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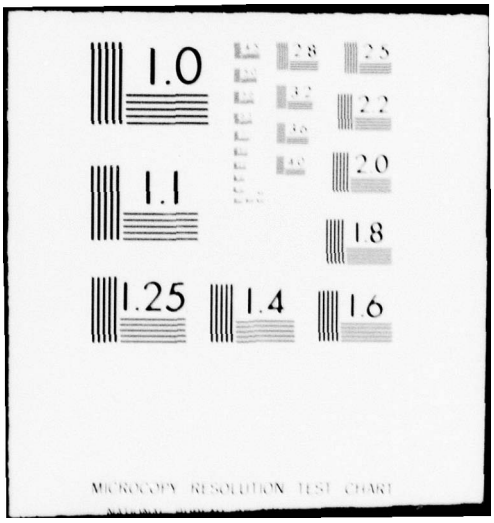
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ABSTRACT

Fifty-two bacteria isolated from sewage, temperate soil, and various tropical soils were tested for their ability to attack 2,4-D and 2,4,5-T. Fourteen caused the disappearance of 35 to 100% of the 2,4-D, and nine brought about the destruction of 20 to 100% of the 2,4,5-T. None of the organisms could use 2,4-D or 2,4,5-T as a sole source of carbon. Degradation of 2,4-D and phenoxyacetic acid in nonsterile sewage and a tropical soil was greatly enhanced by pretreating the sewage and soil with these compounds, suggesting the selection for organisms capable of attacking 2,4-D and phenoxyacetic acid. Cell yields of the three most active 2,4,5-T degraders in a medium with glucose, glycerol, and sodium succinate and in a benzoate-supplement medium with and without 2,4,5-T did not differ, suggesting cometabolic attack. Resting cell suspensions of nine of the isolates cleaved chlorine from the 2,4,5-T molecule while metabolizing more than 40% of the 2,4,5-T, suggesting ring cleavage of the herbicide. Eight isolates produced chlorinated phenol from 2,4,5-T. Studies of the respiratory activity of three isolates also suggested ring cleavage of 2,4,5-T. By use of (^{14}C-ring-UL) 2,4,5-T, it was found that the herbicide was readily metabolized in a tropical soil.

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ANNUAL REPORT

(6) # MICROBIAL DEGRADATION OF PESTICIDES.

by

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Ithaca, New York 14853

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INTRODUCTION

Phenoxy herbicides such as 2,4-D and 2,4,5-T are among the most commonly used herbicides for selective weed control and for defoliation. The metabolic fates of 2,4-D and 2,4,5-T are of obvious concern because of the potential toxicity of the herbicides and their metabolites to nontarget organisms. Although considerable work has been done on the persistence of 2,4-D in the soils characteristic of agricultural lands of continental United States, little attention has been given to the persistence and fate of this herbicide or 2,4,5-T in tropical soils of the Pacific Ocean area. The research in this report was designed to determine the persistence and fate of 2,4-D and the persistence, fate, and role of cometabolism in the biodegradation of 2,4,5-T in such soils.

MATERIALS AND METHODS

Materials. 2,4-Dichlorophenoxyacetic acid (2,4-D) and 2,4,5-trichlorophenoxyacetic acid (2,4,5-T) were obtained from Dow Chemical Co., Midland, Mich.; phenoxyacetic acid (PA.), 2,4-dichlorophenol (DCP), 2,4,5-trichlorophenol (TCP), and catechol from Eastman Organic Chemicals, Rochester, N.Y.; and phenol and sodium benzoate from Mallinckrodt Chemical Works, New York, N.Y. Uniformly ring-labeled [^{14}C] 2,4,5-T (sp act 1.61 mCi/mmol) was purchased from California Bionuclear Corp., Sun Valley, Calif. The purity of the ^{14}C-labeled compound was 98% as determined by thin-layer chromatography. Unlabeled 2,4,5-T and 2,4-D were purified by recrystallizing them twice in benzene. The purity of the compounds was greater than 99% as determined by thin-layer chromatography and melting point determinations. The compounds were prepared at 10,000 ppm in either 95% ethanol (Mallinckrodt) or as the sodium salt in distilled water.

Glassware. Glassware was cleaned by a 24-h immersion in 20% (vol/vol) HNO_3. Nitric acid was removed by multiple washings in tap water followed by distilled water.

Isolation of sewage and soil microorganisms. Bacteria capable of degrading 2,4-D and 2,4,5-T were isolated by the enrichment culture technique using the following sewage and soils at a concentration of 10% as initial sources of inocula: sewage collected at the primary effluent of the Ithaca, N.Y. sewage treatment facility (used within 30 min after collection), a temperate-zone soils mixture, Philippine soil (pH 6.8), Puerto Rico soil (pH 5.8), Nigerian soil (pH 5.9), and Trinidad soil (pH 6.1). The enrichment medium was an inorganic salts medium (3) and contained (per liter): $(NH_4)_2SO_4$, 0.5 g; KCl, 0.2 g; NaCl, 0.1 g; $CaCl_2.2H_2O$, 50 mg; $MgSO_4.7H_2O$, 0.2 g; $FeCl_3.6H_2O$, 20 mg; and buffered with 12 mM potassium phosphate buffer, pH 7.2. When used as the source of carbon, the compounds were added to final concentrations of 250 and 1000 ppm. When used as a substrate for cometabolism, the compounds were added to a final concentration of 100 ppm in the inorganic salts medium containing 0.3 g/liter each of glucose, glycerol, and sodium succinate (basal medium). The medium was sterilized by filtration through sterile 0.2 μm membrane filters (Millipore Corp., Bedford, Mass.). The enrichment cultures (10 ml total volume) were incubated statically in screw-cap tubes at 29°C. 2,4-D, 2,4,5-T and PA disappearance were determined by UV absorbance. Once significant loss and visible turbidity occurred, 1.0 ml of the enrichment culture was transferred to fresh medium. After two successive transfers, the enrichments were streaked on plates containing either the inorganic salts medium with 15 g/liter agar (Difco) and amended with 250 or 1000 ppm of the compound if used as carbon source or basal medium with 15 g/liter agar and amended with 100 ppm of the test compound. Isolates able to attack 2,4-D and 2,4,5-T were subsequently recognized by their ability to degrade the compound in liquid medium. Growth

curves and kinetics of 2,4,5-T disappearance were studied by growing selected organisms in basal medium amended with 50 ppm 2,4,5-T or mineral salts medium with 300 ppm 2,4,5-T at 29°C and 150 rpm. Periodically, portions were removed, and either the optical density determined or the samples were centrifuged at 10,000 X g at 4°C for 15 min and the supernatant fluid assayed for 2,4,5-T disappearance. Nonbiological disappearance of 2,4,5-T was assessed using sterile incubation medium.

Resting cell preparations. To prepare resting cells, cultures were grown in 1-liter Erlenmeyer flasks containing 500 ml of basal medium amended and unamended with 25 ppm 2,4,5-T and incubated at 29°C and 150 rpm for 36 h. The cells were harvested by centrifugation for 15 min at 10,000 X g at 4°C and washed three times with and resuspended in 10 ml of 10 mM phosphate buffer, pH 7.2 to an optical density of 1.5 at 420 nm. To 10 ml of the resting cell suspension was added 25 ppm 2,4,5-T, and the suspensions were incubated for 24 h at 29°C and 150 rpm. The reaction mixtures were centrifuged, and the supernatant fluid was used for the analytical and chemical procedures. Nonbiological degradation of 2,4,5-T was assessed with sterile medium.

Manometry. Standard manometric procedures were used (20). Each flask received either 0.33 ml of a solution with 1.1 μmoles of substrate as 2,4,5-T (sodium salt) in distilled water or inorganic salts medium with glucose or sodium benzoate as sole carbon source. The endogenous flask contained 0.33 ml of 10 mM phosphate buffer, pH 7.2, in the side arm. The main compartment contained 2.67 ml of cell suspension in the phosphate buffer, and the center well received 0.2 ml of 20% KOH. The cells were grown in the same medium as used for respiration studies.

Degradation of (^{14}C-ring-UL) 2,4,5-T. To determine the persistence and degradation of 2,4,5-T in soil, 25 and 75 μg 2,4,5-T (^{14}C-ring-UL)/g soil were added to 5.0 g of Philippine soil (pH 6.8, organic matter about 3%) in 50 ml

screw-cap Erlenmeyer flasks. The screw caps were modified to accommodate teflon-silicone discs (Pierce Chemical Co., Rockford, Ill.). The herbicide was dissolved in 95% ethanol, and the solvent was allowed to evaporate before mixing with the soil. The soil was wetted to 70% of field capacity with distilled water. One flask with each chemical concentration contained a sample of soil irradiated with a total dosage of 6 megarads; this dosage was sufficient to totally inactivate the soil microflora. Periodically, the soil was acidified with 10 N H_2SO_4, and air was passed into the soil for 90 min. The air passing out of each soil was bubbled through filter sticks (Ace Glass, Inc., Vineland, N.J.) into disposable scintillation vials (Kimble, Toledo, Ohio) containing 2.0 ml of carbon dioxide-trapping agent (CO_2 mMet, Amersham/Searle Corp., Arlington Heights, Ill.) and 13 ml of aqueous counting scintillant (ACS, Amersham/Searle Corp.).

Analytical methods. Turbidity was measured at 420 nm in a Bausch and Lomb spectrophotometer, model Spectronic 20. 2,4,5-T, 2,4-D, and PA disappearance were monitored by ultraviolet (UV) absorbance measurements at 292, 280, and 268 nm, respectively, in 1-cm quartz cuvettes in a Beckman grating spectrophotometer, model DB-G.

Chloride release was determined by the technique of Bergmann and Sanik (6).

Phenol production was determined by the method of Chrastil (10).

Catechol production was determined by the method of Arnow (4).

Carbon dioxide-^{14}C activity in the scintillation vials was determined by counting in a Beckman liquid scintillation counter, model LS-100C. All counts were corrected for quenching and background.

RESULTS

Isolation of strains. Enrichment cultures able to degrade 2,4-D and 2,4,5-T were isolated from sewage and soils. During the course of the enrichment studies, the persistence of 2,4-D, 2,4,5-T and phenoxyacetic acid (PA) was determined for sewage and Philippine soil. Sewage and soil was amended with 100 ppm of the compound, and the degradation was followed by a decrease in UV absorbancy. When compared to autoclaved sewage (control), greater than 90% of the 2,4-D and PA disappeared after 7 and 12 days, respectively; however, 2,4,5-T was not attacked after 60 days (Fig. 1). Subsequent additions of 2,4-D and PA to the sewage showed greater than 75% disappearance after 2 and 3 days, respectively, suggesting the selection for organisms capable of attacking the compounds. Similar results were obtained with Philippine soil, except that the time needed to observe 90% disappearance of 2,4-D and PA was 14 and 16 days, respectively, while 3 and 4 days were required for 75% disappearance of subsequently added 2,4-D and PA, respectively (Fig. 2).

To determine the number of isolates from each inoculum that could degrade 2,4-D and 2,4,5-T, the bacteria were grown in basal medium amended with 50 ppm 2,4-D or 2,4,5-T. Disappearance of the compounds was monitored periodically by recording the UV absorbancy of the culture's supernatant fluid, and these data were compared to the disappearance of the compounds in sterile medium (control). A summary of the study to show the number of isolates from each inoculum capable of degrading 2,4-D and 2,4,5-T is given in Table 1. Sewage and Philippine soil provided the most 2,4-D-metabolizing isolates, whereas the number of 2,4,5-T-metabolizing isolates, although highest from sewage, were fairly evenly distributed among the various soils. All enrichments yielded organisms capable of metabolizing 2,4-D and 2,4,5-T. Phenoxyacetic acid, NaB,

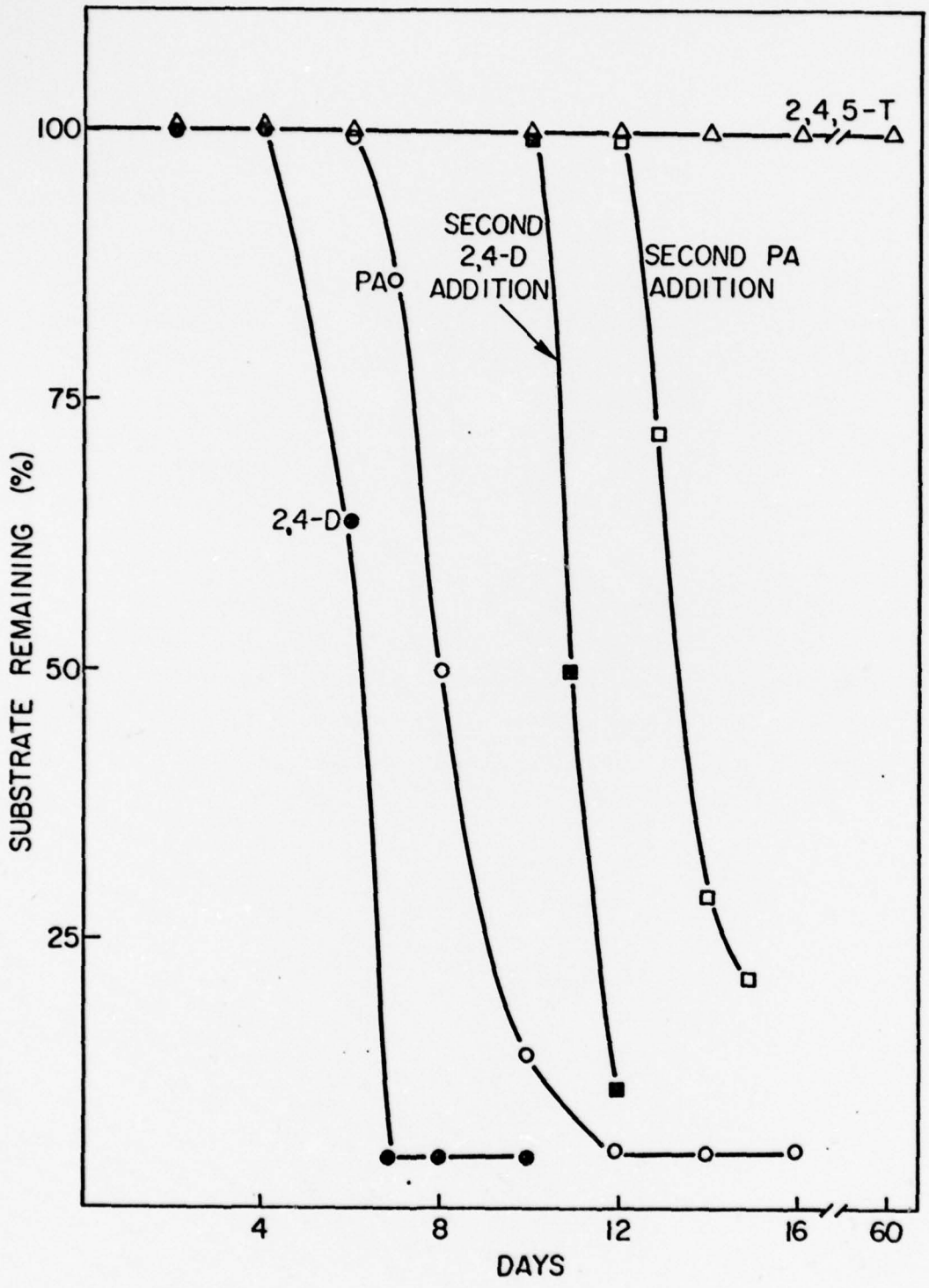

Fig. 1. Disappearance of 2,4-D, 2,4,5-T, and phenoxyacetic acid in sewage amended with 100 µg/ml of each compound.

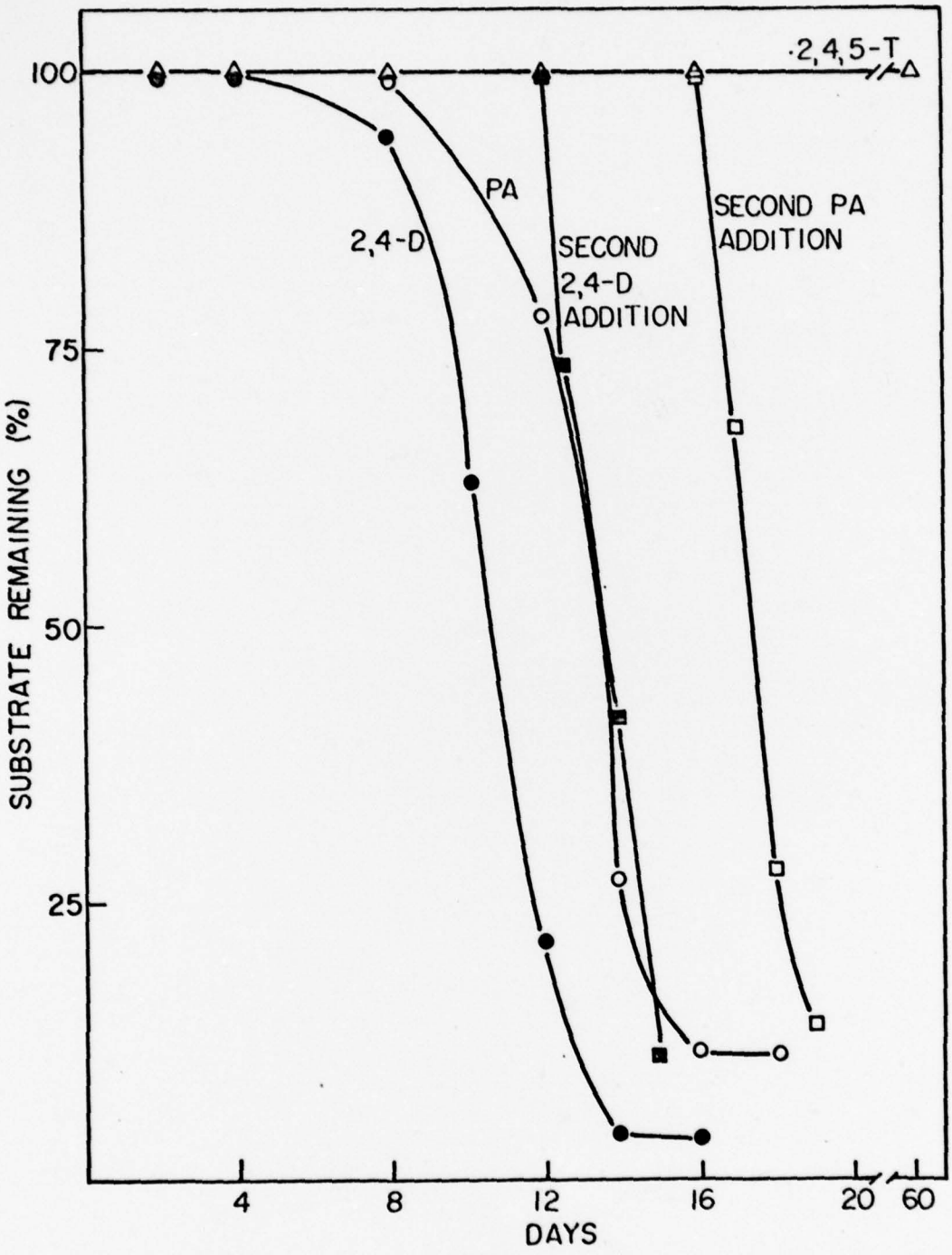

Fig. 2. Disappearance of 2,4-D, 2,4,5-T, and phenoxyacetic acid in Philippine soil amended with 100 µg/mg of each compound.

TABLE 1. Number of 2,4-D and 2,4,5-T-metabolizing bacteria isolated from sewage and soil

Enrichment substrate[a]	2,4-D						2,4,5-T					
		Soil						Soil				
	Sewage	Temperate	Philippine	Puerto Rico	Nigeria	Trinidad	Sewage	Temperate	Philippine	Puerto Rico	Nigeria	Trinidad
2,4-D[b]	25	1	3	1	0	0	1	1	1	1	0	0
2,4-D[c]	0	0	0	0	0	0	0	0	0	0	0	0
2,4,5-T[b]	4	1	2	0	0	0	2	1	1	1	1	1
2,4,5-T[c]	0	0	0	0	0	0	0	0	0	0	0	0
PA[b]	23	0	3	2	2	0	1	2	1	1	1	1
PA[c]	2	1	2	1	1	0	1	1	0	1	1	1
NaB[b]	3	1	3	1	1	1	1	0	2	1	0	1
NaB[c]	2	1	2	1	0	0	1	0	1	1	0	1
Phenol[b]	3	0	2	1	0	1	2	1	1	0	1	1
Phenol[c]	1	0	1	1	0	0	1	1	0	0	1	1
DCP[b]	2	1	1	0	1	1	0	0	0	0	0	0
DCP[c]	0	0	0	0	0	0	0	0	0	0	0	0
TCP[b]	1	1	1	1	0	0	1	1	1	0	1	1
TCP[c]	0	0	0	0	0	0	0	0	0	0	0	0

[a]Abbreviations: 2,4-D, 2,4-dichlorophenoxyacetic acid; 2,4,5-T, 2,4,5-trichlorophenoxyacetic acid; PA, phenoxyacetic acid; NaB, sodium benzoate; DCP, 2,4-dichlorophenol; TCP, 2,4,5-trichlorophenol.

[b]Used as substrate for cometabolism.

[c]Used as carbon source.

and phenol were used as a carbon source and as a substrate for cometabolism, whereas 2,4-D, 2,4,5-T, DCP, and TCP were used as a substrate for cometabolism only.

The isolates were studied further to ascertain their morphological and biochemical characteristics. The bacteria exhibited the following diversity: 88% were Gram-negative, 78% were rod-shaped, 58% were motile, 32% were pigmented and/or fluorescent, 68% were oxidase positive, and 94% were catalase positive (Table 2). A breakdown of the characteristics of the isolates by inoculum indicated that the inocula contained a high percentage of Gram-negative, rod-shaped, catalase-positive bacteria, while the remaining characteristics fluctuated from a low of 5% pigmented and/or fluorescent bacteria from the temperate soil to 88% oxidase-positive bacteria from the Philippine soil.

Metabolism of 2,4-D and 2,4,5-T. Nineteen isolates were capable of metabolizing 2,4-D and 2,4,5-T. Resting-cell suspensions of these bacteria were prepared, and 2,4,5-T disappearance, phenol and catechol production, and chloride release were determined. When compared to sterile controls, eight isolates caused the disappearance of greater than 50% of the 2,4,5-T. Extensive metabolism occurred with isolates 2A3 (88%), 4C3 (88%), and 5D3 (92%) (Table 3). Twelve isolates released chloride in the medium, with isolates 2A3, 4C3, and 5D3 liberating 90% or more. Eight isolates produced phenol from 2,4,5-T. No catechol was detected in the medium after a 36-h incubation period. The loss of UV absorbancy, release of chloride, and absence of phenol and catechol indicated that certain isolates were metabolizing 2,4,5-T by destroying the aromatic ring. The production of phenol with and without chloride release suggested that some of the isolates were converting 2,4,5-T to 2,4,5-trichlorophenol and mono- and dichlorophenol.

TABLE 2. Morphological and biochemical characteristics of bacteria isolated from sewage and soil

Source	Percentage of isolates showing characteristic					
	Gram negative	Rod shaped	Motility	Pigmented, fluorescent	Oxidase positive	Catalase positive
Sewage	90	95	69	50	87	100
Temperate soil	92	60	60	5	40	97
Philippine soil	88	86	71	43	88	91
Puerto Rico soil	73	76	49	28	67	100
Nigeria soil	91	75	50	29	57	85
Trinidad soil	94	76	49	37	69	91
Mean	88	78	58	32	68	94

TABLE 3. Metabolism of 2,4,5-T, release of chloride, and production of phenol by resting-cell suspensions

Bacterium	2,4,5-T lost[a] (%)	Chloride released[b] (%)	Phenol / 2,4,5-T (as %)
1A1	0	24	60
2A1	8	0	60
2A2	0	40	50
2A3	88	94	0
4A3	0	0	35
4A5	44	24	0
5A5	56	0	0
1B7	44	40	0
6B2	24	0	10
2C1	52	0	0
2C2	0	0	75
2C4	64	30	0
4C1	44	50	0
4C3	88	90	0
4C5	8	0	30
4D3	4	24	25
5D3	92	90	0
3E3	72	40	0
5F4	68	50	0

[a] Initial concentration: 25 µg/ml.

[b] Concentration of chloride in 25 µg/ml 2,4,5-T: 10.5 µg/ml.

The three most active 2,4,5-T-degrading isolates (2A3, 4C3, and 5D3) were grown in basal medium amended with 50 ppm of 2,4,5-T or inorganic salts medium amended with 300 ppm 2,4,5-T. Control flasks containing basal medium were incubated with the isolate. Growth, measured by optical density, was correlated with 2,4,5-T disappearance as measured by loss of UV absorbancy. Regardless of the presence or absence of 2,4,5-T in the basal medium, isolate 2A3 demonstrated a sigmoidal growth curve with maximal growth after 30 h (Fig. 3). Disappearance of 2,4,5-T started about 8 h after inoculation, and after 90 h, greater than 90% of the herbicide had disappeared. Sterile controls had less than 2% disappearance after 90 h. No growth occurred in the inorganic salts medium after 90 h. The inability of 2,4,5-T to serve as sole carbon source, the disappearance of the compound in the presence of an external carbon source, and the lack of significant differences in growth between amended and unamended basal medium indicated that the loss of the herbicide resulted from cometabolism. Cometabolism of 2,4,5-T was also indicated for isolates 4C3 (Fig. 4) and 5D3 (Fig. 5).

The rate and extent of O_2 uptake was measured with resting-cell suspensions of 2A3, 4C3, and 5D3 prepared from cultures grown on glucose-inorganic salts medium with and without 2,4,5-T (30 ppm) or inorganic salts medium with sodium benzoate as sole carbon source. The oxygen consumption values are the means of two replicates corrected for endogenous respiration (less than 3 μmol of O_2 in 3 h) and are determined for sodium benzoate or glucose as sole carbon source and 2,4,5-T in the glucose-inorganic salts medium.

The oxidation of 2,4,5-T, glucose and sodium benzoate is presented in Fig. 6. Oxygen consumption was high for sodium benzoate (5 μmol of O_2/μmol of sodium benzoate). Glucose was completely oxidized (6 μmol of O_2/μmol of glucose) by 2A3. Although O_2 consumption for 2,4,5-T was negligible by 2A3 when grown

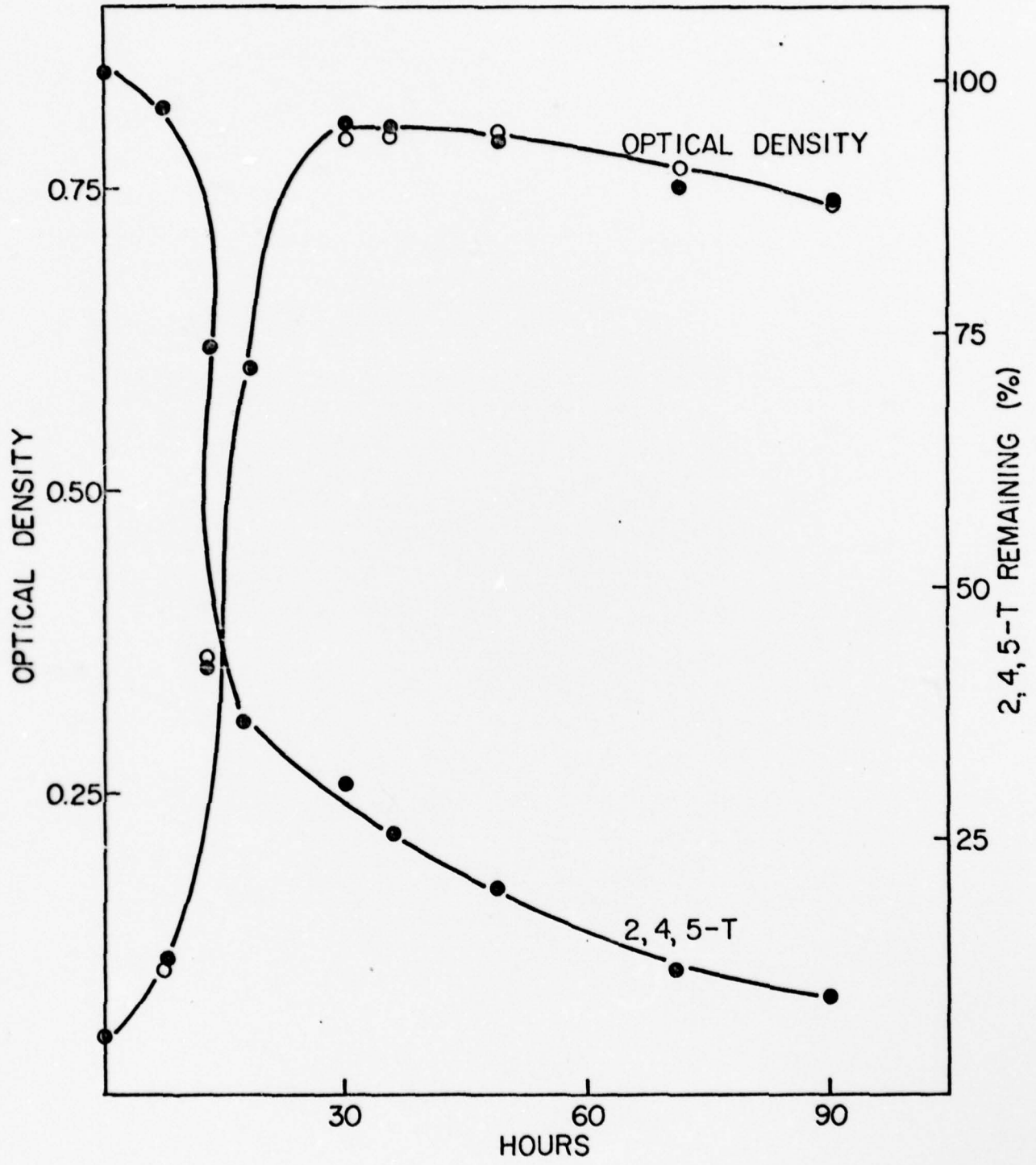

Fig. 3. Growth of isolate 2A3 in the presence (closed circles) and absence (open circles) of 50 µg 2,4,5-T/ml and 2,4,5-T disappearance.

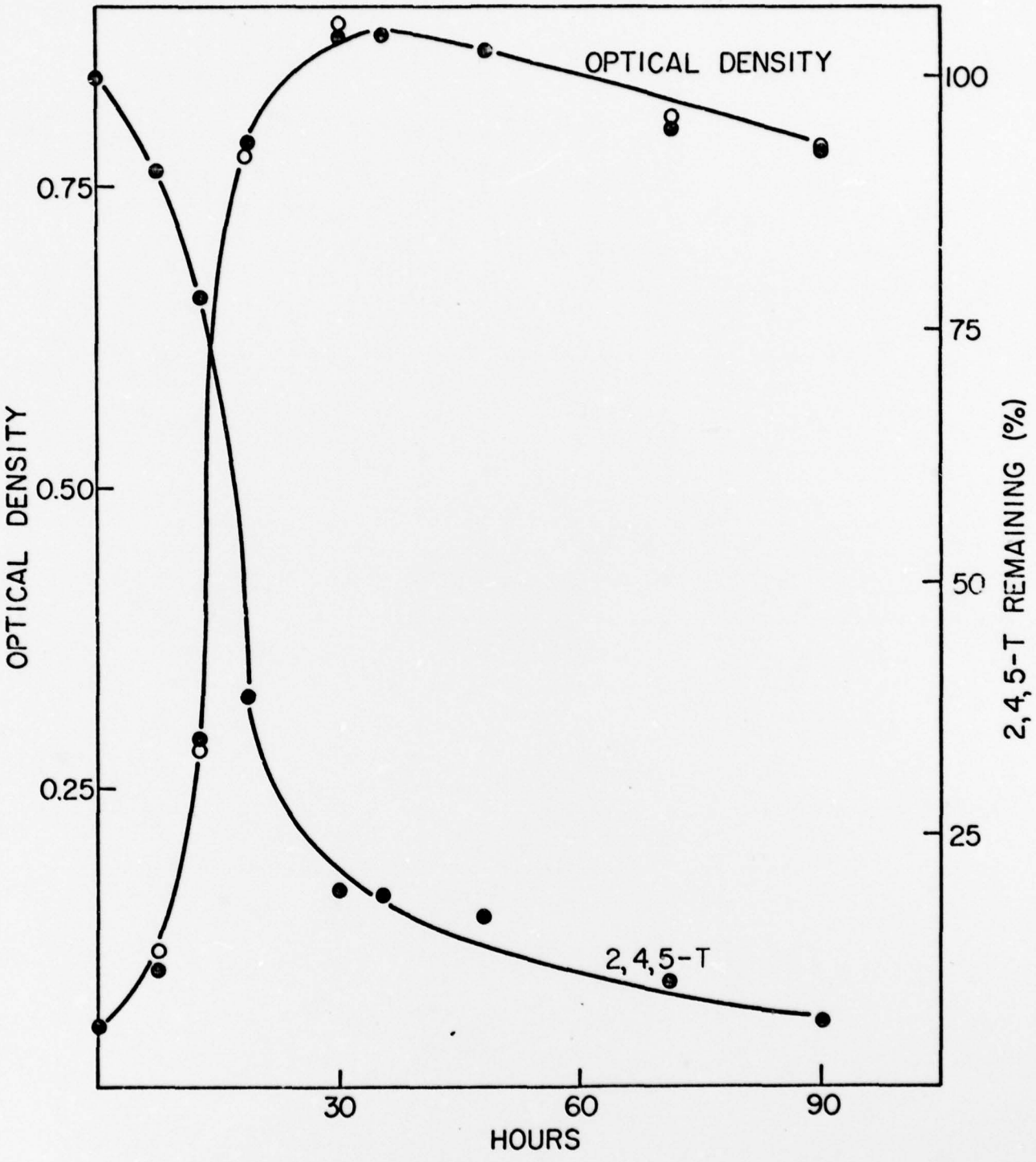

Fig. 4. Growth of isolate 4C3 in the presence (closed circles) and absence (open circles) of 50 µg 2,4,5-T/ml and 2,4,5-T disappearance.

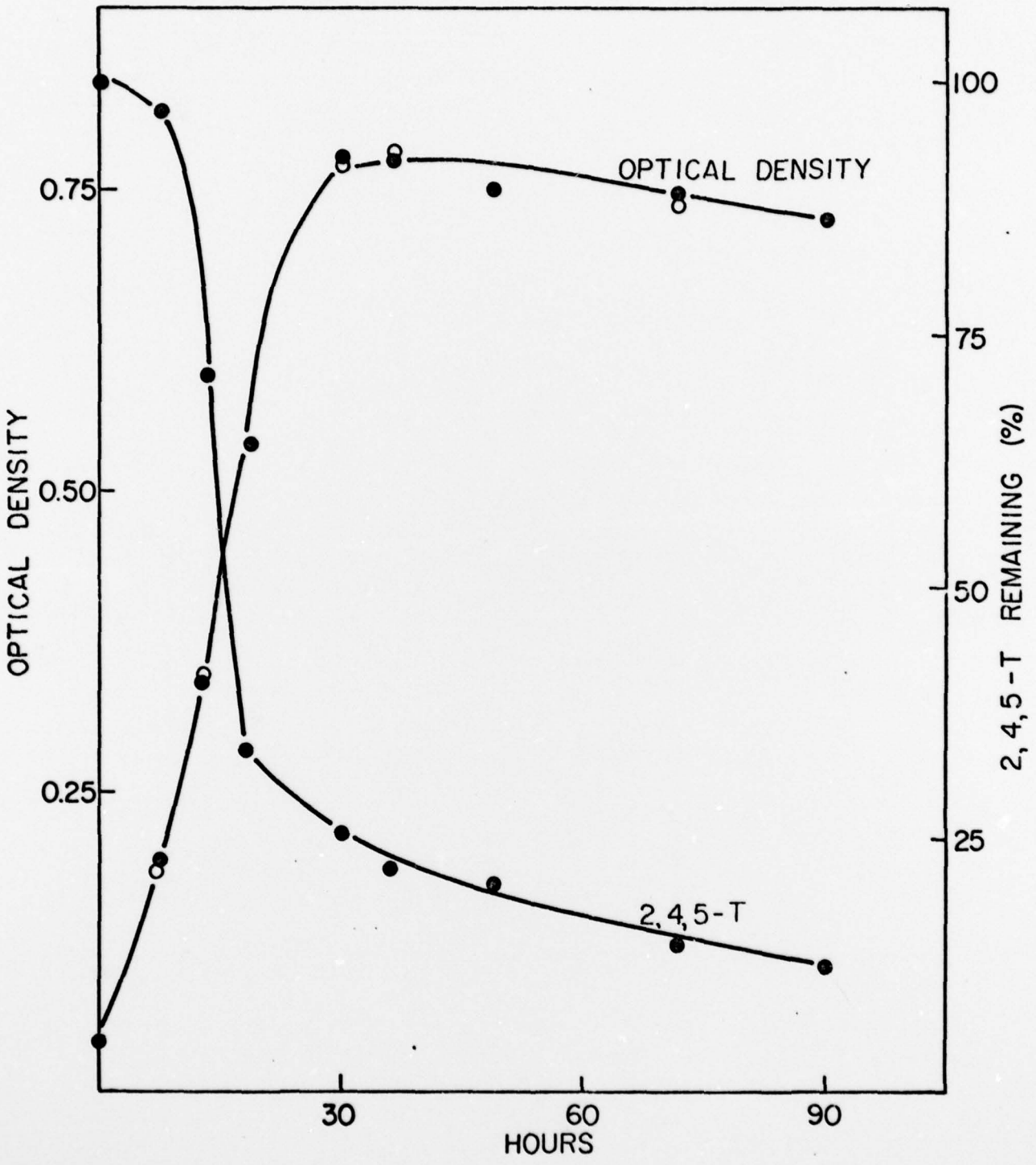

Fig. 5. Growth of isolate 5D3 in the presence (closed circles) and absence (open circles) of 50 µg 2,4,5-T/ml and 2,4,5-T disappearance.

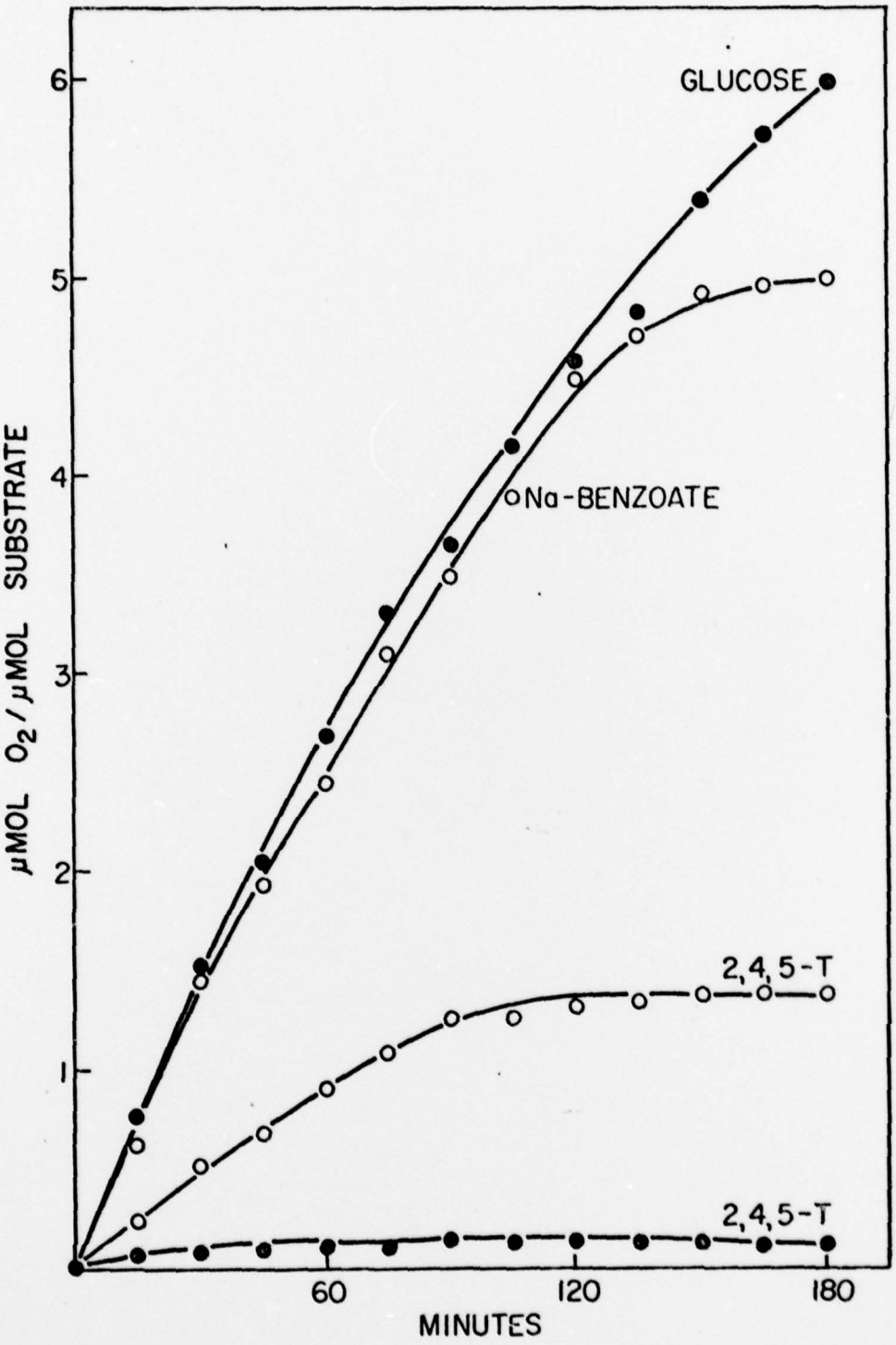

Fig. 6. Metabolism of glucose, sodium benzoate, and 2,4,5-T by resting cells of 2A3 grown in 0.5% glucose (closed circles) or 0.5% sodium benzoate (open circles) inorganic salts broth.

with glucose, the cells consumed about 1.7 μmol of O_2/μmol of 2,4,5-T when grown with sodium benzoate, suggesting a substrate-analog stimulation of respiration. The amount of O_2 consumed was about 25% of the theoretical amount needed to oxidize the 2,4,5-T completely.

Resting cells of 4C3 were prepared from cultures grown in glucose-inorganic salts medium with and without 2,4,5-T. The cells completely oxidized glucose (Fig. 7). Oxygen consumption in the presence of 2,4,5-T by cells not grown in the presence of 2,4,5-T was 2 μmol of O_2/μmol of substrate. However, cells exposed to 2,4,5-T in the growth medium consumed nearly 4 μmol of O_2/μmol of 2,4,5-T, suggesting that the presence of 2,4,5-T stimulated metabolism and ring cleavage of the herbicide.

Resting cells of isolate 5D3 were prepared similar to 4C3, and these cells also oxidized all the glucose (Fig. 8). Cells not exposed to 2,4,5-T in the growth medium consumed nearly 2 μmol of O_2/μmol of 2,4,5-T while "pre-exposed" cells consumed 3 μmol of O_2/μmol of substrate. The enhanced O_2 consumption (about 40% of the theoretical amount for complete oxidation of 2,4,5-T) suggests, similar to 4C3, that the presence of the herbicide during growth stimulated subsequent 2,4,5-T oxidation.

To determine further the persistence and degradation of 2,4,5-T in soil, the Philippine soil was amended with 5 and 15 ppm of 2,4,5-T (^{14}C-ring-UL). The $^{14}CO_2$ evolved was measured periodically by acidifying the soil and trapping the $^{14}CO_2$ in a trapping solution. Compared to gamma-irradiated controls, $^{14}CO_2$ evolution was detected in the soil with 5 ppm 2,4,5-T (Table 4). The amount of $^{14}CO_2$ detected was 16% of the initial radioactivity added after 2 weeks and 23% after 3 weeks. No significant $^{14}CO_2$ evolution occurred in the soil with 15 ppm 2,4,5-T-^{14}C in a 3-week period. Evolution of $^{14}CO_2$ from the soil provided additional evidence of ring cleavage.

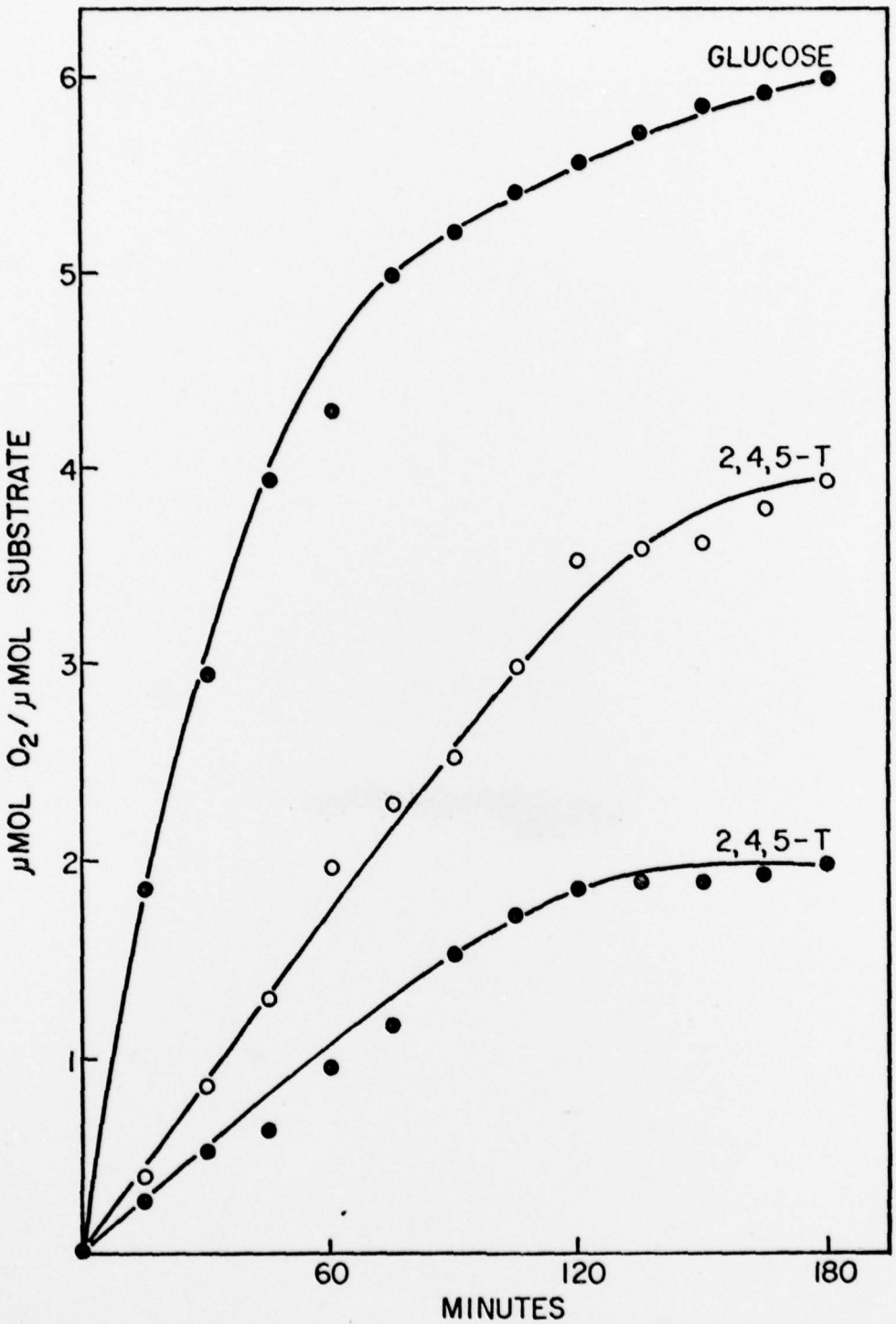

Fig. 7. Metabolism of glucose and 2,4,5-T by resting cells of 4C3 grown in 0.5% glucose-inorganic salts broth (closed circles) and broth amended with 30 µg 2,4,5-T/ml (open circles).

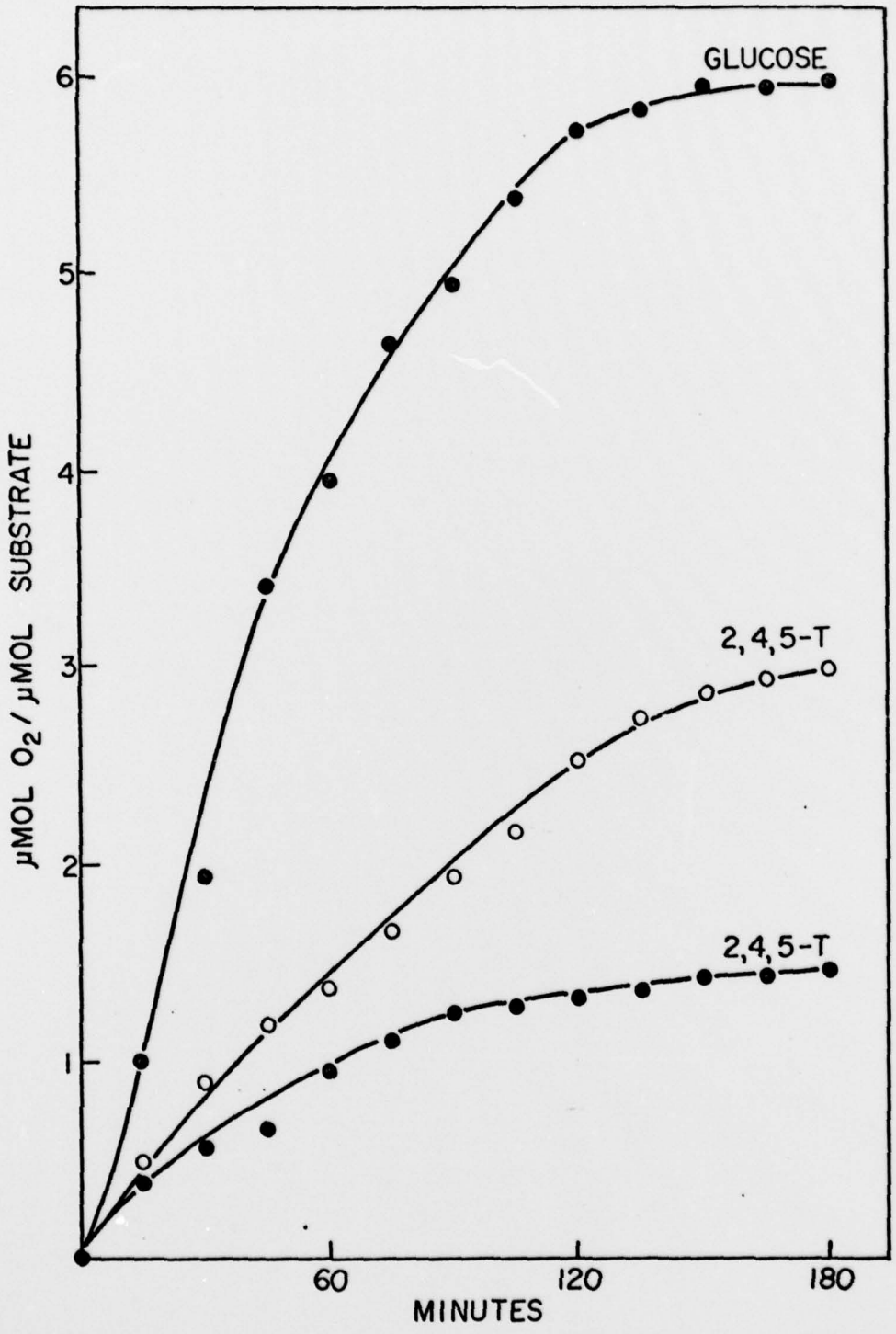

Fig. 8. Metabolism of glucose and 2,4,5-T by resting cells of 5D3 grown in 0.5% glucose-inorganic salts broth (closed circles) and broth amended with 30 µg 2,4,5-T/ml (open circles).

TABLE 4. Degradation of 2,4,5-T(^{14}C-ring-UL) by Philippine soil

Weeks	Degradation (%)[a]	
	5 ppm	15 ppm
1	0	0
2	16.3	< 1.0
3	22.6	1.8

[a] Measured as % of 2,4,5-T(^{14}C-ring-UL) evolved as $^{14}CO_2$.

DISCUSSION

Enrichment-culture techniques using 2,4-D, 2,4,5-T, and analogues indicated that the inocula from natural ecosystems contained many bacteria capable of destroying 2,4-D and 2,4,5-T but only by cometabolism. Studies with resting cell suspensions of the bacteria showed that 74% of the isolates destroyed from 8 to 92% of the 2,4,5-T in the medium, 63% released chloride from 2,4,5-T, and 42% produced phenol. In addition, 64% of the bacteria that degraded 2,4,5-T also released chloride. The liberation of chloride with loss of UV absorbancy indicated ring cleavage of the herbicide. Further, the data from manometric studies suggested ring cleavage of 2,4,5-T by two "induced" bacterial isolates, 4C3 and 5D3. The cleavage of the herbicide agrees with the findings of Ou and Sikka (17), who found extensive degradation of the aromatic ring of a structurally similar molecule, 2-(2,4,5-trichlorophenoxy)propionic acid by aquatic bacteria. In contrast, other workers (2,9) have reported that phenoxy herbicides with a chlorine in the meta position of the aromatic ring were resistant to microbial degradation in soil. The failure to show microbial degradation of these chemicals may have resulted from soil inocula in which the appropriate microorganisms were either absent or not present in sufficient numbers to produce degradation of the compound in the time of the experiment.

In addition to organisms degrading 2,4,5-T, bacteria were also isolated that could produce a phenolic compound from the herbicide. In some cases, phenol production was accompanied by chloride release, suggesting the conversion of 2,4,5-T to the mono- or dichlorophenol. In other instances, phenol was produced without a loss of UV absorbancy and chloride release, suggesting the production of 2,4,5-trichlorophenol. Sharpee (18) showed also the production of 2,4,5-trichlorophenol from 2,4,5-T. The appearance of the chlorinated phenols in the culture medium suggests that 2,4,5-T degradation proceeds via the cleavage

of the acetate moiety as described for 2,4-D degradation (1,7,8,15,19). If the trichlorophenol is dehalogenated, then the molecule may be decomposed by the pathways described for 2,4-D degradation (18,19). The pathway of degradation of the trichlorophenol, however, remains unknown (1). Alternately, 2,4,5-T may be converted to a hydroxylated, dehalogenated organic product such as 3,5-dichlorocatechol as described by Horvath (12). If so, then the catechol may be decomposed as described for 2,4-D since the catechol is also produced during the bacterial degradation of this herbicide (14,18,19).

The data from the enrichment cultures indicated that 100 ppm of 2,4,5-T persisted in sewage and Philippine soil for 60 days. However, a subsequent experiment using a larger sample of Philippine soil amended with only 5 ppm 2,4,5-T (^{14}C-ring-UL) indicated ring cleavage and evolution of $^{14}CO_2$ within 2 weeks. Inasmuch as 2,4,5-T does indeed disappear from soil (1,11) and evidence exists that microorganisms are involved (12), one might expect that microorganisms could be obtained which use it as a source of carbon and energy. However, such an organism had yet to be found. In this study, the three most active bacteria attacked 2,4,5-T by cometabolism. Cometabolism is the metabolism by a microorganism of a compound that will not supply that organism with energy or an essential nutrient. The species thus does not replicate at the expense of the compound; hence, should the initial cell number be small, there will be no increase in cells with the requisite enzymes so that the rate of decomposition will remain low and also will not show the typical increase with time that is characteristic of substrates supporting growth (13). This long persistence coinciding with an apparent microbial transformation (5) is typical of the behavior of 2,4,5-T in soil. The persistence, however, of 2,4-D and 2,4,5-T is dependent on many factors, not the least of which is abundance of 2,4-D and 2,4,5-T-metabolizing bacteria (1,14). Thus, a small soil sample may

not contain the bacteria in sufficient number to degrade 2,4-D and especially 2,4,5-T within the test period. A dependency of 2,4-D and 2,4,5-T metabolism on size of soil samples has been shown (16,21).

Future research will be directed to (a) determining the fate and persistence of 2,4,5-T in Pacific Island soils utilizing gas-liquid chromatography and ^{14}C-tagged herbicide, (b) determining the role of cometabolism in the biodegradation of 2,4,5-T and seeking means to enhance the process, and (c) assessing the effect of selected pesticides on the function of microbial communities of sewage.

CREDIT

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