APPLIED AND ENVIRONMENTAL MICROBIOLOGY, Oct. 1995, p. 3562–3566 Vol. 61, No. 10
0099-2240/95/$04.00+0

Growth of the Fungus *Cladosporium sphaerospermum* with Toluene as the Sole Carbon and Energy Source

FRANS J. WEBER,* KO C. HAGE, AND JAN A. M. DE BONT

Division of Industrial Microbiology, Department of Food Science, Wageningen Agricultural University, 6700 EV Wageningen, The Netherlands

Received 5 May 1995/Accepted 15 July 1995

The fungus *Cladosporium sphaerospermum* was isolated from a biofilter used for the removal of toluene from waste gases. This is the first report describing growth of a eukaryotic organism with toluene as the sole source of carbon and energy. The oxygen consumption rates, as well as the measured enzyme activities, of toluene-grown *C. sphaerospermum* indicate that toluene is degraded by an initial attack on the methyl group.

The complete aerobic biodegradation of toluene to carbon dioxide, water, and biomass by bacteria has been studied extensively. Different initial oxidative reactions have been identified for a variety of bacteria, and the biodegradation of the resulting oxygenated compounds is well documented (6, 16, 21, 22, 26–28, 30). The presently known five different pathways for the utilization of toluene in bacteria are summarized in Fig. 1.

In humans and animals, the initial step in the metabolic transformation of toluene appears to be hydroxylation of toluene to benzyl alcohol (Fig. 1) by a monooxygenase. Benzyl alcohol is further converted to benzoic acid and excreted in urine, either unchanged or as its glycine conjugate, hippuric acid (29).

In contrast, utilization by fungi of toluene as the sole carbon and energy source has not been reported. Partial degradation of toluene by fungi was observed by Holland et al. (19). Cultures of *Mortierella isabellina* and *Helminthosporium* species were pregrown on a rich medium with glucose, and when the mycelium was subsequently incubated in water for 72 h it converted toluene into benzyl alcohol. Degradation of toluene by the white rot fungus *Phanerochaete chrysosporium* has been demonstrated by using ring-labeled [^{14}C]toluene and by measuring the production of $^{14}CO_2$ (32). During these experiments, the fungus was grown for 3 weeks in media containing glucose (1%) and toluene was added at 5 mg/liter. The quantity of toluene degraded was very small, on the order of 1 mg of toluene degraded per g of mycelium per day. Interestingly, the degradation took place under nonligninolytic culture conditions, indicating that lignin peroxidases or other peroxidases were not involved. It is known from many other studies that these enzymes play a key role in the partial oxidation of anthracene to anthraquinone (11) and in the partial oxidation of many other xenobiotic aromatic compounds (12). A number of nonligninolytic fungi have intracellular mechanisms for the partial degradation of complex aromatic structures, as documented by Cerniglia (5), and yeasts also are able to partially oxidize polycyclic aromatic hydrocarbons (18, 23).

Fungal growth with aromatic hydrocarbons other than toluene has been tested for *n*-alkylbenzenes and styrene. Fungi isolated on *n*-alkanes were grown in the presence of *n*-alkylbenzenes to determine the effect of the side chain length (C_1 to C_{12}). The organisms did not grow in the presence of toluene, maybe as a result of the toxicity of this compound, but they grew on other compounds, for instance, on dodecylbenzene, from which the organism accumulated benzoic and phenylacetic acids (10). Two styrene-degrading fungi have been isolated (17), and one strain, identified as *Exophiala jeanselmei*, was subsequently studied in more detail (7). The results obtained with *E. jeanselmei* (7) indicate that the styrene degradation pathway in the fungus was similar to a pathway observed for a styrene-degrading bacterium (17).

The metabolism by fungi of oxygenated aromatic compounds has been amply studied, and reviews of this field are available (4, 24, 31).

During our investigations of the removal of toluene from contaminated air by use of a compost biofilter, we macroscopically observed abundant fungal growth. Microscopic examination confirmed that fungi were predominant in this system, removing toluene from waste gases. On the basis of current knowledge of toluene biodegradation, it might be expected that the fungi only partly metabolize toluene. Alternatively, they might be involved in the degradation of partly oxidized products derived by bacteria from toluene, since it is known that fungi are able to metabolize oxygenated aromatic compounds, as, for instance, benzoate. However, in view of the abundance of the fungi in the biofiltration system, it seemed more reasonable to expect that the fungi were involved in the complete biodegradation of toluene. To test if a eukaryotic toluene-degrading organism was indeed present, we decided to isolate fungi from the biofilter and to investigate one pure culture in more detail.

MATERIALS AND METHODS

Isolation of fungi. Fungi were isolated from a compost biofilter (70 liters) which had been used to remove toluene from contaminated air (≈150 mg/m^3, 7 m^3/h) for 3 months. A suspension of various microorganisms was obtained by washing the biofilter with a 0.8% NaCl solution. From this suspension dilutions were made, and the resulting suspensions were seeded on agar plates with a mineral salts medium (17) and 10 μg of streptomycin per ml to minimize bacterial growth. These plates were incubated at 30°C in a desiccator containing toluene vapor, resulting in a concentration of about 200 mg/liter in the solidified "liquid" phase as calculated by using a water-air partition coefficient of 3.8 (1). Pure fungal cultures were obtained by subsequent transfers to new agar plates.

Growth with volatile aromatic compounds. For growth experiments, 250-ml flasks containing 10 ml of a phosphate-buffered (pH 7) mineral salts medium (17) were used. The bottles were closed with Teflon valves (Mininert; Phase Separations, Waddinxveen, The Netherlands) to prevent evaporation of the various added aromatic hydrocarbons and incubated under stationary conditions at 30°C. Growth was assessed by monitoring the production of CO_2 on a gas chromatograph.

Preparation of washed mycelium suspensions. In order to obtain larger amounts of mycelia, *Cladosporium sphaerospermum* was grown in a flat-bottomed

* Corresponding author. Mailing address: Division of Industrial Microbiology, Department of Food Science, Wageningen Agricultural University, P.O. Box 8129, 6700 EV Wageningen, The Netherlands. Fax: 31 317 484978.

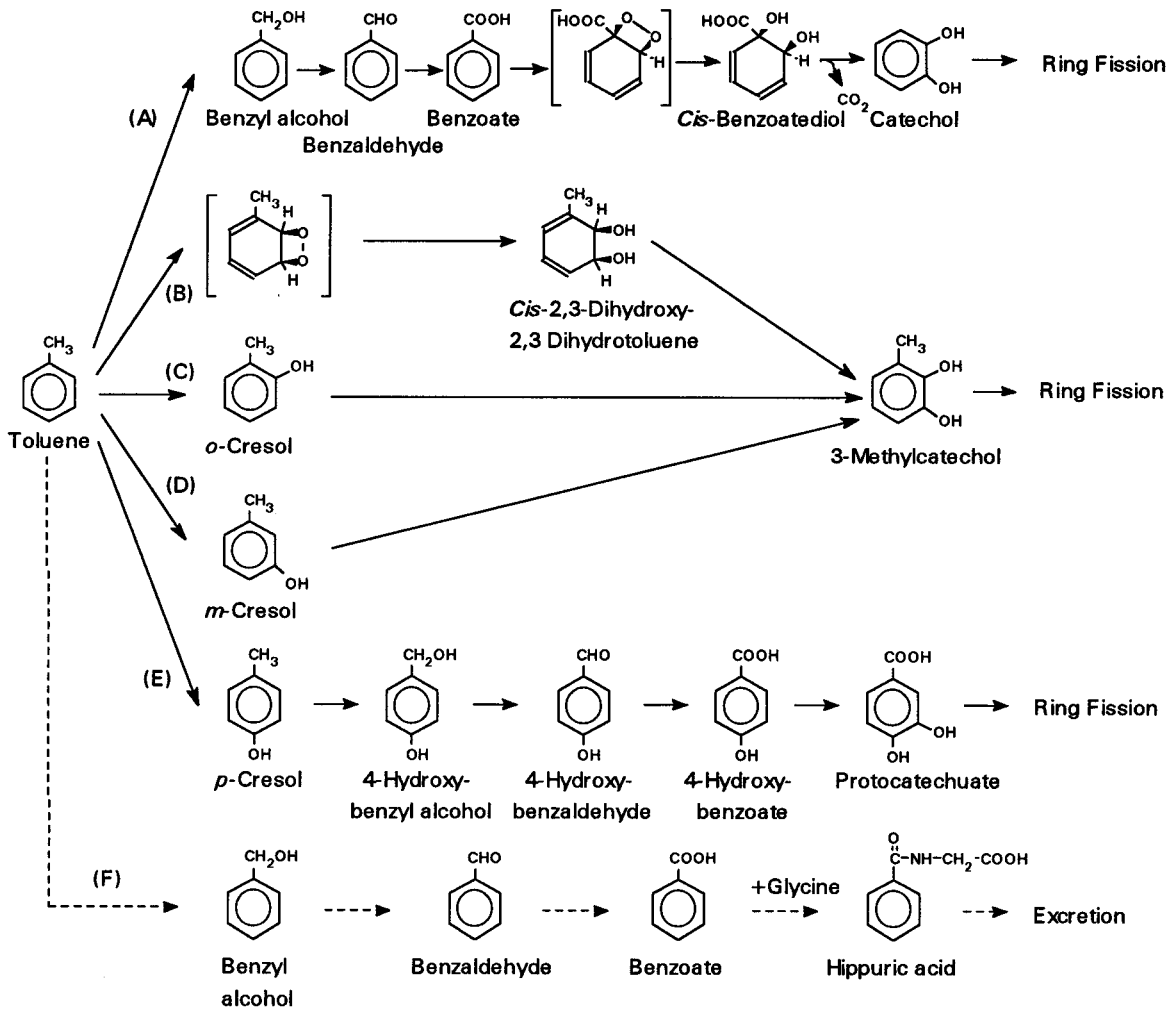

FIG. 1. Toluene biodegradation routes in bacteria (——) and mammals (– –). Data in panels A through F are from references 30, 16, 27, 21, 28, and 9, respectively.

round flask (2 liters) with 500 ml of phosphate-buffered mineral salts medium at 30°C. Air containing approximately 3,000 mg of toluene per m^3 was bubbled through the medium at a flow rate of 125 ml/min. Mycelium was harvested from the growth medium by filtration over cheesecloth, washed with 50 mM potassium phosphate buffer (pH 7.0), and resuspended in the same buffer.

Oxygen consumption experiments. The oxygen consumption of washed mycelium suspensions was determined with a Clark type oxygen electrode. The oxygen consumption of a 4-ml mycelium suspension was monitored for at least 5 min after the addition of 50 μl of a stock solution of 20 mM substrate in *N,N*-dimethylformamide. Addition of *N,N*-dimethylformamide resulted in a minimal increase in the endogenous oxygen uptake rate. After measuring the oxygen consumption rate of the substrate, we checked whether the substrate concentration used was not toxic by measuring the oxygen consumption rate after the addition of 50 μl of the toluene stock solution.

Determination of enzyme activities in cell extracts. Washed mycelium suspensions of toluene-grown *C. sphaerospermum* were frozen at −30°C and disrupted by two passes through a prechilled (−30°C) 5-ml X-press (AB Biox, Göteborg, Sweden). After being slowly thawed, the paste was diluted in 50 mM potassium phosphate buffer (pH 7.0) and centrifuged at 20,000 × *g* for 10 min at 4°C. The supernatant obtained was used for the enzyme activity determinations. The activities of the monooxygenases and dehydrogenases were determined spectrophotometrically. The reaction mixture (total volume, 1 ml) contained cell extract diluted in the phosphate buffer and 0.2 μmol of cofactor (NAD^+, $NADP^+$, NADH, or NADPH). The activities of both 4-hydroxybenzyl alcohol dehydrogenase and 4-hydroxybenzaldehyde dehydrogenase were corrected for the high level of adsorbance of 4-hydroxybenzaldehyde at 340 nm. *p*-Cresol methylmonooxygenase was assayed by monitoring the O_2 uptake as described previously (20), except that a 50 mM potassium phosphate buffer was used. Catechol-1,2-dioxygenase was monitored by measuring the formation of *cis-cis*-muconic acid at 260 nm (14). The activity of protocatechuate-3,4-dioxygenase was measured spectrophotometrically at 290 nm (14). 3-Methylcatechol-1,2-dioxygenase was determined by measuring the formation of the ring fission product at 390 nm.

Analytical methods. Carbon dioxide concentrations were determined by injecting 100-μl headspace samples into a Packard 427 gas chromatograph (Packard/Becker, Delft, The Netherlands) equipped with a Porapack Q column (Chrompack B. V., Middelburg, The Netherlands). Protein was quantified by the Bradford method (3) with bovine serum albumin as the standard. Cell dry weight was determined by weighing dried (24 h, 105°C) cell suspensions.

RESULTS

Isolation of fungi from the biofilter. From a compost biofilter, which had been used to remove toluene from contaminated air, a biomass suspension rich in fungi (>50% as examined by microscopy) was obtained by washing the biofilter with an NaCl solution. From this suspension several pure fungal cultures were isolated, and they grew on mineral agar plates incubated in the presence of toluene vapor.

Growth of fungi with toluene. As these isolated fungi could possibly grow on impurities from the agar plates, the capacity of these fungi to grow with toluene was checked by using liquid medium. One of the isolated fungi grew in liquid medium with toluene as the sole carbon and energy source. This fungus was

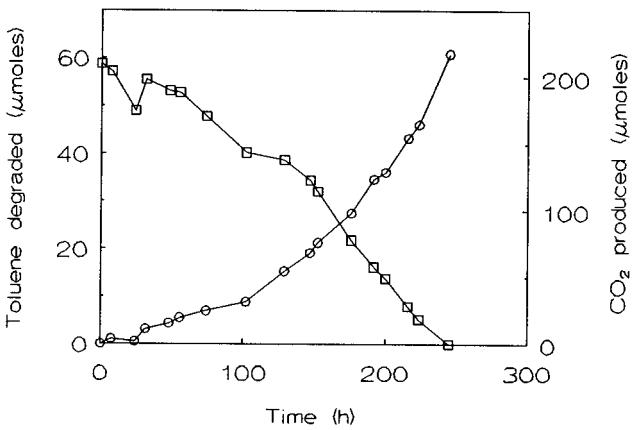

FIG. 2. Growth of *C. sphaerospermum* with toluene in 10 ml of mineral salts medium. □, amount of toluene degraded; ○, total amount of CO_2 produced.

identified by the Centraalbureau voor Schimmelcultures (Baarn, The Netherlands) as *C. sphaerospermum* Penzig.

In Fig. 2, a typical growth curve for *C. sphaerospermum* grown with an initial amount of 6 μl (56 μmol) of toluene in the incubation system (10 ml of liquid medium in a 250-ml culture bottle) is shown. This initial amount of toluene in the incubation system, on the basis of its partition coefficient, results in a concentration of 0.8 mM in the water phase. In about 10 days all the added toluene was degraded and about 210 μmol of CO_2 and about 6 mg (dry weight) of cells was formed. The growth rate was maximal at pH 7.0 under stationary conditions.

Growth of *C. sphaerospermum* with other aromatic solvents. Growth of *C. sphaerospermum* with several other aromatic hydrocarbons was also tested. As the aromatic hydrocarbons used can be toxic even at low concentrations, various amounts of the hydrocarbons were tested (10, 20, and 50 μmol). After 10 days of incubation, growth was assessed by CO_2 determination. Besides toluene, *C. sphaerospermum* could also use styrene, ethylbenzene, and propylbenzene as sole carbon and energy sources for growth at all concentrations tested (>40 μmol of CO_2 produced). No growth was observed with *o*-xylene, benzene, or phenol at any of the concentrations tested.

Whether *C. sphaerospermum* could grow with several intermediates of the known bacterial degradation pathways of toluene was also tested (Fig. 1). Growth was observed with benzyl alcohol, benzaldehyde, benzoate, and catechol. Growth with benzoate and catechol was observed only at 10 and 20 μmol; no growth was observed at 50 μmol. No growth with 3-methylcatechol was observed at any of the concentrations tested.

Oxygen consumption experiments. Oxygen consumption rates of washed suspensions of *C. sphaerospermum* grown with toluene were monitored in the presence of various possible intermediates of the toluene degradation pathway (Table 1). Addition of toluene after measurement of the *p*-cresol-induced oxygen consumption rate resulted in a reduced toluene-induced oxygen consumption rate. This inhibition of the toluene oxygen consumption rate was observed only for *p*-cresol, even when a 10-fold lower concentration of *p*-cresol was used.

The results presented in Table 1 have been corrected for the endogenous oxygen uptake (96 nmol of $O_2 \cdot min^{-1} \cdot mg$ [dry weight] of $cells^{-1}$).

Enzyme activities in cell extracts. Cell extracts of toluene-grown *C. sphaerospermum* were used to measure the activities of various enzymes possibly involved in the degradation of toluene (Fig. 1). 4-Hydroxybenzoate monooxygenase activity could be measured only after the addition of flavine adenine dinucleotide (FAD). Addition of FAD and ferrous ammonium sulfate to the reaction mixture did not enable in vitro monooxygenase activity to be obtained with any of the other substrates tested (toluene, benzoate, 3-hydroxybenzoate, *o*-cresol, or *p*-cresol). As many of these monooxygenases are unstable enzymes and have proven difficult to isolate (14), the inability to measure these enzyme activities is not very surprising. The activities of the measured dioxygenases and dehydrogenases of toluene-grown *C. sphaerospermum* cells are shown in Table 2.

TABLE 1. Rates of oxygen consumption by washed cell suspensions of *C. sphaerospermum* grown with toluene

Assay substrate	Oxygen consumption (nmol of $O_2 \cdot min^{-1} \cdot mg$ [dry wt] of $cells^{-1}$)
Toluene	164
Benzyl alcohol	154
Benzaldehyde	155
Benzoate	13
Catechol	77
3-Methylcatechol	<5
o-Cresol	<5
m-Cresol	<5
p-Cresol[a]	11
4-Hydroxybenzyl alcohol	<5
4-Hydroxybenzaldehyde	128
3-Hydroxybenzoate	6
4-Hydroxybenzoate	7
Protocatechuate	<5

[a] Inhibited the toluene-induced oxygen consumption rate.

DISCUSSION

From a biofilter used to remove toluene from contaminated air, we have isolated a fungus, *C. sphaerospermum*, which is able to grow with toluene as the sole source of carbon and energy. To our knowledge, this is the first report of toluene catabolism by a eukaryotic microorganism.

The spectrum of substrates used for growth by *C. sphaero-*

TABLE 2. Activities of enzymes possibly involved in catabolism of toluene in cell extracts of *C. sphaerospermum*

Enzyme	Cofactor(s)	Enzyme activity (nmol · min^{-1} · mg of $protein^{-1}$)
Dehydrogenases		
Benzyl alcohol	NAD^+	169
	$NADP^+$	<10
Benzaldehyde	NADH	7,180
	NADPH	107
4-Hydroxybenzyl alcohol	NAD^+	15
	$NADP^+$	<1
4-Hydroxybenzaldehyde	NADH	157
	NADPH	9
Monooxygenase		
4-Hydroxybenzoate	NADH + FAD	528
	NADPH + FAD	97
Dioxygenases (intradiol)		
Catechol		118
Protocatechuate		145
3-Methylcatechol		<25

spermum cells indicates that toluene is degraded by an initial attack on the methyl group, leading to benzoate. *C. sphaerospermum* could use benzyl alcohol, benzaldehyde, and benzoate as sole carbon and energy sources.

The route for toluene degradation was further investigated by measuring the oxygen consumption rates of various possible intermediates in toluene-grown mycelium of *C. sphaerospermum*. A prerequisite for measuring a substrate-induced increase in the oxygen consumption rate by whole cells is the ability of the compound to pass through the cytoplasmic membrane. It is expected that substrates like toluene, *o*-cresol, *m*-cresol, and *p*-cresol will enter the cell by diffusion through the cytoplasmic membrane. The fact that no activity was observed with *o*-cresol, *m*-cresol, and *p*-cresol indicates that these compounds are not intermediates of the toluene degradation pathway. Charged compounds like benzoate, however, may require an active transport system to enter the cell. The absence of an increase in oxygen consumption after the addition of these substrates could thus very well be caused by the lack of a suitable membrane transport system. Since an appreciable increase in oxygen consumption was observed after the addition of benzyl alcohol, benzaldehyde, and catechol and no activity was obtained with *o*-cresol, *m*-cresol, *p*-cresol, *p*-hydroxybenzyl alcohol, and 3-methylcatechol, it appears that toluene is degraded by a hydroxylation of the methyl group (Fig. 1).

The results from both the growth and oxygen consumption experiments indicated that toluene is degraded by an initial attack on the methyl group. The high activity rates measured for benzyl alcohol dehydrogenase and benzaldehyde dehydrogenase confirm these results. The activities for the degradation of 4-hydroxybenzaldehyde both in cell suspensions (Table 1) and in extracts (Table 2) are not in keeping with the proposed pathway, but these activities are likely due to an aspecific reaction of the benzaldehyde dehydrogenase. It has indeed been shown that in *Pseudomonas convexa*, 4-hydroxybenzaldehyde and benzaldehyde are oxidized by the same dehydrogenase (2).

In mammals, aromatic hydrocarbons usually are oxidized to an epoxide and subsequently a *trans*-dihydrodiol is formed (13, 15). Such a pathway has been established, for instance, for benzene (13), but toluene was degraded in a different fashion with an initial oxidation at the methyl group (13, 29). Our results indicate that the mammalian and fungal degradations of toluene are similar in that the initial reaction in both cases is with the methyl group and results in benzoate. Benzoate in mammals either is an end product and is excreted or is conjugated to hippuric acid. In bacteria, benzoate is further metabolized via *cis*-dihydrodiol to catechol as the ring fission substrate. In all fungi studied, the sole pathway for benzoate metabolism is by hydroxylation of benzoate to 4-hydroxybenzoate, leading to protocatechuate as the ring fission substrate (31). The measured activities for 4-hydroxybenzoate monooxygenase and protocatechuate-3,4-dioxygenase indicate that benzoate in *C. sphaerospermum* is also degraded via 4-hydroxybenzoate.

In cell extracts of *C. sphaerospermum*, a catechol dioxygenase activity, besides a protocatechuate dioxygenase activity, was also detected, indicating that catechol is a ring fission substrate. As the protocatechuate dioxygenase activity is measured by disappearance of protocatechuate, this activity could also be caused by the conversion of protocatechuate into catechol. It has been shown that in several fungi 4-hydroxybenzoate is degraded via protocatechuate, leading to catechol as the ring fission substrate (4). Whether protocatechuate is the actual ring fission substrate or is converted into catechol is uncertain and will require further investigation.

The discovery of toluene-degrading fungi is of importance for biofiltration. A disadvantage of biofilters used for waste gas treatment is that the pH cannot be controlled, and humidification of the filter can be problematic (25). Both these parameters should be carefully controlled to allow an extended operation of the filter. The application of aromatic hydrocarbon-degrading fungi in these biofilters might have two advantages, as fungi generally require less stringent control of both the pH and the water activity. A reduction of the water activity in the biofilter might also enhance the mass transfer of substrates poorly soluble in water (8).

REFERENCES

1. **Amoore, J. E., and E. Hautala.** 1983. Odor as an aid to chemical safety: odor thresholds compared with threshold limit values and volatilities for 214 industrial chemicals in air and water dilution. J. Appl. Toxicol. **3:**272–290.
2. **Bhat, S. G., and C. S. Vaidyanathan.** 1976. Involvement of 4-hydroxymandelic acid in the degradation of mandelic acid by *Pseudomonas convexa*. J. Bacteriol. **127:**1108–1118.
3. **Bradford, M. M.** 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal. Biochem. **72:**248–254.
4. **Cain, R. B., R. F. Bilton, and J. A. Darrah.** 1968. The metabolism of aromatic acids by micro-organisms. Biochem. J. **108:**797–828.
5. **Cerniglia, C. E.** 1984. Microbial metabolism of polycyclic aromatic hydrocarbons. Adv. Appl. Microbiol. **30:**31–71.
6. **Claus, D., and N. Walker.** 1964. The decomposition of toluene by soil bacteria. J. Gen. Microbiol. **36:**107–122.
7. **Cox, H. H. J., J. H. M. Houtman, H. J. Doddema, and W. Harder.** 1993. Growth of the black yeast *Exophiala jeanselmei* on styrene and styrene-related compounds. Appl. Microbiol. Biotechnol. **39:**372–376.
8. **Cox, H. H. J., J. H. M. Houtman, H. J. Doddema, and W. Harder.** 1993. Enrichment of fungi and degradation of styrene in biofilters. Biotechnol. Lett. **15:**737–742.
9. **Epstein, S., and A. E. Braunstein.** 1931. Über den Einfluβ der sogen. Sauren und basischen Nahrung auf das Schicksal aromatischer Substanzen im Organismus. II. Oxydation von Toluol. Biochem. Z. **235:**328–333.
10. **Fedorak, P. M., and D. W. Westlake.** 1986. Fungal metabolism of *n*-alkylbenzenes. Appl. Environ. Microbiol. **51:**435–437.
11. **Field, J. A., E. de Jong, G. Feijoo-Costa, and J. A. M. de Bont.** 1992. Biodegradation of polycyclic aromatic hydrocarbons by new isolates of white rot fungi. Appl. Environ. Microbiol. **58:**2219–2226.
12. **Field, J. A., E. de Jong, G. Feijoo-Costa, and J. A. M. de Bont.** 1993. Screening for ligninolytic fungi applicable for the biodegradation of xenobiotics. Trends Biotechnol. **11:**44–49.
13. **Gibson, D. T.** 1971. The microbial oxidation of aromatic hydrocarbons. Crit. Rev. Microbiol. **1:**199–223.
14. **Gibson, D. T.** 1971. Assay of enzymes of aromatic metabolism, p. 463–478. *In* J. R. Norris and D. W. Ribbons (ed.), Methods in microbiology, vol. 6a. Academic Press, London.
15. **Gibson, D. T.** 1993. Biodegradation, biotransformation and the Belmont. J. Ind. Microbiol. **12:**1–12.
16. **Gibson, D. T., M. Henley, H. Yoshioka, and T. J. Mabry.** 1970. Formation of (+)-*cis*-2,3-dihydroxy-1-methyl-cyclohexa-4,6-diene from toluene by *Pseudomonas putida*. Biochemistry **9:**1626–1630.
17. **Hartmans, S., M. J. van der Werf, and J. A. M. de Bont.** 1990. Bacterial degradation of styrene involving a novel flavin adenine dinucleotide-dependent styrene monooxygenase. Appl. Environ. Microbiol. **56:**1347–1351.
18. **Hofmann, K. H.** 1986. Oxidation of naphthalene by *Saccharomyces cerevisiae* and *Candida utilis*. J. Basic Microbiol. **26:**109–111.
19. **Holland, H. L., F. M. Brown, B. Munoz, and R. W. Ninniss.** 1988. Side chain hydroxylation of aromatic hydrocarbons by fungi, part 2. Isotope effects and mechanism. J. Chem. Soc. Perkin Trans. II **1988:**1557–1563.
20. **Hopper, D. J., and D. G. Taylor.** 1977. The purification and properties of *p*-cresol-(acceptor) oxidoreductase (hydroxylating), a flavocytochrome from *Pseudomonas putida*. Biochem. J. **167:**155–162.
21. **Kaphammer, B., J. J. Kukor, and R. H. Olsen.** 1990. Cloning and characterization of a novel toluene degradative pathway from *Pseudomonas pickettii* PK01, abstr. K-145, p. 243. *In* Abstracts of the 90th Annual Meeting of the American Society for Microbiology 1990. American Society for Microbiology, Washington, D.C.
22. **Kitagawa, M.** 1956. Studies on the oxidation mechanisms of methyl groups. J. Biochem. **43:**553–563.
23. **MacGillivray, A. R., and M. P. Shiaris.** 1993. Biotransformation of polycyclic aromatic hydrocarbons by yeasts isolated from coastal sediments. Appl. Environ. Microbiol. **59:**1613–1618.
24. **Middelhoven, W. J., M. Koorevaar, and G. W. Schuur.** 1992. Degradation of

benzene compounds by yeast in acidic soils. Plant Soil **145:**37–43.
25. **Ottengraf, S. P. P.** 1986. Exhaust gas purification, p. 426–452. *In* H.-J. Rehm and G. Reed (ed.), Biotechnology, vol. 8. VCH Verlagsgesellschaft, Weinheim, Germany.
26. **Shields, M. S., S. O. Montgomery, S. M. Cuskey, P. J. Chapman, and P. H. Pritchard.** 1991. Mutants of *Pseudomonas cepacia* G4 defective in catabolism of aromatic compounds and trichloroethylene. Appl. Environ. Microbiol. **57:**1935–1941.
27. **Shields, M. S., S. O. Montgomery, P. J. Chapman, S. M. Cuskey, and P. H. Pritchard.** 1989. Novel pathway of toluene catabolism in the trichloroethylene-degrading bacterium G4. Appl. Environ. Microbiol. **55:**1624–1629.
28. **Whited, G. M., and D. T. Gibson.** 1991. Separation and partial characterization of the enzymes of the toluene-4-monooxygenase catabolic pathway in *Pseudomonas mendocina* KR1. J. Bacteriol. **173:**3017–3020.
29. **World Health Organization.** 1985. Toluene. Environmental health criteria 52. World Health Organization, Geneva.
30. **Worsey, M. J., and P. A. Williams.** 1975. Metabolism of toluene and xylenes by *Pseudomonas putida* (*arvilla*) mt-2: evidence for a new function of the TOL plasmid. J. Bacteriol. **124:**7–13.
31. **Wright, J. D.** 1993. Fungal degradation of benzoic acid and related compounds. World J. Microbiol. Biotechnol. **9:**9–16.
32. **Yadav, J. S., and C. A. Reddy.** 1993. Degradation of benzene, toluene, ethylbenzene, and xylenes (BTEX) by the lignin-degrading basidiomycete *Phanerochaete chrysosporium*. Appl. Environ. Microbiol. **59:**756–762.