Biodegradation of the Phthalates and Their Esters by Bacteria

by Paul Keyser,* Basayya G. Pujar, † Richard W. Eaton,* and Douglas W. Ribbons*

Recent studies on the biodegradation of phthalate esters in natural ecosystems, sewage, and laboratory cultures are reviewed. There is ample evidence to demonstrate that bacteria are major elements in the biodegradative processes and that in most situations complete oxidation of the aromatic ring occurs; much less is known about the catabolism of the alcoholic moiety, e.g., 2-ethylhexanol. Evidence is presented to support catabolic pathways in pseudomonads and micrococci that are initiated by successive hydrolyses of the diesters to give the phthalate anion. Thereafter a dioxygenase catalyzes the formation of 4,5-dihydro-4,5-dihydroxyphthalate, which is oxidized by an NAD-dependent dehydrogenase to give 4,5-dihydroxyphthalate. Protocatechuate, formed by decarboxylation of 4,5-dihydroxyphthalate, is the substrate for ring cleavage enzymes. Whereas fluorescent pseudomonads use the β-ketoadipate pathway, the nonfluorescent strains and micrococci examined use a meta-cleavage (4,5-) route. All the intermediates proposed have been accumulated by enzymes purified from Pseudomonas fluorescens. Isophthalate and terephthalate (anions) are readily used as carbon sources by aerobic bacteria, and preliminary evidence is consistent with catabolic routes for these isomers converging at the ring-cleavage substrate protocatechuate. Some possible effects and interactions of synthetic organic chemicals with the natural microflora, and the influence of other vectors, is discussed in relation to the maintenance of the carbon cycle and environmental pollution.

Phthalic acid esters (PAEs) have been synthesized on a massive scale for the last two to three decades, mainly for the formulation of plastics. In 1972 production was in excess of 10⁶ lb/yr (1). A conference, sponsored by the National Institute of Environmental Health Sciences four years ago, focused attention on the accumulation of phthalic acid esters in the environment, and how this might be potentially disadvantageous for the normal development of many life forms, including man. The papers presented were collectively published (1) and two excellent commentary reviews of the meeting appeared in Science (2) and Chemical and Engineering News (3). A statement made by Marx (2) attracted our attention: "A few scientists apparently believe that the phthalates are relatively biodegradable . . . but most consider that the evidence for biodegradation is still inconclusive." Examination of the evidence for the biodegradation of di-2-ethylhexyl phthalate and butyl benzyl phthalate by activated sludge, reported by Graham (4), however, led us to concur with the minority opinion, namely, that these esters are biodegradable. Our position was influenced by the knowledge that o-phthalate (anion) is readily used as sole source of carbon and energy by fluorescent (5) and nonfluorescent (6) pseudomonads, as were the anions of the two isomers, isophthalate and terephthalate (Sherratt and Ribbons, unpublished observations). The isolation and study of the bac-
teria that grew at the expense of o-phthalate led
to the formulation of catabolic pathways that had
common intermediates, 4,5-dihydroxyphthalate
and protocatechuic acid, but which diverged when
3,4- or 4,5-dioxigenases catalyzed the opening
of the benzene ring (Fig. 1). Discovery of the 4,5-
dioxigenative cleavage of protocatechuic acid was
largely due to the studies of Dagley and his
colleagues with a nonfluorescent pseudomonad
(7-9) now known to be Pseudomonas testosteroni
(10), that grows with p-cresol, and a similar
species that was isolated with o-phthalate as sole
carbon source (6,9). Since we knew that o-
phthalate and its two isomers were readily
metabolized by bacteria which are easily isolated
from soils and water, we expected that phthalate
esters would also be readily metabolized, since
esterases frequently possess wide substrate
specificities (11). Indeed, observations made twenty
years ago during studies of biodeterioration of
plastics showed that several plasticizers (includ-
ing phthalate esters) were subject to microbial
degradation (12-14). This topic has
been reviewed by Autian (15) and Mathur (16);
the latter author also observed phthalate ester
utilization by Serratia marcescens.

During the last three years, a number of re-
ports on the biodegradation of phthalate esters have
appeared. These have conclusively shown that
phthalate esters are easily biodegradable in
the laboratory with pure cultures of bacteria and
in several environments with their natural com-
munities of microflora. Most of the studies re-
ported do not allow the conclusion to be drawn

that the entire molecule is mineralized (or
asimilated in the various experimental situations.
This point was clearly realized by Engelhardt,
Wallnofer, and Hutzinger (17), who demonstrated
that either the esterifying alcohol or the aromatic
moieties of phthalate esters could support the
growth of different bacteria or fungi. Several
coryneforms, brevibacteria, and pseudomonads
isolated with o-phthalate (anion), grew with
dibutyl phthalate. Evidence was presented to
show that monobutyl phthalate, phthalate and
protocatechuic acid were intermediates (Fig. 1).
They also made the interesting observation that
pure cultures from dibutyl phthalate (DBP)
enrichments formed mono-n-butylphthalate
almost quantitatively as the only metabolite,
suggesting that the butanol hydrolyzed from one
of the ester groups served as carbon and energy
source for growth. That a single hydrolysis may
be a biologically important event, is supported by
results of studies on the metabolism of phthalate
esters by rats. The major metabolites observed
by Albro and his collaborators (18,19) after ad-
novation of di-2-ethylhexyl phthalate (DEHP)
were oxidized derivatives of the monoester; free
phthalic acid accounted for less than 3% of the
metabolites in the urine. The accumulation of
monesters of the phthalates has also been ob-
served in hydrosols. Johnson and Lulves (20)
showed that two of the esters, DEHP and DBP,
most frequently used, were readily degraded in
hydrosols, but that anaerobic conditions retar-
ded these degradative processes. Of particular
interest in the light of the ease of hydrolysis to give
monoesters was the observation that after
aerobic incubations of [14C-carboxyl]-labeled
diesters, monoesters, and anions in these
hydrosols, mono-n-butyl phthalate and phthalate
anion were completely degraded within 7 days,
whereas only 50% of the 14C was recovered as
carbon dioxide from the diester during the same
period. Since after 1 day exposure of the
hydrosol to the diester about 50% had been
hydrolyzed to the monoester, it is possible that
the diester may exert a partial inhibition of its
own biodegradation under some conditions.

A study of the kinetics of the biodegradation of
five commonly used phthalate esters was made
by Saeger and Tucker (21,22) which convincingly
showed that they readily underwent biodegradation. The data indicated that
phthalate esters with short (or partially oxidized)
alcohol chains, e.g., DBP were more easily
degraded than the long chain esters, e.g. DEHP,
a finding that was also observed in activated
sludge digestions (22) and hydrosols (20).

Figure 1. Catabolic pathways for o-phthalate and its esters.
The purpose of this article is to summarize the present state of knowledge about the biochemistry of the transformations of the phthalates brought about by pure cultures of bacteria. Some of the data produced from this laboratory have been reported previously (23, 24).

Isolation and Identification of the Bacteria

Over 100 enrichment cultures were made with minimal media (25) supplemented with the phthalate carbon source (0.1% for the anions and about 1 ml/100 ml of medium for the esters). The media were inoculated with several sources of natural habitat including soils, plant debris, running and still fresh water, raw sewage and marine and brackish waters. Most of these primary sources gave successful enrichments. Media containing the esters were much slower to develop; however pure cultures of the bacteria isolated from them grew easily with the esters. Marine waters did not yield bacteria able to degrade any of the substrates provided, whereas brackish waters did. This probably reflects the inability of marine bacteria to survive or proliferate in the absence of high salt concentrations, since our colleague, Dr. Barrie Taylor of the Marine Institute of this University has obtained numerous phthalate utilizers from enrichments using synthetic sea water basal media, for which they show a dependence.

Gram-negative rods frequently appeared as the predominant bacterial populations in the enrichments with the phthalate anions. However, Gram-positive cocci were the preponderant type seen when phthalate esters were supplied as the carbon source. A few of the cultures obtained as morphologically pure strains were selected for further studies on the basis of their stability and growth rate. Among the Gram-negative strains, both fluorescent and nonfluorescent species were found; their speciation was based on the biochemical and nutritional criteria provided by Stanier et al. (10). All of the phthalate ester utilizers selected, were strictly aerobic Gram-positive catalase-positive cocci, and appear to be species of Micrococcus (26); this classification is based on their inability to produce acid from glucose anaerobically, which distinguishes them from Staphylococc (27).

Catabolism of o-Phthalate and its Esters: Whole Cell Experiments

The respiratory activities of cells grown with o-phthalate (anion or diester) or glucose were measured to indicate the likely inducible catabolic routes used for oxidation of the phthalates. The results for representative strains are shown in Table 1. In most cases tested, both 4,5-dihydroxypthalate and protocatechu were oxidized at least as fast as o-phthalate when grown with o-phthalate anion; 4-hydroxy-o-phthalate, a possible intermediate, does not stimulate respiration above endogenous rates, nor does it support growth of the bacteria, whereas 4,5-dihydroxypthalate and protocatechu do. None of the substrates stimulate the respiration of glucose-grown cells.

When Micrococcus sp. strain 12B is grown with dimethyl phthalate in the presence of yeast extract, the medium turns purple due to the accumulation of metabolites and their possible reaction with Fe³⁺ ions. Extraction of acidified culture filtrates has allowed the tentative identification of two compounds of o-phthalate catabolism by this strain, protocatechu and 3-carboxy-oxalocrotonate, both of which give purple colors with Fe³⁺ ions. The latter would be an expected oxidation product of protocatechu after 4,5-dioxygenative cleavage (28, Fig. 1). Phthalate esterase activity was detected in cells of the Micrococcus grown with phthalate esters or with succinate. Activity was measured by titration of proton release with phthalate esters as substrates, and therefore, phthalate esterase activity is a constitutive property of this micrococcus. However, oxidation of phthalate anion by this strain is accomplished only after growth of the species with o-phthalate or some of its esters. One remarkable feature of the respiratory pattern of strain 12B grown with dimethyl phthalate, is the failure of cells to oxidize protocatechu. Extracts of these cells, however, possess a very active protocatechu 4,5-oxygenase, which is also inducible.

Enzymic Activities in Extracts of Cells

All of the enzymic activities necessary to account for the reaction sequences proposed in Figure 1 have been demonstrated in extracts of various cells. With extracts of o-phthalate-grown P. fluorescens strain PHK, o-phthalate (in the presence of NADH) is oxidized to β-keto adipate. Approximately two moles of oxygen are consumed and two moles of carbon dioxide are released for each mole of β-keto adipate formed from o-phthalate. Both 4,5-dihydroxypthalate and protocatechu are rapidly oxidized to β-keto adipate by the same extracts, but only one mole of oxygen is consumed in each case.
Table 1. Oxidation of potential intermediates of \(o\)-phthalate, isophthalate, and terephthalate catabolism by washed suspensions of bacteria.

| Substrate supplied | O\(_2\) consumed, nmole/min-mg dry cells |
|---------------------|-----------------------------------------|
|                     | P. \textit{testosteroni} \textit{EN 5a} grown on \(o\)-phthalate | P. sp \textit{EN}, grown on isophthalate | Marine bacterium OP, grown on \(o\)-phthalate |
| None                | 29 | 50 | 10 | 20 |
| \(o\)-Phthalate     | 68 | 194 | 7 | 90 |
| Isophthalate        | 27 | 7 | 50 | 9 |
| Terephthalate       | 27 | 7 | 96 |
| 4,5-Dihydroxyphthalate | 60 | 168 | N.D. | N.D. | 96 |
| 4-Hydroxyphthalate  | 28 | 12 | N.D. | N.D. | N.D. |
| 4-Hydroxyisophthalate | N.D. | 14 | 130 | 36 |
| Protocatechuate     | 64 | 237 | 309 | 7 | 225 |
| Gentisate           | 14 | 40 | 180 | 7 | N.D. |

* N.D. = not determined.

Whereas 2 moles of \(CO_2\) are released when 4,5-dihydroxyphthalate is oxidized, the oxidation of protocatechuate releases one mole of \(CO_2\) (Table 2). Temporal separation of the proposed decarboxylation of 4,5-dihydroxyphthalate to give protocatechuate was observed during simultaneous measurements of \(O_2\) and \(CO_2\) concentrations in reaction mixtures, with specific electrodes (Fig. 2). Release of carbon dioxide, when 4,5-dihydroxyphthalate is the substrate, precedes oxygen consumption; the converse is apparent when protocatechuate is provided as substrate (not shown).

With extracts of \(P.\) \textit{testosteroni} EN5A, pyruvate was found as one end product of protocatechuate and 4,5-dihydroxyphthalate oxidation, and the characteristic yellow absorption spectrum of the product of the 4,5-dioxygenative cleavage was observed (\(A_{\text{max}}\) 410 nm at pH values > 7). A similar intermediate accumulated when extracts of \textit{Micrococcus} sp. strain 12B were incubated with protocatechuate, an observation which was in accord with the suggestion that 3-carboxyoxalocrotonate had accumulated in culture filtrates, mentioned before.

Constitutive esterase activities in the whole cell experiments using \textit{Micrococcus} sp. strain 12B (dimethyl phthalate grown) were demonstrated in extracts of cells but not in the supernatants of culture media. With extracts of cells, hydrolysis of dimethyl, diethyl, and dibutyl phthalates occurred readily; diethyl phthalate was most rapidly hydrolyzed of the three esters provided.

Table 2. Stoichiometry of gas exchange during oxidation of \(o\)-phthalate by extracts and purified enzymes from \(P.\) \textit{fluorescens} PHK.

| Enzyme | Substrate | \(O_2\) consumed, moles/mole substrate | \(CO_2\) produced, moles/mole substrate |
|--------|-----------|---------------------------------------|----------------------------------------|
| Crude extract | \(o\)-Phthalate* | 2 | 2 |
| | 4,5-Dihydroxyphthalate* | 1 | 2 |
| Highly purified | \(o\)-Phthalate* | 1 | 0 |
| Partially purified | \(o\)-Phthalate* | 1 | 0 |
| Crude component | 4,5-Dihydroxyphthalate* | 1 | 2 |
| C of \(o\)-phthalate | Protocatechuate* | 1 | 1 |
| 4,5-Dihydroxyphthalate decarboxylase | Protocatechuate* | 0 | 1 |
| Protocatechuate | 3,4-dioxygenase | 1 | 0 |

*\(\beta\)-Ketoadipate accumulates.
*Diaryl diketones accumulate.
*4,5-Dihydroxyphthalate accumulates.
*Protocatechuate accumulates.
*\(\beta\)-Carboxy-cis-cis-muconate accumulates.
The initial enzyme system of the catabolic sequence, o-phthalate 4,5-dioxygenase, catalyzes the NADH- and O₂-dependent oxidation of phthalate. It has been resolved into two protein components (designated A and C) by (NH₄)₂SO₄ fractionation, both of which are necessary for activity (24,30). Each fraction has been substantially purified by chromatographic separations on DEAE-cellulose, Sephadex, and hydroxylapatite. Fraction A is red-brown with absorption maxima at 465 and 320 nm, and fraction C is yellow with absorption maxima at 400 and 460 nm. Polyacrylamide electrophoresis of the fractions indicated that neither of these protein components were yet homogeneous.

The availability of the proteins of the o-phthalate 4,5-dioxygenase complex, and the decarboxylase and protocatechuate 3,4-dioxygenase has allowed us to show that a dihydrodiol, presumably 4,5-dihydro-4,5-dihydroxyphthalate (Fig. 1), is probably a metabolite between o-phthalate and 4,5-dihydroxyphthalate. When the two purified proteins (A and C) of the o-phthalate 4,5-dioxygenase complex are incubated with phthalate and NADH aerobically, one mole of O₂, and of NADH are consumed for each mole of phthalate supplied, and a compound more polar than phthalate accumulates. This compound is not 4-hydroxyphthalate, since the two are well separated by thin-layer chromatography, and their reaction to Gibb’s reagent is different. Thin-layer chromatography also excludes 4,5-dihydroxyphthalate and protocatechuate as the accumulated intermediate; additionally this compound does not serve as a substrate for homogeneous preparations of 4,5-dihydroxyphthalate decarboxylase, nor those of protocatechuate 3,4-dioxygenase (30). The intermediate does give a positive reaction with triacetylesmate (31), and can be converted into a compound with the same mobility as 4-hydroxyphthalate, by heating in acid. Also, β-ketoacid is formed when crude extracts of P. fluorescens are incubated with the intermediate accumulated from o-phthalate. With less pure preparations of the o-phthalate 4,5-dioxygenase system, 4,5-dihydroxyphthalate has been shown to accumulate. The consumption of NADH is less than equimolar with oxygen under these circumstances. This allows the inference that these preparations contain a dihydrodiol dehydrogenase which can use NAD as electron acceptor (Fig. 1), which has been confirmed in subsequent experiments (P. Keyser, unpublished).

**FIGURE 2. Oxidation and decarboxylation of 4,5-dihydroxyphthalate by cell-free extracts of P. fluorescens PHK.**

**Enzymology of the o-Phthalate Pathway in P. fluorescens PHK**

We have purified some of the enzymes induced during growth of P. fluorescens PHK in the presence of o-phthalate. Two of the enzymes of the o-phthalate catabolic pathway, 4,5-dihydroxyphthalate decarboxylase and protocatechuate 3,4-dioxygenase (Fig. 1) have been obtained fortuitously in pure form as by-products of a chromatographic procedure used for the purification of one component of the initial enzyme system of the catabolic sequence, namely o-phthalate 4,5-dioxygenase. Affinity chromatography was an important final procedure developed for the isolation of homogeneous preparations of the decarboxylase and protocatechuate 3,4-dioxygenase (24,29). Separation of the decarboxylase and 3,4-dioxygenase was first observed during chromatography of an unresolved mixture on Sepharose 4B with 4,5-dihydroxyphthalate as ligand. Similar chromatography on columns of Sepharose 4B (with protocatechuate as ligand) allow the preparation of both enzymes in pure form, as indicated by disc-gel electrophoresis after denaturation with sodium dodecyl sulfate (29).
Catabolism of Isophthalate and Terephthalate

Much less is known about the pathways used by bacteria for the catabolism of isophthalate and terephthalate, and we believe this is the first report of their biological oxidation. They have received much less publicity as potential environmental pollutants—possibly because they are used in lesser quantities than the orthoisomer. Terephthalate is, however, manufactured on a very large scale as a monomer for the textile industry; it is often removed from industrial effluents as a calcium salt, although the acid itself is not very soluble in water.

Isolation of isophthalate and terephthalate (anions) utilizing bacteria presented little difficulty. All of the bacteria obtained were Gram-negative rods and strictly aerobic. The ability to utilize isophthalate terephthalate was also a property of one strain isolated with o-phthalate as carbon source. Protocatechuate was well oxidized by suspensions of cells grown with isophthalate and terephthalate (but not after growth on glucose), whereas other potential ring cleavage substrates (32), namely, catechol(s), gentisate(s), 2,3-dihydroxybenzoate(s) were not oxidized.

One strain, isolated with o-phthalate as carbon source and identified as P. testosteroni, could grow also with either isophthalate or terephthalate. Gentisate markedly stimulated the respiration of this strain, whether or not the cells had been grown at the expense of one of the phthalates. Gentisate 1,2-dioxygenase is known to be a constitutive enzyme in this species (33). The only other potential intermediate of those tested, which stimulated respiration, was 4-hydroxyisophthalate, and this was observed only with isophthalate grown cells. Extracts of isophthalate and terephthalate grown cells always possessed high levels of protocatechuate oxygense activities. Enzyme systems for the oxidation of isophthalate and terephthalate (and 4-hydroxyisophthalate) have not yet been obtained.

These results allow the tentative formulation of catabolic pathways for isophthalate and terephthalate which converge at protocatechuate as the ring cleavage substrate (Fig. 3), like those for o-phthalate (Fig. 1); the position of 4-hydroxyisophthalate as a metabolite is quite uncertain; this compound has been shown to be an intermediate in the catabolism of 2,4-xylenol by Pseudomonas sp. (34). Isophthalate (and terephthalate) may be metabolized by a benzoate-type dioxygenase (35) to give a hydrodiol intermediate, which is decarboxylated concomitantly with rearomatization to give protocatechuate. Much remains to be learned about these catabolic processes.

Concluding Note

That the phthalates and their esters are easily biodegradable is supported by several recent reports of their disappearance in a variety of natural ecosystems and sewage and the delineation of the catabolic pathways used by pure cultures of bacteria capable of growth with them as sole source of carbon and energy. Photochemical transformations of the phthalates may also occur, but we are not aware of detailed reports of them. Nor is it clear how stable the esters are to chemical hydrolysis, possibly catalysed by clay minerals or other agents. The most important mechanism for the mineralization of organic compounds is by the catabolic activities of microorganisms. The oxidation of organic compounds produced mainly by plants, but increasingly by industrial syntheses represents an essential part of the carbon cycle. The importance of the very diverse catabolic processes used to accomplish this, so that the carbon cycle may be maintained has been well documented by Dagley in a number of recent essays (36, 37).

The detection of phthalic acid esters in soils, waters, and animal tissues is not too surprising, in view of the sensitivities of present day analytical methods, and the ease with which they are leached and volatilized from some formulations (1, 4, 38). Whether the levels reported are affecting the balance and development of
natural communities of organisms or not, is quite uncertain. The experiments of Metcalf with his elegant laboratory model ecosystems demonstrated that the phthalate esters are quickly accumulated in plant and animal tissues, as are many other lipophilic compounds of recent industrial origin (39).

In order to demonstrate that phthalate esters are mutagenic or teratogenic, fairly large doses were administered to animals (1, 15, 16); the possible subtle toxic effects of the esters on man after long-term exposure to low doses are unknown (15). These reports clearly indicated that further research on the metabolism of the phthalate esters is necessary in plants, animals and microorganisms. Some attention ought also to be directed towards the effects of synthetic organic compounds on the natural metabolic activities of the microflora. Additionally, knowledge of the effects of synthetic chemicals on the poise of consortia of microbial populations is necessary, as these are the major agents for the return of carbon in the carbon cycle. In this respect the selection of a number of Gram-positive, catalase-positive cocci from enrichments with phthalate esters as carbon source suggests that these compounds may be partially counter-selective for Gram-negative rods normally obtained with phthalate anion enrichments.

Mixed populations of microbes, rather than pure cultures of bacteria selectively isolated in the laboratory, are likely to be more effective for the complete biodegradation of some chemicals in natural habitats, as has been well-documented by Alexander and his colleagues (40), in particular for the degradation of DDT (41). Similar consortia of microbes would appear to be responsible for the anaerobic digestion of carbon compounds in the rumen (42, 43), and other situations where CH₄ is the ultimate end-product of carbon metabolism. This, like the former example, utilizes different microbes to achieve different portions of the overall transformation. Indeed the outcome of events would be entirely different in the anaerobic systems, were methanogenic bacteria absent, as has been amply demonstrated and reconstructed in the laboratory by Wolin and Bryant (43). Furthermore, there is abundant evidence that the course of metabolic processes mediated by the microflora is radically altered by seemingly small and innocuous changes in ionic composition. For example, the presence of sulfate ions tends to depress methanogenesis, not by inhibition of the process, but by allowing other bacteria (sulfate-reducers) to compete for the available reducing power (H₂) produced by fermentation. Similar competitive processes may also occur in aerobic environments during the biodegradation of synthetic chemicals.

Nature has had billions of years to develop mechanisms for the oxidation of compounds which have been biosynthesized. And it is probably true to say that all compounds synthesized by living organisms, are also oxidized by the micro-flora. On the other hand the rapid development of the chemical industry, and in particular that associated with the petroleum industry (44) has placed severe demands on, and also unprecedented benefits to, different forms of life. For microbes to become equipped for the recognition, uptake and transformation of the synthetic compounds, many novel to nature, may require time periods orders of magnitude over the one to three decades that have elapsed since their appearance. Evolution of enzymes for new catabolic processes is a slow process, and catabolic pathway evolution would be expected to be very much slower. It is therefore a remarkable fact that many industrial chemicals, which would be considered rare in nature (and even anti-septic) are easily oxidized by bacteria and fungi. The directed selection and evolution of mutant strains for the oxidation or transformation of compounds, which are not utilized by the parent strains has been studied for a number of years (45,46). The discovery of several systems for gene transfer in the laboratory for the transmission of parts or all of catabolic pathways, inter-species and inter-generic, suggests that this mechanism for acquisition of function may predominate in mixed populations that occur naturally. Clearly, much remains to be learned about the effects, interactions, and fates of synthetic chemicals in natural environments; rational approaches for understanding these processes rests on a more complete knowledge of the catabolic pathways and enzyme mechanisms used by microorganisms, and also the effects that intruding synthetic chemicals have on the normal metabolic activities as they occur in nature.

This work was supported by a research grant to D.W.R. from the National Institute of Environmental Health Science, No. NIH 1 RO1 ES 00986-02.

REFERENCES
1. Environmental Health Perspectives. No. 3 (1973).
2. Marx, J. L. Phthalic acid esters: biological impact uncertain. Science 178: 46 (1972).
3. Anonymous. Phthalate effect on health still not clear. Chem. Eng. News 50: 14 (Sept. 18, 1972).
4. Graham, P. R. Phthalate ester plasticizers—why and how they are used. Environ. Health Perspect. No. 3: 3 (1973).
5. Ribbons, D. W., and Evans, W. C. Oxidative metabolism of phthalic acid by soil pseudomonads. Biochem. J. 76: 310 (1960).
6. Ribbons, D. W. and Evans, W. C. Oxidative metabolism of protocatechuic acid by certain soil pseudomonads: a new ring-fission mechanism. Biochem. J. 83: 482 (1962).
7. Taglay, S., and Patel, M. D. Oxidation of p-cresol and related compounds by a Pseudomonas. Biochim. Biochem. 66: 227 (1957).
8. Trippett, S., Taglay, S., and Stopher, D. A. Bacterial oxidation of protocatechuate. Biochem. J. 76: 9 (1959).
9. Taglay, S., Evans, W. C., and Ribbons, D. W. New pathways in the oxidative metabolism of aromatic compounds by micro-organisms. Nature 188: 560 (1960).
10. Stanier, R. Y., Palleroni, N. J., and Doudoroff, M. The aerobic pseudomonads: a taxonomic study. J. Gen. Microbiol. 43: 159 (1966).
11. Krisi, K. Carboxylic acid ester hydrolases. In: The Enzymes, P. D. Boyer, Ed., Vol. 5, Academic Press, New York, 1971.
12. Stach, W. H., and Pessen, H. The microbial degradation of plasticizers. I. Growth on esters and alcohols. Appl. Microbiol. 1: 30 (1953).
13. Klausmeier, R. E., and Jones, W. A. Microbial degradation of plasticizers. Dev. Indust. Microbiol. 2: 47 (1961).
14. Berk, S., Ebert, H., and Teitel, L. Utilization of plasticizers and related organic compounds by fungi. Ind. Eng. Chem. 49: 1115 (1957).
15. Autian, J. Toxicity and health threats of phthalate esters: review of the literature. Environ. Health Perspect. No. 4, 3 (1973).
16. Mathur, S. P. Phthalate esters in the environment: pollutants or natural products? J. Environ. Qual. 3: (1974).
17. Engelhardt, G., Wallnofer, P. R., and Hutziner, O. The microbial metabolism of di-n-butyl phthalate and related dialkyl phthalates. Bull. Environ. Contam. Toxicol. 13: 342 (1975).
18. Albro, P. W., and Thomas, R. O. Enzymatic hydrolysis of di-(2-ethylhexyl) phthalate by lipases. Biochim. Biophys. Acta 360: 380 (1973).
19. Albro, P. W., Thomas, R. O., and Fishbein, L. Metabolism of diethyhexyl phthalate by rats: isolation and characterization of the urinary metabolism.
20. Johnson, B. T. and Lulves, W. Biodegradation of di-n-butyl phthalate and di-2-ethylhexyl phthalate in fresh water hydrosol. J. Fisheries Res. Board Can. 32: 333 (1975).
21. Saeger, V. W., and Tucker, E. S. Phthalate esters undergo ready biodegradation Plastics Eng. 29: 49 (1973).
22. Saeger, V. W., and Tucker, E. S. Biodegradation of phthalic acid esters in river water and activated sludge. Appl. Environ. Microbiol. 31: 29 (1976).
23. Keyser, P., and Ribbons, D. W. o-Phthalate oxygenase activity from Pseudomonas fluorescens. Bacteriol. Proc. 160 (1975).
24. Pujar, B. G., and Ribbons, D. W. Purification of 4,5-dihydroxyphthalate decarboxylase and protocatechuic 3,4-oxygenease by affinity chromatography. Bacteriol. Proc. K51 (1976).
25. Ribbons, D. W. Metabolism of o-Cresol by Pseudomonas aeruginosa strain T1. J. Gen. Microbiol. 44: 221 (1966).
26. Bergey’s Manual of Determinative Bacteriology. 8th ed., Williams and Wilkins, Baltimore, 1974.
27. Baird-Parker, A. C. The classification of Staphylococci and Micrococcin from world-wide sources. J. Gen. Microbiol. 38: 363 (1965).
28. Taglay, S., et al. Degradation of the benzene nucleus by bacteria. Nature 202: 775 (1964).
29. Pujar, B. G. and Ribbons, D. W. Enzymes of phthalate catabolism: Purification of 4,5-dihydroxyphthalate deca-
30. Keyser, P. Aerobic metabolism of the phthalates by selected pseudomonads. M.S. Dissertation, University of Miami, 1974.
31. Criegee, R., et al. Zur Kenntnis der organischen osmium Verbindungen. II. Mitteilung. Ann. Chem. 556: 99 (1942).
32. Chapman, P. J. An outline of reaction sequences used for the bacterial degradation of phenolic compounds. In: Degradation of Synthetic Organic Molecules in the Biosphere. S. Taglay, Ed., National Academy of Sciences New York, 1973.
33. Wheelis, M. L., Palleroni, N. J., and Doudoroff M. The metabolism of aromatic acids by Pseudomonas testos-
34. Hopper, D. J., and Chapman, P. J. The bacterial metabol-
35. Reiner, A. M. and Hegeman, G. D. Metabolism of benzoic acid by bacteria. Accumulation of (+)-3,5-cyclohexadiene-
1,2-diol-1-carboxylic acid by a mutant strain of Alcaligenes eutrophus. Biochemistry 10: 2530 (1971).
36. Taglay S. Microbial degradation of organic compounds in the biosphere. Am. Scientist, 63: 681 (1975).
37. Taglay S. A biochemical approach to some problems of en-
38. Rouser, G., et al. Laboratory contaminants in lipid chemistry: detection by thin-layer chromatography and infrared spectrophotometry and some procedures minimizing their occurrence. Lipids 1: 107 (1966).
39. Metcalf, R. L., et al. Uptake and fate of di-2-ethylhexyl phthalate in aquatic organisms and in a model ecosystem. Environ. Health Perspect. No. 4: 27 (1973).
40. Alexander, M. The breakdown of pesticides in soils. In: Agriculture and the Quality of Our Environment. N. C. Brady, Ed., Americ Association for the Advancement of Science, Washington, D.C., 1967.
41. Pfander, F. K., and Alexander, M. Extensive microbial degradation of DDT in vitro and DDT metabolism by natural communities. J. Agr. Food Chem. 20: 842 (1972).
42. Hungate, R. E. The Rumen and Its Microbes. Academic Press, New York-London, 1966.
43. Bryant, M. P., et al. Methanobacillus omelianskii, a symbiotic association of two species of bacteria. Arch. Microbiol. 59: 20 (1967).
44. Sampson, A. The Seven Sisters. Viking Press, New York, 1975.
45. Clarke, P. H. The evolution of enzymes for the utilization of novel substrates. In: Evolution in the Microbial World. M. J. Carlile, and J. J. Skehel, Eds., Soc. Gen. Microbiol. Symp. 24: 183 (1974).
46. Mortlock, R. P. Catabolism of unnatural carbohydrates by microorganisms. Advan. Microbial Physiol. 13: 1 (1976).
47. Clarke, P. H., and Richmond, M. H. Genetics and Biochemistry of Pseudomonas. Wiley, New York-London 1975.