Analytical Chemistry
Special Study 74-9
Job No. 1625006

BIODEGRADABILITY OF MONOCHLOROBIPHENYL (MCS 1547)

INTRODUCTION

Biodegradation testing on Aroclor 1221 demonstrated that the monochloro-biphenyl components were readily degraded. In order to provide a more complete picture, biodegradation testing was initiated on MCS 1547 (0.2% biphenyl, 92.5% o-chlorobiphenyl, 1.8% m-chlorobiphenyl, 5.1% p-chlorobiphenyl, and 0.2% dichlorobiphenyl). The type of tests employed were the semi-continuous activated sludge (SCAS) test which simulates secondary sewage treatment and the Thompson-Duthie CO_2 evolution test which measures the extent of ultimate biodegradation.

SUMMARY AND CONCLUSIONS

Primary biodegradation testing of a monochlorobiphenyl mixture (MCS 1547) at an addition rate of 50 mg per 24-hour cycle has been carried out over a nine-month period. The mean disappearance rate observed was greater than 98%. Volatility losses (less than 3%) were not a significant factor in the rate. Moderate inhibition of the normal sludge growth was observed.

The extent of ultimate biodegradation, i.e. conversion of the test material to carbon dioxide, water, inorganic salts, and products associated with normal bacterial metabolism, was determined for monochlorobiphenyl using the Thompson-Duthie CO_2 evolution procedure. At the conclusion of the 28-day test the carbon dioxide produced accounted for 84% of the theoretical carbon content of the monochlorobiphenyl. This level of carbon dioxide production is comparable to that achieved with the reference standard, dextrose.

From the standpoint of both primary and ultimate biodegradation, monochlorobiphenyl does not appear to present any significant problems.

RESULTS AND DISCUSSION

A. Semi-Continuous Activated Sludge Test

The semi-continuous activated sludge (SCAS) test procedure employed here is patterned after the Soap and Detergents Association's standard method /JAOCS 42, 986 (1965)/ using their modified feed /JAOCS 46, 432 (1969)/. A description of the test method, as applied to our work, is given in Analytical Chemistry Method 71-32.

Since previous SCAS data on Aroclor 1221 had indicated that monochlorobiphenyl components were readily biodegradable, a relatively high addition rate of 50 mg per 24-hour cycle was employed. The addition rate was raised to this level over a five-day period in 10 mg increments.

Primary degradation rate data for monochlorobiphenyl was obtained in the following manner. Fifty milliliter samples of the mixed liquor were withdrawn after feeding and at the end of the aeration cycle. The mixed liquor samples were extracted with spectrograde hexane in accord with Analytical Chemistry Method 71-18 and the amount of monochlorobiphenyl in the concentrated extracts determined using UV spectrophotometry (Analytical Chemistry Method 73-24). From the analytical data, the disappearance rate was calculated by the following equation:

$$\% \text{ Disappearance Rate} = \frac{C_o - C_n}{C_o} \times 100$$

where C_o = milligrams of monochlorobiphenyl in unit at beginning of aeration cycle after feeding of test material.

C_n = milligrams of monochlorobiphenyl in unit at end of cycle.

Sampling of the unit was generally carried out during one cycle per week.

To demonstrate the efficiency of the extraction procedure for recovering monochlorobiphenyl, blank mixed liquor samples (50 ml) were spiked in duplicate at the 100, 200, and 300 µg levels and carried through the extraction and analytical procedure. The mean percent recovery obtained was $82.4 \pm 4.5\%$.

In Figure 1 the total amount of monochlorobiphenyl present in the unit at the beginning and end of a cycle is plotted versus the elapsed time in weeks. The disappearance rate is similarly plotted in the same figure. These data indicate that even at the relatively high 50 mg addition rate monochlorobiphenyl degrades rapidly.

To determine the significance of volatility losses in the disappearance rate, the off-gases from the unit were washed with a train of three hexane scrubbers during a complete cycle. Analysis of the scrubber solution showed that the disappearance rate due to volatilization amounted to only 2.3 percent.

Weekly monitoring of the suspended solids concentration indicated that there was moderate inhibition of the normal sludge growth rate. This is not surprising at the relatively high addition rate used.

B. Thompson-Duthie Carbon Dioxide Evolution Test

The carbon dioxide evolved during the biodegradation of monochlorobiphenyl was measured using the apparatus and procedure developed by Thompson and Duthie [J. Water Pollution Contr. Fed. 40, 306 (1968)] and modified by Sturm [J. Am. Oil Chem. Soc. 50, 159 (1973)]. This test is based on the premise that in the utilization of an organic molecule as a carbon and energy source by aerobic bacteria, carbon dioxide, water, inorganic salts, and cellulose material are produced. By measuring the carbon dioxide produced and relating it to the theoretical yield based on the molecular structure and weight of the material, a parameter related to ultimate biodegradability is obtained.

The bacterial inoculum for the test was prepared using the Bench-Chambers die-away procedure [J. Water Pollut. Control Fed. 39, 181 (1967)]. A liter of effluent supernatant from the monochlorobiphenyl-acclimated SCAS unit was allowed to stand for 24 hours. An inoculum flask was prepared containing 50 mg of yeast extract, 100 ml of settled supernatant, 900 ml of BOD dilution water [Standard Method for the Examination of Water and Wastewater, 13th Edition, American Public Health Associates, New York, 1971], and 20 mg of monochlorobiphenyl. The flask was then stored in the dark under quiescent conditions for 14 days.

At the end of the 14-day period, 500 ml of inoculum solution were mixed with 5500 ml of BOD water in two carboys, one for a control and the other for monochlorobiphenyl. A weighed quantity of monochlorobiphenyl was then added to the appropriate carboy.

The carboys were then hooked up to a source of CO_2-free air. The effluent air (50 ml/min) from each carboy passed through a set of three CO_2 scrubbers, each containing 100 ml of 0.05N $Ba(OH)_2$. The evolved CO_2 was trapped as barium carbonate and quantitated by titration of the remaining $Ba(OH)_2$ with 0.1N HCl. During the first week, the CO_2 production was monitored on a daily basis. The intervals between analyses were lengthened as the CO_2 production declined. To compensate for CO_2 either present in solution at the beginning of the test or produced from residual organic material in the inoculum, CO_2 values obtained from the control carboy were subtracted from those obtained from the monochlorobiphenyl carboy.

In Table I the CO_2 evolution data after 28 days are summarized. It is apparent that the CO_2 evolved from the control amounted to less than 10 percent of that evolved from the monochlorobiphenyl carboy.

In Figure 1 the net cumulative CO_2 production expressed as a percent of the theoretical yield is plotted versus the elapsed time after initiation of the test. A representative curve obtained for dextrose in a previous test is shown. The CO_2 evolution for dextrose in a 28-day test will generally fall in the 80-95% range.

The data obtained thus indicates that both the rate and extent of ultimate biodegradation of monochlorobiphenyl do not differ greatly from those observed for dextrose.

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TABLE I

CO_2 EVOLUTION BIODEGRADABILITY DATA

	Control	Monochlorobiphenyl
Weight of monochlorobiphenyl, mg.	--	119.1
Percent carbon	--	76.40
Theoretical CO_2, mg.	--	333.4
Total CO_2 Evolved after 28 days, mg.	26.4	307.6
Net CO_2 Evolved, mg.	--	281.2
Percent of Theory	--	84.3

FIGURE 1

SCAS BIODEGRADATION DATA - MONOCHLOROBIPHENYL (MCS 1547)

50 Mg Feed Level

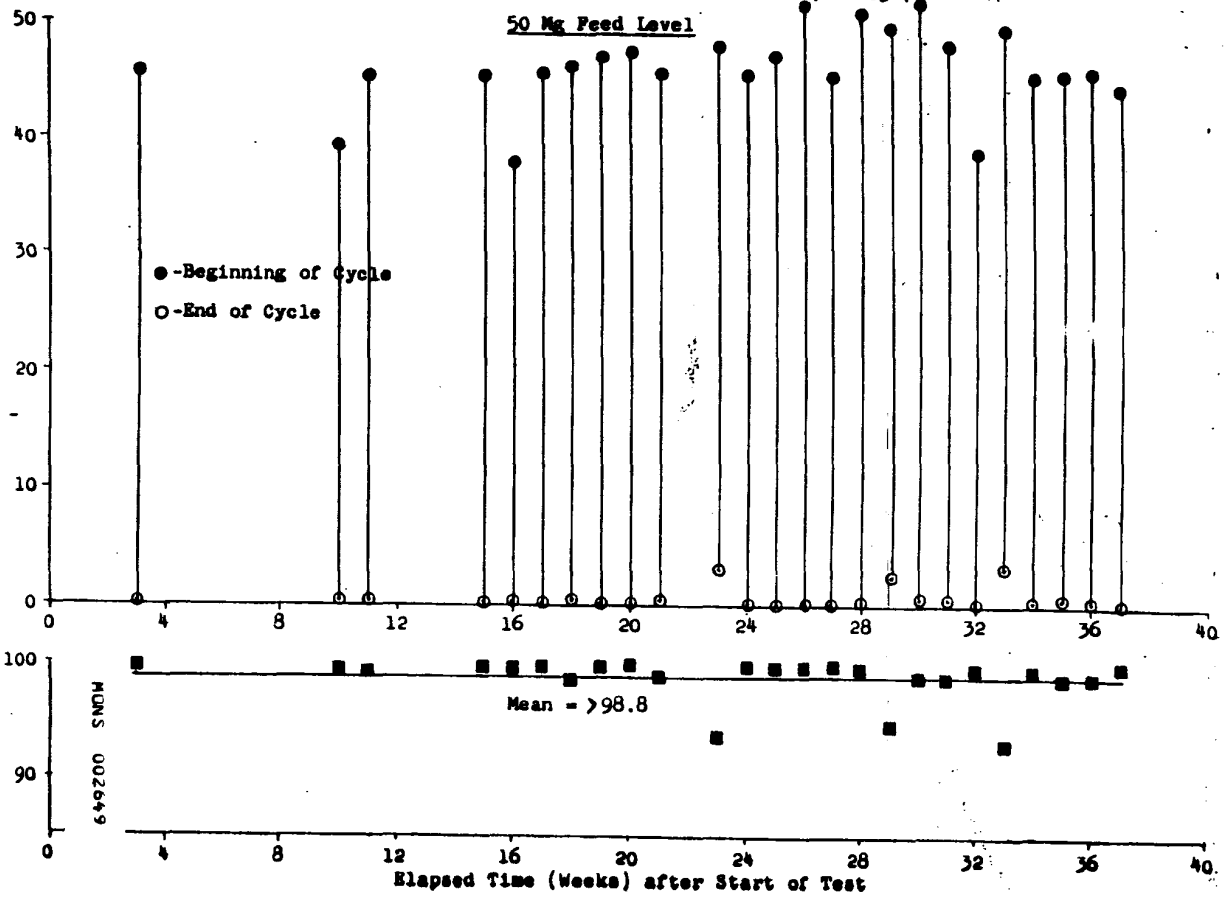

FIGURE 2

MONOCHLOROBIPHENYL (MCS 1547) CARBON DIOXIDE EVOLUTION

