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The effect of decomposition on the lignin of plant materials

John Bruen Bartlett
Iowa State College

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UMI®
THE EFFECT OF DECOMPOSITION ON THE
LIGNIN OF PLANT MATERIALS

By

John Bruen Bartlett

A Thesis Submitted to the Graduate Faculty
for the Degree of

DOCTOR OF PHILOSOPHY

Major Subject Soil Fertility

Approved:

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Dean of Graduate College

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INTRODUCTION

The decomposition of organic matter is a very complex process and presents innumerable problems. One of the most perplexing of these is the question of lignin decomposition. The fact that lignin decomposes so much more slowly than the other constituents of plant materials means that there is a relative accumulation of lignin in the soil with the result that this complex usually forms from 30 to 40 per cent of soil organic matter. This led some of the earlier workers to the belief that lignin does not decompose. More recent work has very definitely shown that this assumption was false. In fact it is obvious that if lignin did not decompose it would soon accumulate in soils to such an extent as to be readily recognizable by chemical means.

A great deal of work has been devoted to the search for specific organisms which attack lignin alone. Attempts to ferment isolated lignin with either mixed or pure cultures have, however, been conspicuously unsuccessful.

One of the major difficulties in studying lignin decomposition is the fact that its constitution is unknown. The various workers are not even in general agreement as to either its constituent groupings or the degree of complexity of the molecule.

The fact that the properties of organic materials undergo marked changes upon decomposition in the soil, coupled with the fact that lignin
...measure of changes in the intensity of the plant material... by the same method with the material studied would give a reasonable work done on the material was made that any changes measured in the intensity of the plant material in the plant tissue... in that event it is well known that no method of intensity measurement will give the... was considered... the changes occurred in the plant material by the part contributed by the changes in the intensity of the plant material and the results... and the results of the plant material and the changes in intensity... as a first step in the study. The intensity was treated from a series of... occur in the intensity as a result of decomposition... work on which was made to find out some of the possible changes which may... the proportion of the changes in the properties of the total material. In this... an important determinant of the decomposition rates...
HISTORICAL

Nature of Lignin

With regard to the nature of lignin the following statement has recently been made (26):

"Despite a great volume of research, many aspects of the chemistry of lignin remain obscure and controversial. Its constitution is unknown and, although many elaborate and superficially plausible formulae have been suggested to account for particular observations and reactions, there is not even general agreement as to the constituent groupings or the degree of complexity of the molecule."

There is even more doubt as to the homogeneity of the substance known as lignin. Three alternatives are possible. In the first place, lignin may be a complex mixture of compounds with similar properties but of unrelated chemical structure, or as a second alternative, lignin may be a mixture of compounds which are similar in structure and have the same basal unit but which vary in minor ways, such as side-chains or substituting groups or in chain-length or degree of polymerization. The third alternative arises from the work of Hilpert (14, 15), who postulates that lignin is merely an artifact or reversion product formed as a result of the action of acid on certain methylated carbohydrates. Thus, according to this third view, lignin as such does not exist in the original plant tissue, but is synthesized during the process of isolation. This theory fails to explain the fact that there are definite similarities between acid and alkali lignins, since products of similar character would hardly be likely to arise
from the action of both alkali and acid on the plant carbohydrates.

Various groups have been postulated as being present in lignin. Of these groups only the presence of the methoxyl group is both easily determined and universally accepted. In addition, from the increase in the methoxyl content of lignin as a result of further methylation, the inference has been drawn that free hydroxyl groups are present (26).

There is also positive evidence for the presence of an aromatic grouping in the molecule, though the yields of phenolic products to be obtained on degradation of lignin are usually quite low. As an illustration of work leading to this conclusion the experiments of Phillips and Goss (32) on the oxidation of alkali-lignin by ozonide may be cited. Methylated alkali-lignin yielded anisic acid (p-methoxy benzoic acid) on oxidation, and ethylated lignin the corresponding ethoxy derivative. This was taken as proof of the presence of a phenolic hydroxy group capable of substitution.

Biological Decomposition of Lignin

Numerous attempts have been made to decompose isolated lignin and lignin derivatives under both aerobic and anaerobic conditions. A survey of this work must inevitably lead to the conclusion that normal isolated lignin is peculiarly resistant to common soil micro-organisms.

Phillips (31) states that both Willstatter lignin (45 per cent H Cl lignin) and 72 per cent sulphuric acid lignin seem to be resistant to microbial decomposition not only by soil micro-organisms, but also by wood destroying Basidiomycetes such as _Trametes pini_ and _Polystictus hirsutus_.

The fact that only very small amounts of CO₂ were evolved from soils to which sulphuric acid lignin had been added led Waksman and Tenney (46) to conclude that isolated lignin is unavailable to micro-organisms. Smith and Brown (36) recently obtained similar results in studies in which considerable quantities of isolated lignin were inoculated with a number of fungi thought to be capable of destroying lignin.

Experiments upon the anaerobic decomposition of isolated lignin have led to conclusions similar to those drawn from the aerobic studies. Boroff and Russell (8) even observed that isolated lignin upon addition to actively fermenting glucose instantly stopped all further gas production. From this they concluded that lignin had a bacteriostatic action on anaerobic processes. More recently Levine, Nelson, Anderson and Jacobs (18) found that alkali-lignin had a very similar inhibitory action upon the fermentation of both cornstalk flour and packing house sludge. Thus isolated lignin seems to be unavailable to micro-organisms under both aerobic and anaerobic conditions.

The only lignin derivative the availability of which has been studied is phenol-lignin. Somewhat unexpectedly Waksman and Hutchings (43) obtained slow but appreciable decomposition of this derivative by both bacteria and fungi. The molecule, however, contains a considerable amount of phenol in combination.

The indications above might suggest that lignin in plant materials would also be unavailable. Results of experiments upon decomposing materials have, however, been extremely contradictory. Some workers have reported fairly extensive decomposition, while others have reported almost quantita-
tive preservation of lignin in decomposing materials. In general, in studies of this kind the lignin content of decomposing plant materials is determined from time to time as the rotting process progresses. Thus the conclusion to be drawn rests largely upon the accuracy with which these lignin determinations are made. Undoubtedly many of the marked differences in the degree of lignin decomposition which have been reported by earlier workers can be attributed to the use of unreliable methods of lignin determination.

The slow utilization of lignin in plant materials is now established without much question for both aerobic and anaerobic conditions. Wakeman, Tenney and Stevens (46) obtained only slight decomposition of the lignin in oak leaves in six months, but after 12 months 35 to 45 per cent removal was recorded. In later experiments over a longer period definite decomposition of the lignin in such materials as cornstalks, rye straw, lucerne plants and oak leaves was obtained. In the case of cornstalks 57.1 per cent of the lignin was removed in 406 days, while in rye straw only 20.5 per cent was removed in 385 days. This work provides perhaps the first definite evidence that the lignin of aerobically decomposing plant materials may be extensively, though slowly, decomposed. Wakeman, Tenney and Diehm (47) obtained only 20 per cent lignin decomposition in horse manure rotted for 10 months. Wakeman and Gerretsen (42), however, found that at 37° nearly 60 per cent of the lignin in the straw composts was lost in 273 days. Osugi and Yoshie (30) obtained no significant losses of lignin in 45 days from soy bean cake, rape seed cake, young vetches and rice straw. Martin (19) failed to obtain any appreciable removal of lignin in decom-
posing lucerne and in clover, tops and roots, even after 270 days. Allen, Abel and Magistad (4) recorded no apparent losses of lignin in rotting pineapple trash within 16 weeks.

The conflicting nature of the evidence on this subject is shown by the fact that, in contrast to the above, a number of workers have reported extensive losses of lignin in comparatively short periods. For example, Phillips, Wiese and Smith (33) obtained as much as 45 per cent loss of lignin in corn cobs in as short a time as 62 days. Under Iowa conditions Smith, Stevenson and Brown (40), and later Smith and Brown (39), recorded losses of 60 to 90 per cent of the lignin of straw composted in the open. The high summer temperature may serve in part to explain this exceptionally extensive attack, since their work would then be in accord with the observation made by Waksman and Gerretsen (42), that lignin decomposition is greater at 40° than at 30° C.

The bulk of the evidence indicates that lignin certainly decomposes under aerobic conditions much more slowly than the other major constituents of plant materials added to the soil, and as a consequence there results a relative accumulation of lignin in the rotting residues. That it undergoes slow decomposition also seems to be proved. However, as the most resistant of the constituents of rotted plants, it undoubtedly is an important part of the soil organic matter or humus.

The information with regard to anaerobic decomposition appears to be less confused. Waksman, Tenney and Stevens (46) obtained less than one per cent removal of lignin from waterlogged oak leaves in six months, but later Tenney and Waksman (41) were able to show losses of from 15 to 32 per cent
from waterlogged cornstalks and rye straw in 16 months. Recent work by Acharya (1, 2, 3) has clearly demonstrated, however, that lignin is slowly removed from plant materials under anaerobic conditions, waterlogged rice straw showing as much as 33 per cent loss of lignin in six months. The anaerobic removal of lignin in biological decomposition seems to be at least as fast, if not faster, than that under aerobic conditions.

Base Exchange Capacity of Plant Materials and Plant Residues

The fact that the cations sorbed from salt solutions by plant materials and decomposed residues are retained in replaceable form has been proved by McGeorge (20). Peat soils were leached alternately with ammonium and barium acetate and the bases retained determined. In each case somewhat higher values were given by the barium salt.

In a study in which 20 different organic soil types were examined, McGeorge (20) found a linear relation between base exchange capacity and carbon content, but no relation between exchange capacity and nitrogen content. He concludes that

"the carbon compounds (largely lignin and carbohydrates) function as the principal absorbing agents in organic soils, and that the complex nitrogen compounds, which are largely proteins or protein derivatives, do not take part in the absorbing property of soil organic matter, at least to any appreciable degree, if the ratio of absorption capacity to nitrogen or to carbon-nitrogen ratio can be taken as a criterion."

Millar, Smith and Brown (23) recently studied the base exchange capacity of 12 mature plant materials and found a highly significant correlation between base exchange capacity and nitrogen content. The fact
For the base exchange property must have occurred at the same time
some quantitative change in the position of the octanoic water responsible
then either the decrease in weight or the increase in weight in connection with
increase in exchange capacity during decomposition was so much greater
increase in exchange capacity with decomposition. These authors suggested that the relative
interaction between the increase in exchange capacity and the decrease in
the internal content. There was on the other hand a noteworthy
exchange capacity and the carbon content of these underoxygenated samples.

Smith and Brown (22) were not able to find any correlation between the
water and brown (22) was apparently a direct correlation to their study in
found that the nitrogen content of the plant materials studied by MITTLE
water of the replacement capacity. Thus the
where the replacement capacity would be located. A much larger
problematic that just the reverse would be true. The internal content of the

the exchange reaction than would the nitrogen, while in the parts to the
interaction direction of the plant materials would play a much larger role in
the nitrogenous fraction of the plant materials would be not so. Thus the
in these nitrogenous plant materials the high nitrogen content is associated

that some of these materials were partitions of a nitrogenous nitrogen

-13-
McGeorge (21) also studied the exchange capacity of several isolated lignin preparations and obtained convincing evidence of their equivalent base exchange property. Corn cob lignin had a much lower exchange capacity (18.6 M.E. per 100g) than alkali-lignin from peat soils, which had a capacity of 160 M.E. per 100g.

Upon examining the exchange capacity of the alcoholic and aqueous alkaline extractives from 11 peat soils McGeorge (21) found that there was considerable variation between individual soils in the case of both preparations, the aqueous extractive being higher in all cases. As an explanation for the variation between the two alkaline extractives he suggests that either the lignin exists in these soils in several different forms, or that organic compounds with exchange properties, soluble in aqueous but not alcoholic sodium hydroxide, are present in these soils.

The fact that lignin is a major constituent of soil organic matter led him to conclude further that of the various forms of lignin suggested above, only those of lower replacement capacity are removed by the alcoholic alkaline extraction, so that the extractive prepared by this method would as a consequence be lower in exchange capacity than that by the aqueous method. The fact that many other workers have shown that the alkaline methods of lignin preparation usually give low yields which may not even be representative of the lignin in situ should be kept in mind in considering this work of McGeorge (21).

He also compared the effect of these two alkaline extractions upon the original peat and found that the aqueous alkaline extraction removed a far larger portion of the replacement capacity than did the alcoholic
extraction. A crude hemi-cellulose mixture, considered by McGeorge to be xylan, was also isolated and found to be very low in exchange capacity. From this he concluded that the exchange capacity of the hemicellulose fraction of peat could not account for the difference in replacement capacity of the two alkaline extractives.

Further work showed that treatment of fresh ground alfalfa with 15 per cent hydrogen peroxide reduced the exchange capacity (from 50 to 35 M.E. per 100 grams), but did not completely destroy the replacement power. The residual organic compounds which still remained, as shown by the high carbon content of the residue, were thought by McGeorge to be lignin-like compounds, and he attributed the residual exchange capacity of the treated material to these compounds. Other experiments in which samples of alfalfa were rotted for three weeks showed that decomposition increased the exchange capacity of the residue three or four times over that of the original material. In addition the exchange capacity of both the alkaline extractives from the undecomposed material was quite large (about 100 M. E. per 100 g). From these data he concluded that lignin-like bodies play a major role in the exchange capacity of undecomposed plant material, that decomposition greatly increases the replacement capacity of such unrotted material, and that the compounds which impart to organic matter its exchange properties are those least readily removed by decomposition. In addition he showed that there was a linear relation between the lignin contents and both the organic and the total replacement capacities of five of the peat soils studied, but no relation between the hemicellulose content and the exchange capacities. His final conclusion was that lignin-like bodies play a domi-
nant role in the exchange capacities of both raw and decomposed plant materials.

Mitchell (24) made an interesting quantitative study of the relative importance of the organic and inorganic constituents in the soil exchange complex. Kerr (17) had found that the Ca-Mg equilibrium constant was quite close to constant for the one peat soil which he studied. Mitchell (24) attempted to determine whether or not this equilibrium value for the organic constituents of the soil would be constant from one soil to another. The amount of calcium and magnesium retained by the organic complexes was determined by the method of difference. In order to remove the organic matter it was found that ignition in an electric furnace at 350 - 400 degrees completely destroyed the organic matter but did not affect the base exchange capacity of the inorganic fraction left behind. The Ca-Mg equilibrium content was determined both before and after ignition, and the loss in exchange capacity due to this treatment was attributed to the organic fraction. The ignition method was compared with the hydrogen peroxide method, and it was found that the former gave very nearly the same values for the exchange capacity after removal of organic matter as did the latter. In addition residues from the former method were much easier to leach.

The equilibrium constants for the inorganic fraction of these soils agreed well with those of Kerr and were approximately constant, but an appreciable variation was found in the values for the organic fraction. Kerr had concluded that the constancy of the equilibrium constants indicated that only one compound was responsible for the inorganic exchange capacity, while Mitchell decided that the variation he obtained in the equilibrium constants of the organic fraction indicates that more than one compound is
Several other workers also do some work on this project.

A study was found to have little or no relation to the exchange capillary
capacity than the inulin. The information content of the exchange
preparation responsible for an appreciable but much smaller fraction of the
response was found that the diffuse radial diffusion fraction was
caused the effect of diffuse radial diffusion upon the exchange capacities of
for the interest portion of the exchange preparation. In addition to the
found that in the four pears studied the exchange seemed to be responsible
these findings as well as the original values were then determined. It was
prepared by extraction from a number of pears. The base exchange capacity of
d Lahore and Islamabad (called "Izmir-names") by the author) was also
exchange substance in this tooted wood

with the height inulin content of the wood, indicates that the life

some of the wood, and for the tooted wood contained

or both the wood inulin and for the tooted wood contained

the results are that the similar presentation come

inulin and found to be about 60% of wood, and of wood partition decomposed by brom formic acid was also done.

The difference in the partition of 72 per cent substance was inulin from spines

Process may be studied for these particular part solute

indicates that the mixture of original compounds responsible for the exchange

compounds of all of the soils studied were, however, taken by me.

that these results were in the neighborhood of three for the original

response for the base exchange capacity of soil organic matter. The
and Iyer (44) observed that the base exchange capacity of "ligno-protein" preparations increased with an increase in the protein content. Muller (25) observed an increase in replacement capacity of manure and straw during decomposition. He considered that the exchange capacity in isolated fractions of such organic matter was due to certain reactive groups such as the hydroxyl and carboxyl groups rather than to specific substances.

This review leaves little doubt that lignin has an important part in contributing to the base exchange capacity of decomposed plant residues and soil organic matter, although no clear explanation has been given for the reason for its activity in this respect.

Methoxyl Content of Plant Materials and of Lignin as Affected by Decomposition

The effect of decomposition on the methoxyl content of plant materials and of lignin has not been very thoroughly studied. Waksman and Smith (45) mention that the determination of the methoxyl groups of plant materials present in ether linkage has been suggested as a measure of the lignin content of these materials, but in view of the variability in amount of methoxyl in the lignin molecule, the method is not a feasible one. Fuchs (11) showed that the methoxyl content of lignin varies from one plant to another. Beckmann, Liesche and Lehmann (6) found that the amount of methoxyl in the lignin of plants increases as maturity is approached. Ritter (35) and Ritter and Fleck (36) showed further that the methoxyl content of the lignin may even vary with its position in the plant. Lignin preparations from mature tissues ordinarily contain from 14 to 21 per cent
methoxyl which must be presumed to be in ether linkage since it is resistant to drastic alkaline treatments. Further, the lignin molecule is not naturally completely methylated, for on treatment with methyl sulphate in alkaline solution the methoxyl content can be increased to as much as 30 per cent. Hibbert, according to Norman (26), has stated that the methoxyl content of lignin is almost the only constant that may be used as an index of the purity of lignin preparations, and for providing quantitative information concerning the changes produced in lignin by additive reactions.

Information as to changes in methoxyl content of lignin as a result of decomposition is scanty. Schwalbe and Ekenstam (37) found the methoxyl content of lignin in wood which has been decomposed by fungi was lower than that of the lignin in normal wood.

Perhaps a more extensive study was made by Waksman and Smith (45), but their work suffers from the objection that it was assumed that all of the methoxyl groups in the plant materials examined were attached to the lignin molecule. The relative amounts of methoxyl in the lignin before and after decomposition were calculated from the total methoxyl contents of the whole and decomposed plant materials. They point out that this procedure is possibly not justified, and that some of the methoxyl may be due to carbohydrate constituents, but argue that "the fact that the carbohydrates are much more rapidly decomposed than the lignin would tend to give weight to the assumption that the methoxyl content of composts is due largely, if not entirely, to lignin." These workers first determined the methoxyl contents of fresh and composted manure and found little difference as a result of the composting process. Next they examined three materials, cornstalks,
rye straw, and oak leaves and the residues therefrom after aerobic and anaerobic decomposition for five years. Anaerobic decomposition brought about a greater reduction of the total methoxyl content of the residue than did aerobic, but even when calculated on the lignin the losses of methoxyl were only of the order of four to five per cent. As already pointed out, the issue is not clearly settled, and doubt still remains as to whether decomposition may be accompanied by partial demethoxylation of lignin.
EXPERIMENTAL

Plan of Procedure

Objectives

The purpose of this investigation may be briefly stated as an examination of the lignin of decomposed materials in order to determine if any changes have been brought about that might influence the properties of rotted plant residues, and to secure information as to the nature of the changes.

Choice of a method of lignin isolation

One of the major difficulties which presents itself in any study of lignin decomposition in rotted plant materials is the fact that the composition of the residues changes materially as decomposition progresses. In the early stages of the process the more readily available constituents, especially the pentose polysaccharides, are rapidly removed. At the same time microbial tissue is being synthesized by the active biological population. In aerobically decomposed materials this synthesized tissue, a considerable part of which is probably protein, may amount to an appreciable fraction of the total mass. The presence of protein causes some disturbance in nearly all the common methods of lignin preparation and determination, due either to precipitation along with the lignin or to
the formation of fission products which condense with the lignin. No
satisfactory means of eliminating this protein disturbance has been found,
and as a result, the presence of this synthesized microbial tissue adversely
affects the purity of lignin preparations from decomposed materials. Thus
the first problem encountered was the selection of a method of lignin
preparation and determination in which the protein disturbance would be at
a minimum.

In general the isolation of lignin may be effected by any one of the
three following general procedures: (a) removal of all other constituents
by strong mineral acids, (b) extraction with alkali, (c) formation of
soluble derivatives.

(a) The methods of lignin isolation in which strong mineral acids are
used to remove all other constituents by hydrolysis leaving lignin behind
as a residue have been extensively used in constitutional work on lignin.
The methods of this group are of two types, the one employing fuming
hydrochloric acid (usually 42 per cent) and the other concentrated
sulphuric acid (usually 72 per cent). It has never been shown that the
former possesses sufficient advantages to outweigh the unpleasantness
associated with its use, and the latter is most generally employed.

The errors involved in the use of the 72 per cent sulphuric acid
method have been extensively investigated by Norman and Jenkins (28, 29)
and more recently by Norman (27). These errors are of two general types,
those caused by carbohydrates, especially the pentose-containing
polysaccharides and those due to protein.
Unless suitable precautions are taken, the presence of some carbohydrates results not only in the formation of insoluble products as a result of the treatment with strong acid, but also in the condensation with the lignin of soluble fission products, such as furfuraldehyde. In either case an impure product or a higher yield of apparent lignin is obtained. This difficulty may be overcome by pretreatment of the material with hot dilute acid, which results in the hydrolytic removal of these carbohydrates.

Bamford and Campbell (5) have questioned the complete efficacy of the acid pretreatment on the grounds that even then some insoluble products may be produced from the partially hydrolyzed carbohydrates.

Cohen and Harris (10) on the other hand, feel that the dilute acid pretreatment removes soluble lignin. Bamford and Campbell found several brief acid treatments gave lower lignin values than one prolonged treatment. In a recent paper Norman (27) has confirmed the fact that intermittent hydrolysis gives slightly lower lignin values than one longer treatment, and suggests that although simple acid prehydrolysis may not be applicable in all cases, the product so obtained is, as far as purity is concerned, far more satisfactory than that obtained in its absence and, as a general procedure for comparative purposes, possesses the desirable feature of simplicity.

Efforts to eliminate the protein disturbances in acid lignin determinations have met with little success. Norman (26) states that,

"The hydrolytic pretreatment with five per cent sulphuric acid for one hour does considerably reduce the protein content of most materials, and while this does not necessarily mean that the nitrogen retained by the lignin will be reduced, such in fact is the case for most materials examined."

The nature of the reaction between lignin and protein in the presence of strong acid is not understood. There appears to be condensation of in-
end on to the ligament complex. In the exceptions of the native compounds seem to
mean of extraction means and monomer of the pure ligaments often reitated to
most of the tissue differential techniques. 

The extraction methods are poor in most of the tissue differential techniques. From soluble differential of tissue, but seem on processed tissue.

In the presence of a certain (certain) ligament compounds such as acetylcoline, epinephrine, etc.

The extraction methods were considered suitable. 

and since the existence of protein combination were increased, none of

Thus, since the later requirement was highly decreased in this work.

Plant material.

the fraction of the main bulk of the ligament compound converted to the

expected (expected) yield. The extraction methods are soluble differential means.

the extraction means and monomer of the native compounds appear orlease.

the main differential from the differential one.

also, considering the extraction has been found in the

problem differential is even greater and more difficult to eliminate than

by virtue of the extraction before or after the extraction, but the

various processes need on extraction by themselves have been

* Extraction of tissue at various pressure or pressure below

*content of the materials from which they are isolated.

Can to be found in the ligament compounds versus directly with the

definite problem frequency of various with the tissue and the amino acid.
yields are obtained but the phenol group seems to add on to the lignin at many points, for phenol lignin preparations may contain up to 50 per cent phenol.

Certain organic acids have recently been shown to condense with lignin to give good yields of soluble products and one of these, thioglycollic acid lignin, has been proposed by Bengtsson (7) as a method for determination. It was thought that in addition to giving a high yield the lignin prepared by this method might be relatively low in nitrogen and thus provide a means of avoiding the protein disturbance.

The thioglycollic method of lignin determination as used by Bengtsson was adopted from that of Holmberg (10). The lignin is considered to combine with the thioglycollic acid according to the following formula:

\[ \text{C}_{40} \text{H}_{40} \text{O}_{12} \cdot \text{nRSCHCONH} \]  

This lignin derivative is insoluble in water and acids but readily soluble in alkalis. In the determination, as devised by Bengtsson, soil containing about 2 g. of organic matter is treated on a water bath with 1 ml. of thioglycollic acid dissolved in 25 ml. of 2 N HCl. After two hours the mixture is thoroughly stirred and allowed to digest for two further hours. The residue is then washed free of acid, about 25 ml. of water being left with the residue when the washing is completed. Next 22.5 ml. of 1 N KOH is added and the mixture allowed to stand overnight. The KOH extract is separated from the alkaline insoluble residue by filtration, made up to 150 ml. and the thioglycollic acid lignin precipitated by the addition of 20 ml. of 5 N HCl. After precipitation the mixture is digested on a water bath for one hour to effect coagulation. Finally the lignin preparation
is filtered out by means of a porous crucible of the alundum type.

The lignin content of both decomposed and undecomposed cornstalks was determined both by Bengtsson's thioglycollic method and, also, by the 72 per cent sulphuric acid method as used by Norrman and Jenkins (28, 29). As the figures in Table 1 show, the thioglycollic acid method gave lower yields than the sulphuric acid method even without adjustment for the presence of thioglycollic groups.

Table 1

Lignin Content of Fresh and Decomposed Cornstalks Determined by the Thioglycollic and Sulphuric Acid Methods

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</tr>
<tr>
<td>Fresh cornstalks</td>
<td>12.2</td>
<td>20.1</td>
</tr>
<tr>
<td>Decomposed</td>
<td>20.2</td>
<td>32.9</td>
</tr>
</tbody>
</table>

In order to determine whether or not the thioglycollic acid method would have the desirable feature of giving a lignin preparation appreciably lower in nitrogen than the acid procedure, preparations were made from the same materials. The nitrogen contents of these four lignin preparations are given in Table 2. It will be seen that the nitrogen content of the thioglycollic acid lignin preparations was not sufficiently lower than that of the sulphuric acid lignins to justify the adoption of this procedure.
Table 2

Comparison of the Nitrogen Content of Lignin Prepared by
the Thioglycollic Acid and 72 Per Cent Sulphuric Acid
Methods from Fresh and Decomposed Corn Stalks

<table>
<thead>
<tr>
<th></th>
<th>Thioglycollic- Lignin</th>
<th>Sulphuric- Lignin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nitrogen %</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fresh corn stalks</td>
<td>1.68</td>
<td>1.30</td>
</tr>
<tr>
<td>Decomposed corn stalks</td>
<td>2.48</td>
<td>2.78</td>
</tr>
</tbody>
</table>

In view of the considerations discussed above, the acid method of preparation was finally adopted in this work, despite its limitations. More is known of the nature and extent of the errors that may be involved and the means that may be employed in meeting them. The 72 per cent sulphuric acid method was chosen in preference to the hydrochloric acid method because, as previously mentioned, the latter offers no known advantage and is much less pleasant to use.

Preparation of the decomposed materials and the isolation of lignin therefrom

Four plant materials, corn stalks, rye straw, oat straw, and wheat straw, were aerobically decomposed in large stone crocks for periods of three to six months. In the case of both corn stalks and rye straw two crocks were employed. One of each of these two pairs of crocks was sampled after three months, while the other in each case was allowed to decompose for six months. Samples of wheat straw and oat straw were taken only after
six months.

Sufficient nitrogen was added initially as ammonium carbonate to bring the total nitrogen up to 1.2 g. per 100 g. of dry matter and water to bring the moisture content up to 75 per cent. A pad of moist cotton was found to be effective in preventing too rapid drying out of these materials, the moisture content of which was maintained by additions from time to time.

After the above specified periods of decomposition the materials were thoroughly air-dried, oven-dried for a short time and the percentage loss of organic matter calculated.

From the four fresh materials and the six decomposed residues, 72 per cent sulphuric acid lignin was prepared according to the method of Norman and Jenkins (28,29). Prehydrolysis for one hour with five per cent sulphuric acid was followed by treatment with 72 per cent sulphuric acid for 16 hours at less than 40°C, the mixture then being diluted to an acid concentration of three per cent and boiled for two hours. The lignin preparations, after extensive washings, were air-dried and finally dehydrated over phosphorus pentoxide.

Methods employed in studying the changes produced in lignin by decomposition

The methods used in the examination of the lignin preparations from these decomposed residues consisted of determination of base exchange capacity and methoxyl content, and quantitative oxidation with alkaline hypelodite. These determinations were carried out upon all 10 of the acid
lignin preparations and in the case of base exchange capacity also upon the original plant materials and the decomposed residues.

**Base exchange capacity.** In all, three different procedures for determining the base exchange capacity were tried, barium and ammonium being the ions employed.

Since the lignin preparations contained only sulphate-insoluble ash, advantage was taken of this fact and sorbed barium was first determined by formation of the sulphate. About 0.5 g. of dry lignin was weighed into an aluminum crucible and leached by suction with 250 ml. of 1 N barium acetate. Each time the crucible was filled, the barium acetate solution was allowed to stand in contact with the lignin for several minutes before being sucked off. The crucibles were then washed free of barium with distilled water. Next 200 ml. of 1 N sulphuric acid was leached through the crucible in a similar manner. In some cases the crucibles were instead allowed to stand overnight filled with acid. The purpose of these treatments was to completely precipitate all of the sorbed barium as barium sulphate. After the barium had been precipitated the crucibles were ashed and weighed. The ash remaining was partly due to the precipitated barium sulphate and partly due to the sulphuric acid insoluble lignin ash. By difference the barium retained could be calculated. The effect of two different strengths of barium acetate, 0.25 N and 1 N were also compared, and it was found that the latter gave the most consistent results. This method, though simple, did not possess sufficient accuracy and was later replaced by the following procedure.
This second barium-sorption method was that proposed by Millar, Smith and Brown (22) and was designed to determine the base exchange capacity of organic materials. Two grams of the parent plant materials, or decomposed residues, or one gram of lignin were leached with neutral normal barium acetate, washed free of excess barium with cold, boiled distilled water, leached with neutral normal calcium acetate, and the barium displaced determined volumetrically as barium chromate by titration with 0.1 N sodium thiosulphate.

The third method tried was an ammonium-sorption method. The materials were leached on filter paper with 250 ml. of neutral normal ammonium acetate and washed free of ammonium ions with distilled water, the filtrate being tested till it showed no color with Nessler's solution. Alternately, if a small amount of ammonium chloride was passed through the filter just after the ammonium acetate treatment, a test for the absence of chloride with silver nitrate could also be used as an indicator of the absence of ammonium. To replace the ammonium the filters were next leached with neutral normal barium acetate, the ammonium in the leachate being determined by distillation into standard acid and subsequent titration.

Methoxyl content. The methoxyl content of the 10 lignin preparations was determined by means of the semi-micro methoxyl method devised by Clark (9) using an apparatus made strictly to the specifications outlined by him.

Oxidation with alkaline hypobromite. The principle of this method was given by Walde and Hixon (49) in a study of the alkaline oxidation of lignin. Their procedure was modified as follows. About 50 mgm. of lignin were weighed into 250 ml. Erlenmeyer flasks and 25 ml. of normal sodium hydroxide
position studies over similar periods of time.

somewhat greater than that shown by most of the previous workers.

The amount of nitrogen decomposition observed in these experiments is

slightly more susceptible to attack.

two materials. On the other hand, the type of nitrogen and what nitrogen remained

and the amount of some of the more composted nitrogen remained on the other

and about 66 per cent decomposition of total plant material occurred in

at a time of three months from 21 to 34 per cent removal of nitrogen.

at a time of three months on the aged plant material took place in

these results were given in Table 5. Very approximate data.

The results obtained on corn root decomposition of the four plant materials

Table of Organic Matter and Nitrogen in Decomposition

Table

Results

come to completion within the one and one-half hour period.

with peak of 114.0 mg of nitrogen. It was practically determined that the reaction

that the results were conditioned in terms of changes of nitrogen

during periods were determined in which all cultivation was

the excess iodine remaining after one-half hour and one and one-half

solutions thiosulfate

in I M thiosulfate were washed and back titrated with 0.1 N

intercepts. 

iodine solution were generated above those that were determined time

were added to each standard containing 60 ml of water and 15 ml of 0.1 N

- 31 -
## Table 3
Aerobic Decomposition of Plant Materials and of Lignin

<table>
<thead>
<tr>
<th>Material</th>
<th>Initial wt. - g.</th>
<th>Cornstalks</th>
<th>Rye straw</th>
<th>Cat straw</th>
<th>Wheat straw</th>
</tr>
</thead>
<tbody>
<tr>
<td>Original</td>
<td></td>
<td>764.</td>
<td>759.</td>
<td>758.</td>
<td>617.</td>
</tr>
<tr>
<td></td>
<td>Lignin content - %</td>
<td>14.8</td>
<td>17.4</td>
<td>17.6</td>
<td>17.8</td>
</tr>
<tr>
<td></td>
<td>Lignin present - g.</td>
<td>115.</td>
<td>132.</td>
<td>133.</td>
<td>92.0</td>
</tr>
<tr>
<td></td>
<td>N in lignin preparation - %</td>
<td>0.69</td>
<td>0.95</td>
<td>0.66</td>
<td>0.76</td>
</tr>
<tr>
<td>Residue</td>
<td>Residue - g.</td>
<td>260.</td>
<td>259.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>at 3 months</td>
<td>Loss of O₃N₃ - %</td>
<td>66.0</td>
<td>65.9</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Lignin content - %</td>
<td>29.8</td>
<td>33.5</td>
<td>34.6</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Lignin present - g.</td>
<td>77.5</td>
<td>86.4</td>
<td>85.8</td>
<td></td>
</tr>
<tr>
<td></td>
<td>N in lignin preparation - %</td>
<td>1.95</td>
<td>1.94</td>
<td>1.95</td>
<td></td>
</tr>
<tr>
<td>Residue</td>
<td>Residue - g.</td>
<td>220.</td>
<td>231</td>
<td>231</td>
<td>147</td>
</tr>
<tr>
<td>at 6 months</td>
<td>Loss of O₃N₃ - %</td>
<td>71.2</td>
<td>69.6</td>
<td>68.8</td>
<td>71.0</td>
</tr>
<tr>
<td></td>
<td>Lignin content - %</td>
<td>33.3</td>
<td>34.5</td>
<td>33.5</td>
<td>37.8</td>
</tr>
<tr>
<td></td>
<td>Lignin present - g.</td>
<td>72.3</td>
<td>79.7</td>
<td>81.1</td>
<td>55.6</td>
</tr>
<tr>
<td></td>
<td>N in lignin preparation - %</td>
<td>35.1</td>
<td>39.8</td>
<td>36.8</td>
<td>39.6</td>
</tr>
</tbody>
</table>

* O₃N₃ = organic matter.
Comparison of the three methods used to determine the base exchange capacity

The values for the base exchange capacity of the lignin preparations and of the plant materials from which they were isolated are given in Table 4, the three procedures enumerated in the previous discussion of methods being used. In the case of the barium ashing method only the values for the lignins from corn stalks and rye straw and from the residues of these materials after three months decomposition were obtained, whereas in the case of the other two methods values for all the materials and all the lignin preparations were obtained, with the exception of the ammonium sorption value for the lignin from oat straw.

The most striking fact brought out by Table 4 is that the exchange capacity values obtained by the barium titration method were very much higher than those given by the ammonium acetate method. In the case of the plant materials the difference is quite appreciable, but in the case of the isolated lignins the barium values are in all cases more than twice as great as the ammonium values. Although it was expected that ammonium acetate might remove soluble material from the rotted residues, it was not anticipated that it would also do so from the acid lignins. McGeorge (20) found that the ammonium values were consistently lower than the barium values for peat soils. He also found that the barium values determined before ammonium acetate leaching were somewhat higher than the barium values determined after ammonium acetate leaching; so there was some indication from his work of the solvent action of the ammonium ion at least upon peat organic matter.
Table 4

Base Exchange Capacity of Plant Materials and Lignin Preparations

<table>
<thead>
<tr>
<th>Plant material</th>
<th>Decomposition method</th>
<th>Lignin method</th>
<th>Lignin, 1% ash-free, M.E. per 100 g.</th>
<th>Plant materials, M.E. per 100 g.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Corn stalks</td>
<td>ashing</td>
<td>titration</td>
<td>15.2*</td>
<td>40.5** 9.2 26.6**</td>
</tr>
<tr>
<td></td>
<td>6 mo.</td>
<td></td>
<td>105.3*</td>
<td>145.5** 44.1 51.5</td>
</tr>
<tr>
<td>Rye straw</td>
<td>ashing</td>
<td>titration</td>
<td>16.2*</td>
<td>38.3** 5.7 16.5**</td>
</tr>
<tr>
<td></td>
<td>3 mo.</td>
<td></td>
<td>106.7*</td>
<td>135.6** 43.3 55.5</td>
</tr>
<tr>
<td></td>
<td>6 mo.</td>
<td></td>
<td>105.7*</td>
<td>155.6** 47.5 73.5</td>
</tr>
<tr>
<td>Oat straw</td>
<td>ashing</td>
<td>titration</td>
<td>10.3*</td>
<td>45.1** 7.1 21.6**</td>
</tr>
<tr>
<td></td>
<td>6 mo.</td>
<td></td>
<td>92.0**</td>
<td>186.4** 47.4 70.6</td>
</tr>
<tr>
<td>Wheat straw</td>
<td>ashing</td>
<td>titration</td>
<td>19.6*</td>
<td>46.4** 8.2 22.1**</td>
</tr>
<tr>
<td></td>
<td>6 mo.</td>
<td></td>
<td>81.2**</td>
<td>154.2** 65.5 89.7</td>
</tr>
</tbody>
</table>

* 0.5 g sample used
** 1.0 g sample used

2.0 g samples used in all other cases.
In order to study this point, two 2g. samples of decomposed oat and wheat straws were first saturated with ammonium acetate; the ammonium ions were then replaced by barium and the barium-sorption capacity determined by the barium titration method. The values obtained, as shown in Table 5, indicate that an appreciable fraction of the replacement capacity has been removed by the solvent action of ammonium acetate.

This, however, only accounts for about half of the difference in the exchange capacity values for the two methods. In the case of the values shown in Table 5, the large volume of water necessary in the ammonium method to remove excess ammonium ions previous to replacement and determination of exchangeable ammonium was not used, since it was only desired to replace ammonium by barium. It may be that this extended aqueous leaching had an additional solvent action which possibly accounts for part of the remaining difference between the two methods. From the above considerations it was concluded that the barium titration method gave a more reliable measurement of the exchange capacity. Consequently, only the barium values are used for comparative purposes in the rest of this section, with the exception of the experiment in which the effect of chemical pretreatment on the replacement capacity of one of the parent materials was measured.
Table 5

Solution Effect of Ammonium Acetate Upon the Base Exchange Capacity of the Decomposed Residues
(Barium Titration Method)

<table>
<thead>
<tr>
<th>Exchange capacity</th>
<th>Cat straw residue, M.E. per 100 g.</th>
<th>Wheat straw residue, M.E. per 100 g.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Before leaching</td>
<td></td>
<td></td>
</tr>
<tr>
<td>with ( \text{NH}_4\text{CH}_3\text{COO} )</td>
<td>70.8</td>
<td>89.7</td>
</tr>
<tr>
<td>After leaching</td>
<td></td>
<td></td>
</tr>
<tr>
<td>with ( \text{NH}_4\text{CH}_3\text{COO} )</td>
<td>57.4</td>
<td>76.4</td>
</tr>
</tbody>
</table>

The effect of decomposition on the base exchange capacity of the lignin and the plant materials

One of the most interesting features of Table 4 is the considerable increase in exchange capacity produced in the lignin as a result of decomposition. The replacement capacity of the original materials was about 40 M.E. per 100 grams, while that of the residues ranged from 135 to 168 M.E. per 100 grams.

In Table 6 the replacement capacities of the original materials and of the residues after six months decomposition are expressed on the basis of 100 grams of the former. These data show that a small increase in the exchange capacity of the residue over that of the unit amount of original material from which it was derived was obtained in three cases, whereas in the fourth case there was an appreciable decrease. This indicates that, although the base exchange capacity of the lignin is much greater in the residues than in the parent materials, the exchange capacities of the
original materials and of the proportionate residues therefrom do not differ greatly.

Table 6

Comparative Base Exchange Capacities of Unit Amounts of Original Materials and the Proportionate Residues Therefrom (Barium Titration Method)

<table>
<thead>
<tr>
<th></th>
<th>Cornstalks</th>
<th>Rye straw</th>
<th>Oat straw</th>
<th>Wheat straw</th>
</tr>
</thead>
<tbody>
<tr>
<td>Exchange capacity of original material M.E. per 100 g.</td>
<td>26.6</td>
<td>18.5</td>
<td>21.6</td>
<td>22.1</td>
</tr>
<tr>
<td>Residue after 6 mo. %</td>
<td>23.3</td>
<td>30.4</td>
<td>33.2</td>
<td>29.0</td>
</tr>
<tr>
<td>Exchange capacity of 6 mo. residue M.E. per 100 g. of original material</td>
<td>16.5</td>
<td>22.5</td>
<td>25.5</td>
<td>26.0</td>
</tr>
</tbody>
</table>

The contribution made by lignin to the exchange capacity of the plant materials and residues was next calculated, the assumption being made that the activity of the isolated lignin is the same as when in situ. This has not been proven, though it is perhaps more probable in the rotted residues than in the parent materials. These calculated values for the decomposed residues and the original materials are given in Table 7. When the figures for the exchange capacity of the lignin fraction are compared with those for the total exchange capacity per 100 grams of material, it can be seen that the lignin appears to be responsible for 65.0 to 96.7 per cent of the sorption capacity of the residues, but only for 22.6 to 39.4 per cent of that of
Table 7

Contribution Made by Lignin to the Exchange Capacity of the
Plant Materials and Residues
(Barium Titration Method)

<table>
<thead>
<tr>
<th>Material</th>
<th>Cornstalks</th>
<th>Rye straw</th>
<th>Oat straw</th>
<th>Wheat straw</th>
</tr>
</thead>
<tbody>
<tr>
<td>Original</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>M.E. per 100 g. ash-free lignin</td>
<td>40.5</td>
<td>58.3</td>
<td>48.1</td>
<td>46.4</td>
</tr>
<tr>
<td>% lignin in the material</td>
<td>14.8</td>
<td>17.4</td>
<td>17.6</td>
<td>17.8</td>
</tr>
</tbody>
</table>
| M.E. contributed by lignin to
  exch. cap. of 100 g. material | 6.0        | 6.7       | 8.5       | 8.5         |
| Exch. cap. of material M.E. per
  100 g. | 26.6       | 13.3      | 21.6      | 22.1        |
| % of exch. cap. due to lignin | 22.6       | 56.2      | 39.4      | 37.6        |
| Residue at 3 mo. |            |           |           |             |
| M.E. per 100 g. ash-free lignin | 145.3      | 135.6     | --        | --          |
| % lignin in the material | 29.6       | 53.3      | --        | --          |
| M.E. contributed by lignin to
  exch. cap. of 100 g. material | 45.3       | 45.2      | --        | --          |
| Exch. cap. of material M.E. per
  100 g. | 51.4       | 58.3      | --        | --          |
| % of exch. cap. due to lignin | 51.4       | 58.3      | --        | --          |
| Residue at 6 mo. |            |           |           |             |
| M.E. per 100 g. ash-free lignin | 166.3      | 168.6     | 168.4     | 154.2       |
| % lignin in the material | 35.3       | 54.5      | 53.5      | 37.8        |
| M.E. contributed by lignin to
  exch. cap. of 100 g. material | 56.4       | 53.7      | 56.4      | 58.3        |
| Exch. cap. of material M.E. per
  100 g. | 57.3       | 73.5      | 70.3      | 69.7        |
| % of exch. cap. due to lignin | 96.7       | 73.1      | 79.7      | 65.0        |

Exch. cap. = base exchange capacity.
the parent materials. Thus in the original materials there must be considerable amounts of nonlignous compounds possessing the property of base exchange. The fact that the greatly increased sorption capacity produced in the lignin by decomposition only slightly changed the replacement capacity of the plant materials can apparently be explained by the removal through decomposition of the greater portion of the nonlignous material capable of base exchange.

Since the lignin preparations all contained considerable amounts of ash, it was thought possible that the inorganic material present might be contributing in some degree to the base exchange capacity. Mitchell (24) has shown that the mineral matter of peats ashed in an electric furnace between 350 and 400 degrees retained all their activity in this respect. Accordingly, 2 g. samples of all the lignin preparations, except that from fresh cornstalks, of which very little was left, were weighed into porcelain crucibles of known weight and ashed within the temperature range suggested by Mitchell. In order to obtain complete destruction of the lignin at this temperature, it was necessary to leave the crucibles in the electric furnace for several days. After ashing was complete the crucibles were weighed and the ash transferred to 250 ml. beakers. The base exchange capacity was then determined by the barium acetate titration method.

All of the values obtained for the base exchange capacity of this lignin-ash, as shown by Table 8, were small, the highest being only 3.6 M.E. in the ash from 100 grams of lignin. This highest value was obtained in the case of decomposed wheat straw lignin, the ash content of which was appreciably higher than that of the rest of the preparations. Since duplicate base exchange determinations on the lignin preparations sometimes showed differences as great as 2 M.E. per 100 g. of lignin, the exchange capacity values obtained for the lignin-ashes are almost negligible in effect. By
### Table 8

**True Base Exchange Capacity of Ash-free Lignin**
*(Barium Titration Method)*

<table>
<thead>
<tr>
<th>Material</th>
<th>Cornstalks</th>
<th>Rye straw</th>
<th>Oat straw</th>
<th>Wheat straw</th>
</tr>
</thead>
<tbody>
<tr>
<td>Original</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total exch. cap. of 100 g. ash-free lignin $= M_{E_{2}}$</td>
<td>--</td>
<td>38.5</td>
<td>48.1</td>
<td>46.4</td>
</tr>
<tr>
<td>Ash content of lignin $= %$</td>
<td>--</td>
<td>7.6</td>
<td>15.1</td>
<td>15.8</td>
</tr>
<tr>
<td>Exch. cap. of the ash in 100 g. lignin $= M_{E_{2}}$</td>
<td>1.8</td>
<td>2.1</td>
<td>2.7</td>
<td></td>
</tr>
<tr>
<td>True exch. cap. of 100 g. ash-free lignin $= M_{E_{2}}$</td>
<td>--</td>
<td>36.3</td>
<td>45.6</td>
<td>45.5</td>
</tr>
<tr>
<td>Residue at 3 mo.</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total exch. cap. of 100 g. ash-free lignin $= M_{E_{2}}$</td>
<td>145.3</td>
<td>155.6</td>
<td>--</td>
<td>--</td>
</tr>
<tr>
<td>Ash content of lignin $= %$</td>
<td>15.0</td>
<td>12.8</td>
<td>--</td>
<td>--</td>
</tr>
<tr>
<td>Exch. cap. of the ash in 100 g. lignin $= M_{E_{2}}$</td>
<td>2.2</td>
<td>1.8</td>
<td>--</td>
<td>--</td>
</tr>
<tr>
<td>True exch. cap. of 100 g. ash-free lignin $= M_{E_{2}}$</td>
<td>142.7</td>
<td>155.5</td>
<td>--</td>
<td>--</td>
</tr>
<tr>
<td>Residue at 6 mo.</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total exch. cap. of 100 g. ash-free lignin $= M_{E_{2}}$</td>
<td>166.6</td>
<td>155.6</td>
<td>168.4</td>
<td>154.2</td>
</tr>
<tr>
<td>Ash content of lignin $= %$</td>
<td>21.7</td>
<td>15.5</td>
<td>17.6</td>
<td>28.3</td>
</tr>
<tr>
<td>Exch. cap. of the ash in 100 g. lignin $= M_{E_{2}}$</td>
<td>3.1</td>
<td>2.5</td>
<td>1.2</td>
<td>3.6</td>
</tr>
<tr>
<td>True exch. cap. of 100 g. ash-free lignin $= M_{E_{2}}$</td>
<td>162.6</td>
<td>152.6</td>
<td>166.9</td>
<td>149.2</td>
</tr>
</tbody>
</table>

Exch. cap. $= \text{Base exchange capacity.}$
use of the exchange capacity values of the lignin-ash and of ash-free lignin itself, the true value for the exchange capacity can be calculated (Table 8). This calculation revealed that the true exchange capacity differed very little from the total exchange capacity, calculated without a consideration of the ash.

The lignin preparations from the decomposed residues were appreciably higher in nitrogen than those from the parent materials (Table 5). This nitrogen cannot be assumed to be present as protein, for experiments have shown (27, 29) that the increments in weight of apparent lignin produced in the presence of known amounts of protein are not as great as would be obtained if simple condensation took place. It is recognized, however, that the presence of nitrogenous material condensed with the lignin is a factor that has to be taken into consideration when discussing changes in properties as a result of decomposition. What effect this condensed nitrogenous material may have on the exchange capacity has not yet been investigated.

Since none of the measurements to be described later indicated any considerable change in the lignin molecule that might account for the large increase in base exchange capacity as a result of decomposition, it was thought that possibly the slow biological removal of carbohydrate material with which the lignin may be originally combined had left free some reactive grouping. Experiments were, therefore, carried out on the hydrolysis and oxidation of one of the original plant materials. Ten 2 g. portions of rye straw were treated in duplicate by the following five treatments:
1. Untreated.
2. Boiled with 3 per cent H₂O₂ till nearly colorless.
3. Hydrolyzed with 5 per cent H₂SO₄ for 1 hour.
4. Hydrolyzed with 5 per cent H₂SO₄ and then treated with 3 per cent H₂O₂ as in 2.
5. Boiled with hot water.

After these treatments all the materials were dried and weighed.

Finally their exchange capacities were determined by the ammonium acetate leaching method. The results given in Table 9 show that instead of increasing the exchange capacity appreciably, these chemical treatments had little effect. From this it can be seen that the increase produced by decomposition in the exchange capacity of either the original materials or of the lignins cannot be duplicated by the mild type of chemical treatments used here.

Table 9
The Effect of Chemical Pretreatment on the Exchange capacity of Rye Straw (Ammonium Titration Method)

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Plant material</th>
<th>Residue after 3 mo.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>M₄E₄ per 100 g</td>
<td>M₄E₄ per 100 g</td>
</tr>
<tr>
<td></td>
<td>orig. mat.</td>
<td>treated mat.</td>
</tr>
<tr>
<td>Untreated</td>
<td>5.4</td>
<td>-</td>
</tr>
<tr>
<td>3% H₂O₂</td>
<td>3.4</td>
<td>4.4</td>
</tr>
<tr>
<td>Hot 5% H₂SO₄ for 1 hr.</td>
<td>1.5</td>
<td>2.4</td>
</tr>
<tr>
<td>Hot 5% H₂SO₄ for 1 hr. followed by 3% H₂SO₄</td>
<td>2.6</td>
<td>4.8</td>
</tr>
<tr>
<td>Hot water on steam plate</td>
<td>5.7</td>
<td>6.3</td>
</tr>
</tbody>
</table>
Table I

Metathetical Content of the Methanol Preparations

Table 10

was observed.

with the lignin, the amount could not account for so great a reduction as
though there be some similarity to the condensation of nitriles, these materials
partly an apparent reduction of this nature might be observed. But, 0.10
from the results were of low
ton of the plant material. * If the lignin from the results were of low
established that partial demethylation of lignin may accompany decompost-
were direct demethylation on treated preparations, it may be taken an
of coal to such an extent as to amount to 40 per cent in wet, after steam lignin. Since these
month period. The losses of methanol varied from 25 per cent in the lignin
ix
The losses of methanol are shown in Table 10. * The effects of decomposition were

methanol content of the lignin preparations determined on an
Oxidation of lignin preparations with alkaline hypoiodite

The amounts of iodine utilized from alkaline hypoiodite solution by the lignin preparations at two time intervals are given in Table 11, expressed in terms of grams of iodine utilized per gram of ash-free lignin.

If the one and one-half hour time interval values in Table 11 are considered, it will be seen that in three cases decomposition has produced no significant change in the susceptibility of the lignin preparations to oxidation, whereas in one case a small reduction was observed.

Table 11
Oxidation of Lignin with Alkaline Hypoiodite

<table>
<thead>
<tr>
<th>Material</th>
<th>Time interval</th>
<th>Cornstalks</th>
<th>Rye straw</th>
<th>Oat straw</th>
<th>Wheat straw</th>
</tr>
</thead>
<tbody>
<tr>
<td>Original</td>
<td>1/2 hr.</td>
<td>1.81</td>
<td>1.61</td>
<td>2.22</td>
<td>1.93</td>
</tr>
<tr>
<td></td>
<td>1-1/2 &quot;</td>
<td>2.09</td>
<td>1.89</td>
<td>2.26</td>
<td>2.21</td>
</tr>
<tr>
<td>Residue</td>
<td>1/2 &quot;</td>
<td>1.70</td>
<td>1.62</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>at 3 mo.</td>
<td>1-1/2 &quot;</td>
<td>2.14</td>
<td>2.07</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Residue</td>
<td>1/2 &quot;</td>
<td>1.79</td>
<td>1.49</td>
<td>1.91</td>
<td>1.62</td>
</tr>
<tr>
<td>at 6 mo.</td>
<td>1-1/2 &quot;</td>
<td>1.97</td>
<td>1.85</td>
<td>2.16</td>
<td>1.78</td>
</tr>
</tbody>
</table>

The nature of the grouping in the lignin molecule which is affected by this reagent is not clear. Walde and Nixon (49) showed that the methylation of lignin prevented alkaline iodine oxidation, with the conclusion that the action is associated with free hydroxyl groups. However, only certain of these must be susceptible since the oxidized product from normal lignin could still be extensively methylated. The oxidation appar-
ently also brings about some demethylation.

The results presented above indicate that no extensive changes in the lignin molecule occurred during biological decomposition, and whatever be its nature, the reactive grouping, which might be expected to be the susceptible point, has been but little affected.
DISCUSSION OF RESULTS

The studies reported here again demonstrate that during aerobic decomposition a considerable removal of the lignin may take place, even though this constituent is more resistant than the carbohydrates, and, therefore, accumulates in the residues. The base exchange capacity of such residues has not been found to be greatly different than that of the plant materials, when calculated on the basis of unit weight of original organic matter. The sorption capacity of the lignin, however, showed a very considerable increase with decomposition. The assumption was made that the activity of the isolated lignin is much the same as that of the lignin in situ, in either the original materials or in the residues therefrom. The validity of this assumption may be somewhat vitiated by possible changes of the keto-enol type, which may take place during the isolation of the lignin (13). Lignin probably occurs in situ in some form of association with carbohydrates of hemicellulosic nature, which may normally shield certain of the reactive groupings. If, however, biological decomposition removes these associated carbohydrates, a similar transformation in the reactivity may be produced thereby. Experiments on acid hydrolyzed material, which might have been expected to produce a like effect, did not in fact bear out this supposition.

Evidence for the change in the replacement capacity of lignin may be to some extent vitiated due to the fact that the purity of the preparations is not above question. The effect of the presence of ash may be discounted
since the activity of the inorganic material was found to be negligible. The fact, however, that some nitrogen is condensed with the lignin is possibly more serious, but this amount must be relatively small since the increase in weight of apparent lignin, produced in the presence of protein, over that of lignin prepared in its absence is less than the nitrogen retained times the protein factor 3.25 (27). On this basis the impurity from nitrogen condensation probably did not exceed 10 per cent in any of the preparations. Such a small fraction could not account for the large increase in the base exchange capacity of lignin observed in this work. This point is, however, capable of experimental verification.

No evidence has been obtained which provides an explanation for the important phenomenon of base sorption by lignin. The groupings active in this respect are likely to be phenolic hydroxy groups and, possibly, carboxyl groups, though it is doubtful whether these latter are present in normal lignin. Simple demethylation of lignin might result in an increase in free hydroxyl groups, but this cannot be the sole explanation unless some other particularly active grouping has thus been exposed. A study of methylated lignin on decomposition might be helpful in this connection.

Reduction in methoxyl content has been established as one of the changes occurring in lignin, since the magnitude of the reduction after six months is greater than could be accounted for by the increasing impurity of the preparations.

The oxidation of lignin with alkaline hypoiodite can be taken, however, as evidence that no very substantial chemical change has occurred in the lignin during decomposition since the amount of iodine utilized by the lignins from the residues was much the same as that by those from the
original materials.

The work presented here has confirmed by direct evidence the conclusion of previous workers, based upon indirect evidence, that lignin plays a much larger role in the sorption capacity of the rotted residues than in that of the parent materials, but no satisfactory explanation for this increased activity of the lignin was obtained.
SUMMARY AND CONCLUSIONS

The object of this work was to determine some of the effects of decomposition on the lignin of plant materials. Cornstalks, rye straw, wheat straw, and oat straw were decomposed aerobically for periods of three and six months. Sulphuric acid lignin was isolated from both the rotted residues and the original materials. The base exchange capacities of the lignin preparations from the residues, as well as of those from the original materials, were determined by the ammonium acetate distillation method and by the barium acetate titration method. The change in the replacement capacity, calculated upon the basis of unit weight of original material, and the contribution of the lignin to the total exchange capacity of the residues and the original materials were determined. The role played by the ash content of the lignin in the base exchange capacity was investigated. Finally, the methoxyl content of the lignin preparations and their reactivity to alkaline oxidation with sodium hypochlorite were measured.

The results obtained in these studies may be summarized as follows:

1. Approximately one-third of the lignin in the four plant materials and about two-thirds of the materials themselves were removed in six months.

2. The exchange capacity of the ash-free lignins from the rotted residues was three or four times as great as that of those from the original materials, but the sorption capacities of the residues and the parent materials did not differ greatly, when both were calculated on the basis of unit weight of original material.
3. A major share of the replacement capacity of the rotted residues was due to their lignin content, but in the parent materials only a small share of the sorption capacity could be attributed to lignin.

4. The fact that the greatly increased sorption capacity of the lignin with decomposition was not accompanied by much change in the replacement capacity of the plant materials can, in all probability, be attributed to a simultaneous removal of nonligninous material possessing base exchange properties.

5. A loss of from 27 to 40 per cent of the methoxy groups of the isolated lignins occurred during six months decomposition.

6. The reactivity of lignin to alkaline oxidation with sodium hypochlorite was not much changed by decomposition.
LITERATURE CITED


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