APPLIED AND ENVIRONMENTAL MICROBIOLOGY, Feb. 1980, p. 436-444
0099-2240/80/02-0436/09$02.00/0

Vol. 39, No. 2

Methanogenic Decomposition of Ferulic Acid, a Model Lignin Derivative

J. B. HEALY, JR.,† L. Y. YOUNG,* AND M. REINHARD

Environmental Engineering and Science, Department of Civil Engineering, Stanford University, Stanford, California 94305

Ferulic acid, a model lignin derivative, was observed to be biodegradable to methane and carbon dioxide under strict anaerobic conditions. This conversion appears to be carried out by a consortium of bacteria similar to that previously described for the methanogenic degradation of benzoic acid. A temporary buildup of acetate in these cultures indicates that it is a likely intermediate and precursor for methane formation. An analog of coenzyme M, 2-bromoethanesulfonic acid (BESA), inhibited gas production and enhanced the buildup of propionate, butyrate, isobutyrate, and isovalerate. Phenylacetate, cinnamate, 3-phenylpropionate, benzoate, cyclohexane carboxylate, adipate, and pimelate were also detected in BESA-inhibited cultures. A pathway is proposed which includes these various acids as possible intermediates in the methanogenic degradation of ferulic acid. This model overlaps previously described benzoic acid degradation pathways, suggesting that this type of anaerobic degradation may be common for aromatic compounds.

Lignin is the second most abundant carbon source in the world, after cellulose. It is an aromatic polymer, and in agricultural and domestic wastes it presents a problem for biological waste treatment and solid waste disposal. During its biodegradation, presumably the complex polymer is broken down to simpler aromatic lignin derivatives, the fate of which is not well understood. In addition, unaltered lignin is generally thought to be refractory under anaerobic conditions (15). Several groups have studied the aerobic metabolism of these aromatic derivative (6, 8, 23). Relatively little attention, however, has been paid to the anaerobic degradation of these types of compounds. So far, the mechanism of the anaerobic degradation of only one aromatic compound, benzoic acid, has been investigated in detail (9-11, 35). The purpose of this investigation was to examine the anaerobic degradation of ferulic acid, an aromatic lignin derivative and a more complex compound than benzoic acid.

Early investigators approach the study of the anaerobic degradation of aromatics by using mixed populations of microorganisms which could ferment the substrates to methane and carbon dioxide (3, 5, 11, 34). Other investigators took a closer look, using isolated species capable of photosynthetic metabolism (7, 14, 28) and nitrate respiration (35, 38). Several reductive pathways have been proposed for the anaerobic degradation of benzoic acid (7, 14, 35, 38), and they cast doubt upon anaerobic pathways which were proposed to be similar to those of aerobic systems (24, 28).

A recent review article by Evans (9) points out the basic similarity among the probable pathways of benzoate degradation during photometabolism, nitrate respiration, and methanogenesis. Available evidence indicates that all three of these different forms of anaerobic metabolism involve ring reduction followed by ring cleavage and result in aliphatic acids. Further conversion of the aliphatic acids to end products depends upon the types of organisms and energy metabolism involved. For example, Ferry and Wolfe (10) identified acetate as a key intermediate in the overall conversion of benzoate to methane by a microbial consortium. For aromatic decomposition during methane production, multiple-species systems are necessary, since methane bacteria use a limited number of substrates and cannot use benzoate (10).

In methanogenic systems similar to that of Ferry and Wolfe (10), others have observed the decomposition of benzoate to yield cyclohexane carboxylate, cyclohex-1-enecarboxylate, heptanoate, valerate, propionate, and acetate (1, 19; C. L. Keith, Diss. Abstr. Int. B **33**:3214-3215, 1972). In addition, a different suite of intermediates, 2-hydroxycyclohexane carboxylate, 2-oxocyclohexane carboxylate, pimilate, caproate, and butyrate, has been reported by Shlomi et al.

† Present address: EAWAG, Swiss Federal Institute of Technology, CH-8800 Dübendorf, Switzerland.

(32). These findings suggest that the aliphatic acids resulting from ring cleavage are broken down into a mixture of simpler volatile acids before methane formation.

We present evidence that ferulic acid is decomposed to methane by a bacterial consortium in a manner similar to that for benzoate, i.e., initial ring reduction and then ring cleavage. In addition, the suggested pathway for degradation appears to overlap and merge into that described for benzoate.

MATERIALS AND METHODS

Culture methods. A serum bottle variation of the Hungate technique (22) was adapted for the maintenance of anaerobic cultures capable of degrading ferulic acid to methane. These mixed cultures were obtained from enrichments which were originally inoculated with seed from an anaerobic digester fed with sewage sludge. A description of the defined media and syringe techniques used with these serum bottle cultures is presented elsewhere (16, 17).

Stock cultures were maintained on 1,000 mg of ferulic acid per liter for more than 18 months by regular replacement of one-fifth of the culture volume with fresh, prereduced medium. Ferulic acid was the sole source of organic carbon in the feed. Substrate concentration was determined by ultraviolet absorbance of a centrifuged sample, diluted appropriately, and measured at 310 nm, a characteristic absorption wavelength for ferulic acid. Gas composition was determined with a Fischer-Hamilton gas partitioner equipped with a 0.5-ml syringe injection port.

Microscopic techniques. Microscopic observations were made on 250-ml serum bottle enrichment cultures, which had been maintained for 6 to 12 months as stock cultures. Observations were made on four different cultures which degraded ferulic acid, vanillic acid, catechol, and phenol.

Wet mounts were prepared from 0.1-ml samples removed from the serum bottles with 1.0-ml syringes and long (3.5 inches; about 8.75 cm) spinal needles. Three different types of samples were used: (i) samples from the turbid supernatant portion above the undisturbed FeS precipitate which rests on the bottom of the serum bottle, (ii) samples from the FeS precipitate, and (iii) samples from a culture which had been shaken into a homogeneous mixture. These different types of samples indicated locations and potential associations of organisms within the serum bottle culture.

A Zeiss phase-contrast microscope was used to observe the wet-mount preparations. Electron microscopy was used to obtain a closer look at the morphology of particular organisms in selected cultures; a model JEOL 100S transmission electron microscope operated at 80 kV was used. Sample preparation involved fixation in 2.5% formaldehyde, drying onto grids, and shadowing in a vacuum evaporator with gold-palladium (40:60) (40).

Cross-acclimations. Portions (7.5 ml) of a culture well-acclimated to ferulic acid were anaerobically transferred to a set of Hungate tubes (Bellco Glass, Inc., Vineland, N.J.). Then 2.5 ml of various substrates, each dissolved in prereduced medium, was added to each of the tubes. A control tube contained no substrate. The onset, rate, and extent of gas production in the tube containing ferulic acid were compared with the gas production characteristics in the other tubes.

Detection and identification of intermediates. Samples for gas chromatography of volatile fatty acids (C_1–C_7) were prepared by acidifying a 20-ml portion of culture with 2 drops of concentrated sulfuric acid and extracting this portion with 1 ml of ethyl ether. One microliter of this ether extract was injected onto a 6-ft (about 18.3-cm) glass column packed with 10% SP-1000/1% H_3PO_4 on 100/120 Chromosorb W AW (Supelco, Inc.). This column was connected to a hydrogen flame ionization detector in a Tracor MT 220 series gas chromatograph (GC) and was temperature programed to increase 15°C/min from an initial temperature of 115°C to a final temperature of 145°C. The helium carrier gas flow rate was 54 ml/min. Acetate levels in the culture medium were determined by comparing the peak heights of culture samples with those of an ether extract from a standard 0.01 M acetate solution.

To separate and detect higher-molecular-weight acid intermediates, it was necessary to convert the acids into their corresponding methyl esters. This esterification was carried out by adding a slight excess of diazomethane to acid/ether extracts of the culture fluid. Diazomethane was prepared with a diazomethane-generating kit (Aldrich Chemical Co., Milwaukee, Wis.; no. 10,159-1) which controlled the reaction of *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine with alkali.

Identifications of esterified ether extracts were performed by using a Finnigan 4000 gas chromatograph/mass spectrometer (GC/MS) computer system. The GC was equipped with a Grob-type injection system. A 50-m UCON HB 5100 glass capillary column (Jaeggi Laboratory, Trogen, Switzerland) with a 0.33-mm internal diameter was coupled directly to the MS. Helium was used as the carrier gas. Three microliters of the ether extract was injected splitless for 30 s onto the 35°C column (13), and after 4 min the temperature of the oven was increased by 3°C up to 180°C. The forepressure of the column was adjusted to 0.75 bar. Injector and interface temperatures were adjusted to 180°C and the ion source was heated to 250°C. Spectra were obtained by cyclic scanning at a rate of approximately 2 s/scan with an ionization voltage of 70 eV. Identifications of the unknowns were based on comparison with a library of known spectra.

Before analyzing the culture fluid of selected cultures with the above GC methods, we inhibited these cultures with 2-bromoethanesulfonic acid (BESA). The inhibitor was dissolved in prereduced defined medium. A range of concentrations (10^{-3} to 10^{-9} M) was examined to select a suitable one allowing the buildup of intermediates. As described in Results and Discussion, 10^{-4} M BESA was chosen as the most appropriate inhibitor concentration.

RESULTS AND DISCUSSION

Ferulic acid-degrading consortium. Under phase-contrast microscopy, short rods (2 by 0.6 μm), curved rods (15 by 0.6 μm), and very

long chains of rods (25 to 80 by 1 μm) comprised more than 90% of the organisms observed in a ferulic acid-acclimated culture. The very long chains were almost always associated with particulate matter and appeared to be sheathed. Occasionally, some of the small rods, which were almost oval in shape, exhibited very rapid motility. A similar mixture of morphological types was also observed in phenol- and catechol-acclimated cultures, although the cell numbers appeared to be much lower than those in the ferulic acid-acclimated cultures. Vanillic acid-acclimated cultures contained the same types of organisms, but few of the very long chains of rods were observed to be attached to the particulate matter. These observations suggest that a morphologically similar consortium of organisms is enriched from ordinary digester effluent regardless of the type of aromatic substrate available to the organisms.

Electron microscopy verified the above observations. Small rods, which appear to be covered with precipitate (Fig. 1A), and a thin curved rod (Fig. 1B) were found. The long chain of rods in Fig. 1B consisted of many small, fat rods (2 by 1 μm) with squared-off ends (Fig. 1C). Occasionally, one or more of these small rods left a gap either in the middle or on the end of the chain. The gaps were observed with phase contrast, but not with electron microscopy.

J. G. Ferry (personal observation and communication) confirmed that the three organism types closely resemble the three found in his benzoate-degrading consortium (10). The small rods are thought to be involved with the initial breakdown of the aromatic ring, whereas the thin curved rod is considered to be a methanogen. The long chains of rods have been observed in many methanogenic systems where acetate is a major compound. It has only recently been studied intensively by A. Zehnder (personal communication). His evidence indicates that this long chain of rods produces methane from acetate and grows extremely slowly. Acetate, which was important in benzoate degradation, also appeared to be an important intermediate in our ferulic acid enrichment. The presence of these acetate-utilizing methanogens, therefore, is not surprising.

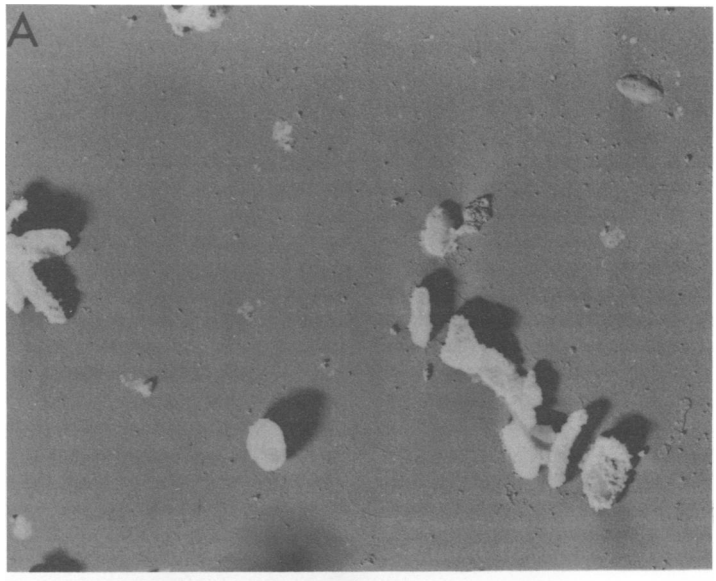
Cross-acclimations. Whether a ferulate-acclimated consortium could also utilize other aromatic substrates was addressed with cross-acclimation studies. Figure 2 presents typical results which compare gas production in identical Hungate tubes fed with different aromatic substrates. Both the control, fed no substrate, and the culture fed phenol produced very little gas. The immediate onset and similar rate and extent of gas production in cultures fed ferulic and cinnamic acids suggest that the ferulic acid culture was simultaneously acclimated to cinnamic acid, but not to phenol. A culture previously unacclimated to cinnamic acid would have required about 13 days before gas production was detectable (17).

Figure 3 summarizes the results obtained from ferulic acid cross-acclimation studies. Substrates to which the ferulic acid culture are simultaneously acclimated are shown in the right-hand columns. There is a degree of structural similarity among the aromatic compounds to which the culture is simultaneously acclimated. For example, ferulic acid and cinnamic acid both have identical propenoic side chains, whereas ferulic acid, vanillic acid, and vanillin all have a methoxy and hydroxyl group in the 3- and 4-positions, respectively, on the aromatic ring. These results suggest that cinnamic acid, vanillic acid, and vanillin may be intermediate ring structures in the degradation of ferulic acid to methane. This is further supported by the fact that neither the cinnamic acid-, vanillic acid-, nor vanillin-acclimated culture was simultaneously acclimated to the more complex ferulic acid compound. Acetate was also observed to be a likely intermediate, since it was readily utilized for gas production over a range of concentrations (100 to 300 mg/liter) (Fig. 4).

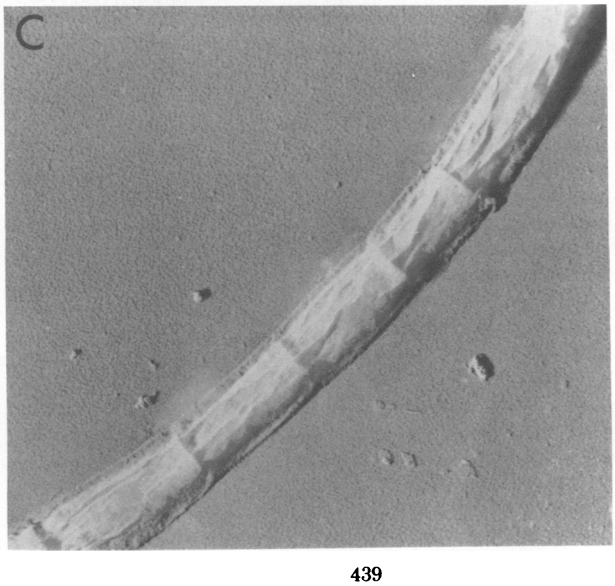
Identification of acetate as an intermediate. Acetate was confirmed as an intermediate with GC procedures. Figure 5 illustrates the temporal relationship of the conversion of substrate carbon to gas in a culture acclimated to 1,200 mg of ferulic acid per liter. Ferulic acid was completely degraded in 3 days, whereas gas production continued for another 8 to 10 days. Significant levels of acetate first appeared after gas production had begun. The levels of detectable acetate did not exceed about 1.5 mmol of carbon throughout most of the gas-producing period and are in the same range as those previously observed during benzoic acid decomposition (10). These data confirm the cross-acclimation results (Fig. 3 and 4) and support the presence of the acetate-utilizing methanogen observed in our consortium (Fig. 1C).

Detection of other volatile acids. Higher-molecular-weight, volatile-acid intermediates,

FIG. 1. *Predominant organisms found in the ferulic acid consortium. (A) Small, short rods, ~2 by 0.6 μm; most appear to be covered with precipitate (~×4,000). (B) Thin curved rod, ~15 by 0.6 μm (~×4,000). (C) Long chain of fat rods, ~2 by 1 μm (~×10,000).*


A


B


C

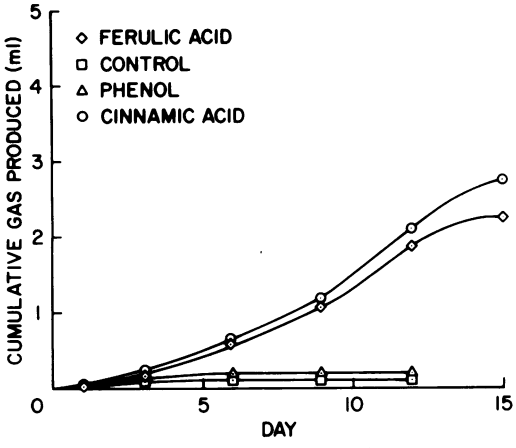


FIG. 2. *Cross-acclimation results for ferulic acid-acclimated consortium.*

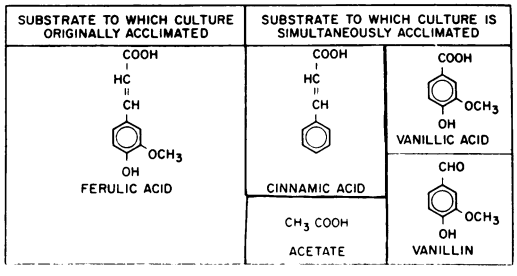


FIG. 3. *Summary of results from cross-acclimation studies with ferulic acid consortium.*

which may have been formed before acetate, were observed on an irregular basis in active cultures. Very low levels (near the detection limit) of both propionate and butyrate were detected by GC several times when much higher levels of acetate were observed. Their irregular appearance and extremely low concentrations may be due to their rapid turnover or conversion to other products. Consequently, in order to enhance the buildup of these volatile-acid intermediates to levels detectable by GC, BESA was chosen as a specific inhibitor of methane production. As a structural analog of coenzyme M (2-mercaptoethanesulfonic acid), BESA is thought to competitively inhibit the methyl transfer reaction at the terminal reductive step during methane formation in methanogens, using H_2-CO_2 (36). A recent report suggests that coenzyme M may also function in methane formation from acetate (33). If the pathway were successfully blocked, many of the intermediates formed before methane would increase to analytically detectable levels.

A range of BESA concentrations was tested for inhibition characteristics. The results show that gas production was inhibited over the concentration range from 10^{-3} to 10^{-9} M. Below 10^{-9} M, BESA appeared to have no effect as compared with a control culture which contained no inhibitor. Of the inhibitory concentrations, only 10^{-3} M BESA completely stopped methane production; greater than 40% of the gas produced at 10^{-5} and 10^{-7} M was methane. As a consequence, 10^{-4} M BESA was selected as a suitable concentration.

Propionate, butyrate, isobutyrate, and isovalerate were detected by GC in acid/ether extracts

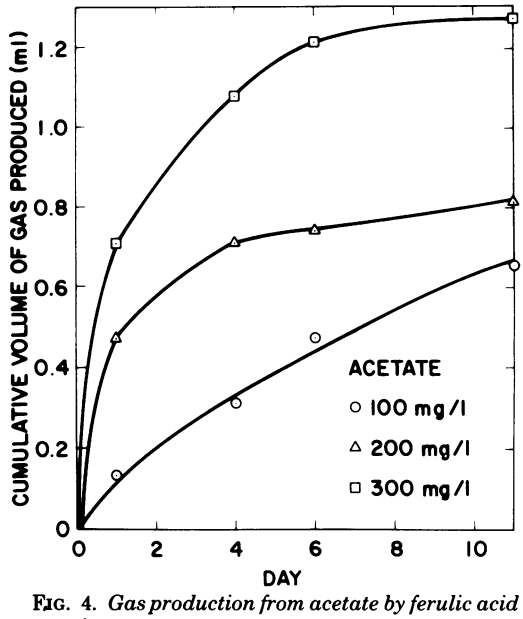


FIG. 4. *Gas production from acetate by ferulic acid consortium.*

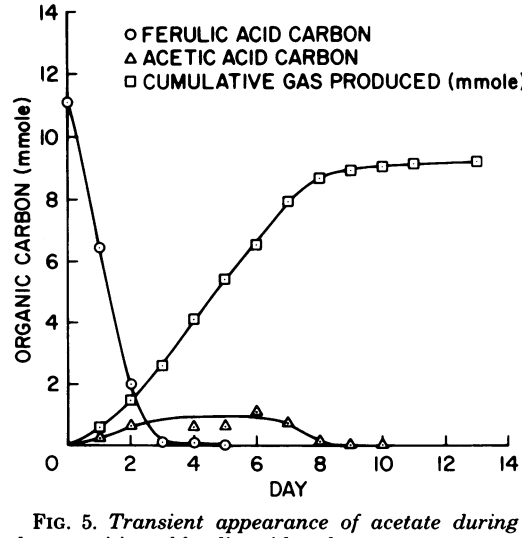


FIG. 5. *Transient appearance of acetate during decomposition of ferulic acid carbon to gas.*

of cultures inhibited with 10^{-4} M BESA. Figure 6 is an example of a GC recording with peaks for the above four volatile acids and acetate. Peaks were identified by comparison with the retention times exhibited by a standard mixture. The acetate peak was usually off-scale at the attenuation necessary to detect the other four volatile acids and, therefore, was quantitatively determined at a much higher attenuation during an additional sample injection.

The effect of BESA inhibition on active ferulic acid cultures is illustrated in Fig. 7A and 7B. Total gas production was less than 20% of that in an uninhibited culture, whereas acetate levels were elevated to concentrations almost four times higher than that in an uninhibited system (see Fig. 5) and did not decrease; this indicates that conversion of acetate to CO_2 and CH_4 was blocked. BESA, however, did not appear to affect the activity of the ring-cleaving member(s) of the consortium, since the added ferulic acid was completely utilized in the usual 4-day period. The four other compounds, propionic, isobutyric, butyric, and isovaleric acids, were detectable only in cultures inhibited with BESA (Fig. 7B), suggesting that when methane formation is not blocked, rapid conversion of these compounds takes place. The buildup of propionate and its subsequent disappearance when acetate buildup leveled off supports previous reports that propionate is readily converted to

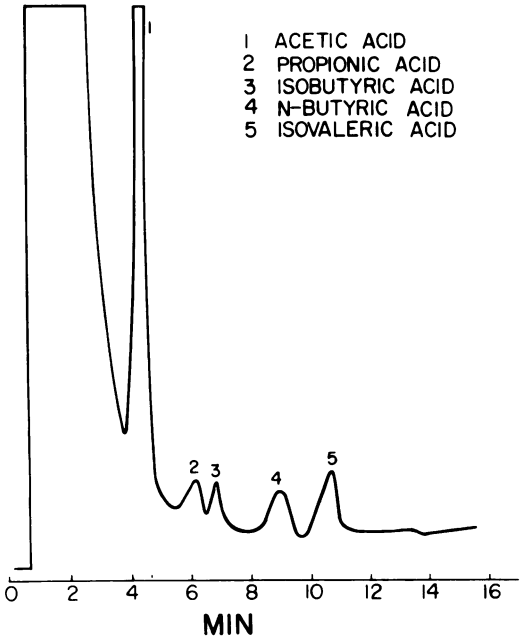


FIG. 6. *Gas chromatogram displaying volatile acids peaks detected in an active ferulic acid culture inhibited with 10^{-4} M BESA.*

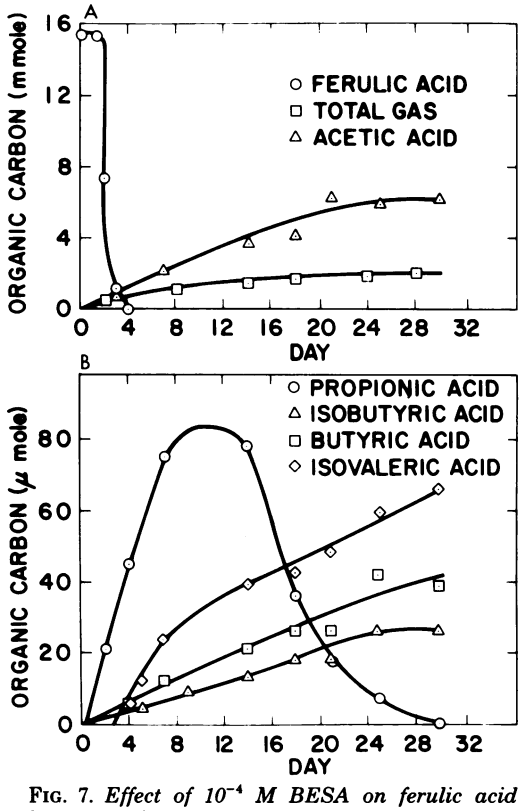


FIG. 7. *Effect of 10^{-4} M BESA on ferulic acid cultures. (A) Total gas production suppressed to 20% of normal activity; acetate levels increased to four times normal. (B) Other volatile acids; temporary increase and decline of propionic acid and appearance of isobutyric, butyric, and isovaleric acids.*

acetate and CO_2 (21), and that it is an intermediate in the degradation of benzoate (1). Butyrate has also been reported as an intermediate in benzoate degradation (9, 19, 32), whereas isobutyrate and isovalerate have not, suggesting that a pathway somewhat different from those previously reported (9, 19, 32) for benzoate may exist. At no time did hydrogen exceed 0.1% of the total gas produced, and it was, for the most part, not detectable.

Identification of higher-molecular-weight compounds. In addition to the buildup of volatile acids in BESA-inhibited cultures, ring compounds were detected in esterified acid/ether extracts by GC/MS techniques. Figure 8 represents a total ion chromatogram showing peaks for the methyl esters of cyclohexane carboxylate, benzoate, phenylacetate, and 3-phenylpropionate. These compounds were not detected in uninhibited cultures or in background controls. These particular samples were taken after inhibition for 24 days. Although levels of these ring compounds could not be accurately

determined at the time, the peak areas indicate relative concentrations. It is thought that 3-phenylpropionate, represented by the largest peak, was present in the BESA-inhibited culture at a concentration lower than 5 to 10 mg/liter.

In addition to the methyl esters of the above ring compounds, the methyl esters of both pimelic and adipic acids also were detected in samples taken after only 2 and 4 days of incubation both with and without BESA inhibitor. The levels of these compounds were two to three times higher in the inhibited samples and corresponded to roughly 10 mg/liter as estimated from external standards. The presence of other intermediates, such as hydroxylated aromatic or cyclohexane derivatives, could not be determined at the sensitivity of the method used.

Proposed pathways. Evans (9) summarized the known information on the anaerobic degradation of benzoate and proposed several similar pathways for its conversion to CH_4 and CO_2. Recent reports (19, 32) have added some detail to the list of intermediates occurring during benzoate catabolism. These pathways basically involve the initial reduction of the aromatic to a cyclohexane ring before ring cleavage. Adipate, pimelate, or heptanoate are then formed as ring fission products and subsequently converted into simple volatile acids. Formate, acetate, and hydrogen, immediate precursors of methane, are produced from these volatile acids (Fig. 9B).

Based on the data presented, Fig. 9 illustrates a tentative model describing the methanogenic degradation of ferulic acid. The previously reported benzoate pathways (9, 19, 32) are summarized in Fig. 9B; the suggested manner in which the ferulic acid is modified before entering into the benzoate scheme is illustrated in Fig. 9A. All the intermediates depicted in Fig. 9A have been observed. The presence of cinnamic acid was confirmed by GC/MS in later experiments, in which it was detected in both inhibited and uninhibited ferulic acid cultures 2 days after incubation. In addition, the detection of benzoate, cyclohexane carboxylate, adipate, propionate, and acetate in our ferulic acid cultures clearly supports the proposed overlap and merging of the ferulic acid degradation scheme with that described for benzoate.

The initial transformations of ferulic acid yielding phenylpropionate or cinnamate entail removal of the hydroxyl and methoxy ring substituents and reduction of the propenoic side chain. These types of transformations are known to occur in anaerobic systems. Dehydroxylation of aromatic ring derivatives has been documented by several investigators to take place in rumen and gut and has been shown to be mediated by the microorganisms in these systems (4, 25–27, 31). Similarly, demethylation is known to occur in these same systems (30, 31). Furthermore, in such reducing environments, saturation of the double bond in an aliphatic side chain has been observed to rapidly occur (18, 37; J. T. O'Rourke, Ph.D. thesis, Stanford University, Stanford, Calif., 1968). Cinnamate can be formed first, if removal of the ring substituents occurs before the side chain reduction. However, formation of phenylpropionate can precede formation of cinnamate if side chain saturation occurs first. Cinnamate could then result as an intermediate during the β-oxidation of phenylpropionate yielding benzoate and acetate. Our procedure does not permit us to distinguish which intermediate occurs first. Balba and Evans, however, recently reported on the latter sequence of events, that is, the β-oxidation of phenylpropionate to cinnamate, benzoate, and acetate (2). Since a consortium of microorganisms is involved, it is possible that several path-

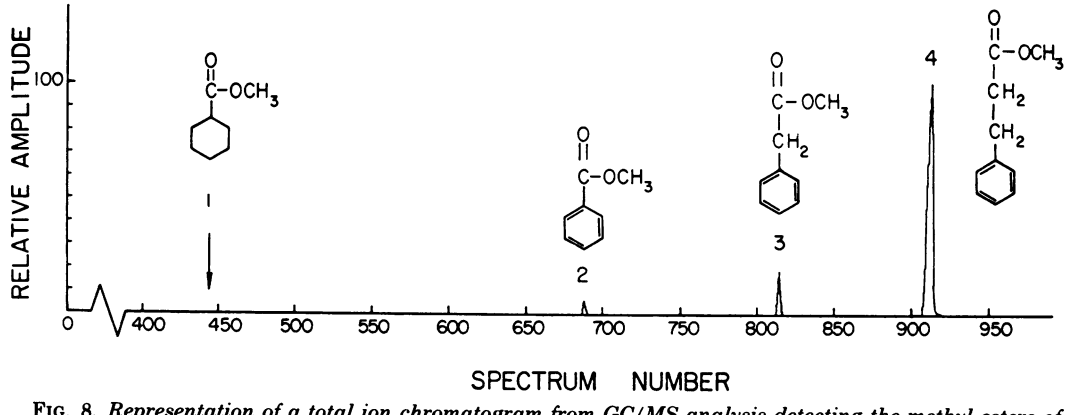


FIG. 8. *Representation of a total ion chromatogram from GC/MS analysis detecting the methyl esters of (1) cyclohexane carboxylate, (2) benzoate, (3) phenylacetate, and (4) 3-phenylpropionate.*

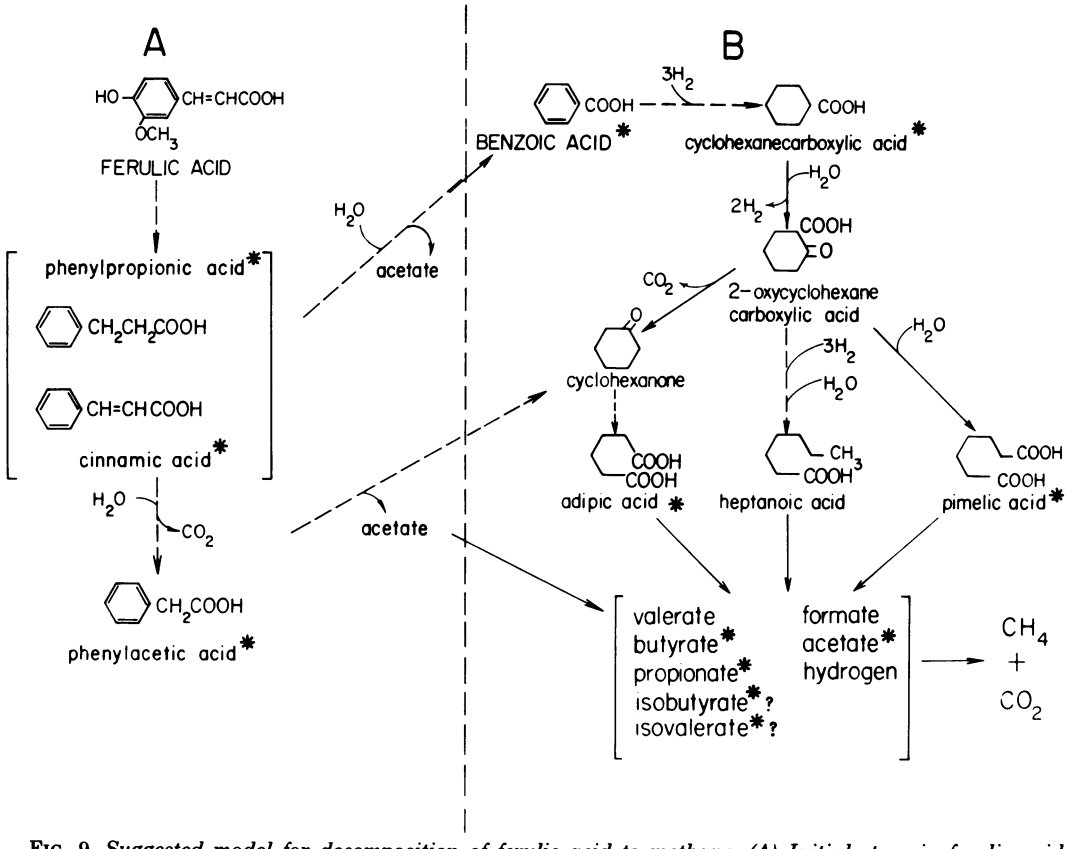


FIG. 9. *Suggested model for decomposition of ferulic acid to methane. (A) Initial steps in ferulic acid decomposition. (B) Decomposition of benzoic acid to methane, summarized from Evans (9). Asterisk denotes intermediates observed in our enrichments.*

ways may contribute to the observed reaction products and intermediates.

Another observed intermediate, phenylacetate, may result by the addition of water across the double bond in cinnamate, decarboxylation, and subsequent removal of hydrogen. Its formation from phenylpropionate by α-oxidation is not likely, since this requires molecular oxygen. There is evidence that a decarboxylation can occur. Ribbons and Evans (29) have reported on an aromatic decarboxylase which can operate anaerobically. In addition, an anaerobic decarboxylase from *Aerobacter* which uses hydroxycinnamic acids as substrates has also been described (12). A similar system, therefore, may be functioning here. The subsequent fate of phenylacetate has been reported as ring reduction, release of acetate, and production of cyclohexanone (2). It therefore appears to enter the known benzoate pathway at a different point than the other observed intermediates.

The detection of both adipate and pimelate suggests that these enrichment cultures may be using more than one of the already described pathways (9, 19, 32) to produce the volatile acids. For our ferulic acid system, further confirmation of these pathways would require identification of other intermediates.

In summary, the model lignin derivative, ferulic acid, is degradable to methane and carbon dioxide by a consortium of bacteria under methanogenic conditions. When viewed as a whole, the results as shown in a conceptual model (Fig. 9) suggest that the methanogenic degradation of ferulic acid occurs in a manner similar to that of benzoic acid, namely, reduction of the aromatic to a cyclohexane ring before ring fission, conversion of the products into simple volatile acids, and subsequent formation of acetate as a primary substrate for methane formation. It is important to note that the observed intermediates clearly show the model to merge with the benzoate pathways. The fate of methoxybenzoate compounds under similar conditions has also shown this to be the case, that is, a merging with the described benzoate pathways (W. C. Evans,

personal communication). Consequently, it is likely that other aromatic derivatives can also be converted in a similar manner. This implies that (i) such anaerobic degradation mechanisms may be common for aromatic compounds, and (ii) aromatic derivatives can be a likely source of methane.

ACKNOWLEDGMENTS

We thank J. G. Ferry for his advice, W. C. Evans for his helpful suggestions, and P. J. Chapman for reviewing the original manuscript.

This work was supported by a grant from the Department of Energy, Fuels from Biomass Program.

LITERATURE CITED

1. **Balba, M. T., and W. C. Evans.** 1977. The methanogenic fermentation of aromatic substrates. Biochem. Soc. Trans. **5**:302-304.
2. **Balba, M. T., and W. C. Evans.** 1979. The methanogenic fermentation of ω-phenyl alkane carboxylic acids. Biochem. Soc. Trans. **7**:403-405.
3. **Barker, H. A.** 1956. Biological formation of methane, p. 3-44. *In* Bacterial fermentations. John Wiley and Sons, New York.
4. **Booth, A. N., and R. T. Williams.** 1963. Dehydroxylation of catechol acids by intestinal contents. Biochem. J. **88**:66.
5. **Clark, F. M., and L. R. Fina.** 1952. The anaerobic decomposition of benzoic acid during methane fermentation. Arch. Biochem. **36**:26-32.
6. **Dagley, S.** 1967. The microbial metabolism of phenolics, p. 287-317. *In* A. D. McLaren and G. H. Peterson (ed.), Soil biochemistry. Edward Arnold, London.
7. **Dutton, P. L., and W. C. Evans.** 1969. The metabolism of aromatic compounds by *Rhodopseudomonas palustris*. Biochem. J. **113**:525-536.
8. **Evans, W. C.** 1963. The microbiological degradation of aromatic compounds. J. Gen. Microbiol. **32**:177-184.
9. **Evans, W. C.** 1977. Biochemistry of the bacterial catabolism of aromatic compounds in anaerobic environments. Nature (London) **270**:17-22.
10. **Ferry, J. G., and R. S. Wolfe.** 1976. Anaerobic degradation of benzoate to methane by a microbial consortium. Arch. Microbiol. **107**:33-40.
11. **Fina, L. R., and A. M. Fiskin.** 1960. The anaerobic decomposition of benzoic acid during methane fermentation. I. Fate of carbons one and seven. Arch. Biochem. Biophys. **91**:163-165.
12. **Finkle, B., J. C. Lewis, J. W. Corse, and R. E. Lundin.** 1962. Enzyme reactions with phenolic compounds: formation of hydroxystyrenes through the decarboxylation of 4-hydroxycinnamic acids by *Aerobacter*. J. Biol. Chem. **237**:2926-2931.
13. **Grob, K., and K. Grob, Jr.** 1978. Splitless injection and the solvent effect. J. High Resol. Chromatogr. Chromatogr. Commun. **1**:57-64.
14. **Guyer, M., and G. Hegeman.** 1969. Evidence for a reductive pathway for the anaerobic metabolism of benzoate. J. Bacteriol. **99**:906-907.
15. **Hackett, W. F., W. J. Connors, T. K. Kirk, and J. G. Zeikus.** 1977. Microbial decomposition of synthetic ^{14}C-labeled lignins in nature: lignin biodegradation in a variety of natural materials. Appl. Environ. Microbiol. **33**:43-51.
16. **Healy, J. B., Jr., and L. Y. Young.** 1978. Catechol and phenol degradation by a methanogenic population of bacteria. Appl. Environ. Microbiol. **35**:216-218.
17. **Healy, J. B., Jr., and L. Y. Young.** 1979. Anaerobic biodegradation of eleven aromatic compounds to methane. Appl. Environ. Microbiol. **38**:84-89.
18. **Heukelekian, H., and P. Mueller.** 1958. Transformation of some lipids in anaerobic sludge digestion. Sewage Ind. Wastes **30**:1108-1120.
19. **Keith, C. L., R. L. Bridges, L. R. Fina, K. L. Iverson, and J. A. Cloram.** 1978. The anaerobic decomposition of benzoic acid during methane fermentation. IV. Dearomatization of the ring and volatile fatty acids formed on ring rupture. Arch. Microbiol. **118**:173-176.
20. **Martin, J. P., and K. Haider.** 1976. Decomposition of specifically ^{14}C-labelled ferulic acid: free and linked into model humic acid type polymers. J. Soil Sci. Soc. Am. **40**:377-380.
21. **McCarty, P. L., J. S. Jeris, and W. Murdock.** 1963. Individual volatile acids in anaerobic treatment. J. Water Pollut. Control Fed. **35**:1501-1516.
22. **Miller, T. C., and M. J. Wolin.** 1974. A serum bottle modification of the Hungate technique for cultivating obligate anaerobes. Appl. Microbiol. **27**:985-987.
23. **Ornston, L. N., and R. Y. Stanier.** 1964. Mechanism of β-ketoadipate formation by bacteria. Nature (London) **204**:1279-1283.
24. **Oshima, T.** 1965. On the anaerobic metabolism of aromatic compounds in the presence of nitrate by soil microorganisms. Z. Allg. Mikrobiol. **5**:386-394.
25. **Peppercorn, M., and P. Goldman.** 1971. Caffeic acid metabolism by bacteria of the human gastrointestinal tract. J. Bacteriol. **108**:996-1000.
26. **Peppercorn, M., and P. Goldman.** 1972. Caffeic acid metabolism by gnotobiotic rats and their intestinal bacteria. Proc. Natl. Acad. Sci. U.S.A. **69**:1413-1415.
27. **Perez-Silva, G., D. Rodriguez, and J. Perez-Silva.** 1966. Dehydroxylation of caffeic acid by a bacterium isolated from rat feces. Nature (London) **212**:303-304.
28. **Proctor, M. H., and S. Scher.** 1960. Decomposition of benzoate by a photosynthetic bacterium. Biochem. J. **76**:33.
29. **Ribbons, D. W., and W. C. Evans.** 1960. Oxidative metabolism of phthalic acid by soil pseudomonads. Biochem. J. **76**:310-318.
30. **Scheline, R. R.** 1966. Decarboxylation and demethylation of some phenolic benzoic acid derivatives by rat caecal contents. J. Pharm. Pharmacol. **18**:664-669.
31. **Scheline, R. R.** 1968. Metabolism of phenolic acids by the rat intestinal microflora. Acta Pharmacol. Toxicol. **26**: 189-205.
32. **Shlomi, E. R., A. Lankhorst, and R. A. Prins.** 1978. Methanogenic fermentation of benzoate in an enrichment culture. Microb. Ecol. **4**:249-261.
33. **Smith, M. R., and R. A. Mah.** 1978. Growth and methanogenesis by *Methanosarcina* strain 227 on acetate and methanol. Appl. Environ. Microbiol. **36**:870-879.
34. **Tarvin, D., and A. M. Buswell.** 1934. The methane fermentation of organic acids and carbohydrates. J. Am. Chem. Soc. **56**:1751-1755.
35. **Taylor, B. F., W. L. Campbell, and I. Chinoy.** 1970. Anaerobic degradation of the benzene nucleus by a facultative anaerobic microorganism. J. Bacteriol. **102**: 430-437.
36. **Taylor, C. D., and R. S. Wolfe.** 1974. Structure and methylation of coenzyme M ($HSCH_2CH_2SO_3$). J. Biol. Chem. **249**:4879-4885.
37. **Wilde, P. F., and R. M. C. Dawson.** 1966. The biohydrogenation of α-linoleic acid and oleic acid by rumen microorganisms. Biochem. J. **98**:469-475.
38. **Williams, R. J., and W. C. Evans.** 1975. The metabolism of benzoate by *Moraxella* species through anaerobic nitrate respirations: evidence for a reductive pathway. Biochem. J. **148**:1-10.
39. **Williams, R. T.** 1964. Metabolism of phenolics in animals, p. 205-248. *In* J. B. Harbonre (ed.), Biochemistry of phenolic compounds. Academic Press Inc., New York.
40. **Young, L. Y.** 1978. Bacterioneuston examined with critical point drying and transmission electron microscopy. Microbiol. Ecol. **4**:276-277.