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Project Summary

Protocol Development for the Prediction of the Fate of Organic Priority Pollutants in Biological Wastewater Treatment Systems

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Many of the organic chemicals classified as priority pollutants can be present in the wastewater entering municipal and industrial biological wastewater treatment plants. A biodegradability test protocol was developed and tested to provide a scientific basis for predicting the fate of organic priority pollutants in typical activated sludge treatment systems and anaerobic digestion processes.

The biodegradability testing procedure focuses on the study of: (1) substrate toxicity to activated sludge systems and anaerobic digestion, (2) partitioning and intermedia transport of pollutants including volatilization and sorption, and (3) substrate biodegradability, including kinetics and effluent quality as a function of solids retention time.

This report describes the evolution of the testing protocol, the development of necessary laboratory procedures, the results of the application of these tests to the priority pollutant pentachlorophenol (PCP), and the utilization of these data to evaluate and predict the fate of PCP in biological wastewater treatment systems.

This Project Summary was developed by EPA's Water Engineering Research Laboratory, Cincinnati, OH, to announce key findings of the research project that is fully documented in a separate report of the same title (see Project Report ordering information at back).

Introduction

Approximately 129 extensively manufactured and widely used organic chemicals are classified as priority pollutants. Since many of these chemicals can be detected in wastewater, questions have, naturally, been raised as to their fate in biological wastewater treatment systems. The degree of hazard posed by a chemical depends greatly on its concentration and exposure time. Thus, the extent of biodestruction in wastewater treatment systems will probably be one of the factors affecting regulatory decisions on the continued manufacture and use of these substances. This research program was initiated to establish and test an experimental protocol for predicting the fate of selected priority pollutants in aerobic and anaerobic biological wastewater treatment systems.

The objectives of the program were (1) to develop and test a biodegradability protocol for determining the toxicity of organic compounds to aerobic biological systems and anaerobic digestion processes, (2) to quantitate the adsorption and stripping losses of the compound, (3) to develop kinetic information allowing prediction of effluent quality as a function of the sludge retention time (SRT) of the biological treatment system, and (4) to estimate the extent of biodegradation (mineralization) of the compound.

The study evaluated the application of the experimental biodegradability

testing protocol to the testing of selected priority pollutants. Pentachlorophenol (PCP) was chosen as the typical priority pollutant because it is known to exhibit high resistance to unacclimated systems, but it is substantially degraded in acclimated systems. Furthermore, PCP shows significant bioinhibitory properties even in systems that lead to its eventual decomposition. These considerations implied that the use of PCP would be a severe test for the proposed methodology. The proposed methodology is also being evaluated with seven additional pollutants that will be the subject of a future report.

Experimental Approach

The study emphasized determining the kinetic relationship between the growth rate of organisms in the system and the concentration of organic compounds. This information can then be used with an appropriate mass balance equation to predict the fate of the substrate for the full-scale system. Since activated-sludge systems are basically large chemostats, the growth rate of organisms in the system is controlled by the SRT. Thus, knowing the SRT of a given full-scale system and the kinetics of biodegradation will permit an estimate of the effluent concentration of the substrate.

The biodegradability studies followed the noninteractive kinetic approach; this implies that the concentration of the priority pollutant in a steady-state continuous feed reactor is governed solely by the specific growth rate of the organisms responsible for its degradation and that the specific growth of these organisms is controlled by the SRT and cell decay rate.

The relationship between specific growth rate of microorganisms responsible for substrate degradation and concentration of organic substrate can be expressed by the equation $\mu = f(S)$, in which the μ (specific growth rate) is a function of substrate (S) concentration. At substrate concentration well below the inhibitory threshold, the equilibrium concentration of a growth-limiting substrate can be related to the specific growth rate of microbial population by the Monod equation,

$$\mu = \frac{\mu m\, S}{K_S + S},$$

where μm (maximum specific growth rate of microorganisms) and K_S (saturation constant for the organic substrate) are empirically determined parameters describing the hyperbolic relationship between μ and S. When S is much lower than K_s, as in the case of most treatment systems, the Monod equation reduces to a simple first order rate equation:

$$\mu = \frac{\mu m}{K_s}$$

or

$$\mu = \left(\frac{\mu m}{K_s}\right) S$$

The relationship between specific growth rate of microorganisms and SRT of a reactor is expressed by the equations:

$$\mu = (1/SRT) + d_s$$

and

$$\mu = (K_s/\mu_m)(1/SRT) + K_s d_s/\mu_m$$

where d_s is the decay rate constant for the organisms degrading the substrate. A plot of S versus 1/SRT should yield a straight line with a slope equal to (K_s/μ_m), an ordinate intercept of $(K_s/\mu_m)d_s$, and an abscissa ordinate as -d. Once μ_m, K_s and d_s values are determined, the concentration of organic pollutant in the reactor may be predicted for a selected SRT.

A multi-tiered approach was used to obtain information needed to describe the fate of a priority pollutant and is outlined briefly in Figures 1 and 2. A three-level approach was used for aerobic studies: Level I (acclimation and abiotic removal), Level II (biodegradation kinetics), and Level III (extent of biodegradation). Anaerobic studies involved a two-level approach: Level I (acclimation and substrate toxicity assay) and Level II (growth kinetics and operational performance of digester systems).

An effective way of establishing the kinetic relationship between the specific growth rate of a microbial culture and the concentration of a growth-limiting substrate is through continuous culture in a continuous stirred-tank reactor (CSTR). The specific growth rate may be controlled by fixing the SRT of the reactor, and under steady-state conditions, the physiological state of the culture reaches a stable condition reflecting the concentration of the substrate in the reactor. Operating several reactors (each at a different SRT) and measuring the steady-state concentration of the compound of interest provides the information necessary to evaluate the kinetic parameters for biodegradation of the compound.

Experimental Results

The efficacy of the proposed protocol was examined experimentally with four priority pollutants—pentachlorophenol, dimethyl phthalate, monochlorobenzene, and bis-(2-ethyl hexyl) phthalate—to determine whether needed information was provided on the fate of these organics when subjected to the outlined levels of testing.

Aerobic Testing

Level I: Acclimation Studies—

Fiber-wall acclimation reactors were operated for 2.5 months with an input flow containing domestic wastewater supplemented with dog food extract to yield a soluble chemical oxygen demand (SCOD) of 200 mg/L. Pentachlorophenol (PCP) was added as the sodium salt at specified concentrations, which were increased periodically from 1 to 20 mg/L. Effluent PCP concentrations were measured quite often in the final month of acclimation and were consistently 100 µg/L or less.

Alternative removal mechanisms of the compound, such as adsorption and volatilization, were tested in Level I. Consequently, a study was made of the possibility that these abiotic removal mechanisms were responsible for the observed PCP removal. The adsorptive capacity of biomass at equilibrium conditions was measured with unacclimated biomass from a control reactor in a manner similar to an adsorption isotherm. The specific adsorptive capacities at the various equilibrium PCP concentrations were calculated, and data were used to estimate the relative importance of the sorptive mechanism for PCP removal relative to the degradation. Sorption was shown to contribute less than 0.1% to PCP removal in the acclimation reactor. A long-term air-stripping test demonstrated that PCP concentration decreased very slightly. A first-order rate constant for the gas stripping ($0.0076\ day^{-1}$) was determined by plotting the natural log of the PCP concentration versus time and measuring the slope. This rate constant indicated that gas stripping made a negligible contribution to PCP removal.

Less than 0.5% of PCP entering the reactor left by means of air stripping

and sorption. Conversely, 99.5% of the PCP eliminated was the result of biodegradation. Consequently, the removal of PCP during Level I testing was primarily attributed to biodegradation and indicated that acclimation had been achieved.

Level II: Biokinetic Studies—

In the second-level aerobic testing to determine the kinetics of biodegradation, continuous-flow reactors without cell recycle (continuous stirred tank reactors, CSTR's) were operated on PCP-spiked wastewater at SRT's of 3, 7, 11, and 15 days. In addition, four control reactors were fed wastewater without PCP while operating at the same SRT's. Only data from the last few months of operation were used to determine the steady-state values. Log normal probability plots of PCP and SCOD data were established for the four reactors. Since concentrations higher than 350 μg/L of PCP were somewhat inhibitory to PCP-degrading organisms, only data with a PCP concentration of 350 μg/L or less were used to calculate the mean values for a given reactor and were used in the kinetic evaluation.

Figure 3 shows a plot of effluent PCP concentration as a function of (1/SRT) for the four reactors. A straight line with a slope equal to (K_s/μ_m) and an ordinate intercept of $(K_s/\mu_m)d_s$ was fitted to the data points by linear regression, and an excellent fit was obtained (correlation coefficient = 0.998). The excellent degree of correlation found indicates that PCP biodegradation is a first-order reaction at growth rates encountered in these reactors. The slope of the line was determined as 593 (μg•day)/L, and the ordinate intercept was 27 μg/L. Using these values, d_s (decay rate of cells) was calculated to be 0.046 day^{-1}.

The use of the above kinetic relationship to describe PCP biodegradation for the purpose of estimating the steady-state performance of a full-scale system should give an accurate prediction for reactors that approach the completely mixed state. The reasons are that the organisms in such reactors all have the same average specific growth rate (determined by the SRT and decay rate for the system) and that the growth equation is based solely on the growth rate. This relationship can be used to predict effluent concentration of PCP from a reactor operation at a given SRT.

The aerobic Level II testing indicated quite certainly that:

a) Substantial biodestruction of PCP occurred at nominal SRT's of 3, 7, 11, and 15 days (true SRT's of 3.2, 7.8, 12.8, and 18.3 days), since input PCP concentration of 20 mg/L dropped to mean steady-state concentrations of 214, 105, 68, and 65 μg/L, respectively.

b) Stable steady-state operation improved with the longer SRT's. Clear evidence of substrate inhibition was exhibited by PCP at the shorter SRT's.

c) The threshold inhibitory concentration of PCP was determined to be 350 μg/L; reactor instability at low SRT's may be associated with transitory excursions into inhibitory PCP concentrations.

d) The first-order PCP degradation rate constant (μ_m/K_s) was shown to be 0.0017 L/μg per day.

e) The lower limit for PCP concentration in a single, completely mixed reactor with infinitely long SRT

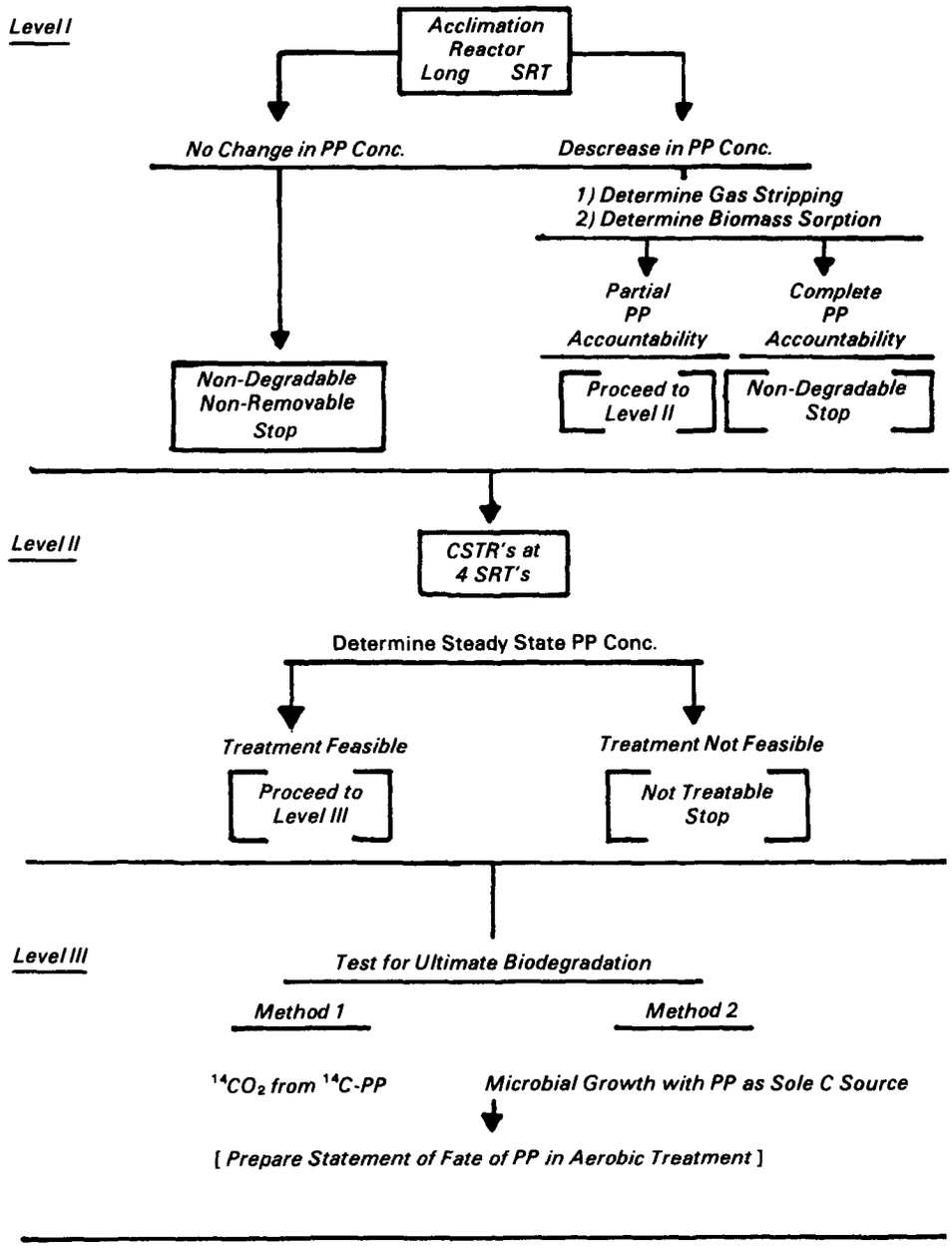

Figure 1. Logic flow diagram for aerobic testing.

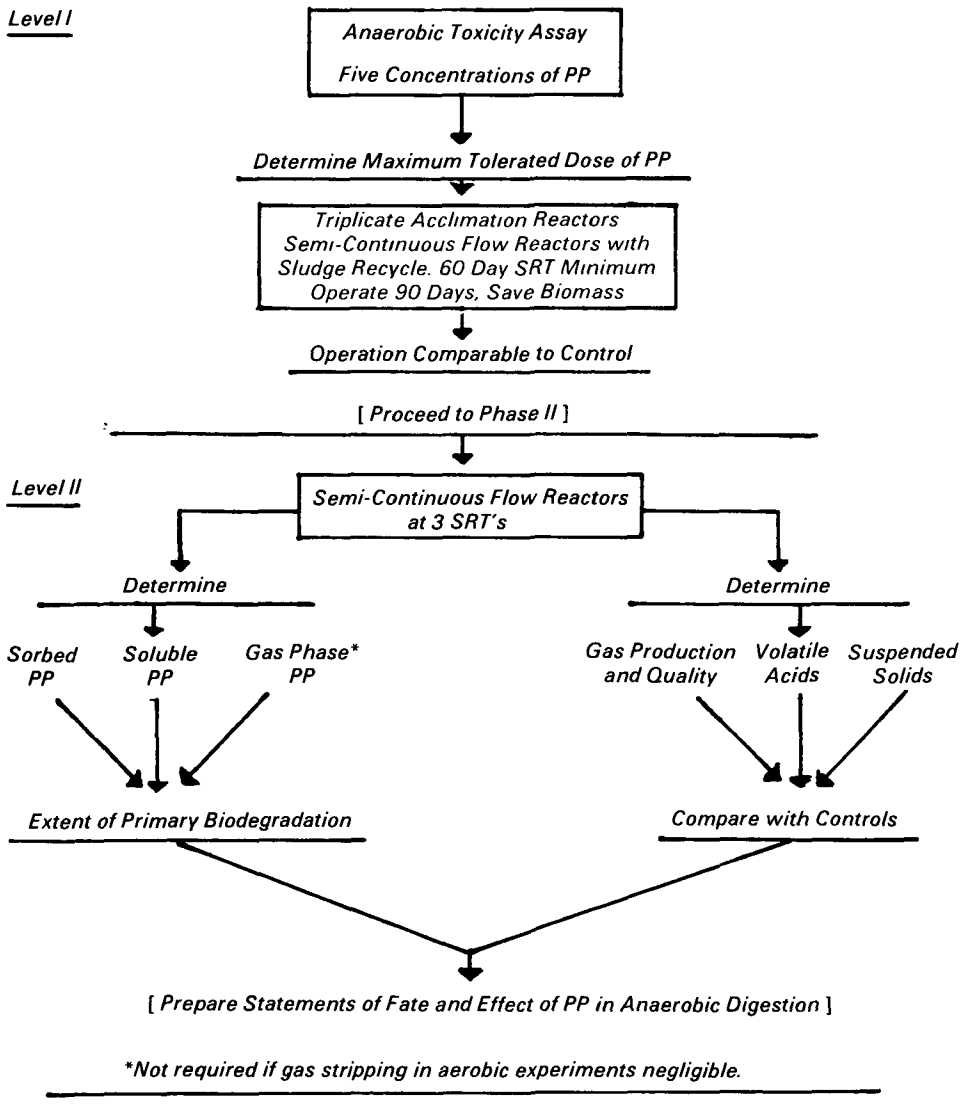

Figure 2. *Logic flow diagram for anaerobic testing.*

was estimated to be 27 μg/L at influent concentration of 20 mg/L of PCP.

Level III: Extent of Biodegradation Studies—

Two types of experiments were used to demonstrate that PCP was mineralized in the Level II reactors. Since randomly labelled ^{14}C-PCP was available, the biologically mediated formation of $^{14}CO_2$ was demonstrated and used as evidence for ultimate biodegradation. Experiments also showed that when PCP was supplied as the sole source of carbon and energy in a continuous-flow system, a new steady state was obtained that could only have been maintained for extended periods of time by the *de novo* growth of PCP-degrading microorganisms. This result implied that PCP acted as a carbon and energy source undergoing ultimate degradation by means of aerobic respiration. These data were interpreted to mean that PCP undergoes ultimate biodegradation and, to a large extent, the disappearance of PCP in acclimated biological reactors is well correlated with its mineralization.

Aerobic Level III testing established that PCP undergoes significant mineralization in the complex reactor environment. A comparison of the radioisotopic method and the microbial growth method indicated that either method used alone would have established that PCP is mineralized.

Anaerobic Testing

Level I: Anaerobic Toxicity Assay and Acclimation Studies—

Level I of the anaerobic protocol was found to require modification. Acclimation of digester biomass to PCP was found to be quite sensitive to PCP concentration. Accordingly, a preacclimation toxicity assay test was inserted to determine the inhibitory levels of the priority pollutant prior to acclimation.

The toxicity assay test suggested that a soluble concentration of PCP in excess of 200 μg/L initiates significant inhibition of gas production by unacclimated organisms. Consequently, the acclimation feeding schedule was adjusted to minimize inhibition.

Since methanogenesis is a critical final step in the complete digestion process, toxicity to this microbial population can result in decreased performance and progressive failure of the system.

The time-related gas production at various PCP concentrations is shown in Figure 4. Slight inhibition was observed at a concentration of 200 μg/L. This result means that the concentration of PCP added daily should not be high enough to result in a digester concentration much in excess of 150 to 200 μg/L. The PCP dosing regime during acclimation was established to avoid toxic concentration effects on digester biomass.

Three acclimation digesters were started at an SRT of 73 days. The operating parameters normally used to assess the performance of anaerobic digesters were not significantly affected by PCP in these systems. Gas production in the test reactors was slightly lower than in the controls, but the difference was not significant. Level I anaerobic reactors were successfully acclimated to a PCP input level of 7.6 μg/L.

Significant biological removal of PCP was observed during Level I anaerobic testing and was accompanied by the appearance of PCP-related decomposition products in gas chromatograms (GC). Direct determination of PCP sorption to digester sludge by solvent extraction and GC analysis indicated that less than 2% of the applied PCP was bound to the sludge.

Level II: Growth Kinetics and Operational Performance Studies—

The SRT values chosen for Level II testing were 10, 20, and 40 days. Since no sludge recycle was used, these values represent the hydraulic retention time (HRT) as well. Reduction of the SRT of an anaerobic digester increases the organic loading rate and stresses the system which, in itself, could cause reactor failure. Thus, a slow transition from Level I to Level II testing is critical.

After the transition period, three reactors with SRT's of approximately 10, 20, and 40 days were fed once daily with raw sludge spiked with 5 mg/L of PCP and compared with control reactors supplied with sludge containing no added PCP. As an indication of operational performance, the gas production data for each of the three reactors are shown in Figure 5 as ratios to the comparable control reactors. After some early adjustment, the mean gas ratio was shown to be close to a value of 1.0, indicating no difference between the controls and the test reactors.

This study indicates that PCP would have no significant effect on gasification if digesters were operated within the SRT limits tested. But an anaerobic digester would probably be susceptible to PCP toxicity if shock loads of PCP were encountered, as evidenced by the experiences with the toxicity test.

Once steady state was achieved in each reactor, the concentration of soluble PCP was below the detectable limit of 10 μg/L. This result indicated that the desired kinetic relationship between pollutant concentration and SRT was not attainable. The data are no less important, however, because the concentration of PCP in the anaerobic reactor with a short SRT of 10 days is lower than the concentration of the substrate in any of the anaerobic reactors, even those with longer SRT values. The data are also interesting because they suggest that PCP was undergoing very complete removal under anaerobic conditions. One can attribute this removal to biodegradation only after evaluating the alternative abiotic mechanisms. When alternative abiotic tests were performed, less than 2% of the PCP removed could be accounted for by abiotic mechanisms. Thus, more than 95% of the PCP removed anaerobically was the result of biotic mechanisms.

Phase II anaerobic testing with reactors at 10-, 20-, and 40-day SRT's and input of PCP concentration 5 mg/L resulted in normal digester performance when compared with unspiked controls.

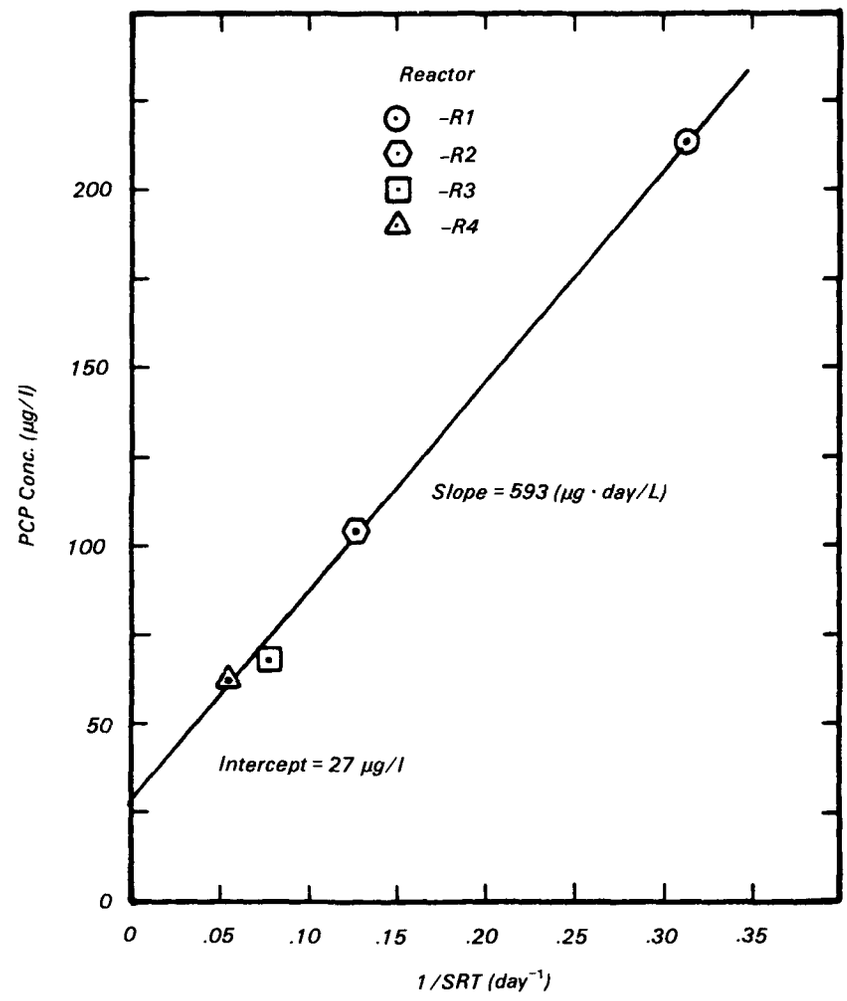

Figure 3. *Kinetic relationship between mean steady state PCP concentrations and solids retention times.*

During the steady state, Level II anaerobic testing soluble PCP concentrations at all SRT's testing were almost undetectable (<10 μg/L). However, oscillations in PCP concentration and gas production were observed earlier in the testing period.

It appears that PCP feed concentrations of less than 5 mg/L would not significantly effect anaerobic digestion if digesters are operated within the SRT limits tested. However, shock loads of PCP would probably result in essentially irreversible inhibition of digestion.

It should be made clear that only primary biodegradation was evaluated and that there was evidence of PCP metabolites accumulating in the reactors. Acclimation was very important to the success of anaerobic digestion and PCP biodegradation. A second study of PCP toxicity toward acclimated biomass clearly revealed that this biomass could tolerate three or more times the PCP concentration than unacclimated sludge could. The slow rate of acclimation combined with the obvious sensitivity of the biomass to PCP would suggest that intermittent shock loads of PCP would have a very deleterious effect on digester performance as well as on PCP degradation.

Conclusions

An experimental protocol for evaluating the fate of priority pollutants in aerobic and anaerobic wastewater treatment systems was developed. The protocol was tested and refined by applying it to a study of the fate of PCP in aerobic and anaerobic bioreactors.

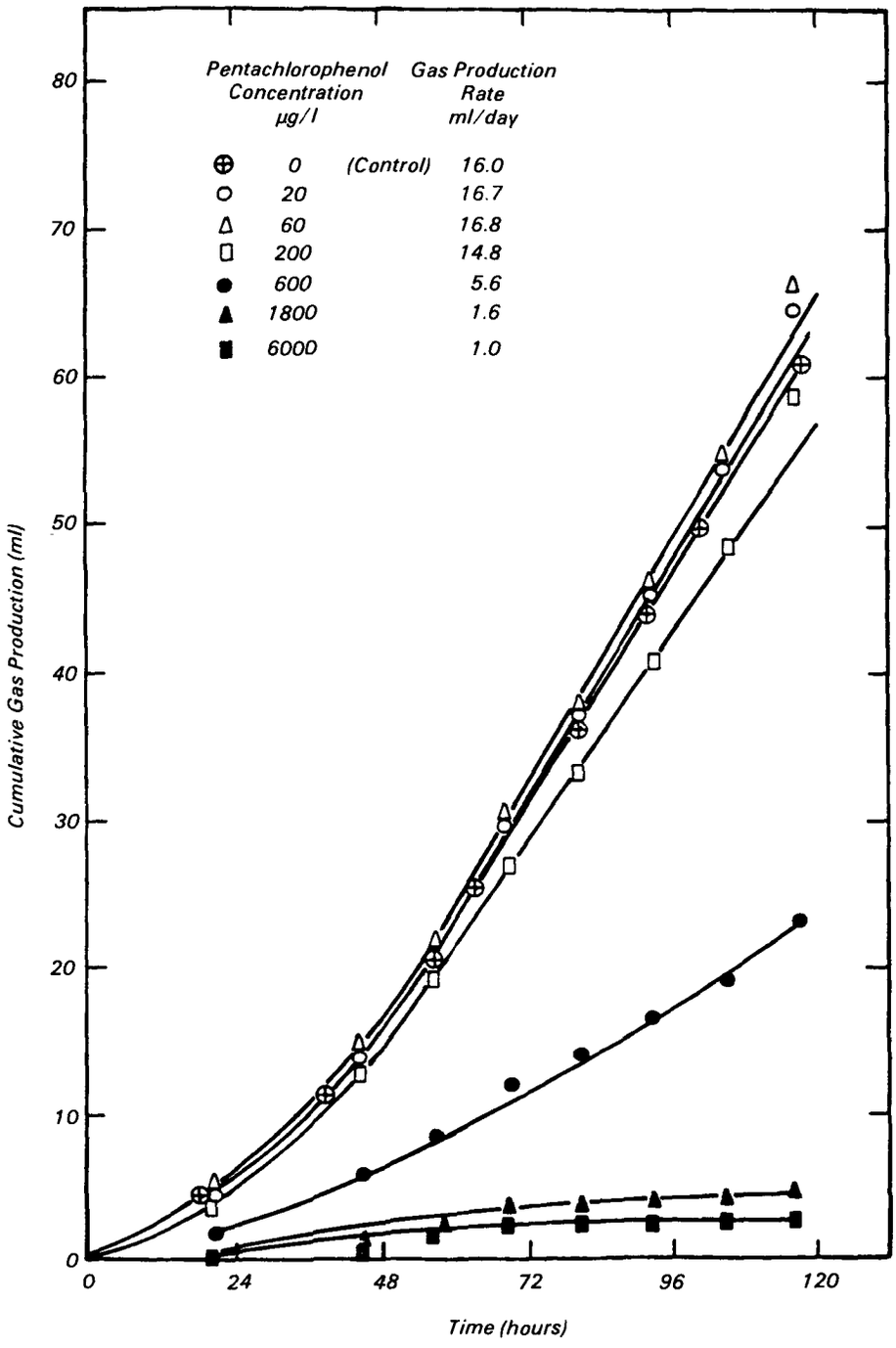

Figure 4. Toxicity of pentachlorophenol to biological methane production.

The aerobic test protocol allows one to predict that PCP can be effectively destroyed in an acclimated municipal wastewater treatment system operated at SRT's of 3 to 15 days to produce effluent PCP levels below 100 µg/L. However, biodegradation kinetics also imply that biodestruction of PCP to levels below 30 µg/L would be extremely difficult to achieve. Biodegradation with mineralization was shown to be the primary mechanism of PCP removal in wastewater treatment.

Neither sorption of PCP to biomass nor gas stripping were significant factors in PCP removal. Sorption accounted for less than 0.1% of the PCP loss, and the gas stripping was responsible for less than 0.17% loss of substrate.

Neither soluble organic carbon nor methylene blue active substance could be correlated with PCP biodegradation. Thus, the use of an internal standard was discontinued.

Generally, the aerobic test protocol was shown to be an effective approach to evaluating the fate and kinetics of PCP removal in aerobic waste treatment systems.

Acclimated anaerobic systems were also shown to biotransform PCP very effectively to levels below 10 µg/L at SRT's as short as 10 days. Here again, biological activity was the primary removal mechanism. PCP did not interfere with normal digester performance at any SRT; however, there were clear indications that PCP toxicity could disrupt performance when a PCP concentration in the reactor exceeded approximately 200 µg/L for unacclimated biomass or 600 µg/L for acclimated biomass. Since intermittent feeding is the norm for digester operation, toxic pulse loading of PCP would be expected to pose a greater hazard to the anaerobic process than to the aerobic process.

Overall, the anaerobic test protocol was shown to be an effective approach in evaluating both the fate of PCP in anaerobic digesters and the influence of PCP on normal anaerobic digestion.

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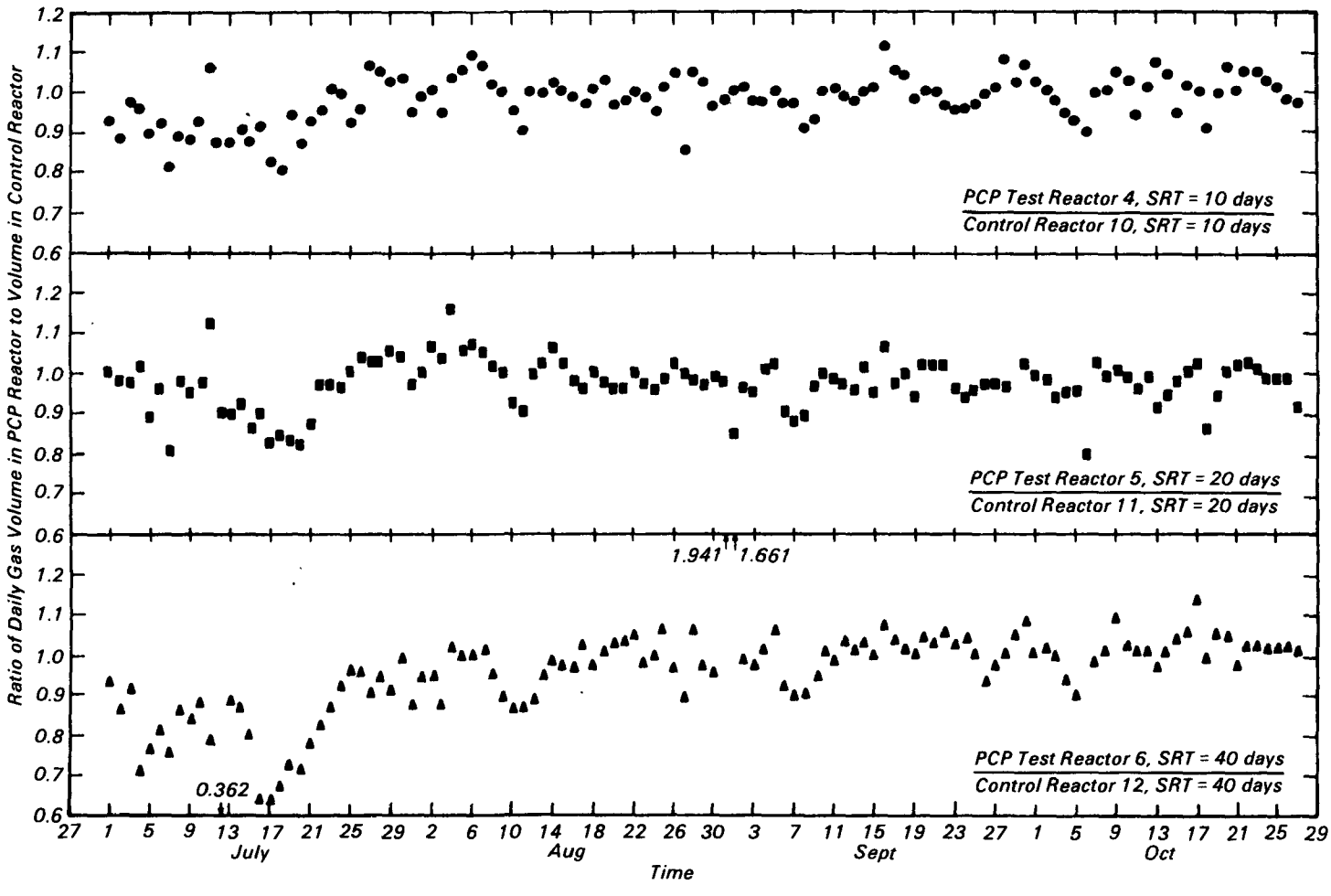

Figure 5. Gas production rate in reactors receiving PCP expressed as a fraction of the rate in the control reactors with the corresponding SRT.

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The complete report, entitled "Protocol Development for the Prediction of the Fate of Organic Priority Pollutants in Biological Wastewater Treatment Systems," (Order No. PB 86-135 654/AS; Cost: $16.95, subject to change) will be available only from:

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