creates a long-lasting pollution problem. Pentachlorophenol frequently invades the aquatic environment via wood treatment plant effluents and runoff waters.

Effluents from wood preserving operations often contain high (milligrams per liter, mg/L) concentrations of PCP. Biological degradation of PCP within the industrial waste treatment plant is essential to effective treatment of this type of waste. Many publicly owned treatment works (POTW's) continuously receive low levels of PCP and many other priority pollutants. Some removal of the toxic pollutants is apt to occur through one or more of the following processes:

1) primary sedimentation (through association with settleable solids)

2) biodegradation

3) volatilization

4) chemical oxidation

5) secondary clarification (through adsorption to wasted sludge)

6) chemical precipitation (where phosphorus removal is practiced)

Several studies of the biological treatment of PCP have been conducted. Most of these investigations have described degradation of PCP by activated sludge microorganisms which have been

selected, through acclimation, for their ability to utilize PCP. Such data are useful in attempting to treat the relatively high concentrations of PCP found in wood-preserving wastes. A wide range of treatment efficiencies have been reported for PCP. Also, some studies have shown PCP removal to be inconsistent. The extent and consistency of PCP degradation by activated sludge was investigated in this study.

Both industrial and municipal wastewater treatment plants must be concerned with toxic effects of priority pollutants on their biological systems. Pentachlorophenol is highly toxic to most aerobic organisms, including activated sludge bacteria. Concentrations of PCP to which the activated sludge is unaccustomed inhibit the degradation of other, easily degradable organics. Thus, the problem of treating priority pollutants is not simply in removing the toxin, but also in maintaining efficient treatment of conventional pollutants.

In the literature, there is a paucity of information regarding the inhibitory effect of PCP on activated sludge. In this study, an attempt was made to assess the acclimation of activated sludge systems to an organic toxin and to determine the effect of shock loads on treatment efficiency.

Finally, it would be helpful to treatment plant operators and others to have a rapid method for assessing the toxicity of chemicals to activated sludge which requires little training or equipment. For instance, an industrial waste treatment plant

operator would want to test any newly proposed process chemicals for adverse effects on the activated sludge system. Municipal operators, likewise, would need to examine the toxicity of a new industrial chemical proposed for discharge to their plant. Early toxicity evaluation of an accidental spill or pretreatment failure might permit diversion to spare the system or adjustment of operating parameters to avoid violation of a discharge permit or standard. Sanitary engineering consultants need to evaluate the toxicity of different process wastes in pilot studies to determine the optimum treatment design.

In this study, an attempt was made to develop a methodology for evaluating the toxicity (or, at least, inhibitory effects) of toxic chemicals to activated sludge.

It was hoped that the results obtained and procedures developed in this study using PCP would be, to some degree, applicable to the other organic priority pollutants.

The objectives of this study were:

1. to determine if activated sludge could acclimate to low levels of PCP so that it exhibited no inhibitory effects,
2. to determine if acclimation to low levels of PCP provided any protection to the biomass against detrimental effects of higher shock loads of that chemical,
3. to determine if acclimation to PCP decreased the inhibitory effect of related priority pollutants,

4. to determine the extent and consistency of PCP degradation in the activated sludge reactors, and
5. to develop a rapid, easy methodology for evaluating the toxicity (or inhibition) of chemicals to activated sludge.

LITERATURE REVIEW

Pentachlorophenol

Uses

Pentachlorophenol and its salts are extensively used worldwide as biocides with a wide range of applications. Wood preservation is by far the most prevalent use for PCP; however, its versatility has warranted registration with the EPA for use as an insecticide, fungicide, herbicide, algicide, disinfectant, and antifoulant for paint (4). The major registered uses of PCP in the United States are listed below (5).

1. Herbicide and desiccant for forage seed crops.
2. Insecticides for beehives, seed plots, greenhouse use.
3. Microbiostat for commercial and industrial water cooling.
4. Postharvest wash for fruit.
5. Microbiocide for burlap, canvas, cotton, rope and twine.
6. Microbiocide for leather.
7. Microbiocide and insecticide for wood treatment.
8. Preservative for oil and water-based paint.
9. Slime control for pulp and paper.
10. Microbiocide for petroleum drilling mud and flood water.
11. Fumigant for shipping-van interiors.
12. Preservative for hardboard and particle-board.
13. Herbicide for non-food vegetation control.

Production

The total world production of PCP has been estimated to be from 50 million kilograms (kg) per annum (5) to over 90 million kg per annum (200 million lbs per year) (6). The PCP production rate in the United States is about 23 million kg per year (7,8), which constitutes it as the second most heavily used pesticide in the country (4,9).

Residues

Contamination resulting from the use of PCP has caused it to become ubiquitous in the environment. Pentachlorophenol levels averaging almost 1.0 microgram per cubic meter ($\mu g/m^3$) were found in the air of a wood treatment plant in Idaho (10) and at low nanogram per cubic meter (ng/m^3) concentrations in the air of more remote areas.

It was estimated in 1970 that 5.5 million gallons per day of PCP-containing wood preservative wastewater was being discharged to the environment (11). The effluent concentration of PCP from a series of wood treatment plants was found to range from 25 to 150 milligrams per liter (mg/L) (12).

A study in Oregon detected PCP concentrations of 1 to 5 micrograms per liter (µg/L) in the effluent of three sewage treatment plants, a mean concentration of 0.41 µg/L in the Willamette River, and 0.06 µg/L in finished drinking water (13). It should be noted that the wood products industry is prominent in the studied region of Oregon. Low levels of PCP were also

found in well water of Northern California (227 µg/L average) (14), and at a mean concentration of 60 µg/L in Hawaiian rainwater (15). The U.S. Food and Drug Administration found PCP at µg/L levels in 2.8 percent of composite food samples in 1973-74 (16) and 5.4 percent of samples in 1974-75 (17). Most of the positive results were from sugars because PCP is registered for pesticidal use on cane fields. Low level residues of PCP were also detected in chicken (18-20), fish (21), and grain products (22).

Because of the widespread distribution of PCP, human exposure is inevitable. Thus, it is not so surprising that PCP has been detected in human tissue (23,24), urine (10,22,25-29), semen (30), blood (10,28,29,31), and fat (32). The ubiquitous nature of PCP was demonstrated by the results of the Health and Nutritional Examination Survey in which PCP was detected in the urine of 85 percent of the samples collected from the general population of the U.S., with a mean concentration of 6.3 µg/L (28).

Chemical Properties

The high degree of chlorination on the PCP molecule strongly withdraws electrons from the oxygen-hydrogen bond and facilitates ionization. This strong electron attraction causes PCP to be unusually acidic and stabilizes the acid anion and salt forms.

Normally, the ring chlorines are resistant to nucleophilic substitution; however, absorption of light energy initiates a

sequence of photolytic reactions. Wong and Crosby (14) proposed a route for photolytic breakdown of PCP to explain their experimental findings: Photonucleophilic replacement of a chlorine atom by a hydroxyl group converts PCP into one of the three possible tetrachlorodiols (33), which are rapidly air-oxidized to the corresponding quinones. The quinones undergo further oxidation, hydration, and ring cleavage forming dichloromaleic acid, and, ultimately, hydrogen chloride and carbon dioxide. A small amount of pentachlorophenol was found to undergo the simpler photochemical reduction to tetra- and trichlorophenols (14,34). Also, ultraviolet light was found to cause a third avenue of PCP destruction when a chlorine atom undergoes photonucleophilic displacement by a chlorinated phenoxide anion (rather than a hydroxyl group) to generate polychlorinated dibenzo-p-dioxins (35,36).

Physical Properties

Some relevant physical properties of PCP are listed in Table I.

Solubility

Because PCP is moderately acidic (pk_a = 4.7) its aqueous physical properties are affected by its degree of ionization, and, therefore, the pH of the surrounding water. For instance, PCP is only one percent ionized at pH 2.7, but better than 99 percent ionized at the near-neutral pH of most natural waters (5). Solubility in water is directly related to pH because the

Table I. Selected physical properties of pentachlorophenol. (References for the data are given in parentheses)

Molecular weight (37)	266.35
Melting point (37)	190°C
Boiling Point (37)	310°C
pKa (38)	4.71
Solubility in water at 20°C (5)	
PCP	0.014 g/L
NaPCP	22.4 g/L
Vapor pressure at 20°C (23)	0.00011 torr
Log octanol/water partition coefficient (39)	5.01
Log hexane/water partition coefficient (39)	2.15
Peak absorbance; maximum at pH 7 (40)	318 nm

ionic form goes into solution more readily than unionized PCP. Pentachlorophenol is soluble in most organic solvents, while its salts - such as NaPCP and KPCP - are soluble in water. This versatility has contributed to the proliferation of its industrial applications (4).

Volatility

The vapor pressure of PCP is inversely related to pH since the more soluble the compound, the less it tends to volatilize. Although an EPA study (40) concluded that volatilization was not a significant transport process affecting the aquatic fate of PCP, Crosby (5) citing Carswell and Nason (41) stated that "even at ambient temperatures PCP must be considered to be relatively volatile." One would expect that a compound with moderate solubility (enhanced by ionization at ambient pH levels) and with such a low vapor pressure would be relatively nonvolatile. However, PCP has been found to evaporate from treated wood (42) and paint (43) as well as from water.

Octanol/Water Partition Coefficient

A partition coefficient of a chemical is defined as its equilibrium concentration ratio between a nonpolar organic solvent and a polar one (such as n-octanol and water). The logarithm of the octanol/water partition coefficient (log K_{ow}) has been used to predict soil adsorption (44), lipophilic storage (45), and bioaccumulation/biomagnification (46-48).

Bioconcentration of chemicals has been shown to be directly proportional to their log K_{ow} (48). The log K_{ow} of PCP is relatively high, and is compared to the coefficients of the related compound used in the present study in Table II.

Adsorption

Adsorption of PCP in soil systems has been found to occur as a function of pH: moderate in acidic soils and slight or absent in neutral or basic ones (40,49). In water systems, adsorption of PCP to suspended organic matter would not be expected to occur to any extent in circumneutral water, but considerable sorption would presumably take place in organic-rich sediments where microbial production of organic acids had lowered the pH. Extensive studies of a freshwater lake in Mississippi following two accidental spills of PCP-containing wood treating waste (50,51) found that high levels of PCP persisted in sediment and leaf litter throughout the periods of study (17 months and 2 years respectively), thus providing a source for chronic contamination of the aquatic ecosystem. After five days of exposure, the distribution of PCP in unacclimated activated sludge was reported to be 36 percent associated with sludge and 47 percent in water (52).

Table II. Log octanol/water partition coefficients of the compounds used in this study (40).

Compound	Log K_{ow}
Phenol	1.46
4-Nitrophenol	1.91
2-Chlorophenol	2.17
2,4,6-Trichlorophenol	3.38
Pentachlorophenol	5.01

Environmental Fate

Degradation in Soil

Because of the extensive use of PCP as a herbicide on Japanese rice paddy fields in the past, many studies were con ducted on its degredation in soils. While degredation of PCP by soil microbes does occur aerobically (53,54), it was found that anaerobic (flooded) conditions accelerated the rate of degradation (55,56). It has been concluded that reductive dehalogenation to less chlorinated phenols is the most important pathway of PCP degradation in soils (57).

Degradation in Water

An EPA study (40) concluded that photolysis and biodegradation appeared to be the main processes determining the fate of PCP in the aquatic environment, while chemical oxidation, volatilization, and hydrolysis did not seem important. Photochemical degradation of PCP in the presence of sunlight is well documented (15,58,59), but, because natural waters quickly absorb the ultraviolet wavelengths required to initiate the reaction, photolysis is probably only important near the surface. Below the surface layer of rapid photolysis, microbial metabolism assumes a more significant role in deciding the fate of PCP. The literature addressing microbial degradation of PCP will be covered in some detail in a later section ("Degradation of PCP by Activated Sludges"). Two investigations using simulated

aquatic environments found that the rate of PCP degradation was associated with the availability of oxygen and light (60) and aerobic conditions and the absence of a second carbon source (61).

Bioaccumulation, Bioconcentration, and Biomagnification

As might be expected from the high octanol/water partition coefficient, PCP bioaccumulate and bioconcentrates in many types of aquatic organisms (21,32,51,52,62-68), creating the potential to biomagnify by entering the food web. Also, the two on-site investigations of a contaminated lake (50,51) revealed that long term PCP persistence in leaf litter and sediments created a source for chronic exposure and biological magnification via detritus and benthic-feeding organisms. Biomagnification of radioactive carbon-labeled pentachlorophenol (^{14}C-PCP) was observed in a model aquatic ecosystem (46). Fortunately, detoxification and depuration have been found to occur in most organisms studied (66,67,69-74).

Toxicology

Imai et al. (75) reported that the toxicity of PCP is due to its role as an uncoupler in biological oxidative phosphorylation in aerobic organisms. A portion of the EPA Ambient Water Qualilty Criteria Document for PCP (76) stated that: "The available data for pentachlorophenol indicate that acute and chronic toxicity to freshwater aquatic life occurs at

concentrations as low as 55 and 3.2 µg/L, respectively, and would occur at lower concentrations among species that are more sensitive than those tested." Pentachlorophenol, especially technical grade, contains impurities such as tri- and tetrachlorophenols and polychlorinated dibenzo-p-dioxins, dibenzofurans, and diphenyl ethers. Some of the impurities are far more toxic than PCP itself, but they are present in relatively low concentration. Pentachloropherol and its impurities were treated as a single entity by the EPA (76).

Degradation of Pentachlorophenol by Activated Sludges

Laboratory Studies

Proof of microbial degradation of PCP in activated sludge has been demonstrated in the laboratory. In the first of a series of studies performed at Purdue University (77), an aerated mixed microbial population which was batch fed 10 mg/L/day of ^{14}C-PCP reached a maximum PCP-oxidative capacity ($^{14}CO_2$ liberation/24 hours/unit cell mass) of 68 percent before beginning to decline and eventually failing. After enrichment by continuous culture, a bacterium was isolated from the heterogeneous population which used PCP as its sole source of carbon and energy (78).

In another study by the same group (79), continuous-flow activated-sludge reactors, featuring extremely long cell residence times, achieved better than 96 percent removal of PCP from

contrived (containing 20-60 mg/L PCP) and authentic (containing 17.6 mg/L PCP) wastewaters. It is of interest that a variety of chlorinated phenols present in the authentic wood-preserving waste were immediately utilized by the PCP-acclimated sludge. For comparison, the contrived wastewater was separately supplemented with reagent grade and commercial grade PCP. Commercial grade PCP showed decreased biodegradability and seemed destined to destroy the PCP-degrading culture (80), presumably due to a buildup of toxic impurities.

Dust and Thompson (81) used bench-scale, activated-sludge units to study PCP biodegradability in treating a mixture of PCP wastewater (5.8-12.5 mg/L) and synthetic sewage (around 2400 mg/L COD). After a period of acclimation, two units initially removed 79 and 92 percent, respectively, of the PCP, but their efficiencies had dropped to 22 and 44 percent, respectively, within a month. The third unit removed only an average of 35 percent of the influent PCP over the first 20 days, so it was fed only sewage for the next five days. The PCP removal efficiency increased to 94 percent when the penta feed was reinstated on day 26 and remained at that level for the final 10 days of study. The fourth reactor maintained an average PCP removal of 93 percent over days 5 through 45. Starting on day 46, the PCP loading was increased by 10 mg/L every two days, reaching 40 mg/L on day 50. The resulting PCP removal percentage were 95 (20 mg/L), 97 (30 mg/L), and 99 (40 mg/L).

Treatment Plant Studies

The practicability of PCP treatment efficiencies obtained in the lab has generally not been upheld in full-scale treatment plants. Treatment of a penta-containing wood preserving effluent by an extended-aeration, activated-sludge system (125 percent sludge recycle) in Thunder Bay, Ontario, achieved only 35 and 58 percent average removals of an 8.5 mg/L nominal PCP influent concentration during two phases of investigation (82). The PCP concentration in the secondary effluent was quite erratic in both phase 1 and 2, varying from 0.79 to 8.6 mg/L and 0.02 to 7.9 mg/L, respectively.

A completely mixed activated sludge system designed for a wood preserving plant at Carbondale, Illinois provided 76 percent treatment efficiency of an average PCP influent of 1.7 mg/L during steady state operation (83). In a shock loading experiment the hydraulic loading rate was doubled. As a result, the PCP removal efficiency dropped from 76 percent to virtually zero.

The EPA examined the fate of priority pollutants in POTW's providing a minimum of secondary treatment (84). At the 14 POTW's registering PCP (out of 20 studied), the influent concentration ranged from 1 to 50 g/L. Eight plants were unable to remove any of the PCP. In six others, PCP removal ranged from six to 87 percent. Pentachlorophenol was detected in the combined waste sludge streams seven times at concentrations up to one mg/L. This raises a question as to what fraction of PCP is

biodegraded and what is simply removed by adsorption to biomass. Association of PCP with the solids could present potential problems in the aeration basin from PCP returned with recylced sludge or in a sensitive sludge disposal process such as anaerobic digestion.

Source and Acclimation of Activated Sludge

The inoculum origin and a period of PCP-acclimation seem critical to the ability of activated sludge to degrade pentachlorophenol. For instance, Liu et al. (61) resorted to an enrichment culture feed of five mg/L of PCP and 20 mg/L sodium monochlorophenate for six months in order to develop an activated sludge which used PCP "after many futile attempts to obtain an active PCP - degrading culture" directly from activated sludge.

Likewise, the ultimate source of the bacteria described by the Purdue University workers (77-80,85,86) was a sample of soil which had been regularly saturated with penta preservative on the grounds of a wood products manufacturer in Terre Haute, Indiana. Before the biodegradation studies were begun, the following rigorous acclimation program was implemented: 1) one month of feeding 2.5 mg PCP/L/day plus phenol with step increases from 20 to 320 mg/L/day; 2) one month of reducing the phenol to zero while stepping the PCP up to 30 mg/L/day; and 3) four more months of maintaining that feed. (Nutrient broth was also fed throughout the acclimation period) (77).

In order to acclimate the activated sludge to PCP, Dust and Thompson (81) fed their units 0.125 to 2.25 mg/L of PCP for two weeks before gradually increasing its concentration in the feed to 10 mg/L over the next 34 days. It is of some interest to note that the source of the two cultures that failed was sludge previously acclimated to creosote wastewater, while the two more successful reactors were innoculated with mud from a drainage ditch containing penta wastewater.

There were no specific acclimation programs performed on the treatment plant activated sludges, but they are consistently subjected to PCP loadings.

Effect of Other Substrates

Because Alexander (87) concluded that multiple halogenation of the carbon ring confers resistance to microbial attack, it seems reasonable that more easily degraded substrates would be preferentially used by a mixed microbial culture. In an early study (88), degradation of chlorophenols occurred only in the complete absence of other organic substrates. More recently, it has been determined that the rate of degradation is merely suppressed by the presence of more readily degraded nutrients such as glucose (53,61,77), sucrose (85), monochlorophenol (61), and nutrient broth (77). Obviously, the wood preserving wastes entering the two studied treatment plants contained a variety of organic substrates. Perhaps, the presence of more readily available organic material, along with the complexity of the

wastewater, explains the reduced PCP treatment efficiencies of those plants. The apparent exception to the trend seems to be the study by Etzel and Kirsch (79) of biological treatment of contrived and industrial wastewaters. Both the authentic wood preserving waste and the synthetic wastewater (supplemented with xylose, glucose, cellobiose, nutrient broth, and sodium acetate) contained a myriad of easily degraded organics, and yet better than 96 percent removal of PCP was obtained.

PCP-Degrading Isolates and Metabolites

The bacterial degraders of pentachlorophenol that have been isolated include a saprophytic coryneform-type bacterium (77,78) and members of the genus Pseudomonas (53,54). The exact avenues of aerobic biodegradation of PCP have not been completely elucidated, but major degradation products apparently include pentachloroanisole, tetrachlorohydroquinone, tetrachlorobenzoquinone, tetrachlorocatechol, and tetrachlorohydroquinone dimethyl ether. Further dechlorination ultimately renders carbon dioxide, chloride ions, and carbon which is incorporated into cell tissues (54,80,85).

Toxicity to Activated Sludge

A number of methods have been proposed for evaluating the toxicity of compounds to activated sludge. Union carbide researchers (89) subjected a series of activated sludge seeds to various doses of a toxicant, along with nutrients, and measured

microbial growth spectrophotometrically. Growth inhibition was associated with toxicity, and the degree of toxicity was determined by means of the concentration series and the amount of inhibition.

Many of the methods described in the literature (90-92) relate toxicity to respiratory inhibition. Evaluation of the toxicity of chemicals by inhibition of respiration con be nonspecific, time consuming, insensitive, and misleading for some compounds. Toxicants which function as uncouplers in oxidative phosphorylation stimulate rather than inhibit respiration (93). Pentachlorophenol is such a compound, and biological oxygen uptake in its presence exceeded the theoretical value by 42 percent (86). Evolution of carbon dioxide was also considered to be a poor method for rapidly determining toxicity because of the slow conversion of organic carbon to CO_2 (94).

In a 1982 paper, Larson and Schaeffer (94) used the quantitative uptake of ^{14}C-glucose over 15 minutes as a measure of the toxicity of chemicals to activated sludge. Glucose uptake was found to be rapid, specific, and dependent on metabolically active sludge. The uptake of glucose by acid-sterilized sludge was found to be negligible (less than one percent of the control), thus ruling out nonspecific adsorption to non-viable cells. Because the cell membrane is impermeable to most polar molecules, glucose must be bound and carried across by active transport via specific macromolecules (permeases) (95).

Therefore, only metabolically active microorganisms would be capable of glucose uptake.

It seems reasonable that toxic effects of chemicals on activated sludge would similarly be reflected in reduced removal of chemical oxygen demand (COD). Etzel and Kirsch (79) found that the 97 percent COD treatment efficiency of a contrived wastewater containing reagent grade PCP fell to 94 percent when commercial grade PCP was substituted, and that it was further reduced to 84 percent when treating an authentic wastewater containing other chlorophenols. The COD removal efficiency of wood preserving waste studied by White et al. (83) was 80 percent under normal operating conditions, but decreased to 72 and 60 percent when the loading rate was doubled in two shock loading experiments. However, the decreased hydraulic detention time may have contributed to the treatment efficiency reduction.

Summary

The vast production and many uses of pentachlorophenol have made environmental contamination inevitable. It is, indeed, ubiquitous in nature - having been found in air, rain, surface water, ground water, drinking water, soil, food, animals (including humans), and, of course, industrial and municipal wastewaters. Fortunately, PCP is destroyed by a variety of processes. It is readily photochemically oxidized by sunlight. It is degraded by reductive dechlorination under anaerobic conditions by microbes, and it is biologically oxidized by a number of

organisms when exposed to non-toxic levels. However, PCP is acutely toxic to most organisms at low levels. Its bioaccumulation potential, which is related to its high octanol/water partition coefficient, poses an increased threat to organisms at higher trophic levels by ecologically magnifying the PCP concentration up through the food web. Regardless of the stated avenues of degradation, PCP still must be considered relatively persistant. Its resistance to decay is a function of its chemical structure; and its tendency to adsorb to organic material increases its persistance in the aquatic environment.

The adverse effects of releasing PCP into the environment are obvious. Therefore, it is imperative that penta containing wastes be efficiently treated before discharge to the environment. Wastewater treatment processes receive PCP directly from industrial sources and indirectly in sewage. High levels of PCP removal have been achieved in laboratory studies. However, even in the lab the difficulty in maintaining a vigorous PCP-degrading culture has been evident. Treatment plants have generally achieved lower PCP treatment efficiencies. This is probably due to the presence of other carbon sources and/or the complexity of the wastewater.

In addition to PCP degradation, it is important to determine the toxicity of the substance to the biological treatment process. Although no literature was found which specifically addressed the issue for PCP, many methods have been described

for evaluating the toxicity of chemicals to activated sludge. Respiration tests were decidedly unsuited to the objectives of this study, but the method based on ^{14}C-glucose uptake contained some pertinent information.

METHODS AND MATERIALS

This chapter will describe the materials and methods used in maintaining activated sludge reactors and analyzing those systems with regard to the effects caused by pentachlorophenol and other priority pollutants. Then, the experiments performed will be summarized.

Reagents

All of the phenols used in this study were of reagent grade quality. Pentachlorophenol and 2-chlorophenol were produced by Eastman Kodak Company (Rochester, NY). The 2,4,6-trichlorophenol was from the Aldrich Chemical Company (Milwaukee, WI). The phenol and 4-nitrophenol, along with the hexanes, nutrient compounds, and test reagent chemicals were products of Fisher Scientific Company (Fair Lawn, NJ). Stock solutions of one milligram per milliliter (mg/ml) were prepared for all of the phenols by raising the pH of distilled-deionized water with one or two drops of sodium hydroxide (50 percent wt/wt) to achieve dissolution.

Reactors

A series of five completely mixed activated sludge reactors were maintained throughout the study. The covered, Plexiglas plastic tanks were innoculated with nine liters of activated sludge from the Blacksburg, Virginia, municipal sewage treatment plant. The activated sludge was aerated and mixed by air blown

through diffuser stones. The sludge temperature was allowed to equilibrate to the ambient room temperature, which ranged from approximately 21 to 27°C.

Feed

The reactors were fed on a daily batch basis. One liter of the suspension was wasted from each basin per day (the volume of sludge used in daily experiments was considered to be part, or all, of that day's wasted volume). The liter wasted each day was replaced by the same volume of the feed mixture plus water, thereby producing a nine day sludge age. In an attempt to approximate municipal plant operating conditions, the reactors were subjected to an organic load (of dextrose) equivalent to treating four aeration basin volumes of 300 mg/L COD waste in a 24 hour period (i.e. a six hour hydraulic detention time). Inorganic nutrients were included in the feed at a COD:nitrogen: phosphorus molar ratio of 100:5:1. Sodium bicarbonate was added for buffering, and tap water was used to supply micronutrients. The actual daily feed mixture was as follows:

Dextrose (glucose monohydrate)	$C_6H_{12}O_6\ H_2O$	10.8 g
Ammonium sulfate	$(NH_4)_2SO_4$	1.08 g
Potassium phosphate monobasic	KH_2PO_4	0.45 g
Soidum bicarbonate	$NaHCO_3$	1.0 g
Pentachlorophenol	C_6Cl_5OH	various levels

This chemical mixture was dissolved in approximately 500 ml of tap water and dripped into the reactors over a period of a few hours to avoid dissolved oxygen depletion. After feeding, the volume of the activated sludge was returned to nine liters by adding tap water. The reactors were fed in the evening after all tests were completed for that day.

Initially, the reactors were fed only dextrose until the cultures became acclimated. The relative inhibition of a range of PCP spikes to the dextrose-acclimated sludge was evaluated by determining the effect on the specific rate of glucose uptake (explained in detail in the next section). The results are plotted as a function of the log of PCP concentration in Figure 1. From these data, PCP concentrations of 0.1, 1.0, and 15.0 were chosen as feed levels for the reactors. Since degradation of PCP was expected to be negligible at first, the substance was fed in at a rate sufficient to just replace the amount wasted. A control reactor containing no PCP was maintained throughout the study. For convenience, the activated sludges exposed to 0, 0.1, 1, and 15 mg PCP/L are, respectively, referred to by the following acronyms throughout this thesis: AS-0, AS-0.1, AS-1, and AS-15. Also, a fifth reactor was stepped up to 300 mg/L of PCP in five days - by adding 100 mg PCP/L/day for the first three days and then 50 mg PCP/L/day for two days - before quickly reducing the PCP concentration in the reactor by removing it from the feed and wasting two liters per day. The purpose of

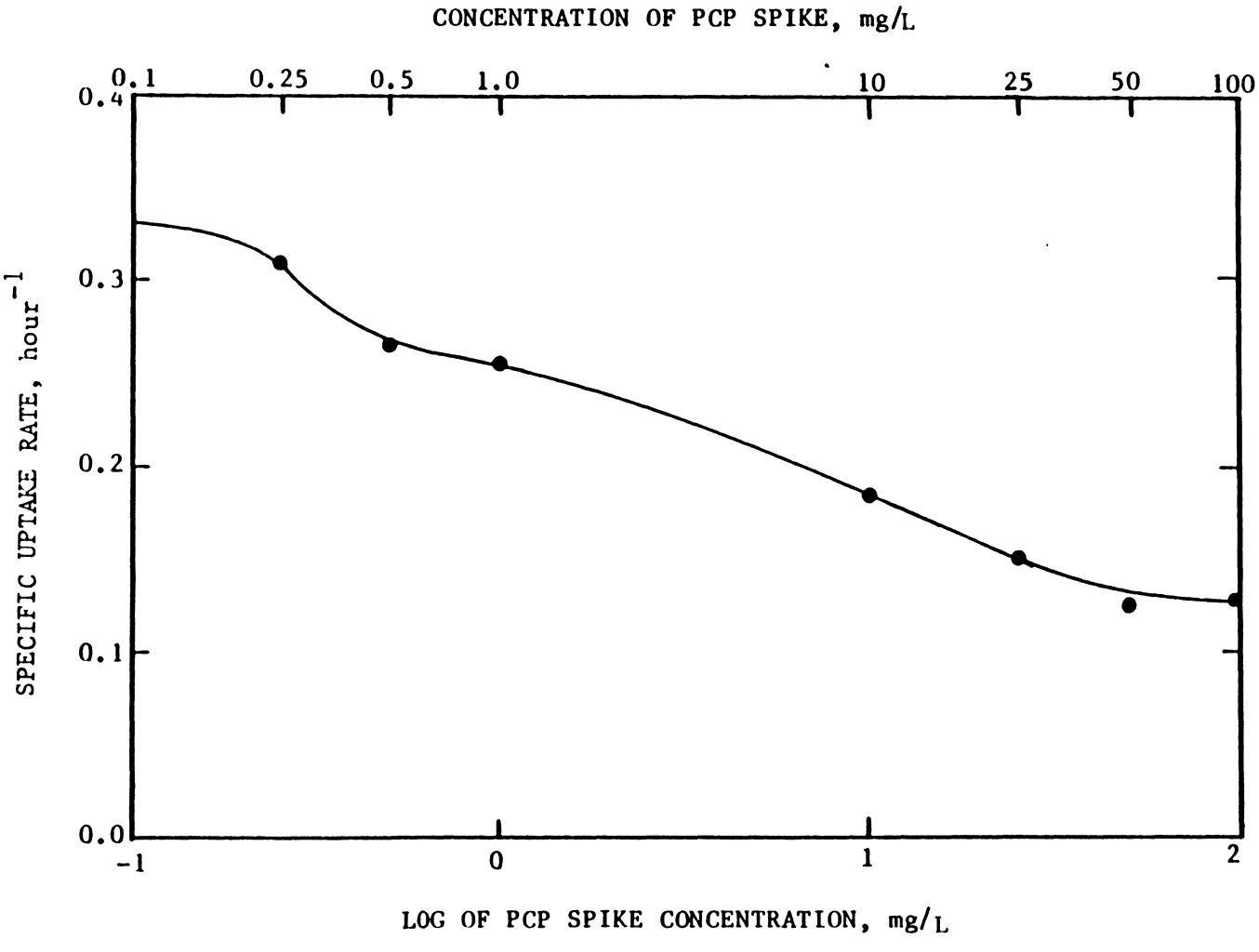

Figure 1. Preliminary investigation of the effect of a range of PCP spikes on the specific uptake rate of dextrose-acclimated activated sludge.

this procedure was to observe the response and recovery of activated sludge that had been exposed to extremely high levels of PCP.

Specific Uptake Rate Test

The rate of COD (dextrose) uptake per unit of biomass was used to measure the degree of metabolic activity of the activated sludge. Comparison of the specific uptake rate (SUR) resulting from a spike of dextrose plus toxin to that of a dextrose-only spike indicated the relative inhibition of the chemical dosage to that activated sludge. The procedure used in determining a specific uptake rate is described below:

1. 250 ml of activated sludge was withdrawn from a reactor and aerated in a 400 ml beaker.
2. The beaker was placed in a water bath and the temperature of the sludge was adjusted to 20-21°C by adding ice or warm water to the bath as required.
3. The sludge is spiked with 500 mg/L COD of dextrose and the desired toxicant dose, simultaneously, and a stopwatch was started.
4. At 5 and 15 minutes, a sample was withdrawn and filtered through a Whatman no. 40 filter paper placed in a 7.0 centimeter (cm) ceramic filtering funnel into a clean 500 ml filtering flask.

5. Ten ml of filtrate was pipetted into a COD flask, and the COD reagents are added. It should be noted that all of the daily SUR testing would be completed through this step, before continuing the procedure, in order to minimize variation in lab conditions.

6. The COD was then determined by the dichromate reflux method described in section 508 of Standard Methods for the Examination of Water and Wastewater (96).

7. After the 15 minute sample was fixed, a known volume was withdrawn from the beaker and filtered through a 5.5 cm Whatman 934-AH glass microfibre filter using a Millipore filter apparatus. The mixed liquor suspended solids concentration (MLSS) was determined according to Section 208c in Standard Methods (96). (The volume used in determining MLSS was dependent on the filtrability of the sludge).

8. The specific uptake rate was calculated by the following equation.

$$SUR, \frac{mg/L\ COD}{mg/L\ MLSS\ hour}, \text{ or simply } hour^{-1} = \frac{\Delta S/\ t}{\Delta X}$$

where

ΔS = COD of the 5 minute sample minus COD of the 15 minute sample

t = 15 minus 5, or 10 minutes

ΔX = MLSS concentration in mg/L

Gas Chromatography

Apparatus

The gas chromatograph (GC) used in determining of PCP concentrations was a Bendix (Lewisburg, WV) model 2600 with an electron capture detector. The two meter by two millimeter (inside diameter) glass column was packed with one percent SP-1240 DA on 100/200 mesh Supelcoport acquired from Supelco, Inc. (Belefonte, PA). The carrier gas was high-purity nitrogen and was delivered at approximately 30 milliliters per minute (ml/min). The temperature controls were set as follows: injection port, 200°C; column (oven), 185°C; transfer, 185°C; detector, 345°C.

Extraction Method

The procedure followed in extracting PCP from activated sludge for GC analysis is described below:

1. 50 ml of activated sludge was transferred from a reactor to a 250 ml separatory funnel.
2. The sample was acidified with four drops of concentrated sulfuric acid to pH 1-2 to reduce the aqueous solubility of PCP.
3. 25 ml of hexane was added to the separatory funnel.

4. The mixture was shaken by hand for two minutes, with venting to relieve pressure, by rotating the wrist crisply so that the vessel oscillated between a one o'clock and five o'clock position.
5. After allowing for separation, the sludge was drawn off and the clean hexane was poured into a screw-capped glass vial for GC analysis. Samples extracted from the 15 mg/l-PCP reactor were diluted 1:5 until the PCP concentration in the reactor decreased markedly. Then, no additions were made. Only the reactors exposed to one and 15 mg PCP/L were analyzed by gas chromatography.

Standard Addition Technique

Because of the relatively high PCP concentrations involved in this study, it was felt that a single, standardized extraction of sludge samples would be sufficient, rather than performing the involved, time-consuming procedure recommended by the EPA (97). However, the consequence of not performing multiple extractions was incomplete extraction efficiency. The extraction efficiency was also found to vary depending on the MLSS concentration and the physical characteristics of the sludge. Therefore, in order to quantify the PCP concentration in the sludge samples, a standard addition technique was performed every few days for both of the analyzed reactors. The standard addition procedure

followed was similar to that described in Instrumental Methods of Analysis (98). The procedure involved making three, standard, PCP additions and subsequent extractions for each reactor. Additions of 5, 10 and 15 mg/L were added to samples from the reactor exposed to 15 mg PCP/L (AS-15), while 1, 2, and 3 mg/L were added to samples of AS-1. The concentration of the extracted samples was plotted as a function of the standard addition, and the best fitting line was drawn through these points, as shown in Figure 2. The x-intercept of this line was taken as zero and the actual concentration of the sludge sample measured along the x-axis.

Summary of Experiments

First, the municipal activated sludge was allowed to adapt to dextrose utilization, and this acclimation was monitored by measuring the uptake rate. For the purposes of this study, complete acclimation was associated with a constant and maximum SUR. The dextrose-acclimated sludge was distributed into five reactors and the PCP feed was initiated. The response and acclimation of the five activated sludges to their respective PCP levels was, likewise, observed. Meanwhile, the effect of dextrose concentration on the SUR was investigated, as was the contribution of PCP to the COD test. The constant-feed activated sludges were spiked with shock loads of PCP (at intervals up to 100 mg/L) to determine if acclimation to PCP provided any protection from shock loads. Afterwards, AS-0 and AS-15 were spiked

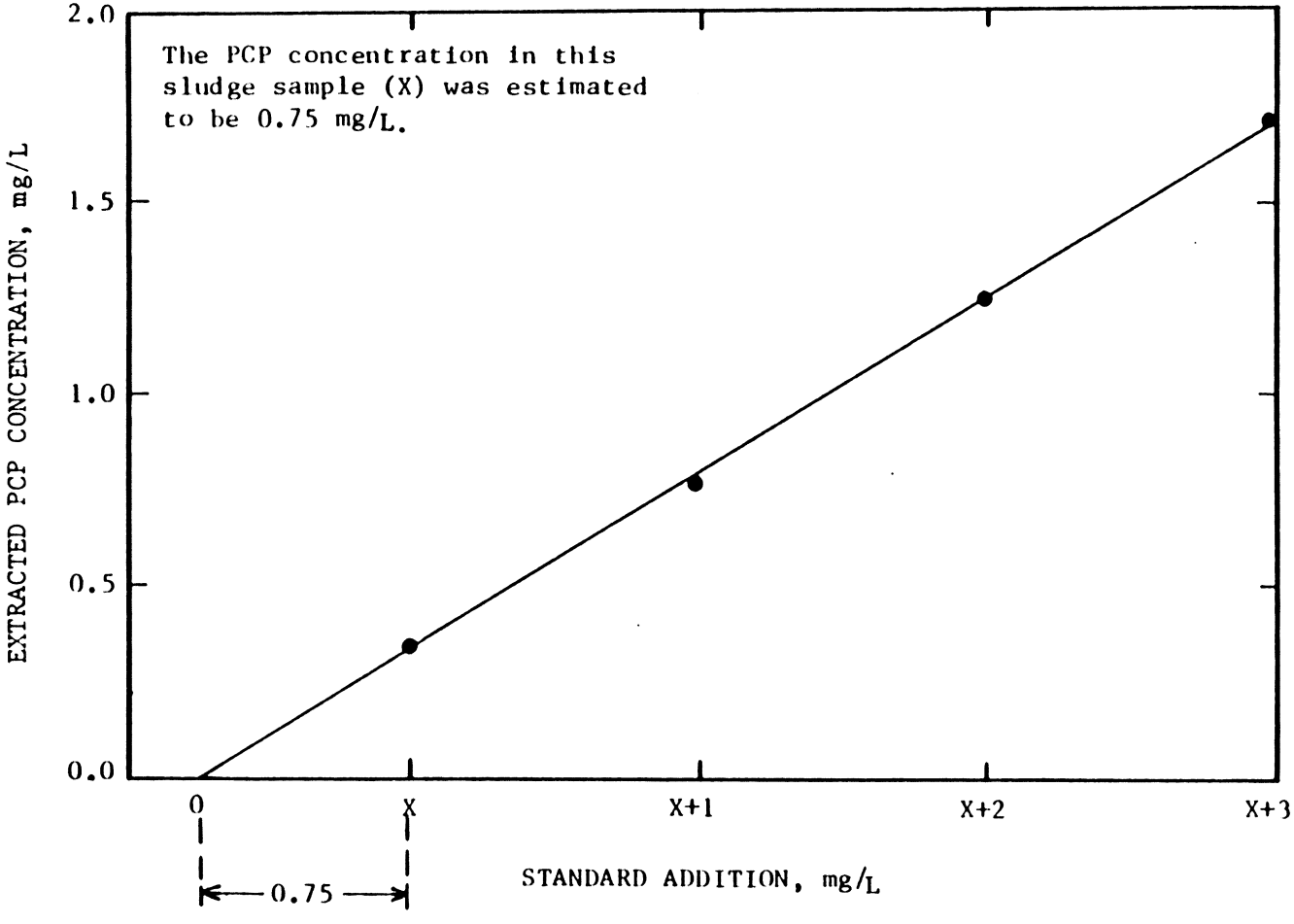

Figure 2. A typical standard addition curve extracted from the AS-1 reactor.

with phenol, 2-chlorophenol, 2,4,6-trichlorophenol, pentachlorophenol, and 4-nitrophenol (25 and 50 mg/L) in order to see if PCP-acclimation would extend any protection from these related compounds. Gas chromatography was used throughout the study to measure the PCP concentration in the AS-1 and AS-15 reactors.

Experiments employing successive extractions and separate extractions of the solid and liquid phases of activated sludge were used to investigate the efficiency of PCP extraction from sludge. In the ultiple extraction experiment, PCP was added to a 50 ml sample of the control sludge (containing no toxicant) to create a total concentration of 10 mg/L. This sample was extracted five times in succession to determine the recovery efficiencies. In the separate phase extraction experiment, a 50 ml sludge sample of AS-15 was centrifuged. The supernatant was poured off and extracted, and the solid plug was resuspended in distilled water (final volume equaled the supernatant volume) and extracted. The relative recoveries were compared.

The standard addition technique was applied to circumvent the problems of incomplete and unknown extraction efficiency. A standard addition curve was constructed for both of the reactors analyzed by gas chromatography every few days. From these curves, the PCP concentration in the reactors was estimated, and the extraction efficiency was calculated.

RESULTS

In this chapter, the results obtained from the five activated sludge reactors studied will be presented. The data displayed in all Figures except Figures 2, 7, and 12 are tabulated in the Appendix.

Adaptation to Dextrose

In Figure 3 it can be seen that acclimation of the activated sludge to dextrose, as indicated by a consistent SUR, required nearly one month. The shaded area represents the range of the apparent maximum SUR under normal conditions (no toxicant present) to be roughly 0.30 to 0.36 $hour^{-1}$.

Response to Pentachlorophenol

Figures 4 through 6 show the effect the PCP feed had on the AS-1, AS-15, and varied PCP feed reactors, respectively. The specific uptake rate of the AS-1 reactor was not affected by the PCP feed (Figure 4). Likewise, the 0.1 mg/L reactor showed no response to that low influent level of PCP (so these data are not shown). However, the SUR of the AS-15 reactor was reduced by about 50 percent by the initiation of PCP feed, but within 15 days it had increased back up to the "normal" range (Figure 5). About day 39, the uptake rate of this reactor began to decrease rapidly, reaching a minimum on day 45, and then returned to the "normal" level over the next five days.

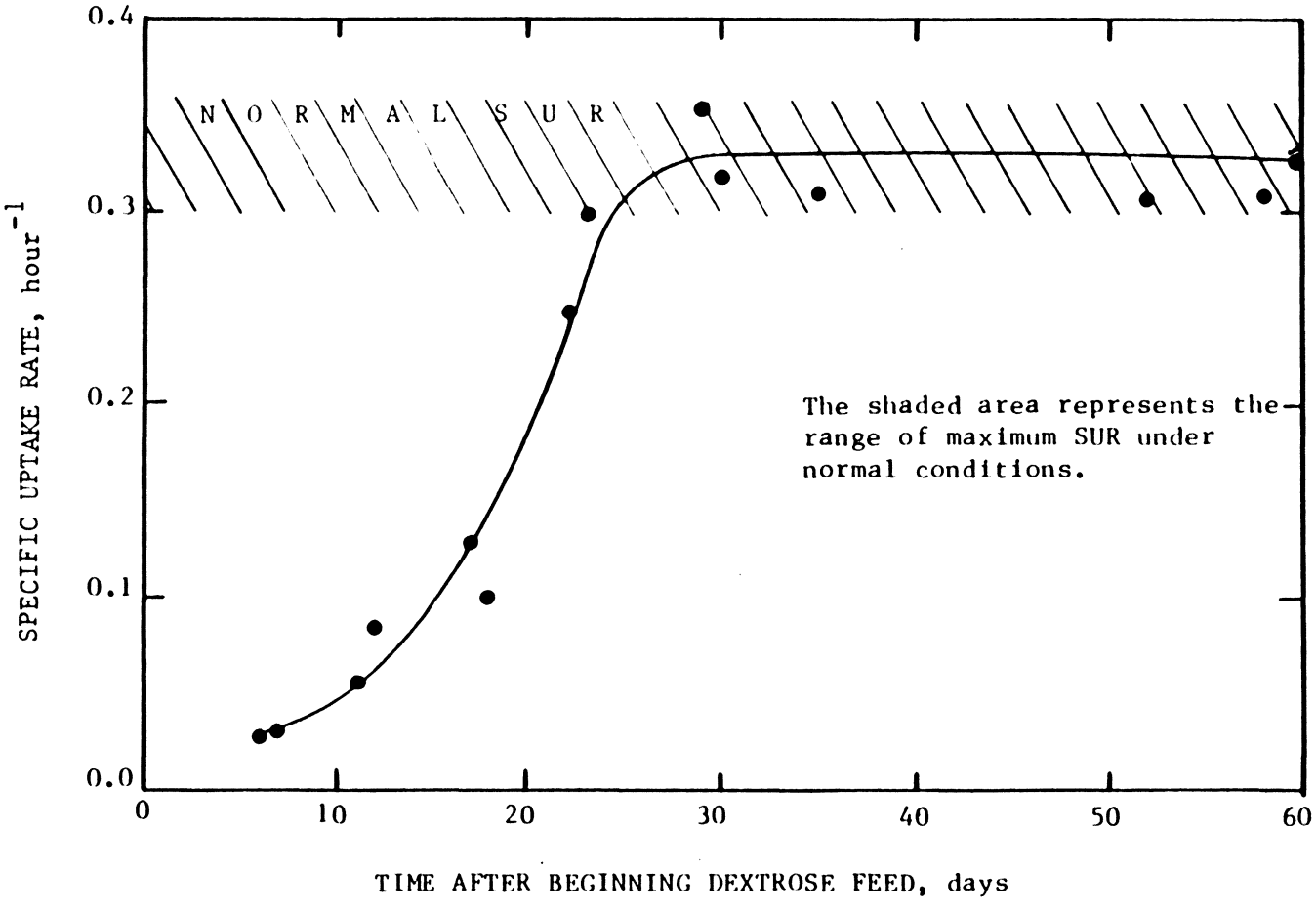

Figure 3. Acclimation of municipal activated sludge to dextrose.

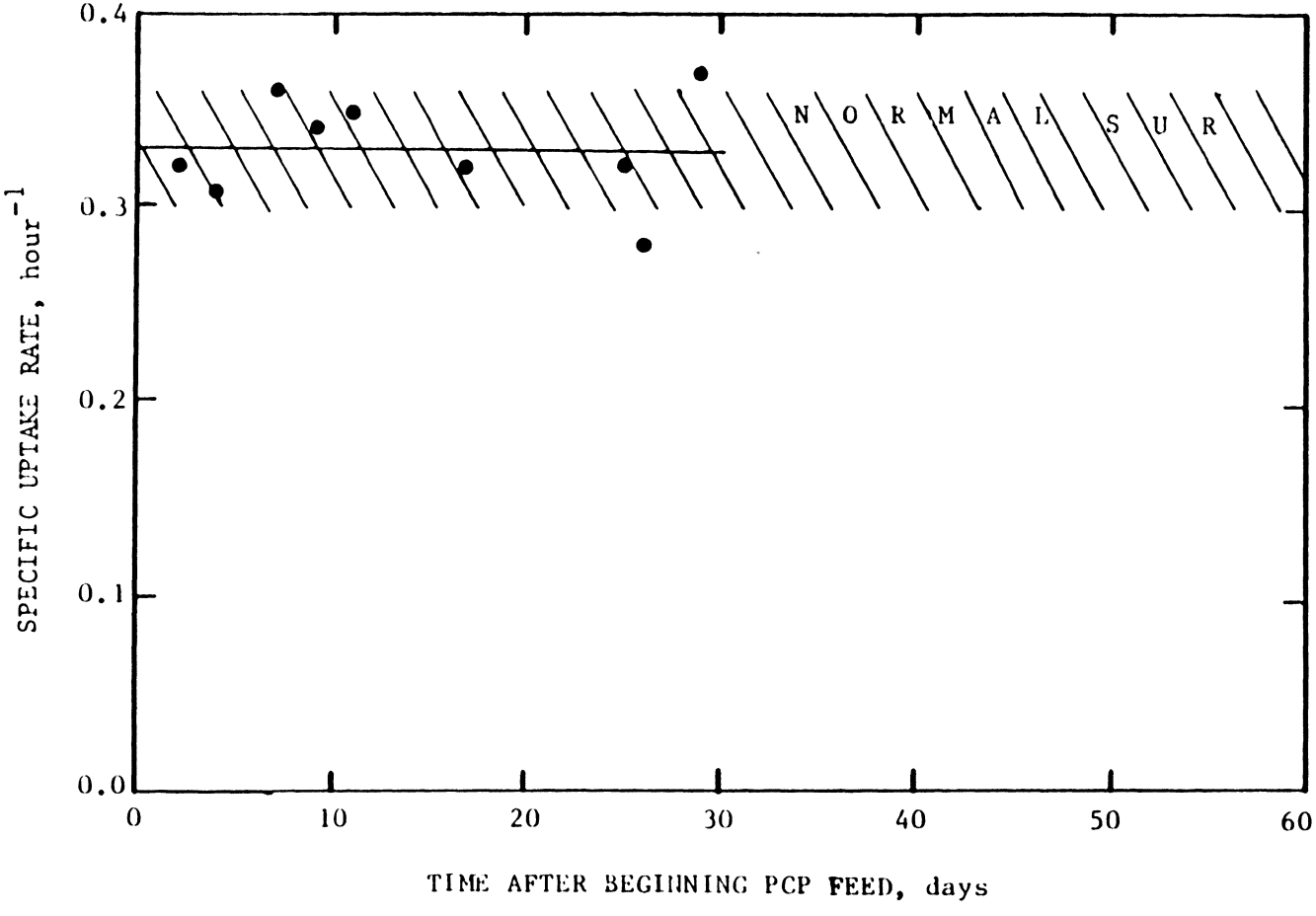

Figure 4. Specific uptake rate for dextrose-only spikes of AS-1.

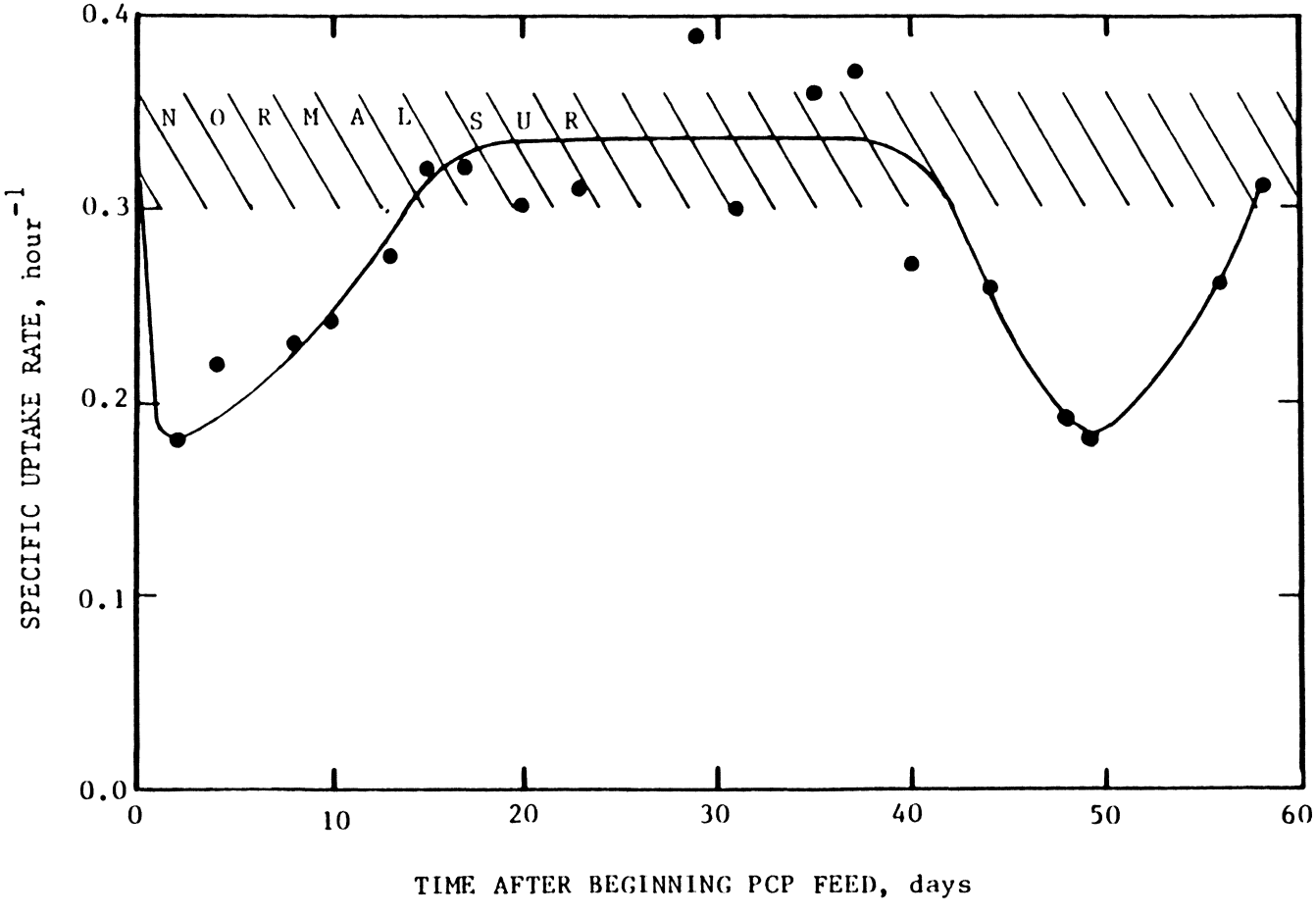

Figure 5. Specific uptake rate for dextrose-only spikes of AS-15.

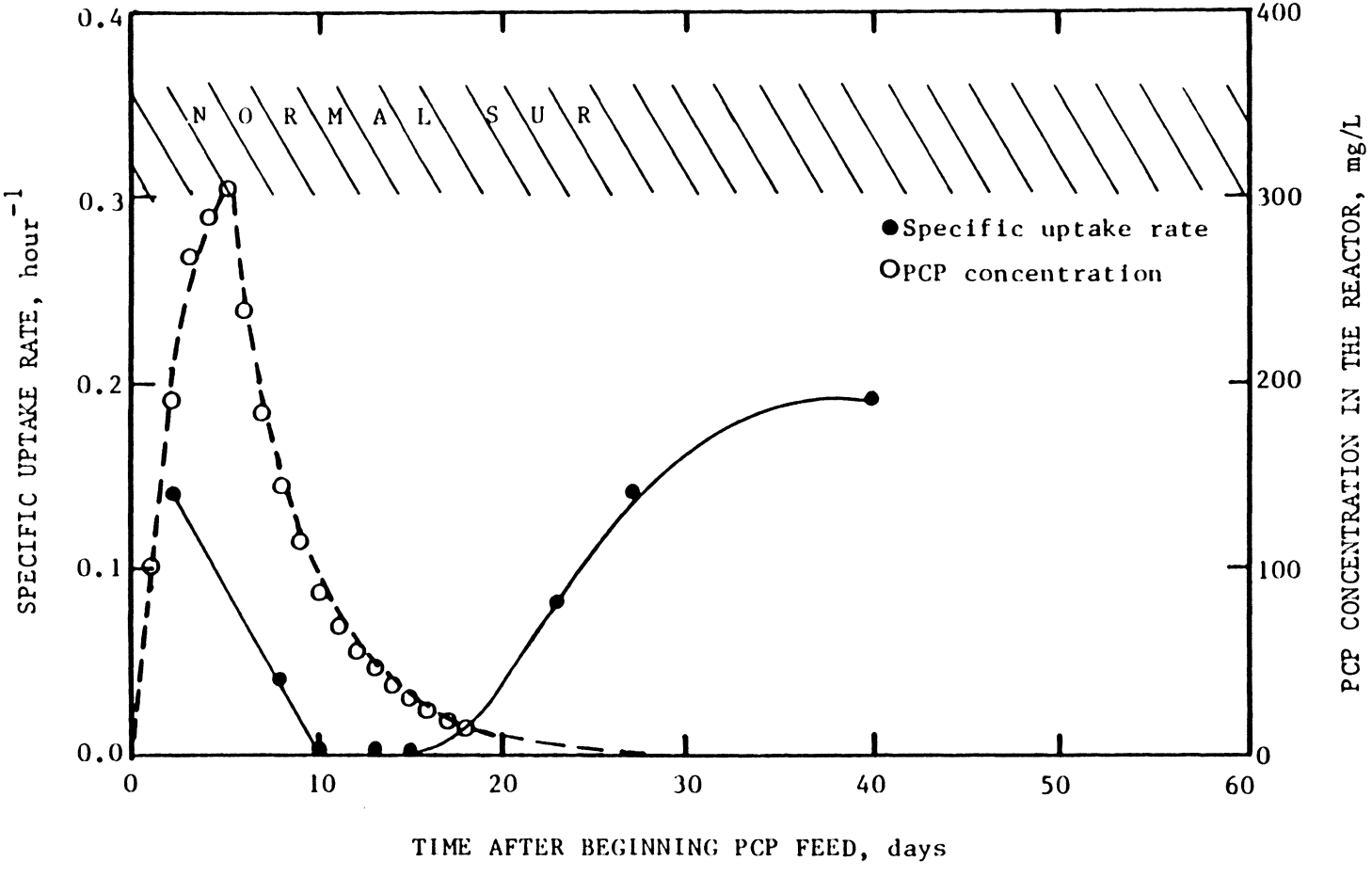

Figure 6. Specific uptake rate for dextrose-only spikes of the reactor containing various levels of PCP.

The calculated PCP concentration in the varied-feed reactor is represented by the dashed line in Figure 6. The PCP concentration reached a maximum concentration of 306 mg/L on day 5 and was then reduced to 10 mg/L by day 20. The SUR, which is represented by the solid line, decreased to virtually zero by the tenth day. During the first 20 days of the study, this reactor was characterized by profuse foaming, foul odors, finely divided flocs, and very high COD. By day 23, however, the uptake rate had begun to increase.

Effect of Dextrose Concentration on Uptake Rate

The relationship between the specific uptake rate and the dextrose concentration is shown in Figure 7. The SUR was apparently consistent at COD concentrations above 400 mg/L.

Chemical Oxygen Demand of Pentachlorophenol

Because Standard Methods (96) states that aromatic compounds are not well oxidized in the COD test, the degree to which PCP contributes to that measurement was investigated. A COD exertion by PCP which is significant in comparison to the dextrose spike could potentially distort the measured specific uptake rate. Table III lists the measured COD, theoretical COD, and the percentage of the theoretical value actually exerted, for the range of PCP concentrations pertinent to this study. It can be seen that 100 mg/L of PCP exerted only 31 mg/L of chemical

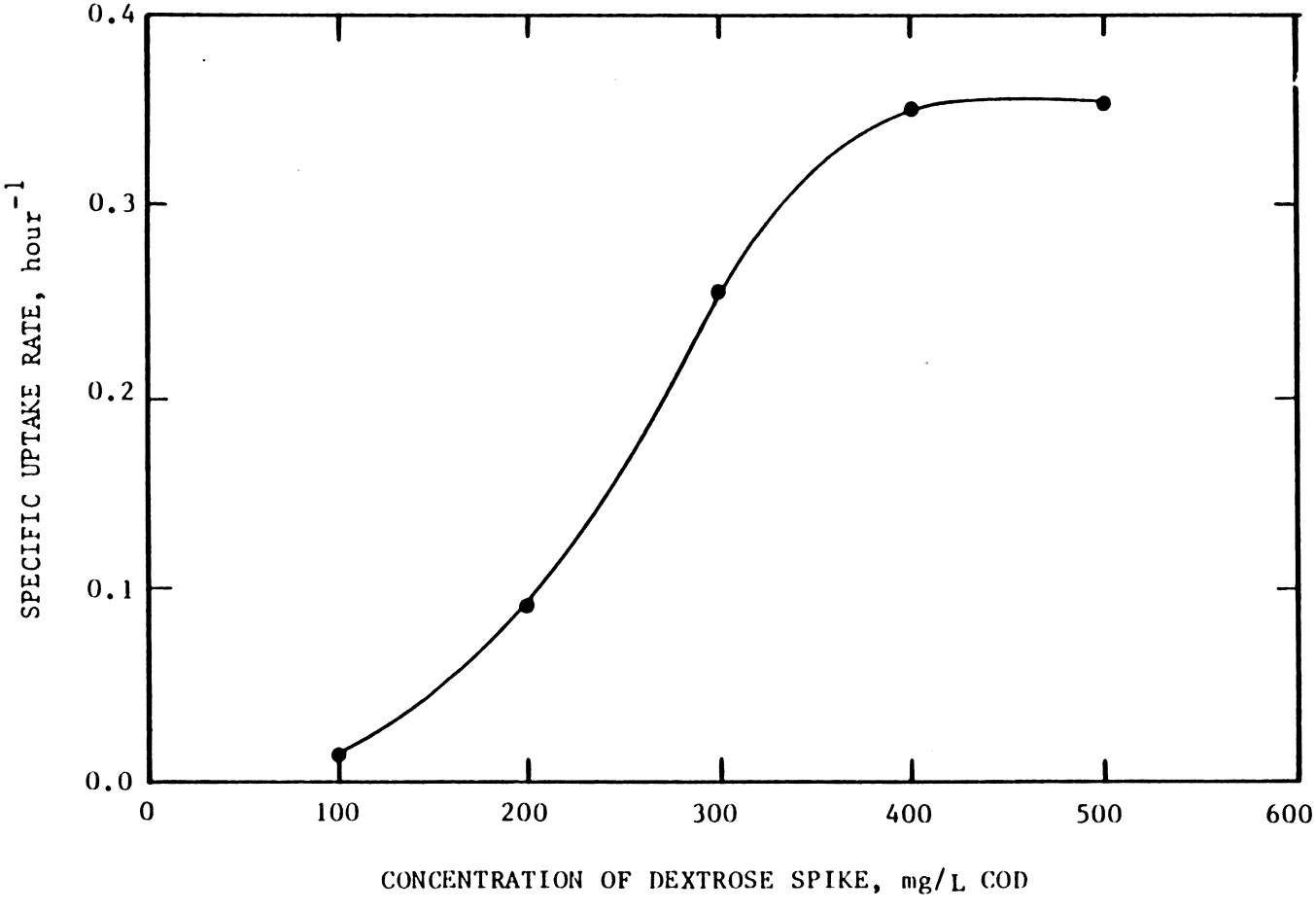

Figure 7. The effect of dextrose concentration on specific uptake rate.

Table III. The chemical oxygen demand exerted by pentachlorophenol.

PCP Concentration mg/L	Measured COD mg/L	Theoretical COD mg/L	$\frac{\text{Measured COD}}{\text{Theoretical COD}}$, %
50	18.2	34.5	53
100	30.9	69.0	45
150	56.4	103.5	54
200	76.4	138.0	55
250	112.8	172.5	65

oxygen demand. An average of 54.4 percent of the theoretical COD was measured for PCP in this experiment.

Effect of Pentachlorophenol on Uptake Rates

The specific uptake rates resulting from 0 to 100 mg/L PCP spikes are plotted in Figure 8 for the control reactor and those exposed to 0.1, 1, and 15 mg PCP/L. The SUR is expressed as a percentage of the uptake rate determined for a glucose-only spike of that particular sludge for two reasons: First, the control reactor developed a fungal growth midway through the study and was discarded because the SUR was no longer typical of the bacteria-dominated systems. A new control reactor was started up, but did not have time to acclimate to dextrose before the other experiments were begun. Second, although the SUR test was reproducible to within a range, it was subject to some variation (Figures 3 and 4). Expressing the SUR as a percent of a control value obtained within minutes of the datum of interest helped to normalize the data. It can be seen from Figure 8 that the higher PCP acclimation concentrations correspond to the higher uptake percentages over the entire range of PCP dosage.

Effect of Related Phenols on Uptake Rates

Figure 9 compares the toxic effect on the SUR of AS-0 (control) and AS-15 caused by five related priority pollutants. In this figure, the white bars represent data from AS-15 while the shaded bars correspond to data from AS-0. Notice that in

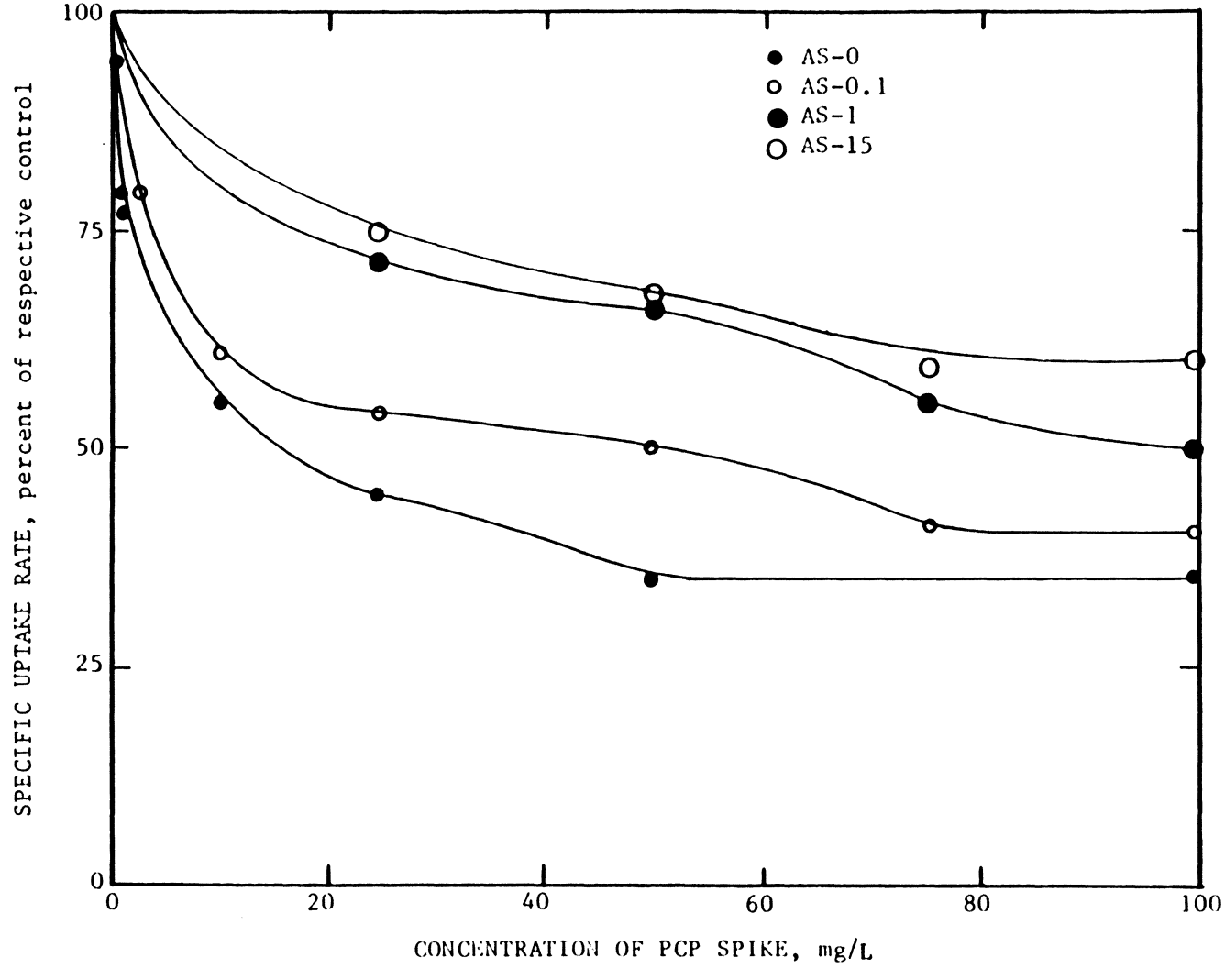

Figure 8. Effect of PCP-acclimation on SUR inhibition caused by shock loads of PCP.

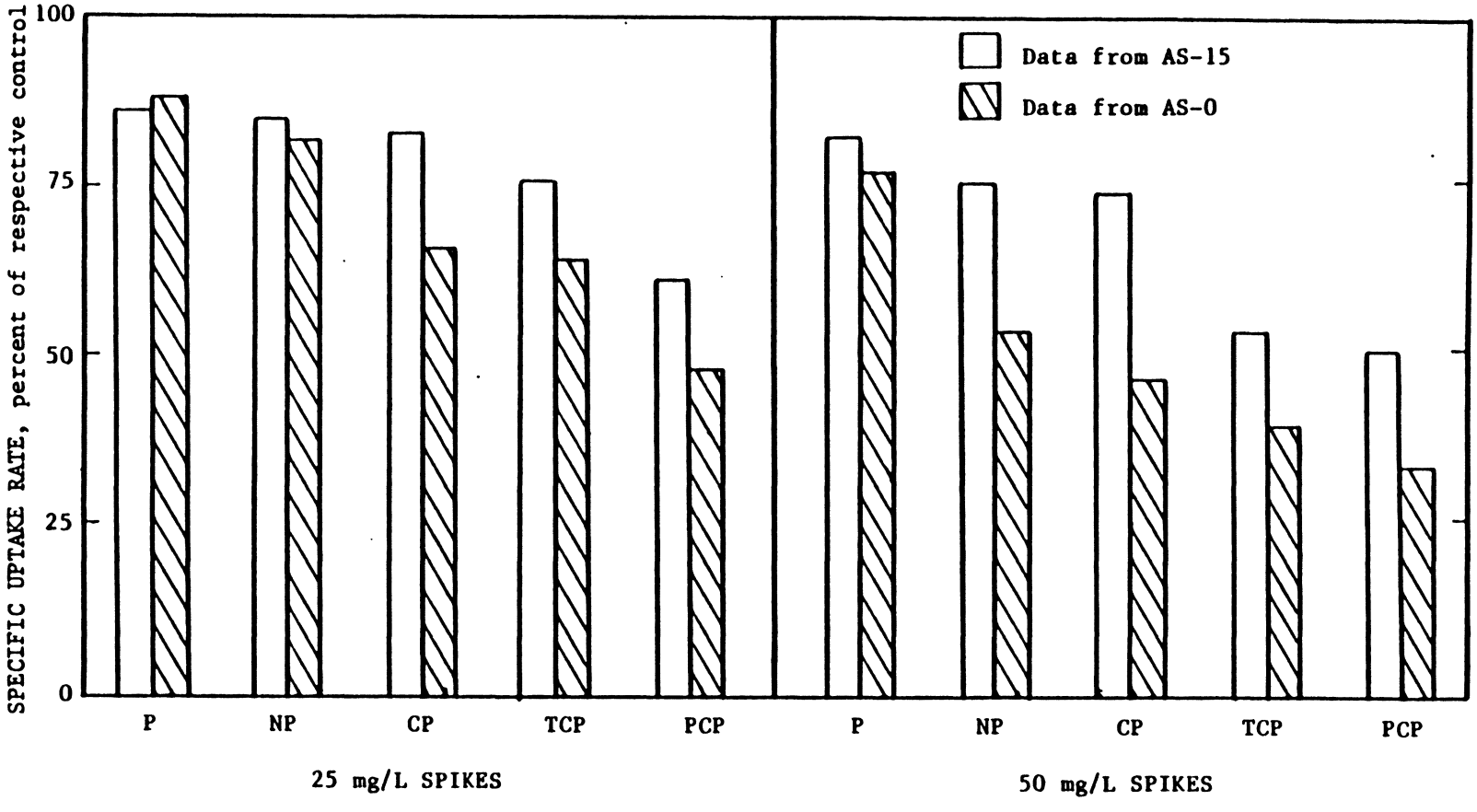

Figure 9. Effect of PCP-acclimation on the SUR inhibition caused by shock loads of related priority pollutants. P=phenol; NP=4-nitrophenol; CP=2-chlorophenol; TCP=2,4,6-trichlorophenol; PCP=pentachlorophenol.

every case except one (namely, the 25 mg/L spike of phenol) the SUR of AS-15 is higher than the corresponding uptake rate of the control sludge. Also, it is interesting to note that the SUR of both sludges decreases as the degree of chlorination on the toxicant molecule increases, and that the data obtained for 4-nitrophenol seem to fall between phenol and 2-chlorophenol in the decreasing trend.

Pentachlorophenol Concentration in the Reactors

The PCP concentrations over time in the one and 15 mg PCP/L reactors, as determined by gas chromatography, are presented in Figures 10 and 11, respectively. Throughout the study, very little change in the PCP concentration occurred in AS-1 (Figure 10). As pictured in Figure 11, the concentration of PCP in AS-15 remained fairly constant for approximately one month before it decreased to virtually zero. After remaining at a negligible level for about a week, the PCP concentration began to gradually increase. The dashed lines on Figure 10 represent the calculated levels of PCP that would be present if no removal occurred following: (a) the initiation of PCP feed and (b) the last day that the PCP concentration was measured at virtually zero. It can be seen that better than 50 percent removal still occurred after the culture stopped totally degrading PCP.

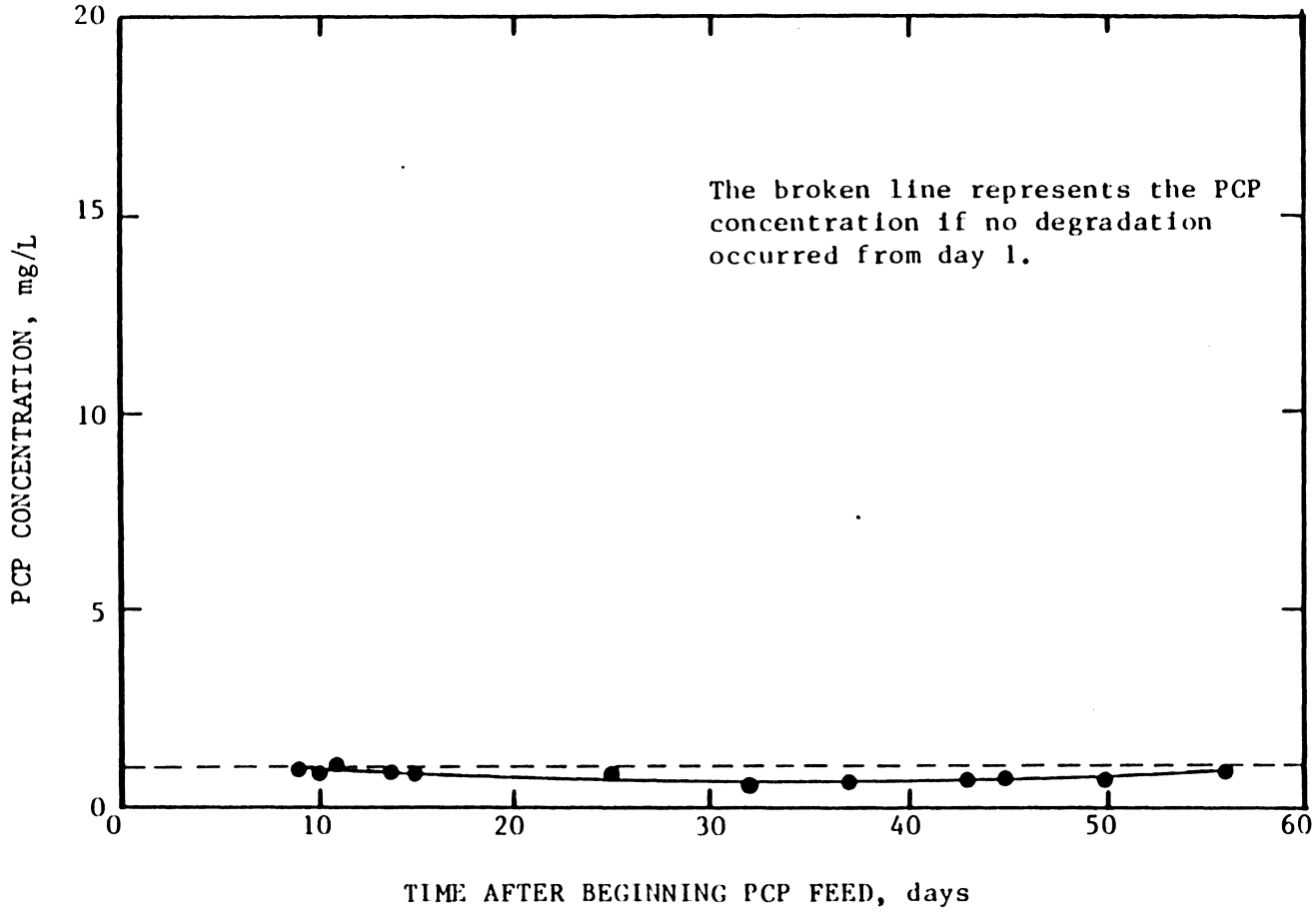

Figure 10. The measured concentration of PCP in the AS-1 reactor.

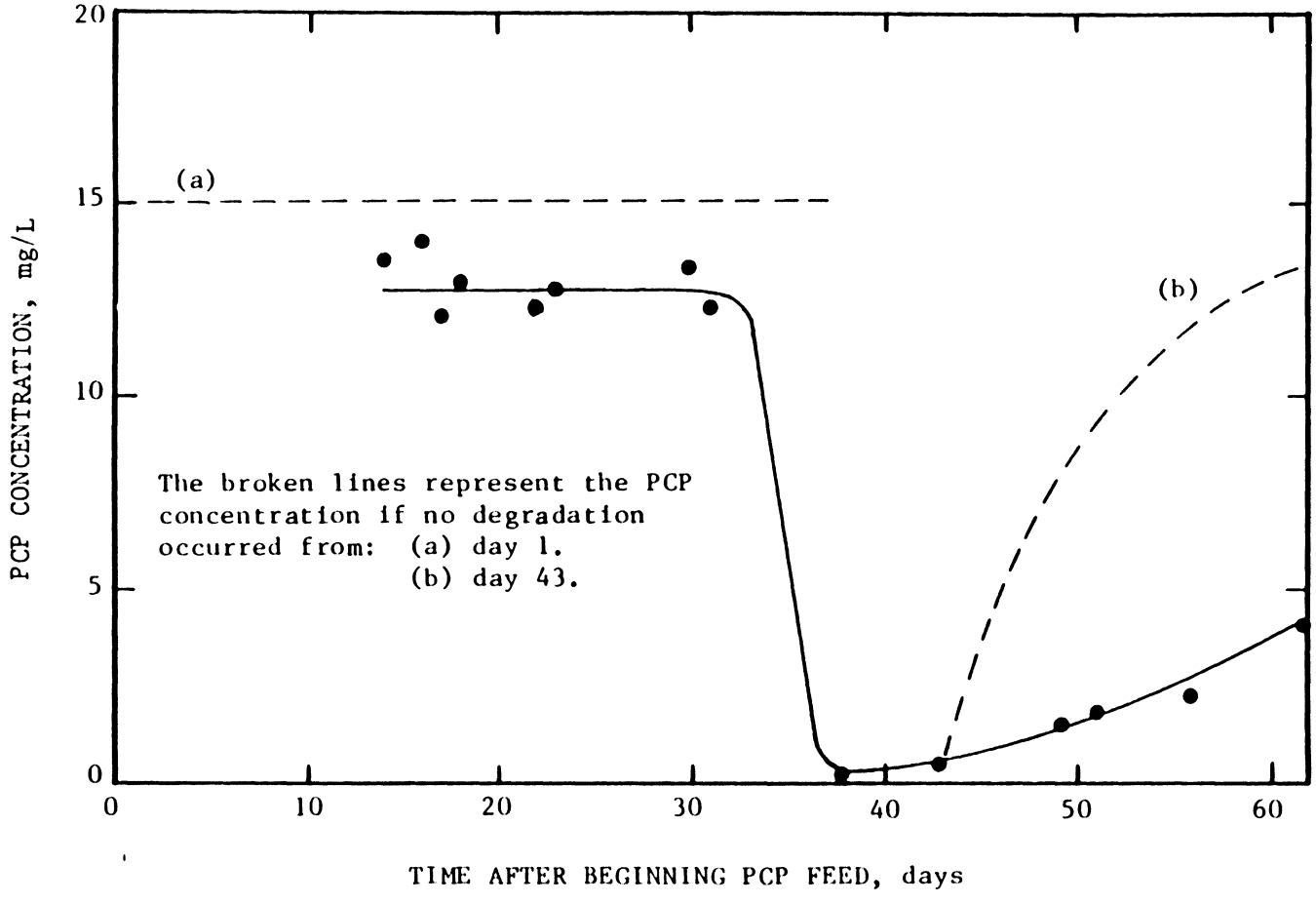

Figure 11. The measured concentration of PCP in the AS-15 reactor.

Multiple Extractions

Figure 12 shows the percentage of PCP recovered by five successive extractions of an activated sludge sample containing a known concentration of penta. After extracting 80 percent of the PCP in the first two extractions, the PCP recovery became almost asymptotic.

Separate Phase Extractions

By performing separate extractions of the solid and liquid phases of activated sludge, it was found that the level of extractable PCP in the supernatant liquor was five times higher than that in the biomass.

Standard Addition Technique and Extraction Efficiency

A typical application of the standard addition technique is presented in Figure 2. The best fitting line is drawn through the data points obtained for the sample (plotted at X) and the three standard additions of PCP (plotted at X+1, X+2, and X+3). The concentration of PCP in the sludge sample is estimated to be 0.75 mg/L for the plotted data.

The extraction efficiency ranged from 47 to 61 percent for AS-1 and from 68 to 71 percent for AS-15.

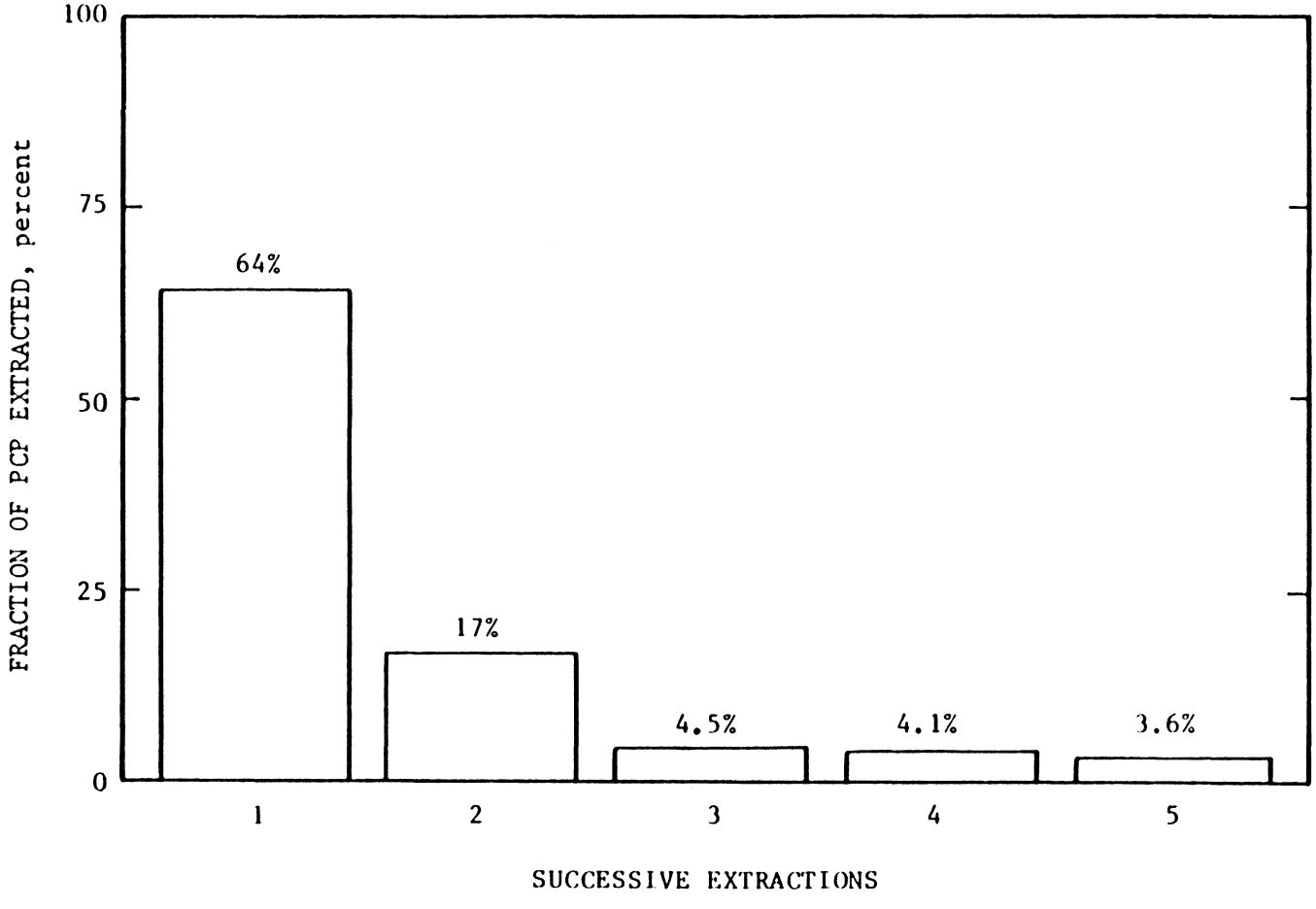

Figure 12. Percentage of PCP recovered by multiple extractions of an activated sludge sample containing a known amount of PCP.

DISCUSSION

Acclimation to Dextrose

Dextrose is considered to be a very readily biodegradible compound. Unexpectedly, the municipal activated sludge required 30 days of acclimation to a dextrose feed before reaching its maximum uptake rate (Figure 3). Delineation of the range of maximum uptake rate for the control sludge was important for the comparison of toxicant-fed sludges. The maximum uptake rate of 0.30 to 0.36 $hour^{-1}$ compares favorably to maximum utilization rates reported by Lawrence and McCarty (99) for the activated sludge process.

Response to the Presence of Pentachlorophenol

The 0.1 and 1.0 mg/L levels of PCP were apparently too low to cause any perturbation of the activated sludges' ability to take up glucose. (Recall that these levels of PCP were selected because they caused little or no response in a preliminary study). The SUR of those two reactors was never deflected below the normal maximum range by PCP addition (Figure 4).

On the other hand, initiation of the 15.0 mg/L PCP feed effected an immediate and sharp reduction in the SUR (Figure 5). Afterwards, the activated sludge went through a 15 day period of acclimation to the presence of PCP in returning to the normal maximum uptake rate. This suggests a type of acclimation different from the lengthy acclimation period required to

develop a vigorous PCP degrading culture described in the literature. The activated sludge apparently underwent a fairly rapid adaptation to tolerate the presence of a toxicant such as PCP. Although PCP was not degraded, its presence did not interfere with the uptake and degradation of other organics. Zahn and Broeker (92) also observed rapid acclimation of activated sludge to the presence of 3,5-dichlorophenol.

At about day 40, a depression of the rate of dextrose uptake was experienced by AS-15. This depression corresponded to the period of active PCP degradation revealed by GC analysis. In Figure 13 a comparison of the SUR and PCP concentration over the discussed time interval is shown. The SUR reduction could possibly be attributed to a species shift to PCP-degrading organisms that were less efficient at taking up dextrose. It is also possible that a buildup of partial degradation products exerted an inhibitory effect on the activated sludge biomass. Another possibility is that the organisms experienced a toxic effect when they began to use pentachlorophenol.

The high PCP concentrations in the varied-feed reactor proved to be extremely inhibitory to the activated sludge (Figure 6). Dextrose uptake ceased, and, judging from the smell and appearance of the culture, considerable cell lysis had occurred. However, once the PCP concentration was reduced to less than 10 mg/L, the sludge once again began to take up dextrose at a significant rate. Although the culture may

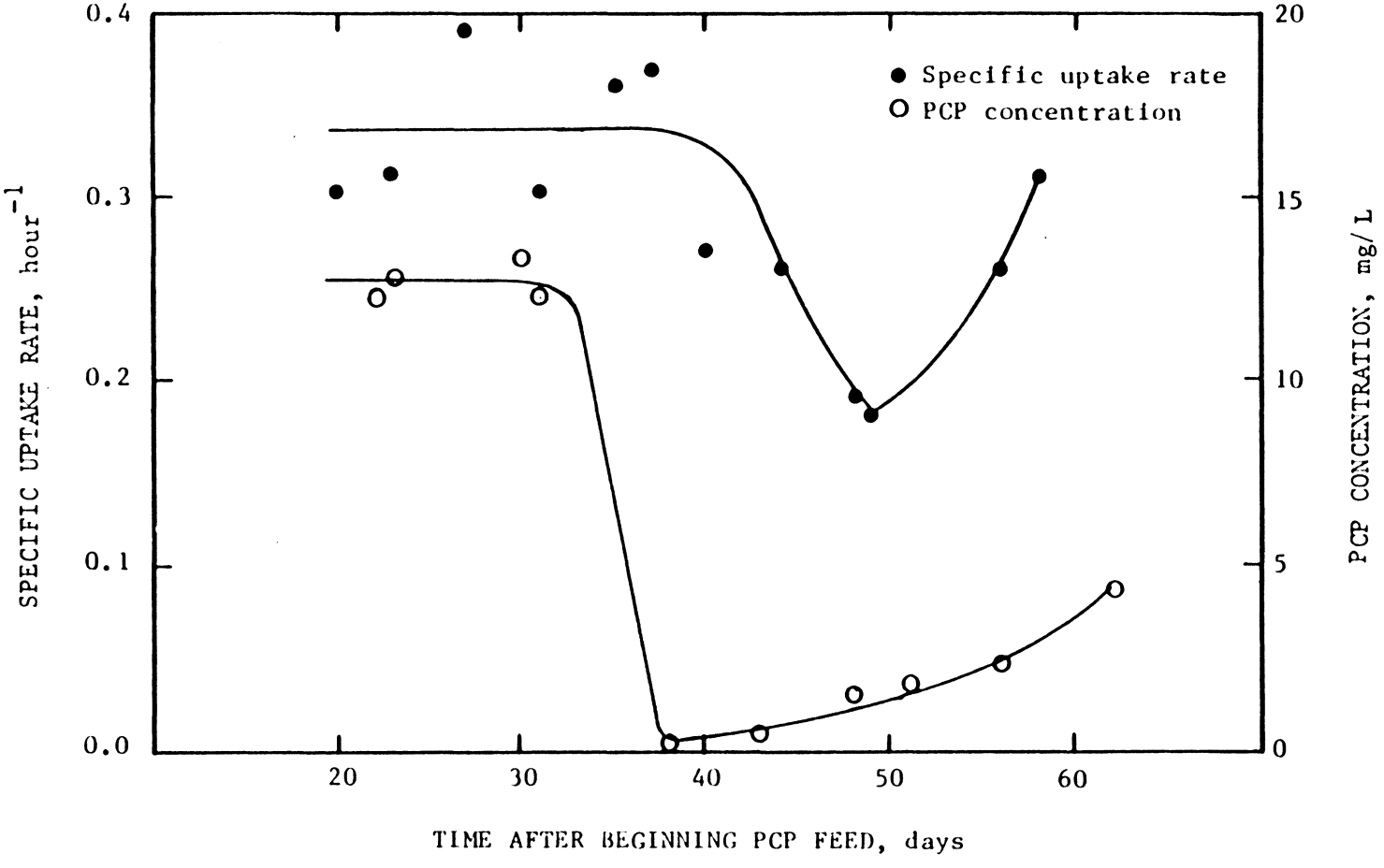

Figure 13. Comparison of SUR depression and PCP degradation in AS-15.

have been damaged, this reactor demonstrated the considerable resiliency of activated sludge. Considering the recovery demonstrated in this reactor, the mechanism of SUR reduction is unclear. The high PCP concentration could have been acutely toxic to a major portion of the biomass, and the uptake rate became measurable again only when the PCP concentration became low enough to allow the proliferation of successive generations of the surviving organisms. Another possibility is that a subacute condition existed in which the high PCP levels chronically inhibited glucose uptake rather than directly killing the activated sludge. Another interesting feature of Figure 6 is that although the PCP concentration peaked at 306 mg/L on day 5, the SUR was not absolutely zero until day 10. This suggests that a period of contact time was required to cause complete inhibition. Therefore, it may be possible to save an activated sludge system by reducing the hydraulic detention time of an especially toxic waste to wash it from the system as quickly as possible (although effluent quality might suffer in the process).

Use of the Specific Uptake Rate to Evaluate Toxicity

Possible Interferences

The significance of Figure 7 is that the 500 mg/L dextrose spike used in the SUR test lies on the asymptotic or substrate saturated section of the curve, and, thus, allows uptake of

dextrose to occur at the maximum possible initial rate. Also, since additional substrate concentration effects no increase in the SUR, contributions from other sources, such as PCP and other phenols, should have little or no effect on the rate of COD uptake.

The PCP spikes used in evaluating toxicity (0-100 mg/L) were found to contribute less than 31 mg/L of chemical oxygen demand (Table III). Therefore, interference with the toxicity test due to COD exertion by PCP was considered to be negligible. The one situation in which the penta concentration was high enough to exert a potentially significant COD effect occurred when measuring the SUR of the varied-feed reactor over the first 10 days. Under those circumstances, the SUR was low enough that a reduction in soluble COD over time due to passive adsorption of PCP to biomass, possibly, could have caused inflated uptake rates.

Strengths and Weaknesses

A drawback of the specific uptake rate test is that the results are subject to some amount of variation. Generally, though, the data obtained by this test were found to be precise within a range of about 0.05 $hour^{-1}$. This variation is probably due to changes in environmental conditions, imprecision of the COD test, and variability of activated sludge. Potential effects on the COD removal rate by organic carbon sources other than dextrose is another possible weakness of the test. As

discussed above, this type of interference by PCP was considered to be minimal under normal conditions. The strengths of the SUR test are that it is specific, rapid, easy (requiring little training and no certification), and requires very little specialized equipment. It is also versatile. With minor variations, the uptake rate can be measured in terms of removal of COD, total organic carbon, radioactive ^{14}C-glucose, or glucose detected by other means. The practicality of the SUR method of evaluating the inhibition of chemicals to activated sludge can easily be expanded. Expressing the specific uptake rate as a percentage of a glucose-only control eliminates the need to acclimate the sludge to dextrose. For the slower uptake rates of unacclimated sludges, the change in substrate concentration (ΔS) can be increased by increasing the duration of the experiment (Δt).

Protection Provided by PCP-Acclimation

In Figure 8, it is evident that acclimation of activated sludge to PCP did, indeed, provide protection against shock loads of pentachlorophenol. Further, the data show a direct relationship between the PCP-acclimation concentration and the amount of protection afforded.

The results presented in Figure 9 indicate that acclimation to PCP also provided a considerable amount of protection to activated sludge against the toxicity of related priority pollutants.

These data imply that it might be beneficial to introduce a low level of toxicant into the biological process of a waste treatment plant as insurance against detrimental effects of future toxic waste loads. Of course, for this type of bleed-in program to be feasible, the treatment system would have to be capable of consistently removing the added toxic chemical.

Another interesting point demonstrated in Figure 9 is that the relative inhibition of activated sludge caused by the compounds evaluated in this study was directly related to both the degree of chlorination and the log octanol/ water partition coefficient.

Pentachlorophenol Degredation

Just as 1.0 mg/L of PCP did not cause any reduction in the specific uptake rate, it likewise was not significantly degraded during the study (Figure 10). Apparently that concentration was simply too low to exert an effect upon the sludge microorganisms and, thus, presented no selective advantage which would create a species shift to PCP-degraders. The concept of a minimum concentration required for degradation (S_{min}) discussed by Kobayashi and Rittman (100) may help to explain the persistence of PCP in AS-1. Two problems to biological treatment of very low substrates concentrations were given: 1) not enough energy is produced by the extremely slow utilization rates to sustain the toxicant-degrading microbes, and 2) the toxicant concentration may be too low to induce production of the enzymes

required for degradation. Typical values of S_{min} for aerobic treatment systems were given as 0.1 to 1.0 mg/L.

The sudden disappearance of PCP from AS-15 (Figure 11), presumably, was due to biodegradation by PCP-utilizing organisms which had become a significant fraction of the culture population. No new selective advantage was evident, but the PCP-degraders simply may have been slow growing organisms. The degradation of PCP was nearly complete for about a week. Afterwards, the substance began to accumulate in the reactor, although some uptake continued, as was evidenced by the deviation from the no-degradation line (b) in Figure 11. Apparently, the PCP-degrading population was beginning to be lost after day 49. A possible reason for the loss of acclimation for the activated sludge would seem to lie in the feed schedule. Remember that the daily feed contained just enough PCP to hold a non-degrading culture at 15.0 mg/L. Therefore, only 1.7 mg/L of PCP was being added to the reactor every 24 hours (because one ninth of the reactor volume was wasted per day). This quantity would quickly be degraded to virtually zero by an active PCP-degrading culture, but it might not be sufficient to maintain such a population. The PCP concentration was probably below S_{min} for most of the week-long period, and the removal efficiency declined. However, as PCP accumulates in the reactor, the selective culture advantage should return and eventually re-establish a PCP-utilizing population. To

speculate further, it is possible that increasing the PCP feed rate to 15 mg/L/day on the first day of total removal would have sustained the PCP-degrading population and prolonged complete uptake of pentachlorophenol.

Other studies (77,81,82) have shown similar difficulty in maintaining an active PCP-degrading activated sludge. Long-term maintenance of an activated sludge that is capable of extensive and consistent PCP-degradation is critical to the feasibility of the bleed-in process described earlier. This type of consistent removal has been described in the literature (79,81).

Extracting Pentachlorophenol from Sludge

The difficulty of extracting PCP from activated sludge was demonstrated by the results of the successive and separate extraction experiments. PCP was extracted relatively well from the clear supernatant, but extracting from sludge forms emulsions which complicate separation. As shown in Figure 12, the amount of extractable PCP seemed to reach some type of equilibrium. Perhaps the percentage of PCP remaining after the second extraction represents that which is associated with the lipid fraction of the cell membranes. Possibly, this remaining PCP is in equilibrium between the biomass and the aqueous phase. As the aqueous PCP is removed by hexane extraction, the lipid-contained PCP once again equilibrates with its surroundings.

The standard addition technique was incorporated to circumvent the extraction problems. The variation in extraction

efficiency is directly related to the variation in the slope of the standard addition curves. The standard addition curves obtained for AS-15 were quite similar. The extraction efficiencies of the AS-1 were lower and more variable. This could, probably, be attributed to the generally lower PCP concentration and to the physical and biological composition of the activated sludge. The AS-1 reactor contained large flocs and was infested with fungal growths, whereas the AS-15 culture had a more "typical" activated sludge appearance.

SUMMARY AND CONCLUSIONS

In this chapter, the study objectives are addressed in terms of the experimental results. Afterwards, the conclusions which seem warranted are listed.

First of all, the activated sludge was able to acclimate to the presence of low levels of PCP. The 0.1 and 1.0 mg/L concentrations of PCP caused no inhibition of glucose uptake. Initiation of the 15.0 mg/L PCP feed sharply depressed the SUR, but the rate returned to the normal maximum range within 15 days.

Secondly, acclimation of low levels of PCP provided protection to the activated sludge from inhibitory effects of higher shock loads of PCP. As the PCP-acclimation concentration increased (0, 0.1, 1.0, 15.0 mg/L) the amount of SUR depression decreased, over the entire range of PCP dosage (0-100 mg/L).

Thirdly, PCP-acclimation also provided protection to activated sludge from inhibitory effects of related priority pollutants. The utilization rate of the control sludge was consistently lower than that of the 15 mg/L sludge when spiked with 25 and 50 mg/L loads of the related toxins.

Forthly, extensive and consistent PCP degradation was not achieved in either of the GC-analyzed reactors. The PCP concentration in AS-1 reactor never decreased significantly. The concentration of PCP in AS-15 decreased to virtually zero for about a one week period, but then it began to increase.

Finally, regardless of the drawbacks caused by variation and interferences, determining the relative rate of dextrose uptake seemed to be an adequate way to evaluate the detrimental effect of chemicals to activated sludge.

Based on the results of the experiments described in this study, the following conclusions seem appropriate:

1. Activated sludge can acclimate to the presence of at least 15 mg/L of PCP with no lasting inhibition of the specific rate of dextrose uptake.
2. Acclimation to low levels of PCP provides protection to activated sludge from inhibitory effects of higher shock loads of pentachlorophenol. There is a direct relationship between the PCP-acclimation concentration (from 0 to 15 mg/L) and the amount of protection afforded to the biomass from shock loads of PCP (from 0-100 mg/L) over the ranges shown.
3. Acclimation to PCP also provides protection from inhibition caused by the related priority pollutants tested.
4. Although nearly complete removal of PCP was obtained temporarily, consistent and effective biodegradation was not achieved in the PCP-fed reactors.
5. The specific uptake rate procedure described in this study seems to be a rapid, easy method of evaluating the relative inhibition of chemicals to activated sludge.

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APPENDIX

Table A-1. Acclimation of municipal activated sludge to dextrose.

Date	Time After Beginning Dextrose Feed days	Specific Uptake Rate $hour^{-1}$
June 22	6	0.031
23	7	0.033
27	11	0.058
28	12	0.086
July 2	17	0.130
3	18	0.100
7	22	0.250
8	23	0.300
14	29	0.355
15	30	0.320
19	35	0.310
August 5	52	0.310
11	58	0.310
13	60	0.325
16	63	0.280
18	65	0.240
19*	66	

*Reactor contents were discarded on this date. Declining SUR was attributed to the developing fungal growth. A new control reactor was initiated on 3 September.

Table A-2. Specific uptake rates after beginning to feed PCP to four reactors.

Time After Beginning PCP Feed days	Specific Uptake Rate, $hour^{-1}$			
	AS-0.1 Reactor	AS-1 Reactor	AS-15 Reactor	Varied Feed Reactor
2		0.32	0.18	0.14
4		0.31	0.22	
6	0.23*			
7		0.36		
8	0.20		0.23	0.04
9	0.18	0.34		
10			0.24	0.00
11	0.22	0.35		
13			0.28	0.00
14	0.20			
15			0.32	0.00
17		0.32	0.32	
20			0.30	
22	0.24			
23			0.31	0.08
25		0.32		
26		0.28		
27				0.14
29		0.37	0.39	
31	0.22		0.30	
35			0.36	
37			0.37	
40			0.27	0.19
44			0.26	
48			0.19	
49			0.18	
56			0.26	
58			0.31	

*The inoculum for the AS-01 reactor was obtained from the Blacksburg municipal treatment plant almost one month after the seed for the other reactors, and the activated sludges were obviously different (the AS-0.1 sludge was yellow-orange whereas the others were dark brown). Although fed dextrose for a month before initiating PCP feed, the AS-0.1 reactor only achieved a normal maximum SUR range of about 0.20-0.24 $hour^{-1}$.

Table A-3. Specific uptake rates of each activated sludge for various doses of pehtachlorophenol

	Concentration of PCP Spike, mg/L								
	0.25	0.5	1.0	2.5	10.0	25.0	50.0	75.0	100.0
SUR for the	93	90	76	73	48	41	48		42
control	97	76	68	75	42	40	39		36
activated	93	80	76		58	55	24		30
sludge,		76	76		70	45	33		
(AS-0),			90		58	39	33		
percent*						53	33		
						42			
average	94	80	77	74	55	45	35		36
SUR for				73	61	50	55	42	42
AS-0.1,				77	65	58	49		
percent*				87	60	54	46		
average				79	62	54	50	42	42
SUR for						66	66	53	54
AS-1,						64	57	56	53
percent*						84	62	55	49
							71		42
							64		
							82		
average						71	67	55	50
SUR for						86	70	60	66
AS-15,						70	60	67	56
percent*						73	73	58	42
						68	58	62	61
						73	70	63	65
							72	45	63
							65		69
average						74	67	59	60

*In this table, the specific uptake rates obtained for the PCP spikes are expressed as a percent of the SUR for a dextrose-only spike of that sludge on that day.

Table A-4. Specific uptake rates of AS-0 and AS-15 for 25 and 50 mg/L spikes of related priority pollutants.

	P	NP	CP	TCP	PCP **	P	NP	CP	TCP	PCP
SUR for	100	67	58	74	53	67	42	42	34	33
AS-0,	93	80	73	60	42	93	53	47	40	33
percent*	71	100	67	58	48	71	63	50	42	33
average	88	82	66	64	48	77	53	46	39	33
SUR for	75	83	69	83	50	83	72	70	39	55
AS-15,	95	86	86	72	69	83	68	78	55	54
percent*	89	85	93	72	63	81	70	74	53	54
average	86	85	83	76	61	82	70	74	53	54

*The specific uptake rates are expressed as a percent of the SUR for a dextrose-only spike of that sludge on that day.

**P = phenol; NP = 4-nitrophenol; CP = 2-chlorophenol; TCP = 2,4,6-trichlorophenol; PCP = pentachlorophenol.

Table A-5. The PCP concentration in the AS-1 and AS-15 reactors determined by gas chromatography.

Time After Beginning PCP Feed days	PCP Concentration, mg/L	
	AS-1	AS-15
9	0.92	
10	0.88	
11	1.00	
14	0.92	13.5
15	0.84	
16		14.0
17		12.0
18		13.0
22		12.2
23		12.8
25	0.80	
30		13.3
31		12.2
32	0.45	
37	0.57	
38		0.1
43	0.60	0.5
45	0.77	
49		1.5
50	0.75	
51		1.8
56	0.92	2.3
62		4.3

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THE ACCLIMATION OF ACTIVATED SLUDGE TO PENTACHLOROPHENOL

by

Gary T. Hickman

(ABSTRACT)

Bench scale activated sludge reactors were acclimated to dextrose and then to low levels of pentachlorophenol. The metabolic activity of activated sludge was evaluated by its specific rate of dextrose uptake, $\Delta s/\Delta t/X$ (measured by COD removal). Depression of the specific uptake rate, resulting from batch experiments in which the activated sludges were spiked with priority pollutants, indicated the relative inhibition caused by that toxin dosage. This study intended to determine if: (1) acclimation to low levels of PCP would provide any protection to the biomass against detrimental effects of higher shock loads of pentachlorophenol; (2) PCP-acclimation would decrease the inhibitory effect of related priority pollutants; (3) PCP would be consistently and efficiently degraded in the reactors.

The practicality of this study was twofold. First, to determine the feasibility of introducing small concentrations of a toxin to the biological system of a treatment facility in order to gain protection against shock loads of that and related toxic chemicals. Secondly, to develop a rapid and easy method for evaluating the effects of a chemical load on activated sludge.

The procedure was found to be applicable and it showed that acclimation of activated sludge to PCP provided protection against shock loads of pentachlorophenol as well as phenol, 4-nitrophenol, 2-chlorophenol, and 2,4,6-trichlorophenol. Gas chromatography analysis showed very little disappearance of PCP in the 1 mg PCP/L reactor; however, in the 15 mg PCP/L reactor, the penta concentration decreased to virtually zero for about a week and then it began to gradually increase.