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Catechol and Phenol Degradation by a Methanogenic Population of Bacteria

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An anaerobic population of bacteria became acclimated to catechol and phenol in 32 and 18 days, respectively. Evidence from carbon balance measurements indicates that the aromatic ring is cleaved and that the products are stoichiometrically fermentable to methane and carbon dioxide.

Although several groups have extensively studied the aerobic degradation of aromatic compounds (3, 5, 9), relatively little attention has been paid to the anaerobic degradation of aromatics. Several aromatic compounds have been reported to be biodegradable under the anaerobic conditions present during photosynthetic metabolism (4, 7), nitrate respiration (10, 12), and methane fermentation (1, 6, 11). However, both catechol and phenol have been found to be highly refractory. Although a variety of different anaerobic culture conditions involving both pure and mixed cultures have failed to demonstrate the anaerobic biodegradability of either of these compounds (2, 4, 10, 12), one previous investigation (1) did succeed in demonstrating the anaerobic biodegradability of phenol by methanogens. The anaerobic biodegradability of catechol, however, has not been observed previously. This report provides evidence that both catechol and phenol are totally degraded under anaerobic conditions and converted to methane and carbon dioxide.

A serum bottle variation of the Hungate technique for growing anaerobic bacteria was adapted from Miller and Wolin (8) for methanogenic enrichment cultures. These cultures consisted of 150 ml of culture fluid contained in 250-ml serum bottles. Prereduced defined nutrient medium (Table 1) was inoculated with a 10% (vol/vol) methanogenic population from methane digester effluent. The digester was fed daily with primary settled sludge on a 15-day turnover time. The serum bottle enrichments were set up as batch cultures and incubated in the dark with intermittent shaking. The aromatic substrate was the sole source of carbon. The medium was buffered at pH 6.8, with bicarbonate in solution in 30% CO_2 in the gaseous atmosphere (CO_2-N_2). Resazurin was used as an indicator of reduced conditions, while sodium sulfide was present as a reducing agent.

The enrichments were monitored with a syringe (8) for the cumulative volume of gas produced, and analyses of gas composition were made with a Fisher Hamilton model 29 gas partitioner. The concentration of the aromatic substrate was monitored by measuring the supernatant portion of a sample for ultraviolet absorbance at 277 nm for catechol and at 270 nm for phenol. The above gas and ultraviolet measurements were corrected for background levels taken from a control enrichment in which no aromatic substrate was added. Carbon mass balances were calculated for catechol and phenol after the cultures became acclimated to the compound.

Figure 1 illustrates the utilization of phenol over time under methanogenic conditions and the concomitant production of gas. The temporal correlation between the onset of gas production and substrate disappearance is clearly evident. Phenol decomposition began after a 2.5-week acclimation to the initial concentration of substrate. An additional 2-week period was required for the complete disappearance of the compound. Evidence for the enrichment of phenol decomposers is shown by the fact that little lag occurred after a second spike of substrate was added. After the initial gas production leveled off, a second increase in gas production also closely corresponded to the disappearance of the substrate spike.

Figure 2 illustrates similar characteristics for the utilization of catechol. In this case, an exceedingly long acclimation period of 4.5 weeks was required before any decomposition took place. In addition, a shorter lag of about 1 week occurred before the utilization of the second substrate spike began. Similar to the phenol system, gas production coincides closely with the degradation of both the initial concentration and the additional substrate spike.

Complete phenol utilization initially took 14 days and was reduced to 10 days after the additional substrate spike. This indicates a more

TABLE 1. *Media composition for methanogenic enrichments*

Compound	Concn (g/liter)
Aromatic	0.3
Resazurin	0.001
$(NH_4)_2HPO_4$	0.04
$CaCl_2 \cdot 2H_2O$	0.25
NH_4Cl	0.2
$MgCl_2 \cdot 6H_2O$	1.8
KCl	1.3
$MnCl_2 \cdot 4H_2O$	0.02
$CoCl_2 \cdot 6H_2O$	0.03
H_3BO_3	0.0057
$CaCl_2 \cdot 2H_2O$	0.0027
$Na_2MoO_4 \cdot 2H_2O$	0.0025
$ZnCl_2$	0.0021
$FeCl_2 \cdot 4H_2O$	0.368
$NaHCO_3$	2.64
$Na_2S \cdot 9H_2O$	0.5

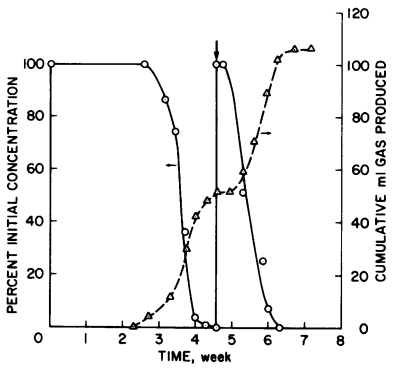

FIG. 1. *Metabolism of phenol under methanogenic conditions. 100% initial concentration corresponds to ~300 mg/liter. Symbols: ⊙, phenol; △, cumulative milliliters of gas produced; ↓, point in time when culture was spiked with additional substrate.*

rapid rate of decomposition after enrichment. However, this did not occur in the case of catechol.

Figure 3 illustrates the carbon balance during the decomposition of phenol to CO_2 and CH_4. As phenol carbon decreases, it is converted totally to gaseous carbon end products, with more than half in the form of methane. The carbon conversion for catechol occurs in a similar manner as for phenol, although requiring a longer period of time. Most of the catechol carbon is converted to gas with methane, in this case, accounting for a little less than half.

The fermentation of phenol and catechol to CO_2 and CH_4 can be stoichiometrically described as follows:

$$\text{phenol } C_6H_6O + 4H_2O \rightarrow 2.5CO_2 + 3.5CH_4$$

$$\text{catechol } C_6H_6O_2 + 3.5H_2O \rightarrow 2.75CO_2 + 3.25CH_4$$

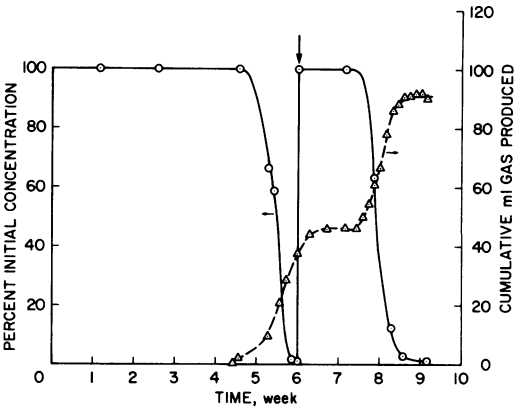

FIG. 2. *Metabolism of catechol under methanogenic conditions. 100% initial concentration corresponds to ~300 mg/liter. Symbols: ⊙, catechol; △, cumulative milliliters of gas produced: ↓, point in time when culture was spiked with additional substrate.*

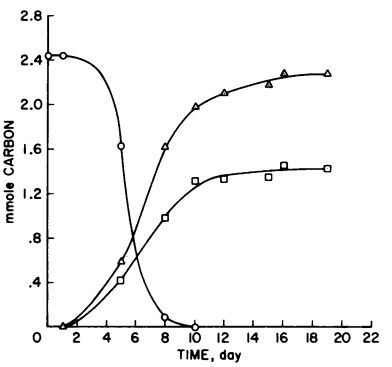

FIG. 3. *Phenol carbon balance. Symbols: ⊙, millimoles of phenol carbon; △, cumulative millimoles of total gas produced; ⊡, cumulative millimoles of methane produced.*

Based on the initial substrate concentration and the above stoichiometry, theoretical yields of gas can be calculated and compared with the measured values (Table 2). For both compounds, actual values reasonably reflected theoretical ones. For both anaerobic populations, the total gas produced was 79 and 85% of the theoretical values for phenol and catechol, respectively. The corresponding methane production was 89 and 99% of the theoretical values.

These data clearly demonstrate that for these compounds the aromatic ring is cleaved during the fermentation to CO_2 and CH_4. In addition, one-half or more of the substrate carbon can be converted to methane.

Catechol and phenol, therefore, are substrates that can be biodegraded by a methanogenic population of bacteria. Our results support the

TABLE 2. *Summary of phenol and catechol gas production*

Substrate	Total gas produced (mmol)		Total methane produced (mmol)	
	Actual[a]	Theoretical	Actual[b]	Theoretical
Phenol	1.92 ± 0.046	2.43	1.26 ± 0.085	1.42
Catechol	1.91 ± 0.081	2.25	1.21 ± 0.262	1.22

[a] Actual values are reported as mean ± standard error and are based on six sets of enrichments.

[b] Actual values are reported as mean ± standard error and are based on three sets of enrichments for phenol and two sets of enrichments for catechol. The large standard error for catechol can be partially explained by the fact that the seed populations were obtained from digesters maintained on different feed sources.

previous report of phenol conversion to methane (1). In addition, we believe that this is the first reported case of anaerobic degradation of catechol. Chemielowski et al. (1) and Clark and Fina (2) used enrichment conditions similar to those of this study, but observed no catechol degradation. Since our enrichment required 4.5 weeks before any evidence of degradation appeared, long acclimation periods appear to be necessary. The 3-week enrichment period used by Chemielowski et al. (1) may not have been sufficiently long. The source of the seed population may also affect the time necessary before the onset of degradation. For one case, in which we used a different inoculum source, 7 weeks was required before catechol decomposition began. The source of inoculum plus the needed long acclimation period may also account for the refractory nature of catechol reported by Clark and Fina (2).

The methanogenic biodegradability of these compounds indicates that aromatic ring compounds are not refractory under strict anaerobic conditions and that reductive pathways in heterotrophic bacteria exist for their decomposition.

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