reaction velocities of the corresponding primary alcohols in benzene at 26°. Other experiments, carried out in this Laboratory, indicate that the solvent is without effect upon the relative velocities of these reactions. The circumstance that the amines are compared with ammonia and the alcohols with methyl alcohol need cause no concern since it is the relative magnitude of the numbers in each series which is at issue. One series is reported at one temperature, the other series at another, but the agreement of the numbers in the third column suggests either that temperature does not affect *relative* velocities, as seems probable, or that the effect in these particular experiments is small. The constancy of the ratio of the corresponding fignres of the two series is excellent, the deviations from the average being less than the probable error in the determinations of the relative reaction velocities of the amines. As far then as these four alkyl groups are concerned, their effect upon the reactivity of the primary alcohols toward phenyl isocyanate is directly proportional to their effect upon the reactivity of the primary amines toward the same reagent. It seems probable that the same law holds for other alkyl groups and for other reagents beside phenyl isocyanate.

Summary

The relative reaction velocities of a number of amines with phenyl isocyanate in dry ether at 0° have been measured.

The relative reaction velocity of aniline is about half and those of ethyl-, *n*-propyl-, *n*-butyl- and *n*-amylamine between eight and ten times that of ammonia.

The effect of the ethyl, *n*-propyl, *n*-butyl and *n*-amyl groups upon the reactivity of the primary alcohols toward phenyl isocyanate is proportional to their effect upon the reactivity of the primary amines toward the same reagent.

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[CONTRIBUTION FROM THE ILLINOIS STATE WATER SURVEY]

The Anaerobic Fermentation of Lignin

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In 1928, while studying the anaerobic fermentation of fibrous materials, the writers noted that the pith in materials such as cornstalks was quickly and very completely fermented under anaerobic conditions to carbon dioxide and methane.[1] From data obtained in numerous studies, it was apparent that the lignin fraction in the pith and in the general body of the cornstalks was furnishing part of the gas recovered.[2] The large amount of organic matter used to start these earlier fermentations, however, made it difficult to definitely establish this fact. Since the gasification of lignin had not been previously observed,[3–12] the work was repeated under conditions designed to give quantitative data on lignin.

(1) Boruff and Buswell, *Ind. Eng. Chem.*, **21**, 1181 (1929).
(2) Boruff and Buswell, *ibid.*, **22**, 931 (1930).
(3) Waksman and Tenney, *Soil Science*, **22**, 395–406 (1926).
(4) Fischer and Lieske, *Biochem. Z.*, **203**, 351 (1928).
(5) Waksman and Stevens, THIS JOURNAL, **51**, 1187 (1929).
(6) Waksman, *Soil Science*, **22**, 323 (1926).
(7) Thaysen and Bunker, "The Microbiology of Cellulose, Hemicelluloses, Pectin and Gums," Oxford Press, 1927.
(8) Thiessen, *et al.*, *Ind. Eng. Chem.*, *Anal. Ed.*, **1**, 216 (1929).
(9) Phillips, Weike and Smith, *Soil Science*, **30**, 383 (1930).
(10) Tenney and Waksman, *ibid.*, **30**, 143 (1930).
(11) Langwell, *J. Soc. Chem. Ind.*, **51**, No. 49, 988 (1932).
(12 -Fowler and Joshi, *J. Indian Inst. Sci.*, **3**, 39 (1920).

Studies on Isolated Lignin

The anaerobic fermentation of lignin isolated from cornstalks by four different methods, namely Kalb, Friedrich, and the two different Phillips procedures, has been investigated. Lignin prepared by the Kalb procedure[13] when inoculated with organic matter from cultures of carbon dioxide–methane producing bacteria gave only small volumes of gas over that from the control (5 g. of lignin gave 133 cc. of methane and 43 cc. of carbon dioxide in one experiment and 368 cc. of methane and no carbon dioxide in another; both experiments were incubated at 25–30° for thirty-three days). Friedrich[14] lignin also gave small volumes of gas, namely, 140 cc. of methane and 6 cc. of carbon dioxide from 2.0 g. of lignin in thirty-three days. In these three studies no correction was made for the carbon dioxide retained in the mother liquor. This undoubtedly averaged about 1000 cc. Lignin prepared by hydrochloric acid[15] and by sodium

(13) Kalb, *Ber.*, **61**, 1007 (1928).
(14) Friedrich, *Z. physiol. Chem.*, **176**, 127 (1928).
(15) Phillips, THIS JOURNAL, **51**, 2420 (1929).

hydroxide[16] treatment, in 235 days at 25–30°, gave somewhat more gas, but still the carbon recovered in the gas (corrected for dissolved carbon dioxide), namely, 1.31 g. and 1.51 g., respectively, represented only 43 and 25%, respectively, of the weight of carbon added as lignin, assuming the formula for the lignin to be $C_{40}H_{46}O_{16}$.[17] The methane to carbon dioxide ratios obtained, namely, 1 to 2.2 and 1 to 2.1, indicate too great a recovery of carbon dioxide to represent complete and normal fermentation of a material having an approximate formula of $C_{40}H_{46}O_{16}$.[13]

Asbestos fiber inoculum[19] was placed in three two-liter anaerobic fermentation tanks held at 25 to 30° and fed glucose. Normal fermentation was soon established as evidenced by the one to one carbon dioxide to methane ratio characteristic of glucose.[18] To the fermentations were then added 5 g. of lignin isolated from corncobs and furnished through the courtesy of Max Phillips of the U. S. Department of Agriculture. Gasification stopped at once. Further additions of glucose failed to revive the fermentation.

From the above data it is evident that isolated lignin is incompletely fermented and is undoubtedly somewhat bacteriostatic to the flora responsible for the production of carbon dioxide and methane. Waksman[20] and Schrader[21] have each reported they were unable to get soil organisms to attack isolated lignin. The present writers, however, are reluctant to state that *isolated lignin* will not ferment. Many times in the past other materials have acted in a similar manner until the proper inoculating, feeding and incubating conditions were discovered. The high carbon dioxide content noted in the lignin gas is characteristic of abnormal or incomplete fermentation.[18]

Even though *isolated lignin* cannot be fermented by a natural anaerobic flora, this is no definite proof that lignin in its *natural* state will not ferment, since it is general knowledge that the methods commonly used for the isolation of lignin bring about certain modifications in its general constitution.[9,22]

Fermentation of Lignin in its Natural State

In August, 1929, a series of eight one-liter anaerobic fermentation cultures were set up and to each was added 15.0 g. dry weight of chopped cornstalks. Each bottle also contained 2.24 g. of inoculum solids (1.40 g. volatile on ignition). The cornstalks and inoculum liquor were analyzed separately for cellulose, pentosans, lignin, etc. The inoculum liquor was practically 100% water soluble and contained no pentosans, cellulose or lignin. Four of these fermentations were permitted to run for 136 days. As the volume of gas developed in each was practically the same, the residues were combined, filtered, washed and the filtrate and washings combined. The analysis of the residue is given in Table I. The filtrate and washings from the four bottles contained 12.89 g. of total solids, 7.7 g. of which was volatile on ignition and 9.0 g. of which was water soluble. The solids contained no pentosans, cellulose or lignin. The remaining four bottles of the series were permitted to run for 600 days, then their contents were combined, filtered, washed and analyzed. The filtrate and washings contained 9.1 g. of total solids, 5.0 g. of which was volatile on ignition, 9.0 g. being water soluble. The solids contained no pentosans, cellulose or lignin. From the data given in Table I, it is apparent that 47% of the volatile matter was gasified in 136 days and 63% in 600 days. It is also noted that 54% and 57% of the pentosans and cellulose, respectively, disappeared in 136 days, while at the end of the 600-day

TABLE I

TOTAL WEIGHTS OF MATERIALS ADDED TO AND REMOVED FROM CORNSTALK FERMENTATIONS AT 25–30°[d]

	Cornstalks added, g.	Total added, g.[a]	Residue removed: After 136 days, Cornstalks	Residue removed: After 136 days, Total[b]	Residue removed: After 600 days, Cornstalks	Residue removed: After 600 days, Total[b]	Fermented: In 136 days, Total	Fermented: In 136 days, %	Fermented: In 600 days, Total	Fermented: In 600 days, %
Total solids	60.0	69.0	26.11	39.0	21.4	30.5	30.0	44	38.5	56
Total volatile matter	57.6	63.2	25.67	33.4	18.7	23.7	29.8	47	39.5	63
Ash	2.40	5.8	0.44	5.6	2.7	6.8	0.2	3	1.0	17
Water soluble[c]	3.65	12.6	.74	9.7	1.8	10.9	2.9	23	1.7	14
Ether soluble	1.39	1.4	.17		0.2					
Ethyl alcohol–benzene soluble	3.92	3.9	1.74		.0					
Pentosans, total	12.66	12.7	5.93	5.9	3.7	3.7	6.8	54	9.0	71
Cellulose C. & B.	22.32	22.3	9.61	9.6	3.4	3.4	12.7	57	18.9	85
Lignin	15.06	15.1	8.75	8.8	7.1	7.1	6.3	42	8.0	54
Protein	1.43	3.9	1.22		2.7					

[a] Includes materials in inoculum liquor. [b] Includes materials in filtered mother and wash liquors. [c] Not corrected for ash. [d] Analysis made according to Bray, *Paper Trade J.*, **87**, 59 (1928).

(16) Phillips, THIS JOURNAL, **50**, 1986 (1928).
(17) Phillips, *ibid.*, **49**, 2037 (1927).
(18) Symons and Buswell, *ibid.*, **55**, 2028 (1933).
(19) Breden and Buswell, *J. Bact.*, **26**, 379 (1933).
(20) Waksman, *Soil Science*, **22**, 323 (1926).
(21) Schrader, *Ges. Abhandl. zur Kenntnis Kohle*, **6**, 173 (1921).
(22) Sherrard and Harris, *Ind. Eng. Chem.*, **24**, 103 (1932).

period, 71 and 85% had been removed, respectively. Although the lignin content of the residue was 33% as compared with 25% in the original stalks, the total weight of lignin was reduced from 15.06 g. to 8.75 g. in 136 days and to 7.1 g. in 600 days.

On January 17, 1931, an autoclave of 16 liters capacity was charged with 575 g. of chopped cornstalks and 12.5 liters of inoculum liquor from a cornstalk fermentation tank. The inoculum liquor and cornstalks were analyzed separately as was also the residue and mother liquor left after the 644-day fermentation period (see Table II). The gas generated during the fermentation was collected in the autoclave until a pressure of 25 to 35 pounds per square inch was reached, then it was withdrawn, measured and analyzed. As noted in Table II, 416.1 g., or 75% of the total weight of volatile (organic) matter, was converted to gas during the anaerobic fermentation; 147.6 g. or 88% of the hemicelluloses, 167.8 g. or 93% of the cellulose added, and 58.5 g. or 52% of the total lignin added, were converted to gas. That no constituents other than those listed in Table II were probably present at the end of the experiment is borne out by the fact that 101.1 and 101.2% of the materials were accounted for in the analysis.

TABLE II

ANALYSIS OF CORNSTALKS ADDED TO AND RECOVERED FROM PRESSURE FERMENTATION AT 25–30°[d]

Constituents[d]	Cornstalks added composition %	Inoculum liquor 12.5 liters Total, g. A	Total, g. B	Cornstalk residue recovered after 644 days Composition %	Total, g. C	Solids in mother liquor 12 liters Composition %	Total g. D	Materials digested A + B − C − D, g.	%
Total		575.0	32.8		144.0		51.6	412.2	68
Volatile matter	93.6	538.2	15.0	79.4	114.4	44.0	22.7	416.1	75
Hot water soluble[e]	12.8	73.5	10.0	9.7	14.0	12.7[a]	6.6	62.9	75
Ether soluble	2.5	14.4	Trace	2.4	3.5	Trace		10.9	76
Ethyl alcohol–benzene (1:2) sol.	0.3	1.7	Trace	1.6	2.3	0.6	0.3	+0.9	
Hemicelluloses	29.1	167.2	Nil	13.6	19.6	Trace		147.6	88
Cellulose	31.3	180.0	Nil	7.7	11.1	2.1	1.1	167.8	93
Lignin[b]	19.5	112.0	Nil	33.0	47.5	11.6	6.0	58.5	52
Protein[c]	4.4	25.3	7.8	12.5	18.0	18.2	9.4	5.7	18
Ash	6.4	36.8	17.8	20.6	29.6	56.0	28.9	+3.9	
Accounted for by analysis	106.3			101.1		101.2			
Total carbon (wet combustion)[f]	42.7	245.5	8.2	41.8	60.3	21.5	11.1	182.3	72

[a] Corrected for ash content. [b] Lignin also determined as per Ritter, Seborg and Mitchell, *Ind. Eng. Chem., Anal. Ed.*, **4**, 202 (1932). [c] (Total nitrogen–ammonia nitrogen) × 6.25 = protein. [d] Analysis made according to Waksman and Stevens, *Ind. Eng. Chem., Anal. Ed.*, **2**, 167 (1930). [e] Not corrected for ash. [f] Larson, Boruff and Buswell, *S. Wks. J.*, **6**, No. 1, 24 (1934).

Gas Recovery Data.—The carbon in the 168 liters of carbon dioxide and the 182 liters of methane recovered amounts to 187 g. as compared with 182 g. of total carbon lost from the digestion mixture during the fermentation (see Table II). These data further establish the fact that a quantitative account has been kept of all materials added and withdrawn and that the lignin lost from the digestion was recovered as gas.

Discussion of Results

Since a complete analysis of all the constituents added to and withdrawn from an anaerobic fermentation of cornstalks shows a material decrease in the weight of lignin present, it is apparent that a large portion of the lignin must have been materially altered or removed entirely from the substrate. That it was not altered so that it would not appear in the analysis is indicated by the fact that 101% of the materials present was accounted for in the analysis. The lignin lost in the fermentation must have been recovered as gas because a carbon balance was obtained (Table II). Furthermore, the fermentation of all the other constituents (less the 58.5 g. of lignin decomposed) could not account for the total volume and weight of gas formed. The production of 350 liters of carbon dioxide and methane, weighing 462 g., as the result of complete fermentation of 416 g. of material, represents a recovery of 1.11 g. of gas per gram of volatile matter fermented. One might have expected this latter fignre (1.11) to have been a little higher, in view of the fact that the values for cellulose, pentosans and lignin are 1.11, 1.14 and 1.47, respectively,[18,23] but careful consideration of the data shows that about four-fifths of the total gas was formed by the fermentation of cellulose and hemicelluloses which give the lower yields of gas per gram of material fermented.

Conclusions

1. Isolated lignin ferments very slowly and incompletely under anaerobic conditions.

2. Complete analytical data show that appreciable quantities of lignin in its natural state in cornstalks, ferment anaerobically to carbon dioxide and methane.

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(23) Buswell and Boruff, *Sewage Wks. J.*, **4**, 454 (1932).