BIODEGRADATION OF ^{14}C-PHENOL BY ACTIVATED SLUDGE

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Phenol, C_6H_5OH, is in widespread use as a raw material for manufacturing synthetic resins, medical, and industrial compounds. Various phenols (substituted hydroxy derivatives of benzene) are waste products of oil refineries, coke plants, some chemical plants, and human and animal refuse. The presence of such compounds in natural waters can affect fish. The threshold limit of toxicity for some fish is a few mg/l and at even lower concentrations fish can acquire an obnoxious taste. Phenolic materials can impart a bad taste to drinking water. "The removal of phenolic tastes from a water supply offers a serious challenge at the treatment plant" (1). McKee and Wolf (2) note that "despite the fact that it (phenol) is used as a bactericide in strong concentrations, weak phenol solutions are decomposed by bacterial and biological action in streams". Responsible manufacturing practice and good product stewardship cause management to increasingly call upon us to establish the ultimate fate of such compounds which may find their way into the environment. Contact with activated sludge from a waste treatment plant provides a rapid test of aerobic biodegradability.

The Dow Midland Division in its Phenolic Wastewater Treatment Plant commonly removes phenol from a waste stream at 80 ppm as judged by the 4-aminoantipyrene test (1). A trickling filter system takes the concentration down to about 30 ppm. Then, activated sludge reduces it to less than 1 ppm. Chemical oxygen demand (COD) and bio-chemical oxygen demand (BOD) and total oxygen demand (TOD (3)) data indicate that the phenol is consumed.

A C-14 radiotracer study has now been carried out to confirm these observations, to follow this decomposition more directly, and to establish a method useful for examining compounds of widely varying biodegradability. By radioassay, the fate of the ^{14}C-labeled phenol including the distribution of C-14 between evolved CO_2 and cell material has been determined after various contact times. Reaction rates under these conditions have been measured for unacclimated sludge and for specially acclimated sludge.

Methods

A laboratory system designed to contain the activated sludge culture plus the degradable substrate is shown in Figure 1. The oxidation cylinder is aerated through a fritted glass bottom with CO_2-free air at 60-100 ml per minute. Air from the cylinder is dried; the respired CO_2 passes through the infrared (IR) detector and into the scrubbers to be absorbed.

Return sludge (the settled solids at about 2% concentration) from the Dow Phenol Waste Treatment Plant for a preliminary trial or from the Dow General Waste Activated Sludge Plant (for other trials) was the starting culture. This was placed in a 2-liter cylinder fitted with an air sparger. The solids were settled, the free liquid decanted off, then an equal volume of fresh water was added. Experimental trials 1 and 2 were run on this sludge with only its inherent acclimation. Additional acclimation was given for a preliminary experiment and for Experiment 3 as follows: Phenol was added to a concentration of 100 ppm and the culture was aerated overnight. This procedure was repeated daily to provide a fresh supply of phenol to the sludge.

On the ninth day a test run was carried out in the oxidation cylinder. An IR CO_2 curve was obtained on the preliminary experiment. Measurements of phenol by the 4-AAP Method (1) on filtered, single distilled samples were made COD and TOD (1,3) were run on the filtered supernate. All these tests showed no phenol after the CO_2 peak.

For each run, sufficient settled return sludge to give a mixed liquor volatile solids (MLVS) concentration of approximately 3,000 mg/l was placed in the oxidation cylinder. Labeled phenol was added. Then water was added to bring the volume to 1.7 liters. The uniformly-labeled ^{14}C-phenol was obtained from New England Nuclear Corp. with a specific activity of 1.57 mCi/millimole. Its chemical purity by gas chromatography was 93.4% and by thin-layer chromatography 98.2%. For use, a working solution of 30.07 mg/ml in water was prepared. From this stock, 1.5 ml, 5 ml or 10 ml were added to the oxidation cylinder to obtain 27 ppm, 88 ppm and 177 ppm for Experiments 1, 2, and 3 respectively. A 200-ml sample was removed at the start to measure volatile solids and to do radioassays.

After the initial sampling, the oxidation cylinder contents was sampled at various time intervals over a 1 day period for ^{14}C-analysis. A hypodermic syringe (#17 needle) was used to withdraw 20 ml of sludge from a rubber tubing connection near the base of the cylinder. Formalin (1.0 ml) was added as a preservative. Respired CO_2 was collected in a mixture of 40% monoethanolamine in Dowanol® EM[a] ethylene glycol methyl ether. The total trap contents was removed as a sample and the absorbent replaced at each sampling time.

Radioassays were done by liquid scintillation counting using a Packard TriCarb[b] 3380 liquid scintillation spectrometer. Samples of trap contents were prepared by diluting a 3 ml aliquot in 15 ml of Econofluor[c] scintillation solution/methanol (60:90 v/v). Total sludge samples (0.2 ml) were combusted using a Harvey Biological Oxidizer, then counted. Supernate samples were obtained by centrifuging the sludge suspension for 2 minutes and removing a 0.2 ml aliquot. This was mixed with 10 ml Aquasol[c] water solubilizing liquid scintillation fluid and then counted. All ^{14}C quantities are calculated and reported as the equivalent amount of ^{14}C-phenol.

A test of the nature of the activity remaining in total sludge samples after a period of biodegradation was made. Samples of Experiment 2 at 2 and 8 hours were acidified and extracted twice with ethyl ether. Data from radioassay of this ether was converted to the equivalent ppm phenol that was extractable from the sludge. A water control was also run.

Results

The radioassay results from all the experiments are summarized in Table I. CO_2 given off by the respiration of the sludge system rose to a maximum in 1-2 hours after dosing with phenol as shown by the IR data and the corresponding respired $^{14}CO_2$ data in Figure 2. Some of the IR response is due to endogenous respiration of original non-radioactive cell material, whereas the $^{14}CO_2$ must all come from the ^{14}C-phenol.

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a Trademark of the Dow Chemical Company.
b Trademark of the Packard Instrument Co.
c Trademark of the New England Nuclear Corp.

In the unacclimated cases as shown in Figures 2 and 3 there is a lag time of about 0.5 to 1 hour before very much $^{14}CO_2$ is respired. There was only a 0.25 hour lag with the acclimated sludge system. Even during these initial lag times, however, the compound was being removed from solution and taken up by the solids. Compare Figures 3 and 4. After 6 hours less than 25% remained in solution. A major part of the $^{14}CO_2$ release occurred within 8 hours (39-45% of original compound released as $^{14}CO_2$). Figure 3.

The peak rates for the unacclimated runs were 0.95 and 2.04 mg phenol/g MLVS/hr for Experiments 1 and 2 respectively at 2.5 and 3.5 hours. The highest rate observed was 16.9 mg phenol/g MLVS/hr from the acclimated sludge in Experiment 3.

After 1 day of exposure the distribution of the initially supplied radioactivity was:

	Radioactivity Found %			
Experiment	In Solution	Sludge Solids*	CO_2	Recovery
preliminary	0.8%	29.5%	62.1%	92.4%
1	3.0	43.0	62.1	108.1
2	2.6	37.8	54.2	94.6
3 (8 hours)	17.6	30.6	38.6	86.8

The activity in the solids does not behave like phenol in solution. It is scarcely accessible to ether extraction. The extraction recoveries from 2 and 8 hour total sludge samples of Experiment 2 were only 44% and 0.2% (72% and 4.9% if from supernate alone). The water control gave a 98% extraction recovery of the phenol.

Discussion and Conclusions

The mode of disappearance of phenol during biodegradation by activated sludge seems to be that it is first absorbed into the cell mass, then metabolized with a major portion going to CO_2 and the rest to cell material.

The biodegradation built up to a rapid rate then decreased. It approached the original endogenous respiration rate of the activated sludge after 8-10 hours. The phenol itself was being removed from solution even before much $^{14}CO_2$ respiration had occurred. Most of the ^{14}C-material left in the solids and in solution after 8 hours was probably not phenol.

The distribution of the phenol's carbon atoms between CO_2 and solids as observed in this ^{14}C-phenol biodegradation is in general agreement with data from standard BOD tests. H. C. Alexander has previously measured a BOD_5 of phenol as 1.82 parts O_2 per part phenol. The theoretical oxygen demand of phenol is calculated from the following balanced reaction:

$$C_6H_5OH + 7\ O_2 \longrightarrow 6\ CO_2 + 3\ H_2O$$

$$\frac{224\ \text{M.W. } O_2 \text{ consumed}}{94.1\ \text{M.W. phenol}} = 2.38 \text{ parts } O_2\text{/part phenol}$$

The BOD_5, if considered as being due to phenol oxidation to CO_2, represents 76% of the phenol present, the remaining 24% going into new cell growth. As noted previously the ^{14}C-phenol after 1 day of biodegradation showed 54-62% respired CO_2 and 30-43% retained in solids.

* By difference: total sludge minus supernate.

Because of the relatively rapid biodegradation of the phenol, it was possible to observe the CO_2 release by both IR and by radioassay. The radiotracer technique, however, has much greater sensitivity so that it can be used to get data on compounds degrading over a month long period rather than several hours. This extends even further the experimental capability of this relatively rapid activated sludge biodegradation technique.

Summary

The ultimate biodegradation of phenol by activated sludge was confirmed by collecting and measuring $^{14}CO_2$ released from a ^{14}C-labeled phenol. Infrared detection confirmed that the evolved material was CO_2. In 1 day, 54-62% of the supplied compound was converted to $^{14}CO_2$, 30-43% was found in the sludge solids. Three percent or less remained in the solution. For unacclimated sludge a peak degradation (at about 3 hr.) proceeded at a rate of (1-2) mg ^{14}C-phenol per gram mixed liquor volatile solids per hour. An acclimated sludge reached a rate of 17 mg ^{14}C-phenol per gram mixed liquor volatile solids per hour. Lag times of 0.5 to 1 hour were evident for the unacclimated sludges but only 0.25 hour for an acclimated one. The ^{14}C-activity left in the sludge after 8 hours did not freely extract with ether as did known phenol in solution.

This procedure is a useful tool in evaluating the biodegradability of an organic compound. Where needed, sensitivity is available for observing biodegradation of compounds whose degradation rate is several thousand times slower than that of this phenol.

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TABLE I - RESULTS OF TRAP, SLUDGE AND SUPERNATE RADIOASSAYS

Experiment Number	Sampling Time, Hr	$^{14}CO_2$ Collected (as μg phenol)		^{14}C-Activity Concentration (as ppm phenol)	
		Trap 1	Trap 2	Total sludge Suspension	Supernate
Preliminary (100 ppm)	1.5	21,400			
	3.0	31,900			
	8.0	22,100			
	24.0	14,400		30.3	0.8
1 (26.5 ppm)	0			32.2	25.6
	0.5	18	.0	24.3	20.6
	1.0	151	-.1	22.9	13.5
	1.5	1612	-.1	19.5	5.2
	2.0	2228	.4	17.2	4.0
	3.0	4217	.6	14.8	3.4
	4.0	2047	.6	14.9	2.4
	6.0	3767	1.6	18.5	2.5
	8.0	2668	0.5	13.7	1.8
	22.0	7056	16.6	12.2	0.8
2 (88.4 ppm)	0			73.0	82.1
	0.5	169	0.2	64.3	74.6
	1.0	1672	0.4	64.1	64.6
	1.5	3444	0.5	66.2	55.4
	2.0	3991	0.4	65.2	50.0
	2.5	2998	0.5	70.5	47.1
	3.0	4017	0.7	61.4	38.2
	3.5	4558	1.9	64.4	29.9
	4.0	4207	0.8	55.6	24.0
	6.0	17332	6.8	48.9	2.8
	8.0	8759	8.5	43.7	2.8
	22.0	14148	21.8	35.7	2.3
3 (176.9 ppm)	0			87.8	147.4
	0.25	116	-0.5	79.2	122.0
	0.50	7174	-0.6	135.0	67.8
	0.75	17536	-0.5	87.2	84.1
	1.0	15275	-0.4	122.3	80.2
	1.25	7260	-0.5	124.6	75.2
	1.5	5159	-0.4	116.7	76.8
	1.75	3763	-0.3	108.8	64.4
	2.0	3343	2.1	120.1	69.3
	2.5	5212	36.4	109.5	58.9
	3.0	4838	-0.3	107.7	46.3
	4.0	4661	-0.1	93.5	44.2
	5.0	5126	-0.5	91.8	44.3
	6.0	5543	0.2	92.4	36.1
	8.0	8782	1.1	85.4	31.2

Figure 1

LABORATORY ACTIVATED SLUDGE BIODEGRADATION SYSTEM

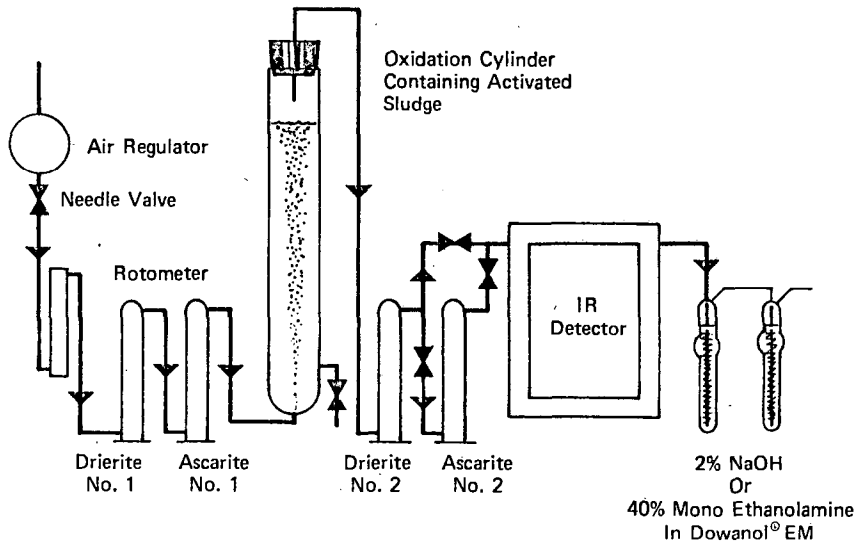


Figure 2

CO_2 AND $^{14}CO_2$ RESPIRED

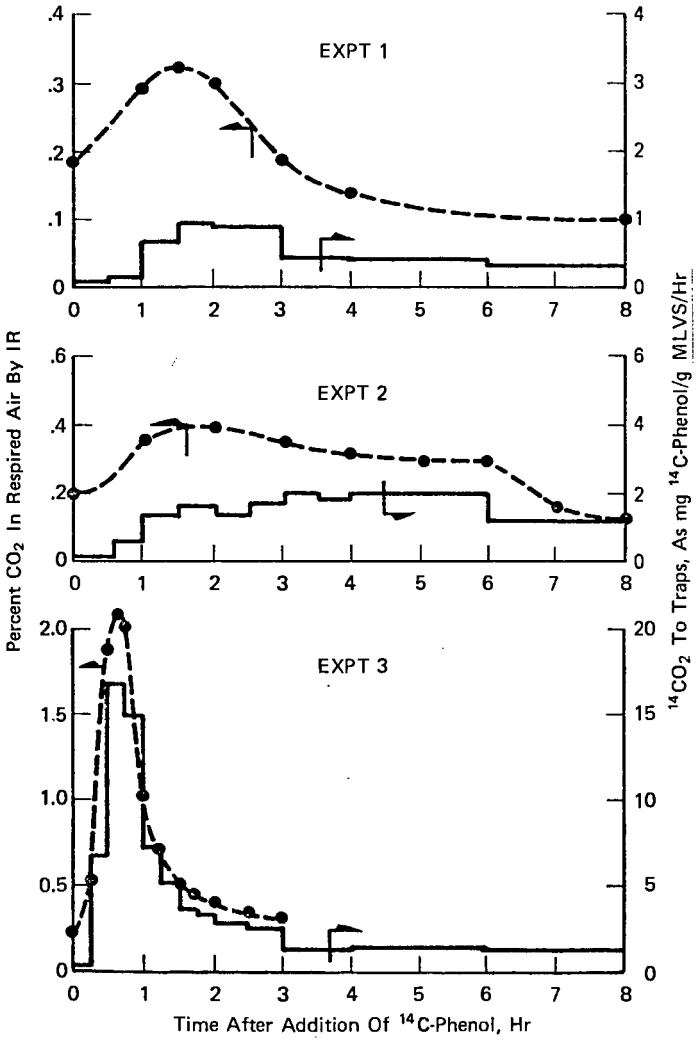


Figure 3

CUMULATIVE $^{14}CO_2$ COLLECTED IN TRAPS

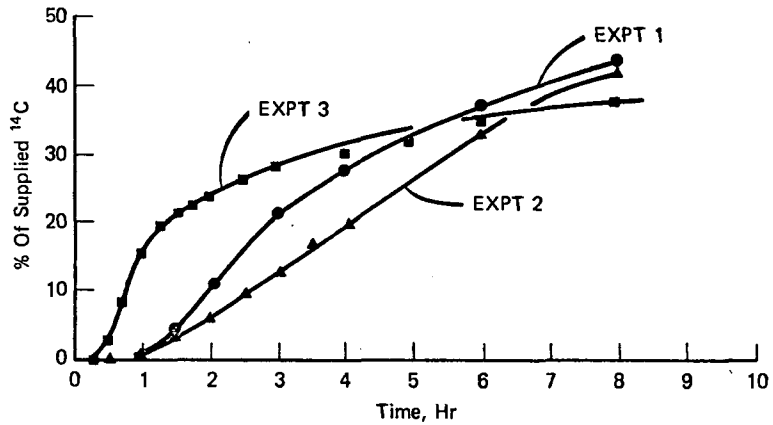
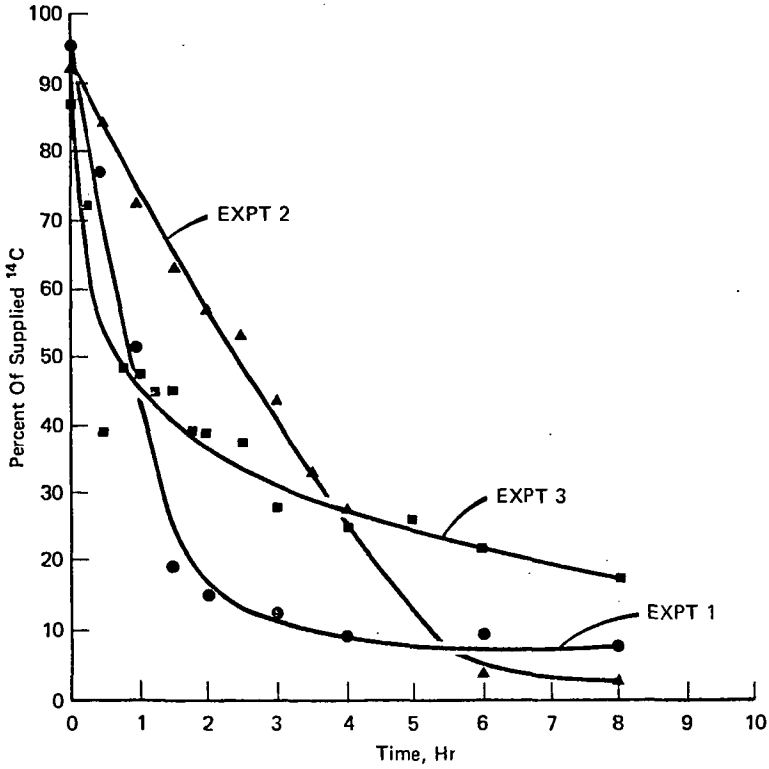


Figure 4

^{14}C-ACTIVITY REMAINING IN SUPERNATE