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MICROBIAL PRODUCTION OF METHANE FROM WOOD AND INHIBITION BY ETHANOL EXTRACTS OF WOOD[1]

RONALD MINK[2] and PATRICK R. DUGAN, Department of Microbiology, Ohio State University, Columbus, OH 43210

Abstract. Mixed cultures of anaerobic bacteria fermented both coniferous and deciduous wood sawdust, with concomitant methane production. A consistently greater lag in methanogenesis was observed on coniferous as compared to deciduous wood. Arabinose, glucose, galactose, mannose, rhamnose and xylose when added to enrichment cultures had either no effect or a slight stimulation of CH_4 production in the absence of added methane precursors (acetate, formate, CO_2, H_2). In the presence of added acetate, formate, CO_2 and H_2; arabinose, rhamnose and xylose appeared to stimulate mixed culture methanogenesis; whereas, xylose retarded methanogenesis in pure cultures of *Methanobacterium formicicum*. Alcohol extracts of either deciduous or coniferous wood were inhibitory to methanogenesis from either mixed cultures or from *M. formicicum*. The greater amount of alcohol extractives in coniferous wood may explain the greater lag in methanogenesis when compared to that of deciduous wood.

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The production of CH_4 from the microbial fermentation of wood and other cellulosic materials observed since the last century suggested that cellulosic waste might become a minor alternative energy source through the microbial production of methane (Maugh 1972). Knowledge of the biological ecosystem responsible for fermentation of wood to methane is limited. Our report investigated the role of 2 types of wood as ultimate sources of methane in a mixed culture system and the influence of wood fractions on the ultimate genesis of methane in both the mixed culture system and pure cultures of methanogenic bacteria.

MATERIALS AND METHODS

Cultures

A mixed cellulolytic culture was obtained from a carboy of fermenting deciduous sawdust that was actively producing CH_4. This system was originally inoculated both with fresh sheep rumen fluid and with a sample of actively fermenting sawdust from the natural environment. Mixed cultures were enriched by successive transfers of the culture in the **MS medium** of Ferry *et al* (1974). *Methanobacterium formicicum* strain JF was obtained from R. S. Wolfe, University of Illinois, Urbana. Cultures were maintained in **MS medium** either in broth or on agar slants under $H_2 + CO_2$ (4:1). We estimated relative numbers of methanogenic bacteria by CH_4 formation as determined by gas chromatography and by fluorescent microscopy (Mink and Dugan 1977).

Culture Medium

The growth medium used for maintenance of all cultures and for experiments described later, was based on that devised by Ferry *et al* (1974).

MS medium

Distilled H_2O	840.0 ml
Mineral Solution I	37.5 ml
Mineral Solution II	37.5 ml
Yeast Extract (Difco)	2.0 g
Typticase (BBL)	2.0 g
Sodium Formate	3.0 g
Sodium Acetate	1.5 g
0.1% Resazurin Solution	1.0 ml
Vitamin Solution	10.0 ml
Mineral Elixir	10.0 ml
8% Na_2CO_3 Solution	25.0 ml
$FeSO_4$ Solution	2.5 ml
1 N HCl	14.0 ml
Reducing Agent	40.0 ml

When solid medium was required, 20 gm of agar was added to the above medium.

Mineral Solution I

K_2HPO_4	6.0 g
Distilled H_2O	to 1.0 liter

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[2]Present address: Dept. of Dairy Science, Univ. of Illinois, Urbana, IL 61801.

Mineral Solution II

KH_2PO_4	6.0 g
$(NH_4)_2SO_4$	6.0 g
NaCl	12.0 g
$MgSO_4 \cdot 7H_2O$	2.4 g
$CaCl_2 \cdot 2H_2O$	1.6 g
Distilled H_2O	to 1.0 liter

Vitamin Solution

Biotin	2.0 mg
Folic Acid	2.0 mg
Pyridoxine HCl	10.0 mg
Thiamine HCl	5.0 mg
Riboflavin	5.0 mg
Nicotinic Acid	5.0 mg
DL Ca Pantothenate	5.0 mg
Cyanocobalamin	0.1 mg
p-Aminobenzoic Acid	5.0 mg
Distilled Water	to 1.0 liter

Mineral Elixir

Nitrilotriacetic Acid	1.5 g
$MgSO_4 \cdot 7H_2O$	3.0 g
$MnSO_4 \cdot H_2O$	0.5 g
NaCl	1.0 g
$FeSO_4 \cdot 7H_2O$	0.1 g
$CoCl_2 \cdot 6H_2O$	0.2 g
$CaCl_2 \cdot 2H_2O$	0.1 g
$ZnSO_4 \cdot 7H_2O$	0.2 g
$CuSO_5 \cdot 5H_2O$	0.01 g
$AlK(SO_4)_2$	0.01 g
H_3BO_3	0.01 g
$Na_2MoO_4 \cdot 2H_2O$	0.01 g
Distilled H_2O	to 1.0 liter

The stock solution of $FeSO_4$ was prepared by adding 2 g $FeSO_4 \cdot 7H_2O$ to one liter of distilled water. Additionally, 3 drops of concentrated HCl were added to keep the $FeSO_4$ reduced. The reducing agent was prepared as follows: (185 ml) distilled water was boiled under 100% nitrogen (Liquid Carbonic, Chicago, Ill.) which has been passed through a copper column. Subsequently, 13.4 ml of 3 N NaOH was added, the solution was cooled and 2.5 g cysteine HCl and 2.5 g $Na_2S \cdot 9H_2O$ were added.

Wood Fermentation Systems

Four 20 ℓ glass carboys were set up as follows:

Carboy A contained the equivalent of 1000 g dry weight of coniferous wood sawdust (mixture of pine and fir) in 20 ℓ water.
Carboy B also contained the equivalent of 1000 g dry weight of coniferous wood sawdust in 20 ℓ water, with chloroform added at a concentration of 25 μM.
Carboy C contained the equivalent of 1000 g dry weight of deciduous wood sawdust (predominantly oak) in 20 ℓ water, with chloroform added at a concentration of 25 μM.
Carboy D contained 1000 g deciduous sawdust without chloroform.

Twenty g of NH_4Cl was added to each carboy as a supplemental nitrogen source. Dry weight of the sawdust was determined after drying 4 representative samples of the sawdust for 6 hr in an oven at 105 °C (Schorger 1917). We then added the calculated wet weight of wood corresponding to the desired dry weight to each carboy. Chloroform was used to inhibit the reduction of carbon dioxide to methane (Bauchop 1967).

We adjusted carboys to a pH of 7 with 1 N NaOH and allowed them to equilibrate for a period of 48 hr, with the pH routinely checked and adjusted as needed. After 48 hr, the carboys were inoculated with 50 ml of the mixed cellulolytic culture plus 50 ml of fresh sheep rumen fluid. The carboys were then sealed with a rubber stopper through which a tube ran to an inverted graduated cylinder filled with water used for gas collection and quantitation. Gas headspace in the carboys was determined beforehand by water volume displacement. Samples for CO_2 and CH_4 analyses were taken by means of a septum-capped T tube between the carboy and the collection cylinders and routinely assayed by gas chromatography over an 8-week period.

Inhibition and Stimulation of Methanogenesis by Carbohydrates

D-glucose, d-galactose, d-mannose, ℓ-arabinose, d-xylose, ℓ-rhamnose and cellobiose were investigated in terms of their ability to inhibit or stimulate methanogenesis in mixed, enriched and pure cultures of methanogenic bacteria. Carbohydrate-supplemented media were prepared by adding 30 mg of carbohydrate to 5 ml of MS medium. When 1 ml of inoculum was added, the carbohydrate was at a final concentration of 0.5%. Studies using the mixed cultures obtained directly from fermenting sawdust employed the MS culture medium in which the appropriate carbohydrate was substituted for acetate and formate. Mixed cultures were maintained at 20 °C under a 1:4 CO_2:N_2 gas mixture. Five mls of the medium dispensed in Hungate tubes were inoculated by the syringe variation of the technique of Macy *et al* (1972) with 1 ml of mixed culture inoculum. Mixed culture tubes were not gassed again after the initial gassing with CO_2+N_2. The mixed culture was used for inhibition and stimulation studies with complete MS medium prepared with the appropriate carbohydrate. Five ml of the medium was dispensed into Hungate tubes, and 1 ml of a 48 hr enrichment culture was added by the syringe method. These cultures were incubated at 20 °C and maintained and gassed every 24 hr with a CO_2+H_2 gas mixture. Pure culture medium was prepared as described for the mixed culture tubes, and 5 ml of medium was inoculated with 1 ml of a 96 hr culture of *M. formicicum* using a modification of the Macy technique (Bryant 1972) and incubated at 37 °C under $CO_2 + H_2$ and gassed daily. In all experiments 5 replicates of each carbohydrate as well as carbohydrate-free controls were prepared. Mixed cultures were assayed for methane by gas chromatography 4 days and 6 days post inoculation.

M. formicicum cultures were assayed for methane at 1, 4, and 6 days post-inoculation. Due to an apparent inhibition of methanogenesis observed from *M. formicicum* by xylose, 10 replicates each of xylose supplemented pure cultures and carbohydrate-free controls were

inoculated with 72 hr cultures and assayed for methane daily for 7 days post-inoculation.

Extraction of Wood

Extracts of wood were prepared by the procedure of Kurth (1939) in which 20 g of air-dried (20 °C, 48 hr) wood samples were steam distilled for 1.25 hr then successively extracted for 8 hr each with ethyl ether, methanol and water in a 500 ml Soxhlet extractor. The wood residue was air-dried at 55 °C between each extraction. Wood residue from the final water extraction was dried in an oven at 105 °C and stored in a dessicator until used. The steam-distilled fractions contained few apparent extractives and were not retained for study. The ether, alcohol, and water extractives in their respective solvents were allowed to evaporate to a volume of 100 ml. We determined the dry weights by placing 2 replicates of 1 ml samples in tared aluminum pans. Water extraction samples were dried at 250 °C, alcohol extraction samples at 105 °C, and ether extraction samples at 55 °C. The dry weight of the wood residue was calculated by difference and is referred to as the lignocellulose fraction (see table 1).

TABLE 1

Dry weight (g) of wood fractions per gram of wood.

Fraction	Deciduous	Coniferous
Total Wood Wt.	1.0000	1.0000
Ether Soluble	0.0370	0.0305
Alcohol Soluble	0.0038	0.0485
Water Soluble	0.0160	0.0175
Lignocellulose	0.9432	0.9035

Inhibition and Stimulation of Methanogenesis by Wood Fractions

Complete MS medium (5 ml) was dispensed along with wood fractions (in the amounts listed in table 2) into Hungate tubes using the techniques described previously. Wood fraction concentrations were in proportion to the amounts extracted from either coniferous or deciduous wood with 5 replicates of each fraction as well as fraction-free controls. Inoculations (1 ml) were made by the syringe method with a 48 hr mixed culture. Pure culture tubes containing wood fractions (in the amounts listed in table 2) were prepared using the Bryant (1972) technique and inoculated with a 96-hr culture of *M. formicicum*. All tubes were gassed daily with $H_2 + CO_2$ (4:1) and incubated at 20 °C. Tubes were assayed for CH_4 by gas chromatography on days 4 and 6 post-inoculation.

Gas Analysis

The wood fermentation systems were assayed for CO_2 and CH_4 with a Carle 8004 gas chromatograph equipped with thermistor detectors and a ⅛-inch by 8 ft stainless steel column packed with 30/60 mesh silica gel. The column temperature was 80 °C. High Purity Grade Helium (Matheson Gas Products, East Rutherford, NJ) was used as the carrier gas at a flow rate of approximately 12 ml/min and results were recorded with a Linear Instruments Model 252A recorder equipped with an electronic integrator.

All other gas assays were done on a Varian Aerograph 200 gas chromatograph equipped with a hydrogen flame ionization detector and a ⅛-inch by 5-ft glass column packed with 30/60 mesh silica gel, maintained at a temperature of 30 °C. High Purity Grade Helium (first passed through molecular sieve 5A) served as the carrier gas with a flow rate of approximately 22 ml/min. Hydrogen for the detector was provided by an Aerograph generator at a flow rate of 25 ml/min. The recorder used in conjunction with this gas chromatograph was a Westronics Model LD11A.

Gas samples were collected with either a 50 µl or 100 µl gas-tight syringe (Precision Sampling, Baton Rouge, LA). Gas peaks obtained from the chromatographs were quantitated by comparing them to standards containing a known quantity of the appropriate gas.

RESULTS AND DISCUSSION

Wood Fermentation Systems

The effect of the chloroform on the inhibition of methanogenesis in the carboys containing both deciduous and coniferous wood was readily apparent (fig. 1). Both of the chloroform-free systems produced methane, but the lag period was approximately 2 weeks longer in the coniferous wood system. During the lag periods in both of these systems, types

TABLE 2

Weight of wood fractions per tube with MS medium.

Fraction		Mixed Culture		*M. formicicum* Culture	
		Deciduous	Coniferous	Deciduous	Coniferous
Ether soluble	(mg)	44.4	36.6	36.6	36.6
Alcohol soluble	(mg)	4.5	58.2	58.2	58.2
Water Soluble	(mg)	19.2	21.0	21.0	21.0
Lignocellulose	(g)	1.13	1.08	1.08	1.08

and amounts of gas almost identical to the chloroform-inhibited controls were produced, *i.e.*, the methane precursors, CO_2 and H_2, although the H_2 gas was not quantitated. A tendency existed for gas pockets to be trapped in the sawdust and not immediately released into the carboy headspace, although the carboys were physically agitated prior to sampling. This tendency did not significantly affect long term results since the gas was eventually released.

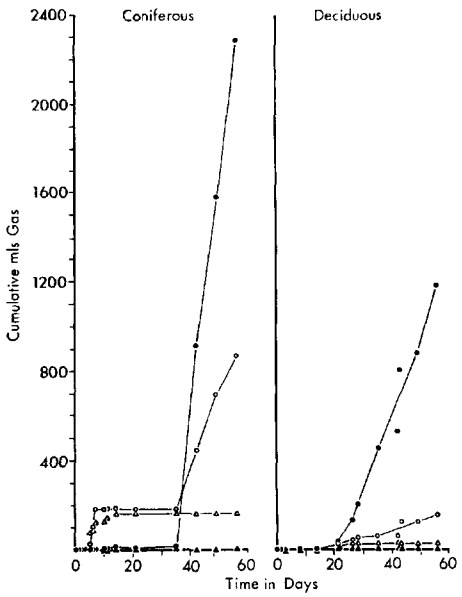

FIGURE 1. Cumulative gas production in coniferous and deciduous wood fermentation systems. • CH_4 production in $CHCl_3$-free systems; ○ CO_2 production in $CHCl_3$-free systems; ▲ CH_4 production in $CHCl_3$-inhibited systems; △ CO_2 production in $CHCl_3$-inhibited systems.

Inhibition and Stimulation of Methanogenesis by Carbohydrates

An initial study of the effect of wood components on bacterial methanogenesis involved addition of carbohydrates, known to be components of holocellulose, to the mixed cellulolytic culture obtained from fermenting sawdust. These data are shown in table 3 as total mls of methane accumulated in test tubes after 4 days and 6 days, and the final pH of the tubes after 6 days. Since no H_2, acetate or formate was provided in these tubes, all of the H_2 used for methane production ultimately came from the carbohydrates or other organics in the growth medium.

TABLE 3

Cumulative methane production by mixed culture in carbohydrate-supplemented MS medium without formate and acetate, under $CO_2 + N_2$.

Carbohydrate	Day 4	Day 6	Final pH (Day 6)
Arabinose	92±18*	103±13	5.34
Cellobiose	95±23	97±10	5.21
Galactose	81±20	119±25	5.06
Glucose	58± 6	85± 9	5.37
Mannose	62±15	91±15	5.53
Rhamnose	87±25	99±13	5.54
Xylose	68±13	104±11	5.26
Control**	70± 9	70±12	6.75

*µl/tube (n=5) mean ± SD.
**No carbohydrate.

The daily methane production from *M. formicicum* in xylose-supplemented medium is presented in figure 2. The average pH of the xylose-supplemented tubes after 7 days was 7.33 compared to 7.4 for unsupplemented controls. The average cell dry weight was 0.880 mg (±.407 S.D.) in the presence of xylose and 2.575 mg (±.661 S.D.) in the absence of xylose.

In all the systems studied, one would expect the rate of methane production to increase with time due to continued growth of the microorganisms. In many cases this did not occur, possibly because the heavy inoculum used as a precaution against oxygen contamination had rapidly depleted the formate and acetate substrates in the medium. Thus, after 1 or 2 days, the sole source of methane was the CO_2 and H_2 provided daily. Since less total substrate was avilable, less methane was produced.

The observations of gas production from the wood fermentation systems (figure 1) are interesting because of the high degree of similarity between the chloroform-free systems and the chloroform controls during the initial incubation periods. Since chloroform is known to inhibit the reduction of carbon dioxide to methane and consequently to cause

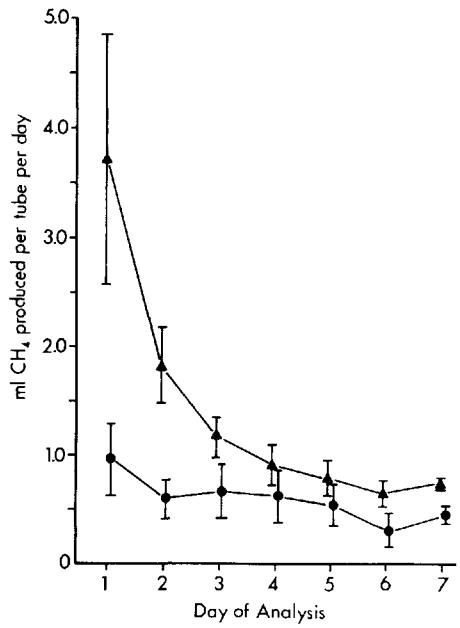

FIGURE 2. Daily CH_4 production from *M. formicicum* cultures in xylose-supplemented MS medium ●; unsupplemented controls ▲. Each point is the mean of 10 replicates.

the accumulation of CO_2, the same initial results in the chloroform-free systems suggest an inhibition by wood at this stage of the fermentation. If the source of inhibition could be determined, subsequent removal of that inhibitor might allow immediate production of methane to occur. Alternatively, these inhibitors may have utility by allowing the accumulation of low molecular weight organic by-products formed during the first stage of fermentation (*i.e.*, alcohols or acids) by preventing their conversion during a second stage of fermentation to methane.

We investigated the carbohydrates known to be wood components as a possible source of this inhibition. The first carbohydrate study, which used a mixed cellulolytic culture (table 3), did not establish a clear preference of any carbohydrate as an ultimate hydrogen source. The methane production was more likely limited by the drop in pH in the systems than by the lack of substrate. This same problem was also encountered with the enrichment culture experiment in carbohydrate-supplemented media (table 4). Arabinose, rhamnose, xylose and to a lesser degree, galactose, appeared quite stimulatory to methane production after 4 days. Although this response might result from a pH effect, (it is noted that these cultures also had the least shift in pH after 6 days) some of these sugars apparently ultimately served as methanogenic substrates because daily methane production was significantly higher than the control in some cases (*i.e.*, arabinose and rhamnose at day 4).

TABLE 4

Methane production by mixed culture in carbohydrate-supplemented MS medium (complete) under $CO_2 + H_2$.

Carbohydrate	Day 4	Day 6	Final pH Day 6
Arabinose	748±262*	406± 82	6.54
Cellobiose	156± 51	42± 30	5.71
Galactose	269± 72	191± 35	6.42
Glucose	11± 35	24± 31	5.53
Mannose	171±103	30± 17	5.83
Rhamnose	524±191	492± 57	6.57
Xylose	406±106	171± 20	6.43
Control**	227± 74	476±277	7.21

*μl/day (n=5) mean ± S.D.
**No carbohydrate.

The influence of holocellulose carbohydrates on methane production by *M. formicicum* (table 5) indicated that these carbohydrates generally have no effect on methanogenesis. The one exception appears to be xylose, which exhibited some inhibitory effect on methane production. This phenomenon was verified by the daily analysis of a xylose-supplemented system (fig. 2). The differences in methane production may be attributed wholly or in part to the differences in growth between the control tubes and the xylose-supplemented tubes.

The results of the experiments carried out with mixed cultures in growth medium supplemented with the dried wood fractions (table 6) were fairly consistent with the observations made on the wood fermentation systems. Generally, less methane was produced in the presence of deciduous wood fractions. The ether-soluble and particularly the alcohol-

TABLE 5
Methane production by M. formicicum in carbohydrate-supplemented MS medium (complete) under $CO_2 + H_2$.

Carbohydrate	Day		
	1	4	6
Arabinose	532±225*	269±153	412±268
Cellobiose	576± 62	705±257	485± 46
Galactose	912±505	934±345	469± 64
Glucose	837±125	771±215	493± 57
Mannose	971±135	646± 69	503± 15
Rhamnose	501±193	537±197	390±185
Xylose	105± 76	20± 19	171±186
Control**	792±140	565±142	678± 40

*µl/day (n=5) mean ± S.D.
**No carbohydrate.

soluble fractions of the coniferous woods exerted a significant inhibition on methanogenesis.

The values for coniferous lignocellulose tubes may be of questionable accuracy because the initial and subsequent gassing of these tubes may not have removed all oxygen, since some of the wood residue tended to float to the surface of the medium, where traces of oxygen could have been retained. This phenomenon was not observed in the deciduous lignocellulose tubes. Whole wood controls were not used due to difficulty in keeping the wood in the growth medium.

The data from the wood-fraction supplemented cultures of *M. formicicum* (table 7) corroborates fairly well the observations made with mixed cultures. Both deciduous and coniferous alcohol-soluble fractions exhibit inhibitory effects. The inhibition by the coniferous alcohol-soluble fraction is probably a concentration effect since the coniferous wood contains more than 10 times the amount of alcohol extractives of the deciduous wood (table 1). The ether- and water-soluble extracts had no significant effect after day 1.

Cellulose degradation is carried out by a large number of microorganisms whereas lignin is more recalcitrant. Although some bacteria are capable of carrying out lignin degradation to a limited extent (Kirk 1971, Stranks 1952), most lignin degradation in nature is carried out by fungi under anaerobic conditions (Crawford 1976, Kirk *et al* 1975). The stimulatory nature of the water-soluble fractions in mixed cultures is understandable in that this fraction contains all the free carbohydrates and salts in the wood. Thus, these components could provide additional nutrients ultimately including CO_2 and H_2. The alcohol-soluble wood fractions contain various pigments and tannins. Tannins are complex polyhydric alcohols with considerable variation in structure, and are well known microbial inhibitors (Benoit *et al* 1968a, Grant, 1976). Most wood chemists believe that tannins and resins protect wood from microbial attack (Wise and Jahn 1952). It is also known that enzymes are inactivated by tannins and that microbial substrates such as polysaccharides and nonenzyme proteins become resistant to microbial attack after binding to tannins (Benoit *et al* 1968a, b). It appears likely that tannins could be responsible for much of

TABLE 6
Methane production by mixed culture in wood fraction-supplemented MS medium (complete) under CO_2+H_2.

Wood Fraction	Day 4		Day 6	
	Coniferous	Deciduous	Coniferous	Deciduous
Ether Soluble	0	89± 24	230±137	428±189
Alcohol Soluble	0	167± 46	8± 15	0
Water Soluble	287±142*	485±218	599±163	671±240
Lignocellulose	600±252	730±377	17± 30	412± 71
Control**	227±74		476±277	

*µl/day (n=5), mean ± S.D.
**MS medium but no wood fractions.

TABLE 7
Methane production by M. formicicum in wood-fraction-supplemented MS medium (complete) under CO_2+H_2.

Wood Fraction	Day 1		Day 4		Day 6	
	Coniferous	Deciduous	Coniferous	Deciduous	Coniferous	Deciduous
Ether Soluble	0	0	533±353	1144±170	230±137	428±189
Alcohol Soluble	6± 11*	41	0	65	8± 15	0
Water Soluble	2397±423	2764±363	888±155	1032±111	599±163	671±240
Lignocellulose	206±143	384±499	45± 74	1787±934	17± 30	412± 71
Control**	3938±806		855±110		563±96	

*μl/day (n=5), mean ± S.D.
**MS medium but no wood fraction.

the temporary inhibition of methanogenesis in our system (lag in fig. 1 and in tables 6 and 7) and that the longer lag in production of methane from coniferous wood as compared to deciduous wood is related to the greater amount of alcohol solubles in coniferous than in deciduous wood (table 1).

With the exception of xylose, the lack of effect of the carbohydrates on *M. formicicum* might be anticipated since the bacterium is not saccharolytic. Although the role of exogenous xylose, if any, in the metabolism of *M. formicicum* is unknown, the possibility exists that it may serve either as an inhibitor for a metabolic pathway or as a chemical or biological sink for a methane precursor. Although the mechanism of inhibition remains to be determined, it is doubtful that xylose plays a significant role in the direct inhibition of methanogenesis during wood fermentation since xylose did not appear to be inhibitory in the mixed culture system.

The wood resins are found in the ether soluble fractions as are fats and fatty acids; however, there are more ether solubles in deciduous than coniferous wood (table 1). Although the ether solubles also initially retarded methanogenesis in both pure and mixed cultures, methane was being produced by day 6 in mixed cultures (table 6) and by day 4 in pure cultures (table 7).

The alcohol soluble fraction of wood appears to be the primary inhibitor of methanogenesis in this fermentation system. It is likely that the inhibitory components are biologically degraded or neutralized in a mixed culture system over a period of time, allowing methanogenesis to occur after a lag period. The degree of inhibition and length of lag period is most likely a factor of both the concentration and chemical nature of the components.

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LITERATURE CITED

Bauchop, T. 1967 Inhibition of rumen methanogenesis by methane analogues. J. Bacteriol. 94: 171–175.

Benoit, R. E., R. L. Starkey, and J. Basaraba 1968a Effect of purified plant tannin on decomposition of some organic compounds and plant materials. Soil Sci. 105: 153–158.

———, and ——— 1968b Inhibition of decomposition of cellulose and some other carbohydrates by tannin. Soil Sci. 105: 291–295.

Bryant, M. P. 1972 Commentary on the Hungate technique for culture of anaerobic bacteria. Amer. J. Clin. Nutr. 25: 1324–1328.

Crawford, D. L., and R. L. Crawford 1976 Microbial degradation of lignocellulose: the lignin component. Appl. Environ. Microbiol. 31: 714–717.

Ferry, J. G., P. H. Smith, and R. S. Wolfe 1974 *Methanospirillum*, a new genus of methanogenic bacteria, and characterization of *Methanospirillum hungatii*, sp. nov. Int. J. Syst. Bacteriol. 24: 465–469.

Kirk, T. K. 1971 Effects of microorganisms on lignin. Ann. Rev. Phytopathol. 9: 185–210.

———, W. J. Connors, R. D. Bleam, W. F. Hackett, and J. G. Zeikus 1975 Preparation and microbial decomposition of synthetic (^{14}C)-lignins. Proc. Nat. Acad. Sci. (U.S.A.) 72: 2521–9.

Kurth, E. F. 1939 Separation of wood extractives into simpler components. Ind. Eng. Chem. Anal. Ed. 11: 203–205.

Macy, J. M., J. E. Snellen, and R. E. Hungate 1972 Use of syringe methods of anaerobiosis. Amer. J. Clin. Nutr. 25: 1318–1323.

Maugh, T. H. 1972 Fuel from wastes: a minor energy source. Science 178: 599–602.

Mink, R. W., and P. R. Dugan 1977 Tentative identification of methanogenic bacteria by fluorescence microscopy. Appl. Environ. Microbiol. 33: 713–717.

Schorger, A. W. 1917 The chemistry of wood. I. Methods and results of analysis of some American species. Ind. Eng. Chem. 9: 556–561.

Stranks, D. W. 1952 Microbiological degradation of lignocellulose material. Pulp Paper Mag. Canada 53: 220–224.

Wise, L. E., and E. C. Jahn (ed.) 1952 Wood Chemistry, Vol. I. Reinhold Publishing, New York.