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Anaerobic Degradation of *m*-Cresol by a Sulfate-Reducing Bacterium

KATHLEEN L. LONDRY,[1] PHILLIP M. FEDORAK,[2] AND JOSEPH M. SUFLITA[1]*

Department of Botany and Microbiology, University of Oklahoma, Norman, Oklahoma 73019,[1] and Department of Biological Sciences, University of Alberta, Edmonton, Alberta, Canada T6G 2E9[2]

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***m*-Cresol metabolism under sulfate-reducing conditions was studied with a pure culture of *Desulfotomaculum* sp. strain Groll. Previous studies with a sulfate-reducing consortium indicated that *m*-cresol was degraded via an initial *para*-carboxylation reaction. However, 4-hydroxy-2-methylbenzoic acid was not degraded by strain Groll, and no evidence for ring carboxylation of *m*-cresol was found. Strain Groll readily metabolized the putative metabolites of a methyl group oxidation pathway, including 3-hydroxybenzyl alcohol, 3-hydroxybenzaldehyde, 3-hydroxybenzoic acid, and benzoic acid. Degradation of these compounds preceded and inhibited *m*-cresol decay. 3-Hydroxybenzoic acid was detected in cultures that received either *m*-cresol or 3-hydroxybenzyl alcohol, and trace amounts of benzoic acid were detected in *m*-cresol-degrading cultures. Therefore, we propose that strain Groll metabolizes *m*-cresol by a methyl group oxidation pathway which is an alternate route for the catabolism of this compound under sulfate-reducing conditions.**

Cresols are phenolic contaminants of fuel processing wastewaters that are of environmental concern because of their toxicity and their mobility in subsurface environments. The anaerobic biodegradation of all three cresol isomers has been demonstrated in field and laboratory studies (6). A variety of mechanisms for the metabolism of cresols under anaerobic conditions have been reported. *p*-Cresol is degraded by anaerobic oxidation of the methyl group in methanogenic (18) and sulfate-reducing (20) consortia, by nitrate-reducing isolates (4, 16), and by an iron-reducing isolate (10). *o*-Cresol is apparently degraded by either *para*-carboxylation (2) or methyl group oxidation (11) under methanogenic conditions, by methyl group oxidation under sulfate-reducing conditions (20), and by *para*-carboxylation followed by dehydroxylation under nitrate-reducing conditions (16).

The metabolic pathway by which *m*-cresol degradation proceeds under anaerobic conditions depends on the available electron acceptor. Under nitrate-reducing conditions, studies with the isolate S100 have indicated that *m*-cresol is likely degraded by a methyl group oxidation mechanism (3, 16). In methanogenic consortia, the methyl group is not oxidized (14); *m*-cresol is transformed by an initial *para*-carboxylation (15) followed by an apparent demethylation and dehydroxylation to yield benzoic acid (8). Previous studies have indicated that in a sulfate-reducing consortium, *m*-cresol is likewise degraded by an initial *para*-carboxylation mechanism (13).

We investigated the metabolic pathway for *m*-cresol degradation by using a sulfate-reducing organism previously isolated for its ability to degrade other aromatic compounds. *Desulfotomaculum* sp. strain Groll is a gram-positive, spore-forming, mesophilic, strictly anaerobic, rod-shaped bacterium. It is versatile, growing under freshwater or saltwater conditions, and can use a variety of electron acceptors, including sulfate but not nitrate (5). This organism was reported to degrade a wide variety of aromatic compounds, including *m*-cresol. We confirmed that this is the case, and we have elucidated the initial steps in the metabolism of *m*-cresol by this organism. Our findings indicate that there is a second pathway for *m*-cresol degradation under sulfate-reducing conditions that involves methyl group attack.

* Corresponding author. Mailing address: Dept. of Botany and Microbiology, University of Oklahoma, 770 Van Vleet Oval, Norman OK, 73019. Phone: (405) 325-5734. Fax: (405) 325-7541. E-mail: JSUFLITA @ou.edu.

MATERIALS AND METHODS

Growth of strain Groll. *Desulfotomaculum* sp. strain Groll was obtained from the Deutsche Sammlung von Mikroorganismen, Braunschweig, Germany (catalog no. 7213). The organism was cultivated in an anaerobic medium as previously described (5) but with only 1 mM sulfide as the reductant. The medium was dispensed in an anaerobic chamber to incubation vessels which were sealed with butyl rubber stoppers and aluminum crimp seals. The headspace of each vessel was then exchanged to N_2-CO_2 (80:20). The cultures were amended by addition of substrates from neutralized, sterile, anoxic stock solutions. Strain Groll was incubated at 32°C in the dark without shaking. Cultures were maintained by periodically adding substrate (250 μM) and by repeated transfer. Samples of culture fluid were withdrawn under strictly anaerobic and aseptic conditions and stored frozen until analyzed. Prior to high-performance liquid chromatography (HPLC) analysis, samples were thawed and centrifuged at 10,000 × *g* for 5 min.

Metabolite degradation studies. The degradation of putative metabolites was tested in a medium prepared as described above and inoculated (10%, vol/vol) with a culture of strain Groll, which depleted the *m*-cresol. All compounds were added to a concentration of 500 μM unless otherwise indicated, and sterile controls were autoclaved. Degradation was monitored by following the depletion of test compounds over time. Sulfate was added in nonlimiting concentrations (5 to 20 mM), and reduction of sulfate was confirmed at the end of the incubations. For dual-substrate studies, *m*-cresol and a test compound were added at equimolar concentrations and the degradation of both was monitored. Fluorinated analogs were added at 50, 100, and 250 μM concentrations to duplicate cultures containing 250 μM *m*-cresol. Rates of biodegradation were calculated during substrate decay but did not include lag periods.

To test the effects of carbon dioxide availability on degradation of compounds, the medium described above was prepared, but it was modified by addition of 50 mM *N*-tris(hydroxymethyl)methyl-2-aminoethanesulfonic acid (TES) buffer (pH 7.3). This medium was prepared two ways: by adding bicarbonate and providing an N_2-CO_2 headspace, as described above, or by excluding bicarbonate and providing a 100% N_2 headspace. Cultures were inoculated from cell suspensions that had been washed three times with carbonate-free medium.

Analytical techniques. Concentrations of aromatic compounds were determined by HPLC (Beckman Instruments, Inc., Berkeley, Calif.) with an Econosphere reverse-phase C_{18} column (250 mm by 4.6 mm; particle size, 5 μm; Alltech Associates, Inc., Deerfield, Ill.). The isocratic mobile phase consisted of a mixture of 50 mM sodium acetate buffer (pH 4.5) and 20 to 40% acetonitrile delivered at 1 ml/min. A variable-wavelength UV absorbance detector (model 165; Beckman Instruments) was used to monitor substrates and metabolites at 254 or 280 nm. Compounds were identified by comparison of their retention times to those of authentic standards and were quantified by comparison to standard curves prepared the same day.

Butyrate was analyzed with a Beckman HPLC system, using an Aminex HPX-

57H organic-acid column (Bio-Rad Laboratories, Richmond, Calif.) with a 0.016 N H_2SO_4 mobile phase delivered at 0.9 ml/min and UV detection (214 nm). This system was also used to detect and identify metabolites in culture fluids. Sulfate concentrations were determined by using a Dionex IC system with an AS4A-SC 4-mm particle-size column, a model CD20 conductivity detector, and a mobile phase of 1.8 mM sodium carbonate and 1.7 mM sodium bicarbonate delivered at 2 ml/min (Dionex, Sunnyvale, Calif.). Toluene and *m*-xylene were determined by gas chromatography (GC) with a Hewlett-Packard (Palo Alto, Calif.) 5890 series II gas chromatograph equipped with a flame ionization detector and a 30-m-long Carbograph VOC capillary column (Alltech Associates), operated isothermally at 70°C.

To identify metabolites, culture fluids were first alkali treated (pH 12 for 20 min) to cleave thioester bonds, acidified to <pH 2 with 10 M H_2SO_4, and extracted three times with diethyl ether. The ether extracts were filtered through anhydrous sodium sulfate, pooled, and concentrated. Trimethylsilyl (TMS) derivatives of compounds in culture extracts were prepared with *N*,*O*-bis(trimethylsilyl)acetamide in acetonitrile in accordance with the manufacturer's instructions (method 5; Pierce Chemicals, Rockford, Ill.). Aqueous solutions of authentic standards were extracted and derivatized in the same way. The ether extracts and the derivatized extracts were analyzed by GC-mass spectrometry (GC-MS) with a 5890 series II gas chromatograph equipped with a 5970 MS detector (Hewlett-Packard) and a 30-m-long DB-5 fused silica capillary column (J&W Scientific, Folsom, Calif.). The oven temperature was initially held at 50°C for 1 min and then raised at a rate of 6°C/min to 170°C.

Chemicals. 4-Hydroxy-2-methylbenzoic acid and 6-fluoro-3-methylphenol were synthesized as described by Londry and Fedorak (8). Sodium benzoate, *m*-cresol, and *p*-cresol were obtained from Sigma Chemical Co. (St. Louis, Mo.); all other chemicals were obtained from Aldrich Chemical Co. (Milwaukee, Wis.). The chemicals obtained were of the highest purity available (>96%) and were used without further purification.

RESULTS

Degradation of *m*-cresol is linked to sulfate reduction. Strain Groll was initially propagated on benzoate, which was previously reported to be a preferred substrate (5). After an initial lag period of 8 weeks, strain Groll was able to metabolize *m*-cresol when transferred to fresh medium containing this substrate (250 μM). The organism subsequently degraded repeated additions of *m*-cresol (250 to 500 μM), resulting in increased culture turbidity and sulfate reduction. The stoichiometry of sulfate depletion coupled with *m*- or *p*-cresol metabolism was 110% of the theoretically expected amount based on equation 1, which is not corrected for incorporation into biomass:

$$C_7H_8O + 4.25SO_4^{2-} \rightarrow 7CO_2 + 4.25S^{2-} + 4H_2O \quad (1)$$

Strain Groll could metabolize up to 2 mM levels of *m*-cresol. There was always a lag (7 to 21 days) prior to *m*-cresol decay, regardless of whether the substrate was added to pregrown cultures or fresh transfers. When sodium dithionite was used in the medium, the lag was shortened, but this reducing agent was not required for metabolism. Strain Groll could metabolize aromatic substrates in media with sulfide concentrations up to 15 mM. Thus, the organism had a high tolerance for toxic substrates as well as products.

Tests for *m*-cresol degradation by a *para*-carboxylation pathway. We tested 4-hydroxy-2-methylbenzoate, 4-hydroxybenzoate, benzoate, and 2-methylbenzoate as substrates for the sulfate-reducing isolate because these compounds were previously identified as metabolites in a sulfate-reducing consortium. Strain Groll did not metabolize 4-hydroxy-2-methylbenzoate or 2-methylbenzoate, even in dual-substrate experiments in which *m*-cresol was degraded (Table 1). 4-Hydroxybenzoate and benzoate were readily degraded; in dual-substrate studies, 4-hydroxybenzoate had no effect on *m*-cresol degradation by the isolate whereas benzoate inhibited *m*-cresol decay (Table 1). This suggested that only the latter compound was involved in the parent substrate metabolism.

Previous studies with this organism indicated a potential for carboxylation of aromatic compounds (5), and we found that *m*-cresol was not degraded in the absence of CO_2 (data not shown). However, CO_2 was also required for the degradation of benzoate, 3-hydroxybenzoate, and 4-hydroxybenzoate, indicating a more general requirement for CO_2 for the degradation of aromatic compounds. However, degradation of butyrate was independent of CO_2 availability (data not shown).

TABLE 1. Aromatic compounds tested as potential substrates for strain Groll

Compound added (500 μM)	Compound degraded?	Lag period[a]	Rate (μM/day)[b]	Effects on *m*-cresol[c]
Hydrocarbons				
Toluene	No			None
m-Xylene	No			ND[d]
Cresols				
o-Cresol	No			None
m-Cresol	Yes	3 wk	28	
p-Cresol	Yes	2 wk	≥30	None
4-Fluoro-3-methylphenol	No			Slight[e]
6-Fluoro-3-methylphenol	No			Slight
3-Hydroxybenzyl trifluoride	No			Slight
Phenolic compounds				
Phenol	Yes	6 wk	32	ND
Methyl hydroquinone	No[f]			None
4-Methylcatechol	Yes[g]	4 wk	16	None
2-Fluorophenol	No			Moderate[h]
3-Fluorophenol	No			Moderate
4-Fluorophenol	No			Moderate
Hydroxylated benzoates				
3-Methylsalicylate	No			None
4-Methylsalicylate	No			ND
5-Methylsalicylate	No			None
4-Hydroxy-2-methylbenzoate	No			None
4-Hydroxyphthalate	No			None
4-Hydroxybenzoate	Yes	2 wk	≥25	None
Methyl-group oxidation compounds				
3-Hydroxybenzyl alcohol	Yes	1 wk	48	Inhibited
3-Hydroxybenzaldehyde	Yes	1 day	≥36	Inhibited
3-Hydroxybenzoate	Yes	11 days	32	Inhibited
Benzoates				
Benzoate	Yes	7 days	27	Inhibited
2-Methylbenzoate	No			None
3-Methylbenzoate	Yes	3 wk	11	None
4-Methylbenzoate	No			None
2-Fluorobenzoate	Yes	1 wk	10	Inhibited
3-Fluorobenzoate	No			Inhibited
4-Fluorobenzoate	Yes	1 wk	7	Inhibited

[a] The lag period indicates the time prior to degradation of >10% of the available substrate.
[b] The rate refers to substrate depletion following the lag period.
[c] Effects on *m*-cresol degradation were determined by monitoring the degradation of both compounds in dual-substrate experiments.
[d] ND, not determined.
[e] The rate of *m*-cresol degradation was inhibited slightly.
[f] Abiotic transformation occurred, but products were not further degraded.
[g] Abiotic transformation occurred, followed by degradation of products.
[h] The rate of *m*-cresol degradation was reduced by half.

Substrate utilization studies. Since *m*-cresol was most likely not *para*-carboxylated, we investigated other possible routes of degradation. Carboxylation of *m*-cresol *ortho* to the hydroxyl group would yield 4-methylsalicylate, but this compound was not degraded. In fact, none of the methylsalicylate isomers

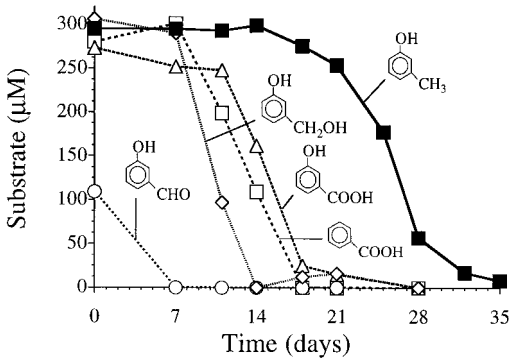

FIG. 1. Degradation of individual proposed *m*-cresol metabolites by strain Groll. Symbols: *m*-cresol, ■; 3-hydroxybenzyl alcohol, ◇; 3-hydroxybenzaldehyde, ○; 3-hydroxybenzoate, △; benzoate, □. No transformation of these compounds was observed in autoclaved sterile controls.

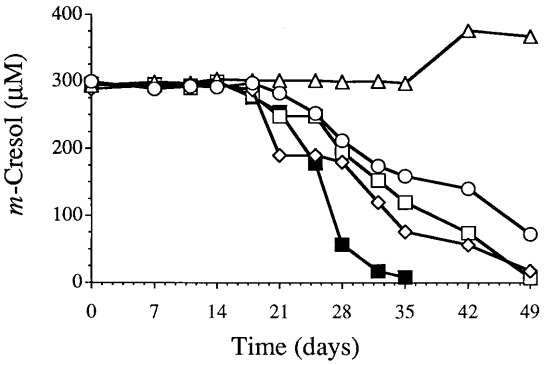

FIG. 2. Inhibition of *m*-cresol degradation by the addition of equimolar amounts of proposed *m*-cresol metabolites. Symbols: *m*-cresol only, ■; *m*-cresol with 3-hydroxybenzyl alcohol, ○; *m*-cresol with 3-hydroxybenzaldehyde, △; *m*-cresol with 3-hydroxybenzoate, ◇; *m*-cresol with benzoate, □.

were transformed by this organism (Table 1). When methyl hydroquinone and 4-methylcatechol were tested as substrates, both were found to be abiotically transformed; the products of the former accumulated, but the products of the latter were degraded after a 6-week lag. This degradation occurred after *m*-cresol was depleted in dual-substrate incubations, and 4-methylcatechol had no effect on *m*-cresol decay (Table 1). Thus, it is unlikely that the initial attack on *m*-cresol involved ring hydroxylation. Methylbenzoates were included in the study because 2-methylbenzoate was proposed to be a dead-end metabolite of *m*-cresol (15). Only the 3-methylbenzoate isomer was degraded by this organism (Table 1). This metabolism occurred after a longer lag period and had no effect on the degradation of *m*-cresol. In all of the dual-substrate studies, the addition of *m*-cresol neither stimulated nor inhibited the degradation of any of the test compounds. *m*-Cresol was degraded in all cultures, indicating that the test compounds were not inhibitory at the concentrations added.

The degradation of the putative *m*-cresol metabolites predicted by a methyl group oxidation pathway is shown in Fig. 1. All four of these compounds were metabolized more rapidly by strain Groll than was *m*-cresol (Fig. 1). 3-Hydroxybenzaldehyde was completely degraded only at concentrations $\leq$100 μM; at higher concentrations it was only partially metabolized. In dual-substrate studies, 3-hydroxybenzyl alcohol, 3-hydroxybenzaldehyde, 3-hydroxybenzoic acid, and benzoic acid each caused an increase in the length of the lag period and slowed the rate of *m*-cresol degradation (Fig. 2). With the exception of the fluorinated analogs, the other test substrates did not affect the lag period or the rate of *m*-cresol decay. In cultures containing 3-hydroxybenzaldehyde at a concentration of >100 μM, *m*-cresol depletion was completely inhibited, indicating that this aldehyde is likely toxic to this bacterium.

Addition of analogs to *m*-cresol-degrading cultures. A variety of analogs and inhibitors were tested for their effect on *m*-cresol degradation and the accumulation of metabolites. Molybdate (0.5 mM) and 2-fluoroacetate (10 mM) precluded *m*-cresol decay by strain Groll. The fluorinated cresols had a minimal effect on *m*-cresol degradation (Table 1). The fluorinated phenols moderately inhibited *m*-cresol metabolism in a concentration-dependent manner. The effect of the fluorobenzoates varied by isomer. Inhibition of *m*-cresol degradation by 2- and 4-fluorobenzoate was concentration dependent, but 3-fluorobenzoate completely precluded *m*-cresol decay at all concentrations tested. Of all the fluorinated analogs tested, the fluorobenzoates were the only ones metabolized (Table 1). The addition of fluorinated analogs did not result in the accumulation of *m*-cresol metabolites that could be detected by either of two different HPLC procedures.

Detection of metabolites in culture fluids. Cultures of strain Groll were analyzed for the presence of intermediates that would suggest the mechanism by which *m*-cresol was metabolized. Cultures fluids were analyzed directly by two HPLC methods; in some cases, culture fluids were extracted with an organic solvent, derivatized, and analyzed by GC-MS. All analyses were performed with culture fluids from cell suspensions of *m*-cresol-degrading strain Groll, and the results were compared with those from analyses of cultures utilizing benzoate, as well as of sterile and uninoculated control cultures. We were unable to detect 4-hydroxy-2-methylbenzoate, 2-methylbenzoate, or other compounds indicative of *m*-cresol metabolism by any pathway other than methyl group oxidation.

Cultures amended by addition of the putative methyl group oxidation metabolites were also analyzed by these methods. 3-Hydroxybenzoic acid was detected as a metabolite of 3-hydroxybenzyl alcohol. Figure 3 illustrates that as 3-hydroxybenzyl alcohol was degraded, a transient accumulation of 3-hydroxybenzoic acid was observed. 3-Hydroxybenzoic acid accumulated transiently regardless of whether *m*-cresol was also added. No other metabolites were detected in these cultures or in cultures receiving 3-hydroxybenzaldehyde, 3-hydroxybenzoic acid, or benzoic acid.

HPLC analysis of strain Groll suspensions which degraded over 10 amendments of *m*-cresol revealed the presence of a

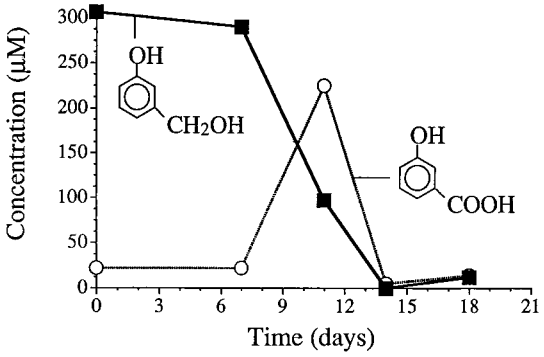

FIG. 3. Accumulation of 3-hydroxybenzoate (○) in cultures of strain Groll degrading 3-hydroxybenzyl alcohol (■).

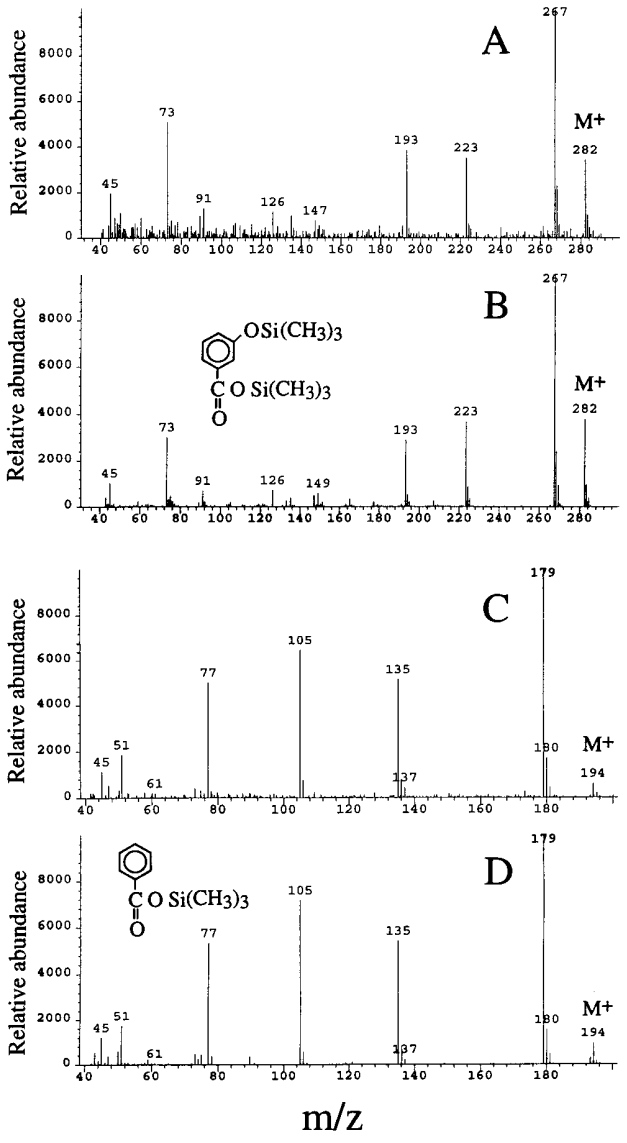

FIG. 4. Mass spectra of TMS-derivatized metabolites of *m*-cresol (A and C) and of the TMS derivatives of 3-hydroxybenzoic acid (B) and benzoic acid (D), as determined by GC-MS.

peak with a retention time corresponding to that of authentic 3-hydroxybenzoic acid. This peak was not detected in benzoate-supplemented cultures. This compound was detected only in *m*-cresol-grown cultures that were stressed, presumably by the accumulation of sulfide; microscopically, the cells were shrivelled and sporulating. GC-MS analyses of the extracted and derivatized culture fluid confirmed the presence of 3-hydroxybenzoic acid in cultures of *m*-cresol-degrading strain Groll (Fig. 4). This compound was not detected in derivatized extracts of cultures in which butyrate or benzoate served as the starting substrate or in uninoculated culture fluids containing *m*-cresol. The TMS derivative of benzoic acid was also detected in culture fluids of *m*-cresol-degrading strain Groll. Figure 4 compares the mass spectra of a peak detected by analysis of extracted culture fluid and an authentic derivatized benzoate standard. The detection of 3-hydroxybenzoate and benzoate in culture fluids provides further evidence for their role as metabolites of *m*-cresol degradation by strain Groll.

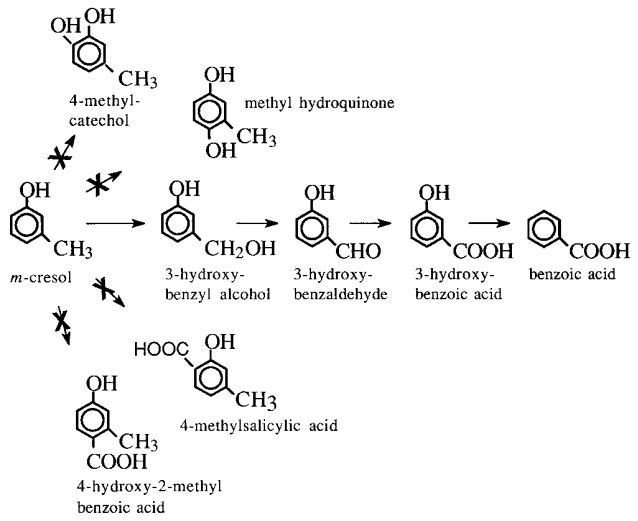

FIG. 5. Possible reactions for the initial transformation of *m*-cresol under sulfate-reducing conditions by *Desulfotomaculum* sp. strain Groll. *m*-Cresol is hydroxylated on the methyl group to give 3-hydroxybenzyl alcohol, which is oxidized to 3-hydroxybenzaldehyde and then to 3-hydroxybenzoate. 3-Hydroxybenzoate is subsequently dehydroxylated to form benzoate, which is further metabolized. *m*-Cresol was not metabolized via ring carboxylation or hydroxylation, as the products of these potential reactions were either not degraded by strain Groll (4-hydroxy-2-methylbenzoate, 4-methylsalicylate, and methylhydroquinone) or were degraded only after a longer lag period than *m*-cresol's (4-methylcatechol).

DISCUSSION

Of the possible routes for *m*-cresol decay by strain Groll, our evidence is most consistent with the oxidation of the methyl group to yield 3-hydroxybenzoic acid. The latter is subsequently converted to benzoic acid (Fig. 5). This pathway has been suggested previously for *m*-cresol metabolism by a denitrifying pseudomonad (3, 16). However, previous studies of *m*-cresol degradation by a consortium under sulfate-reducing conditions suggested an initial *para*-carboxylation of the substrate to form 4-hydroxy-2-methylbenzoic acid (13). Subsequent studies extended these observations in that both 4-hydroxybenzoic acid and benzoic acid were found as *m*-cresol metabolites (9). Thus, the presumed pathway for *m*-cresol degradation was considered identical to that proposed to occur under methanogenic conditions (8). However, *m*-cresol metabolism by strain Groll differs from that of the sulfate-reducing consortium. With the consortium, 4-hydroxy-2-methylbenzoic acid and 4-hydroxybenzoic acid were readily degraded but 3-hydroxybenzyl alcohol and 3-hydroxybenzoic acid were not metabolized in either the presence or absence of *m*-cresol (13). The degradation of 4-hydroxybenzoic acid preceded and delayed the metabolism of *m*-cresol by the consortium (13). In contrast, strain Groll did not degrade 4-hydroxy-2-methylbenzoic acid yet did metabolize the presumed methyl group oxidation metabolites. Strain Groll also metabolized 4-hydroxybenzoic acid, a common intermediate in the degradation of aromatic compounds (6), but in contrast to the consortium, the addition of this compound had no effect on *m*-cresol decay.

The sulfate-reducing consortium exhibited a CO_2 requirement for *m*-cresol carboxylation but not for benzoate metabolism (13). Strain Groll had a less specific requirement for CO_2 for the degradation of *m*-cresol, benzoate, and other carboxy-

lated aromatic compounds. However, strain Groll did not require CO_2 for butyrate decay. Our results are somewhat different from those of Kuever et al. (5), who reported that degradation of benzoate and butyrate by strain Groll was CO_2 independent while catechol could not be degraded in the absence of CO_2. At present, it is not clear why CO_2 is required for the degradation of carboxylated aromatic compounds by this organism.

Simultaneous adaptation studies provided initial indications for a *m*-cresol degradation pathway. Degradation of the proposed metabolites was preferred to and faster than the degradation of *m*-cresol. Benzoic acid could likely be formed from 3-hydroxybenzoic acid by a dehydroxylation reaction, which has been shown for this and other hydroxybenzoate isomers (for a review, see reference 6). 3-Hydroxybenzoic acid and benzoic acid probably exist as intracellular coenzyme A derivatives, but they are referred to as free acids, the form used and detected in our experiments.

Further evidence that these compounds represent putative *m*-cresol metabolites comes from substrate competition experiments. Each of the four suggested metabolites increased the length of time required for *m*-cresol degradation compared to cultures which only received *m*-cresol. Other aromatic compounds, with the exception of the fluorinated analogs, did not affect *m*-cresol decay regardless of their rate of degradation.

In previous studies, fluorinated analogs were useful tools for elucidating the pathway of *m*-cresol metabolism (8). In this study, the effects of the fluorinated analogs depended on the position of the fluorine, the other substituents, and sometimes the concentration of the analog. However, in contrast to previous studies (3, 8), none of the analogs caused an accumulation of metabolites. It thus appears that *m*-cresol degradation by strain Groll is tightly regulated and that the requisite enzymes may have excluded the fluorinated analogs. 2-Fluorobenzoate and 4-fluorobenzoate were metabolized by strain Groll, but the biodegradation mechanism is unknown. Anaerobic degradation of fluorobenzoates by nitrate-reducing bacteria has been reported previously (17, 21).

Evidence for a methyl group oxidation pathway for *m*-cresol degradation was obtained by the detection of metabolites in culture fluids. 3-Hydroxybenzoic acid was detected as a metabolite in cultures degrading *m*-cresol or 3-hydroxybenzyl alcohol. Similarly, trace amounts of benzoic acid were also detected in *m*-cresol-degrading cultures. The detection of these two putative intermediates suggests a progression of oxidation reactions as indicated in Fig. 5. 3-Hydroxybenzyl alcohol and 3-hydroxybenzaldehyde, while readily metabolized by strain Groll, were not detected in spite of a variety of attempts to cause the accumulation of these compounds. It may be that these presumed intermediates are transformed so rapidly that they effectively escape detection.

We also tested a variety of aromatic compounds as possible substrates for strain Groll. Strain Groll degraded 4-methylcatechol, a potential product of lignin hydrolysis. Kuever et al. (5) reported the degradation of 3,4-dihydroxybenzaldehyde and 3,4-dihydroxybenzoic acid, which would be consistent with the oxidation of the methyl group of 4-methylcatechol. Our studies, combined with the original report by Kuever et al. (5), indicate that at least 28 different aromatic compounds, as well as many nonaromatic compounds, can be degraded by this metabolically diverse anaerobe.

The degradation of 3-methylbenzoic acid (*m*-toluic acid) by strain Groll was intriguing as there are few reports of anaerobic metabolism of toluic acids. Londry and Fedorak (7) demonstrated that *o*-toluic acid was degraded under methanogenic conditions after a relatively long acclimation period, but we are unaware of additional reports of toluic acid biodegradation. Toluic acids are environmentally significant as they accumulate as products of xylene biodegradation under anaerobic conditions (1, 12, 19). Strain Groll does not degrade toluene or *m*-xylene (Table 1), but Kuever et al. (5) reported that benzyl alcohol and benzaldehyde are metabolized by strain Groll. Therefore, only an initial hydroxylation of toluene would be required for its degradation by this organism. It may be possible to combine the organisms that transform xylene isomers with strain Groll, or to modify the methyl group-hydroxylating enzyme(s) of strain Groll, to achieve complete mineralization of toluene or *m*-xylene under sulfate-reducing conditions.

Our studies indicate that there are at least two pathways for the degradation of *m*-cresol under sulfate-reducing conditions. Both pathways converge on benzoic acid, a common metabolite of the anaerobic degradation of aromatic compounds (6). Under sulfate-reducing conditions, the three cresol isomers can be initially transformed by methyl group oxidation. *p*-Cresol can be degraded by methyl group oxidation under many anaerobic conditions, while *m*-cresol and *o*-cresol have been shown to undergo either methyl group oxidation or an initial *para*-carboxylation, depending on the electron acceptor available. It is interesting that *m*-cresol metabolism has been suggested to occur by methyl group oxidation in pure cultures (this study and reference 3) and by *para*-carboxylation in mixed consortia (8, 13). This leads us to question whether community dynamics can affect the mechanism by which aromatic compounds are degraded in anoxic environments. Further research will be required to conclusively establish the pathway for *m*-cresol metabolism by strain Groll by measuring enzyme activities responsible for these conversions and to determine the environmental significance of this mechanism for degradation of aromatic compounds in anaerobic environments.

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