1974

Peroxyacetic Acid Delignification of Wood and Bark from Ponderosa Pine (Pinus ponderosa)

Danny C. Ronning

PEROXYACETIC ACID DELIGNIFICATION OF WOOD AND BARK FROM PONDEROSA PINE (PINUS PONDEROSA)

BY

DANNY C. RONNING

A thesis submitted
in partial fulfillment of the requirements for the
degree Master of Science, Major in
Animal Science, South Dakota
State University
1974

PEROXYACETIC ACID DELIGNIFICATION OF WOOD AND BARK FROM PONDEROSA PINE (PINUS PONDEROSA)

This thesis is approved as a creditable and independent investigation by a candidate for the degree, Master of Science, and is acceptable for meeting the thesis requirements for this degree. Acceptance of this thesis does not imply that the conclusions reached by the candidate are necessarily the conclusions of the major department.

Thesis Adviser Date

Head, Animal Science Dept. Date

ACKNOWLEDGEMENTS

The author wishes to express his gratitude and appreciation to his major adviser, Dr. L. D. Kamstra, who provided the necessary guidance and counsel throughout my course of study and in the preparation of this thesis. Appreciation is also extended to fellow graduate students for their helpful suggestions and support.

To the typist of this manuscript, Anna Baumiller, sincere appreciation and thanks is given for her excellent cooperation and assistance.

Special recognition is given to my wife, Vera M. Ronning, and my children for their sacrifice, encouragement, and understanding throughout the course of this study.

DCR

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

INTRODUCTION

The timber industry produces large quantities of unused wood fiber residues each year through its removal of 14 billion cubic feet of growing stock from the United State's 500 million acres of commercial timberland. The most recent figures that are available (Grantham and Ellis, 1974) indicate that 1.6 billion cubic feet of wood residue is left behind after logging operations each year. Unused residues from primary manufacturing sites amount to an additional 1,000 million cubic feet of wood and 600 million cubic feet of bark. When calculated on the basis of tons of dry matter, logging residues total 23.9 million tons, primary plant residues total 14.9 million tons of wood and 9.4 million tons of bark. Economic factors and increasingly stringent environmental pollution standards have stimulated the industry to seek new methods for utilization of these residues or find more acceptable means for their disposal.

Coinciding with this situation is the growing concern about providing adequate food supplies for the world's population. It is becoming increasingly evident that human competition with animals for the high quality food sources will become more prevalent in the future. This occurrence will necessitate the use, by animals, of feed materials not heretofore extensively considered.

Ruminant species, which include cattle, sheep and goats, have the unique capability of digesting cellulose and hemicellulose, the cell-wall carbohydrates that constitute 70 to 80 percent of wood

fiber. By their conversion of these higher polysaccharides, which are indigestible by humans and other monogastrics, to animal products the ruminant species offer a potential means for solving the residue disposal problems of the wood industry while concomitantly extending the food supply for human nutrition.

The ability of ruminants to digest the cell-wall carbohydrates depends on the microbial digestion that occurs in the reticulo-rumen. This organ lies between the esophagus and the abomasum or true stomach and contains large numbers of bacterial and protozoal organisms in an anaerobic atmosphere. Many of these microorganisms possess enzyme systems which are capable of hydrolyzing the beta configuration of the glycosidic linkages which join the monomeric subunits of cellulose and hemicellulose. Through this process of enzymatic hydrolysis cellulose and hemicellulose are gradually degraded to their basic sugar units which are further metabolized to volatile fatty acids, primarily acetic and propionic. These fatty acids are absorbed through the reticulo-rumen wall and serve as the main source of energy to the animal (Blaxter, 1962).

As a potential food source wood has several advantages. It is one of the few renewable resources that is available in abundant supply and can be obtained year round. Because its growth occurs slowly over a period of many years short term fluctuations in supply are not likely to be a problem. Its ability to grow on land area that is not well suited for human habitation or domestic crop cultivation makes it very noncompetitive for land use.

Many researchers have investigated the inclusion of raw wood fiber into ruminant rations and numerous physical and chemical treatments have been conducted in attempts to improve its nutritional quality. In spite of these efforts a desirable processing technique has yet to be achieved.

One of the major limitations is the nature of the raw material itself. Wood is a heterogeneous substance that varies in physical and chemical composition among genera and species. Within a single tree, composition varies cross-sectionally and longitudinally and is influenced by seasonal changes.

This investigation will review previous work concerning the use of wood fiber as a ruminant feed and will evaluate the effectiveness of peroxyacetic acid treatment of wood fibers to improve their digestibility.

REVIEW OF LITERATURE

Wood Composition and Chemistry

The elementary chemical composition of wood is quite uniform. The principle chemical elements are: carbon, 49-50 percent; hydrogen, about 6 percent; oxygen, 44-45 percent; and nitrogen, about 0.1 to 1 percent (Tsoumis, 1968). The major organic components are cellulose, hemicellulose, and lignin. Figure 1, taken from Browning, (1967), shows the various component fractions that constitute all wood fiber. The major components are present in all woods with some differences in proportion according to species. Small variation is found from tree to tree and between different parts of the same tree. The kinds and amounts of the minor components present are largely dependent upon the species.

Table 1 was constructed from the values reported by Wenzl (1970) on the gross chemical composition of North American hardwoods and softwoods.

Cellulose, the major constituent of wood, is a linear polymer of glucopyranose units linked by B-(1→4)-glycosidic bonds. Molecular weight determinations have found that the number of glucose units per molecule ranges from as few as 15 or less, to as high as 10,000 to 14,000, with an average of about 3,000 (Cowling and Brown, 1969).

The hemicelluloses in wood consist of relatively short heteropolymers of glucose, xylose, galactose, mannose, and arabinose. Linkage of these monosaccharide units and derivatives is by 1→3,

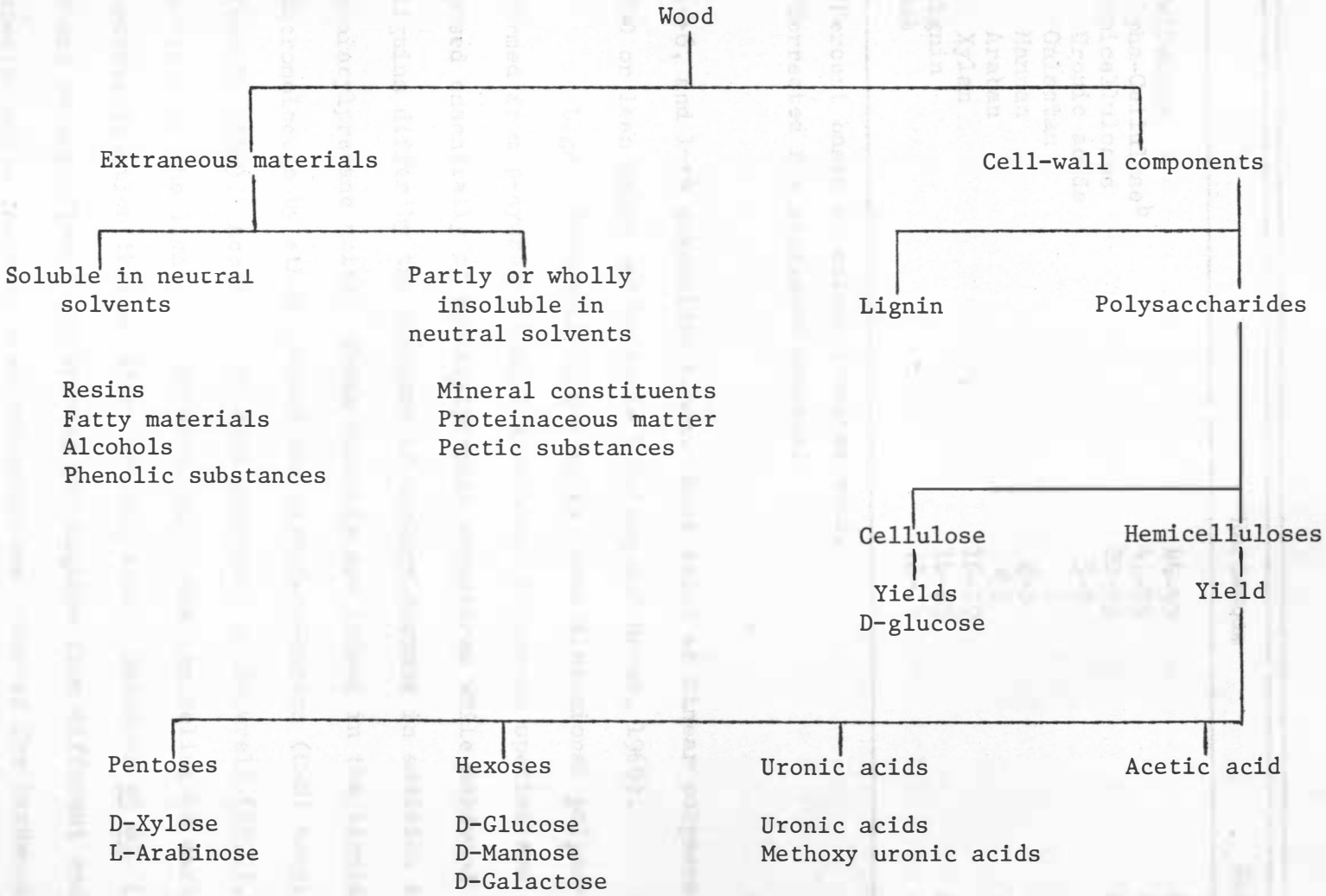


Figure 1. The Composition of Wood (Browning, 1967)

TABLE 1. CHEMICAL COMPOSITION OF WOOD[a]

	Hardwoods	Softwoods
Cellulose	44-57	44-49
Alpha-Cellulose[b]	41-53	40-45
Hemicelluloses	20-35	20-35
Uronic acids	3-5	2-5
Galactan	1	1-2
Mannan	2-3	8-13
Araban	<1	.5-1.5
Xylan	16-17	4-7
Lignin	16-25	25-35
Ash	.2-.4	.2-.4

[a]Percent based on extractive-free wood.

[b]Corrected for nonglucan material.

1→6, and 1→4 glycosidic bonds. Most exist as linear polymers of 200 or less units per molecule (Cowling and Brown, 1969).

Lignin from wood is a complex three dimensional polymer formed from p-hydroxycinnamyl alcohols. Softwoods species are composed essentially of quaiacylpropane structures while hardwood lignins differ by the presence of syringylpropane in addition to quaiacylpropane units. These subunits are joined in the lignin macromolecule by ether (C-O-C) and carbon-to-carbon (C-C) bonding (Wenzl, 1970). According to data presented by Brownell (1971), a portion of the lignin may be attached to the hemicellulose carbohydrate fraction through ether bonding also. Sarkanen et al. (1967) found no significant differences for lignins from different conifer species while large species variation was observed for hardwood

lignins, with the ratio of syringylpropane to quaiacylpropane groups being the largest variable.

The principal mineral elements found in wood ash are calcium, potassium, and magnesium along with traces of other elements found in the soil where the tree is grown. Some tropical species also contain large amounts of silica (Wenzl, 1970).

Raw Wood Materials as Feedstuffs

Interest in the feeding of wood has been expressed for many years; as evidenced by Kellner's evaluation in 1913 of the feeding value of sawdust, twigs, leaves, and brushwood based on their chemical analysis. The more recent investigations have been primarily concerned with the use of wood fiber as a roughage substitute in high concentrate rations or as a diluent in maintenance-type diets. Results of some of the work have been promising but most studies have shown a limited potential for wood as a feed ingredient unless it is modified by some means to allow greater availability of its carbohydrates.

Slyter and Kamstra (1974) reported results of a study that compared ponderosa pine (Pinus ponderosa) sawdust and alfalfa hay as roughage sources in an 85 percent corn grain based beef finishing ration. The inclusion of sawdust at 10 percent of ration showed no significant differences in growth performance, feed conversion, or carcass characteristics when compared to the 15 percent alfalfa control diet. Sawdust as the sole roughage source (15 percent of total ration) resulted in significantly less ($P < .05$) average daily gain

than was obtained on the 15 percent alfalfa diet. Nutrient conversion, based on TDN per kg of gain, was most efficient when sawdust provided two-thirds of the roughage in the ration.

Oak sawdust was used by Anthony and Cunningham (1968) in sheep and cattle finishing trials. When included at 2.5 percent or 10 percent of the diet the sawdust supported daily gains equal to, or slightly better, than that obtained from the all concentrate basal diet.

A similar study by Anthony et al. (1968) was conducted to compare sawdust and Coastal bermuda grass hay as roughage sources. Lambs were fed rations that contained either 10 percent sawdust or 10 percent ground Coastal hay. Those fed the sawdust containing ration equaled those fed the Coastal-containing ration in daily gain and were only slightly less efficient in feed conversion. A second trial compared 15 percent sawdust and 15 percent bermuda grass hay in steer rations. The animals fed the hay rations consumed more feed, had a faster rate of gain, and were more efficient than those fed the sawdust. The incidence of rumen parakeratosis was greater in the sawdust-fed animals than in the hay fed group.

Kinsman et al. (1969) reported that a 20 percent hardwood sawdust diet fed to lambs produced adequate growth (0.25 kg per day), feed consumption (1.67 kg per day), and feed efficiency (6.64 kg). No control groups were used for comparison.

Vara et al. (1968) compared rations containing 14 and 28 percent ground cottonwood to a ground corncob control ration. Average

daily gain was less for bulls receiving the cottonwood diets but their better feed efficiency resulted in no differences in cost of production.

Kitts et al. (1969) conducted two in vivo feeding trials with beef steers to determine the utilization of unprocessed ground alder wood. The first experiment compared wood and hay when both were fed at levels of about 10 percent of a barley based finishing ration, either with a high-urea protein supplement or a natural protein supplement. Weight gain was not significantly different for any of the treatments although the animals fed sawdust plus either of the two supplements gained less than those receiving hay with either supplement.

Examination of the data reveals that the lower performance of the sawdust fed steers may be partially explained by differences in nutrient composition between the hay and sawdust containing rations. Diets containing barley, hay, and either of the two supplements contained about one percent more protein than the comparable sawdust rations (13.25 and 13.49 percent versus 11.88 and 12.02 percent) and about 8.6 percent more digestible energy (1470 and 1462 kcal/lb versus 1358 and 1341 kcal/lb).

The second experiment by these same researchers was designed to study the extent to which the non-processed, raw wood was utilized by the animal. The same amount of a barley basal ration was fed to each of four treatments with raw sawdust added to three of the treatments at levels of 13.3, 27.2 or 35 percent of total ration. Differences in daily gain were not significant for any of the treatments.

Feed conversion, when expressed as the amount of basal diet consumed per unit of gain, slightly favored the animals receiving 13.3 percent sawdust which indicated a slight degree of actual utilization of the sawdust.

The effects of sawdust particle size and dietary level were examined by El-Sabben et al. (1971). Both fine and coarse particles of oak sawdust were included in beef finishing rations at levels of 5 percent and compared with a control ration containing 5 percent timothy hay. Steers fed the 5 and 15 percent sawdust, either coarse or fine particle size, gained less than those receiving 5 percent timothy hay; however only those animals receiving the 5 percent fine sawdust gained significantly less ($P < .05$). In a second trial the level of hay in the control ration was increased to 15 percent with the sawdust levels remaining at 5 and 15 percent. Fine sawdust at both 5 and 15 percent resulted in significantly lower gains ($P < .05$) as compared to the control. The coarse sawdust fed steers gained more than the steers fed fine sawdust but none of the sawdust fed cattle gained as well as those fed the timothy hay control ration. Steers fed the control ration and those fed the 15 percent coarse sawdust exhibited the lowest incidence of rumen parakeratosis. Gilbert et al. (1973) also reported that when lamb finishing rations containing 15 percent sawdust or 15 percent hay were compared, the sawdust diets resulted in lower daily gain and feed efficiency ($P < .05$).

Dinius et al. (1970) evaluated the potential of several wood residue materials of varying particle size for use as roughage

substitutes in high concentrate rations. Aspen sawdust, oak sawdust, mixed hardwood shaving, and oak flooring waste were fed to wether sheep at a rate of 10 percent of the complete ration to determine acceptability and digestibility of rations containing these materials. Dry matter intake and digestible energy intake for the rations containing the wood by-products were not significantly different from that of the no roughage control ration. The authors also reported very little preferential selection of ration components in the wood containing rations unless particle size approached 3 cm in length.

Raw wood fiber has also been considered for use as a major component in maintenance type ruminant rations. Slyter and Kamstra (1973) reported the use of ponderosa pine (Pinus ponderosa) in rations of first-calf beef heifers during their last trimester of pregnancy. A treatment ration of 25 percent ground sawdust was compared to a control ration of alfalfa-grass hay. Intake of both rations was restricted to 20 lb per head daily. The sawdust containing ration supported adequate weight gains, caused no consumption or toxicity problems and calf birthweights were normal. Marion et al. (1959) maintained beef cows for 140 days on a mesquite wood ration. The cows reportedly calved normally and were in better condition than a comparison group of cows fed cottonseed hulls.

A wood silage from poplar bark was evaluated by Enzmann et al. (1969) in a feeding and digestion trial using mature sheep. When the ensiled bark was fed ad libitum plus a daily supplement of 0.23 kg soybean meal the sheep lost weight and required the addition of

0.34 kg of oats to meet their maintenance requirement. Total digestible nutrients (TDN) value of the bark silage was reported as 31.7 percent and digestible energy (DE) to be 1.6 kcal per gram.

The potential use of wood fiber for regulation of feed intake and growth in dairy animals was investigated by Cody et al. (1968). No adverse health effects in heifers were observed by feeding pine sawdust at levels up to 45 percent of the diet. They found that levels of sawdust from 25-45 percent were effective in limiting grain intake and satisfactory gains were obtained on a ration composed of 65 percent concentrate and 35 percent sawdust. A second study, (Cody et al., 1972) with young dairy bulls resulted in ruminal and omasal impaction when sawdust from the shortleaf southern pine (Pinus echinata) was fed at dietary levels above 25 percent. Adequate growth rate was maintained when sawdust levels did not exceed 10-25 percent of the ration.

Ellis and Durham (1968) conducted a similar trial in which 6.8 kg of mesquite wood and 1.125 kg of concentrate were provided each day to mature beef cows during and after pregnancy. The researchers concluded that the wood did not supply sufficient energy for maintenance and milk production after calving, but was adequate for maintaining their weight during pregnancy.

Satter et al. (1970) investigated the possibility of using bigtooth aspen (Populus grandidentata Michx.) sawdust as a partial roughage substitute for hay in lactating dairy rations. Cows received 2.3 kg of long hay and about 17 kg of a pelleted ration containing 32

percent sawdust. Normal milk production and milk fat were maintained by this diet. Cows on a similar ration but without the sawdust produced milk having only half as much fat. Two similar experiments were conducted later by the same researchers (Satter et al., 1973) to determine if aspen sawdust could serve as the sole roughage source at dietary levels lower than those used in the previous study. The results showed that milk fat increased with increases in dietary sawdust levels up to 30 percent. Feed intake at the 30 percent sawdust level could not be maintained unless supplemental hay was provided.

The results of in vitro digestion experiments are generally in agreement with the in vivo work that has been conducted. A modified in vitro rumen technique developed specifically for wood materials by Mellenberger et al. (1970) was used by Millet et al. (1970) to study 24 different species of wood. Digestibility of the hardwoods ranged from 2 percent for red alder and sweetgum to 35 percent for aspen. The softwoods were essentially indigestible (0 percent for pine, hemlock, and spruce to 5 percent for Douglas-fir). Using the same digestion procedure, Feist et al. (1970) found similar variations among species.

Baker (1973) studied the effect of lignin on the in vitro digestibilities of two hardwood (birch and oak) and two softwood (pine and Douglas-fir) species. Pulps having different lignin contents were assayed for digestibility by the in vitro method of Mellenberger et al.

In general, as the lignin content decreased, digestibility increased, and the hardwood species showed greater response in digestibility increases than the softwoods at the same degree of delignification. At lignin contents of less than 7 percent, however, all pulps had about the same digestibility (80 to 90 percent). This same inverse relationship between lignin content and digestibility for wood and wood derived materials was observed by other investigators (Woodman and Stewart, 1932; Homb, 1949; Saarinen et al., 1959; and Mertens and Van Soest, 1971) and has also been reported with plants and forages as well (Crampton and Maynard, 1938; Kamstra et al., 1958; and Tomlin et al., 1965).

Van Soest (1968) found that the effects of lignin on digestibility are restricted to the cellulose and hemicellulose carbohydrate fractions of the cell wall. He concluded that since these components constitute the largest portion of wood fiber that has nutritional value, a delignification method that did not have a detrimental effect on them would be valuable.

Alkaline Chemical Treatments

Much of the work with chemical treatment of wood and other fiberous materials to improve nutritive value has involved the use of various alkali salts. One of the most widely known is a process for the sodium hydroxide (NaOH) treatment of straws (Beckmann, 1922) that has been modified for use with wood and other fiberous plant materials as well. The original procedure consisted of steeping the material

to be treated in eight times its weight of 1.5 percent sodium hydroxide solution for 4 hours. The material was then washed free of alkali and fed wet or dried. Increase in digestibility of straws treated by this method was about two-fold. Wilson and Pigden (1964) treated wheat straw and poplar wood with sodium hydroxide at levels up to 15 grams NaOH per 100 grams of wood or straw. They reported a linear increase in *in vitro* digestibility as the NaOH concentration increased from 0 to 9 percent; above this level no further increases in digestibility could be obtained. With this treatment the *in vitro* digestibility of the poplar wood increased from 3 to 40 percent while the wheat straw increased in digestibility from 30 to 70 percent.

Feist *et al.* (1970) investigated the effect of NaOH treatment on *in vitro* digestibility and found the response to be highly species dependent. Red oak (*Quercus rubus*) increased in *in vitro* digestibility from 3 percent before treatment to 14 percent after treatment while the digestibility of American basswood (*Tilia americana*) increased from 5 to 56 percent and Quaking aspen (*Populus tremuloides*) increased from 35 to 50 percent. Red oak and quaking aspen were also treated with increasing concentrations of NaOH to find the level that resulted in maximum digestion response. Both species reached a plateau digestibility at NaOH treatment levels of 5 to 6 grams per 100 grams of wood. Another study by Millet *et al.* (1970) confirmed the findings of Feist *et al.* on the response of hardwoods to NaOH treatments, but two softwoods studied, Douglas-fir and Sitka spruce, did not respond to the alkali treatment. Huffman *et al.* (1971) treated several species of

both hardwoods and softwoods with NaOH. Their results indicated a response of hardwoods similar to that obtained by Feist et al. and Millet et al.; however, the softwood species demonstrated essentially zero digestibility whether treated or untreated.

Alkali treated aspen sawdust (5 g NaOH per 100 g of wood) was incorporated into roughage (alfalfa meal) and concentrate (corn) based rations at levels of 0, 15, 30, 45 and 60 percent by Mellenberger et al. (1971). As the percentage of treated aspen increased in the ration, the over-all digestibility of the ration decreased. Digestibility of the aspen was found to be increased by at least 25 percent as a result of NaOH treatment.

Anhydrous liquid ammonia, used by Millett et al. (1970) increased the digestibility of aspen wood over 50 percent (from 33 to 51 percent) and the nitrogen content from 0.08 percent to 1.44 percent. Treatment with gaseous ammonia resulted in digestibilities not significantly different than those from the liquid ammonia treatments. Earlier work reported by Tarkow and Feist (1969) gave similar responses.

The mechanism by which alkali treatments increase digestibility was shown by Tarkow and Feist (1969) to be a breaking (by saponification) of intermolecular ester lignin to lignin and lignin to carbohydrate bonds. As a result of this chemical action the swelling capacity of the wood is increased beyond normal water saturated dimensions; thus it provides for improved enzymatic and microbial penetration into the wood. Millet et al. (1970) confirmed their view and suggested that when

alkali amounts exceed the 5 to 6 percent level usually required to reach plateau digestibility the yields of product obtained decrease because of the high degree of hemicellulose solubility in alkali solutions.

Ball Milling

Reduction of wood fiber particles to extreme fineness can be achieved by grinding in a ballmill for extended time periods. Bender et al. (1970) reported that ball milling for 24 hours increased the in vitro digestibility of aspen from 23.4 to 42.8 percent and of white birch from 15.1 to 50 percent. Ball milling of three softwoods (fir, hemlock, and spruce) was not effective. Millett et al. (1970) progressively increased ball milling time from zero to 240 minutes and obtained concomitant increases in 48 hour in vitro digestibilities of red oak (from 5 to 45 percent) and aspen (from 20 to 55 percent). In agreement with the work of Bender et al. (1970) they found that ball milling had little effect on softwoods.

The ball milling treatment technique is considered to be of limited value due to highly selective species response, its high cost of operation, and the possibility that finely ground wood will not function as effectively in the rumen as it does in the in vitro assay. Fine grinding of forages has been shown to decrease in vivo digestibility of cellulose rather than increase it, Moore (1964) and Meyer et al. (1965).

Acid Hydrolysis

Early work with acid hydrolysis was done by Sherrard and Blanco (1921). In their procedure white pine sawdust was digested with 1.8 percent sulfuric acid for 15 minutes under a steam pressure of about 120 psi and then neutralized with lime. This hydrolyzed product contained 20 percent less cellulose, 16 to 18 percent more reducing sugars, 12 percent less crude fiber and about the same lignin content (about 30 percent) as compared to the original sawdust. Feeding this material to 3 dairy cows reportedly gave good results although no data was presented by the authors.

Archibald (1926) used the process of Sherrard and Blanco (1921) to make hydrolyzed products from white pine and Douglas-fir sawdusts. In a digestion trial with sheep he found the average dry matter digestibility to be 46 percent for the hydrolyzed white pine and 33 percent for the Douglas-fir hydrolyzed sawdust. Based on calculations of net energy by formula, the author found the white pine hydrolyzed sawdust to have an apparent net energy value of 18.6 therms/100 lbs, but the Douglas-fir hydrolyzed sawdust had a negative net energy value, indicating that more energy was used in the digestive process than was released from the material.

Mild hydrolysis conditions were achieved by Heaney and Bender (1970) by steaming aspen chips for 1.5 to 2 hours at pressures of 100 to 115 psi and reaching temperatures of about 160 to 170 degrees C. Results of a digestion trial with lambs found the steamed aspen wood to have digestion coefficients of 48 percent for dry matter and 45 percent

for gross energy. Digestible energy of the material was calculated to be 2.17 kcal per gram. Bender et al. (1970) used the treatment procedure of Heaney and Bender (1970) and reported a 48 hour in vitro digestibility value for treated aspen of 56.6 percent. Six other hardwood species treated also showed considerable increases in digestibility but treated conifers; Black spruce (Picea mariana), Balsam fir (Abies balsamea), and Eastern hemlock (Tsuga canadensis) showed no response.

Kitts et al. (1969) fed an "extruded" wood product to fattening steers at 15 and 20 percent of a barley based ration and compared it to rations containing hay or sawdust at equivalent levels. The product was formed by a high friction grinding process which subjected the wood to pressures of 1500 to 2000 psi and temperatures of approximately 350 degrees F. Results of the feeding trial showed that the steers receiving hay at either of the two levels (15 or 20 percent) gained significantly faster than animals fed either the extruded wood or untreated sawdust. There was no significant difference in rate of gain for animals fed either form of wood as the roughage source although the extruded wood rations did support slightly better growth and feed efficiency than did the sawdust containing diets.

Pfander et al. (1969) evaluated a commercial hemicellulose product (trademark, Masonex, Masonite Corporation, Chicago, Illinois) as a ruminant feed source. This product is formed by subjecting wood chips to live steam at 42 kg/cm^2 pressure for one minute and then washing in counter current washers to obtain a solution of about four

percent dry matter. The washings are eventually evaporated to a 65 percent solids product having a pH of about 3.7. This product can also be neutralized to about pH 5.5 or spray dried. Through numerous trials using sheep the researchers found this hemicellulose product to be relatively palatable and to be equivalent to molasses in feeding value. No adverse effects from long term feeding of the hemicellulose were observed in a trial in which wethers were maintained five years in confinement on rations containing 30 percent of this material.

Erlinger (1974) evaluated a hydrolyzed wood product formed by digesting 2.35 percent sulfuric acid treated sawdust for 40 seconds under a steam pressure of 42.182 kg/cm^2. He found this product to be less efficiently utilized as an energy source than corn cobs, to have little roughage characteristic, and possibly contained toxic substances.

Extreme wartime shortages of available feed supplies prompted the Scandinavian countries to use wood pulp materials in ruminant rations. Hvidsten (1949) described a cellulose feed made primarily from spruce by the sulfite method. Organic matter digestibilities by sheep and cattle of sulfite pulped forest thinnings with bark averaged 75 to 86 percent. By comparison, a mechanical pulp was only 9 percent digestible and a steam hydrolyzed sawdust product digested 48 percent. Nordfeldt (1951) reviewed the preparation and use of wood pulp materials for ruminant feeds and emphasized the need for protein and mineral supplementation of these purified carbohydrate feeds. Peacetime economic factors and availability of conventional feedstuffs of

better quality made the use of wood pulps for feed uneconomical and their use was discontinued.

A revival of interest in the use of wood pulp materials for feed has occurred in recent years. Saarinen et al. (1959) evaluated 40 different high yield birch pulps by conducting digestion trials with sheep. The authors reported that the dry matter digestibility of the various pulps ranged from 28 to 90 percent; with the alkali pulps generally having higher digestibilities than pulps prepared by acid or chlorite methods.

A wood-extract molasses (82 percent soluble carbohydrates) by-product resulting from the manufacture of cellulose from wood pulp was evaluated by Cullison and Ward (1965). Feeding the product to steers in liquid form at 9 percent of a growing ration and as a dried powder at 15 percent of the diet resulted in feed intakes and weight gains not significantly different from a control ration containing 9 percent blackstrap molasses.

Clarke et al. (1971) reported the results of a 70 day feeding trial in which steer rations containing 50 percent and 70 percent Douglas-fir wood pulp were compared to a control of 82.5 percent barley. Variable intakes were observed as the animals fed 50 percent wood pulp consumed more daily feed than the control animals (9.40 kg versus 8.73 kg) but those receiving 70 percent wood pulp consumed less (7.86 kg). Feed efficiency of the group receiving 70 percent wood pulp was comparable, however, with the control steers (5.76 versus 5.30); poorest efficiency resulted from the 50 percent wood pulp ration

(7.15 kg feed/kg of gain). Average daily gain decreased with increasing dietary level of sawdust.

A feeding trial by Clarke and Dyer (1973) compared equalized intakes of a 70 percent sulfite Douglas-fir pulp ration with a 78 percent barley ration. From the feedlot performance data the authors concluded that the wood product was not equivalent to barley in feeding value but was approximately equivalent to hay.

The possibilities of using pulp and papermaking residues as ruminant feeds was investigated by Millet et al. (1973). In vitro dry matter digestibilities of 15 pulp residues varied from zero for southern pine and spruce groundwood fines to 95 percent for bleached, mixed hardwood, kraft processed pulp fines. In vivo digestibilities of some selected pulp residues were lower than the in vitro values for the same material. The authors suggested that the small particle size of the residues increased their rate of passage and consequently reduced bacterial degradation.

Peroxyacetic Acid Treatments

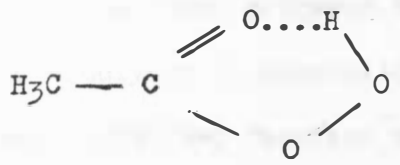
Peroxyacetic acid (CH_3CO_2OH), also named peracetic acid, is an organic peroxycarboxylic acid used widely as an oxidizing agent. The "per" prefix is often used in industry but in scientific usage the preferred prefix is "peroxy" (Mageli and Sheppard, 1967). In this text the peroxy prefix is used except when citing the work of other authors who used the "per" prefix.

Before discussing the potential of peroxyacetic acetic for modifying wood fibers to form ruminant feedstuffs, information about its chemical and physical properties and characteristics is necessary. Therefore, the following description of peroxyacetic acid according to Swern (1970a) seems appropriate.

Peroxyacetic acid is a colorless liquid that melts at approximately 0 C and undergoes violent exothermic decomposition when its calculated boiling point of 110° at 760 mm pressure is reached.

Peroxyacetic acid has a sharp, irritating unpleasant odor. It causes severe skin and eye damage and, on inhalation, its vapors have been shown to be lethal to rats within four hours at a concentration of 1000 ppm (0.1 percent).

In the liquid state peroxyacetic acid exists as an intramolecularly chelated monomer of the following structure. As a



consequence of the intramolecular hydrogen bonded condition peroxyacetic acid is about 1/3000 as strong an acid as its parent carboxylic acid, acetic acid. The pK values are 8.2 and 4.74, respectively, at 20 C.

Peroxyacetic acid is completely miscible with water, ethyl acetate, chloroform and other halogenated solvents, acetone, acetic acid, hydrocarbons and many other organic solvents which are not

oxidized by it. It readily oxidizes cork and rubber, and plasticized polyvinyl chloride undergoes plasticizer extraction when contacted by peroxyacetic acid. Glass is the most suitable material for handling peroxyacetic acid but polyethylene, teflon, polystyrene, and other inert plastics can be used, as well as high purity stainless steel.

The presence of impurities, especially metal salts, catalyze the decomposition of peracetic acid, usually to acetic acid and hydrogen peroxide; with oxygen, carbon monoxide and dioxide being liberated. In general, alkaline substances lower the stability of peroxyacetic acid but acids have little effect.

Peroxyacetic acid solutions in water or acetic acid can be stabilized by addition of transition metal chelating agents at concentrations of about 100 to 1000 ppm. Examples are sodium pyrophosphate, sodium hexametaphosphate, sodium tetrametapyrophosphate.

Peroxyacetic acid has been prepared by a number of methods; the most important and widely used, according to Swern (1970b), is the direct sulfuric acid-catalyzed reaction of 30 to 98 percent hydrogen peroxide with acetic acid by the following reaction: $CH_3CO_2H + H_2O_2 \leftrightarrows CH_3CO_3H + H_2O$. This reaction is an equilibrium reaction and the final concentration of peroxyacetic acid is determined by initial concentration of acetic acid and hydrogen peroxide and the molar ratios of these reactants.

When acetic anhydride is employed instead of acetic acid in the preparation of peroxyacetic acid, water free solutions can be obtained in less reaction time. Since the water is removed by reaction

with acetic anhydride the conversion of hydrogen peroxide to peroxyacetic acid is very high and is often quantitative.

Azeotropic removal of water by vacuum distillation has been used to shift the equilibrium of the acetic acid-hydrogen peroxide reaction to favor the formation of peroxyacetic acid. As a consequence approximately stoichiometric quantities of reactants can be used.

Peroxyacetic acid is widely used as an oxidizing agent in organic chemistry; Swern (1970c) lists over 800 compounds that were reported to have been epoxidized with peroxyacetic acid since 1952. Major industrial uses of peroxyacetic acid have been the epoxidation of fatty oils and fatty acid esters to form plasticizers and stabilizers for polyvinyl chloride and its copolymers. The diverse number of applications and reaction mechanisms of peroxyacetic acid are beyond the scope of this text and will not be discussed except as they pertain to its reaction with wood fibers.

Early investigations on the application of peroxyacetic acid to wood were conducted by Poljak (1948) for the determination of cellulose in woods and to find a new pulping process. By treating fir wood chips for 2-4 hours at 60-80 C with ten times their weight of 10-15 percent peracetic acid he reported obtaining a lignin free cellulose product in 60 percent yield. Some hemicelluloses were removed by the action of peracetic acid and about 4 percent of the carbon in the wood was converted to carbon dioxide.

Haas et al. (1955) used a modification of Poljak's method to study the oxidative dilignification by peracetic acid by hardwood

and softwood species. Five gram samples of woodmeal or shavings were treated with 250 ml of 10 percent peracetic and placed in a 90 C water-bath for varying time periods. After 15 minutes of reaction time beechwood was almost totally delignified as the 75 percent yield of holocellulose contained only 0.5-1 percent residual lignin. Delignification of spruce or pine under the same conditions required longer reaction times (30-50 minutes). In accordance with the findings of Poljak (1948) the researchers found some loss of hemicelluloses under the treatment conditions indicated above (about 4 percent of the pentosans were rendered soluble). Although no details are available, extended reaction times or more drastic treatments were reported to cause serious hemicellulose losses (especially the pentosans).

In 1961 Leopold presented data on the peroxyacetic acid treatment of loblolly pine. By treating one gram of wood with 5 grams of peracetic acid (buffered with 2.5 grams of sodium acetate) for two hours at 70 C he obtained a 75.3 percent yield of holocellulose. Based on the weight of the original wood, the peracetic acid oxidation was reported to decrease lignin content from 28.5 to 2.6 percent; the retention of carbohydrates was good, with some loss of mannan occurring.

Sarkanen and Suzuki (1965) studied the mechanism of oxidative delignification of Douglas-fir meal by peracetic acid. Infrared absorption measurements and ultraviolet absorption characterization of the water soluble lignin product revealed that practically all of

the lignin aromatic nuclei were converted to muconic acid structures that contained some of the original methoxyl as methyl ester groups. Side-chain oxidation of the lignin was found to be small. Based on these results, the authors proposed the following mechanism for peracetic oxidation of lignin:

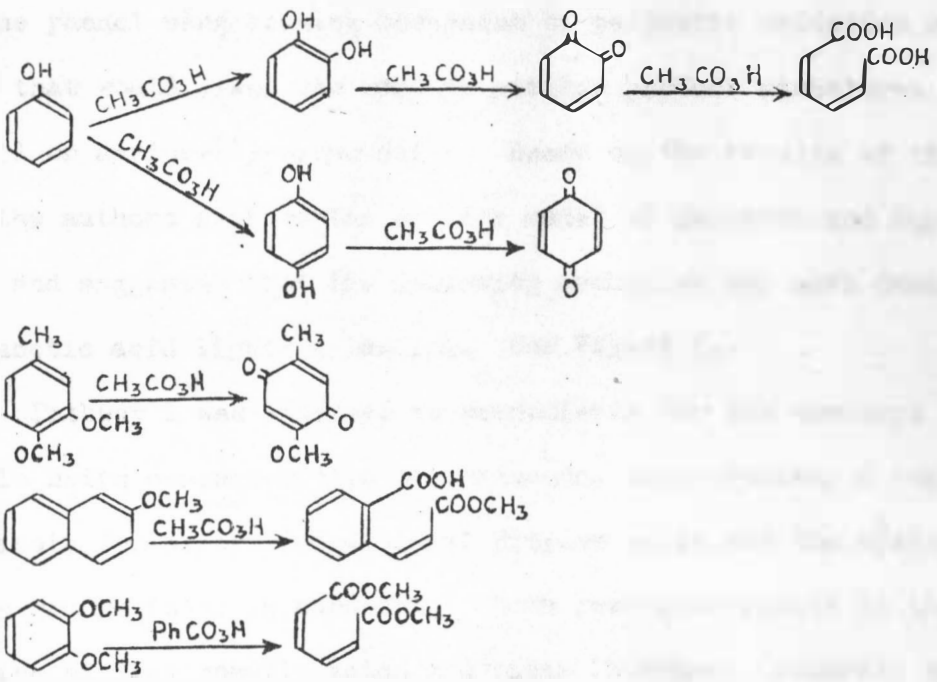


Figure 2. Peracetic Oxidation of Model Compounds (Sarkanen and Suzuki, 1965)

Ishikawa et al. (1966) studied the oxidative degradation by peracetic lignin of several model compounds similar to the vanillyl alcohol elements present in softwood lignin. The main decomposition products were found to be quinones with small amounts of muconic,

maleic, and oxalic acids also formed. Results of a comparative study of hardwood (red alder) and softwood (Douglas-fir) lignin isolations from peracetic acid treatments were reported by Lai and Sarkanen in 1968. The researchers found that peracetic lignins from softwood or hardwoods were so similar that the lignin source could not be recognized by chemical or physical analytical methods. They concluded that the phenol ring opening mechanism of peracetic oxidation must be a type that would yield the same or similar product structures from quaiacyl or syringyl propane units. Based on the results of this study the authors revised the earlier model of Sarkanen and Suzuki (1965) and suggested that the following mechanism was more descriptive of peracetic acid lignin oxidation. See Figure 3.

Pathway 1 was reported to predominate for the quaiacyl phenolic units characteristic of softwoods, while Pathway 2 reactions predominate for etherified quaiacyl propane units and the syringyl propane groups found in hardwoods. Both reactions result in the formation of dicarboxylic acids and their lactones; primarily maleic and fumaric acid derivatives. Species variation in the rate of lignin oxidation was observed by these investigators; who proposed that the higher methoxyl content of syringyl propane units in hardwoods permitted greater reactivity with peracetic acid.

Sakai et al. (1972) investigated the reaction of peracetic acid with the β-aryl ether linkage that involves 50-66 percent of the lignin unit. By oxidizing model compounds with peracetic acid and identifying the resulting reaction products the researchers determined

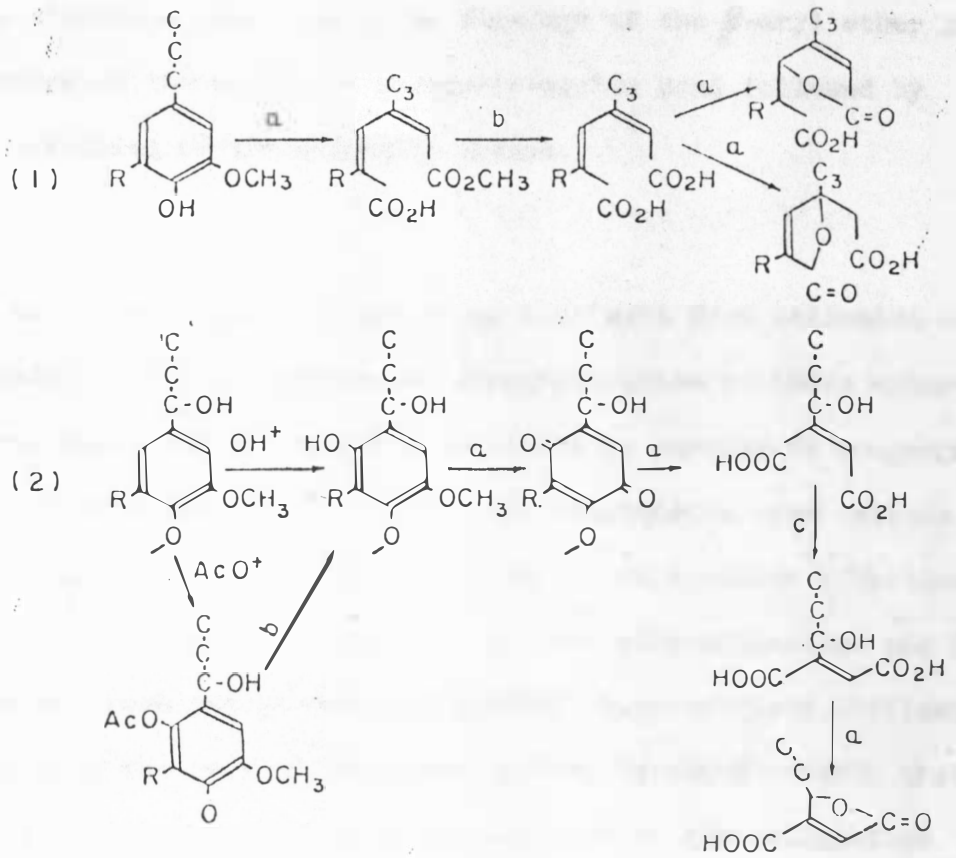


Figure 3. Proposed Oxidation Mechanism of Lignins by Peracetic Acid. (a) Oxidative Ring Opening by Peracetic Acid, (b) Hydrolysis, (c) Isomerization. R = H, OCH_3 or Alkyl Group (Sarkanen and Suzuki, 1965)

the main sidechain reactions to be cleavage of the β-aryl ether linkage and cleavage of the alkylaryl carbon-to-carbon bond followed by further oxidation of the aldehydes formed.

Summary

Any significant utilization of nutrients from untreated wood is precluded by the low dry matter digestibilities of these materials; therefore, their use as feeds is restricted to serving as roughage in high concentrate diets or as diluents in maintenance type rations.

It has been shown that lignin serves as a rather effective barrier to the enzymatic degradation of wood polysaccharides and that some form of treatment is required to facilitate nutrient utilization. Examination of the various treatment methods reviewed reveals that response is highly species specific as hardwoods are delignified more easily than softwoods and their digestibility increases more rapidly as lignin is removed. These differences are explained by Baker (1973) to result from one or more of the following factors: (1) softwoods contain 25 to 50 percent more lignin than hardwoods, (2) there are differences in the lignin-carbohydrate association between the two woods, and (3) the lignin structure of softwoods differs from that of hardwoods.

For a physical and/or chemical treatment of wood fiber to effectively improve nutrient utilization Kitts et al. (1969) indicate the following prerequisites: (1) cost of treatment should be economical enough to allow the finished product to compete effectively.

with the cost of other roughages, (2) the treatment method should increase nutrient availability without causing excessive polysaccharide depolymerization or micronutrient loss, and (3) the treatment should remove any natural inhibitors of polysaccharases and should not leave any toxic residues.

While peroxyacetic acid is effective as a delignifying agent there are no reports in the literature regarding its use for improving nutrient availability of wood fiber; therefore a number of peroxyacetic acid treated wood samples were examined by chemical analysis and *in vitro* rumen fermentation assay to determine its effectiveness.

METHODS OF PROCEDURE

Selection and Preparation of Substrates for Peroxyacetic Acid Treatments

Ponderosa pine (Pinus ponderosa) which occupies 87 percent of South Dakota's 1.33 million acres of commercial forest land, is clearly the species of major economic importance to the state's lumber industry (Choate and Spencer, 1969). Results of preliminary studies by the author also found this material to be essentially unresponsive to treatment by alkali, steam, pressure or combinations of these methods. Therefore, ponderosa sawdust and bark were selected as substrates for treatment by peroxyacetic acid.

Wood chips and bark were furnished by Whitewood Post and Pole, Whitewood, South Dakota. Following transport to the laboratory these materials were air-dried at room temperature until they reached moisture equilibrium with the atmosphere. The air-dried samples were then ground successively through a Wiley mill to pass 4.0 mm and 2.0 mm screens and stored in polyethylene bags until used for treatment and analysis.

Preparation and Analysis of Peroxyacetic Acid

The peroxyacetic acid (CH_3CO_3H) used for treatment of the sawdust and bark substrates was prepared in the laboratory according to the unpublished procedure of Schroeder and France (1974). As this method of synthesis is not widely known it is described below.

I. Preparation of Peroxyacetic acid

A. Special equipment

1. 6,000 ml Pyrex boiling flask for reaction vessel
2. mechanical stirring device and glass rod stirrer

B. Chemicals

1. Hydrogen peroxide—Technical grade (stabilized or unstabilized) 30-35 percent H_2O_2 is adequate
2. Acetic anhydride—Use reagent grade $(CH_3CO)_2O$
3. Sodium pyrophosphate—Used as a stabilizer for H_2O_2 and CH_3CO_3H

C. Procedure

1. Place the reaction vessel into an ice bath. Add 2 volumes of H_2O_2 and allow to cool to about 10 C. (If using unstabilized H_2O_2 add 10 g of sodium pyrophosphate per 1500 ml of H_2O_2).
2. Add 3 volumes of $(CH_3CO)_2$ to the reaction vessel dropwise with continual agitation of the flask contents.

Precautions:

1. Always add acetic anhydride to hydrogen peroxide, and not the reverse, to prevent conversion of peroxyacetic acid to the highly explosive diacetyl peroxide.
2. Rate of $(CH_3CO)_2O$ addition must be controlled so as to maintain a reaction temperature below

80 C, which is approximately the lower temperature boundary at which peroxyacetic acid solutions of 30 to 40 percent concentration become shock sensitive. Conversely, if the reaction temperature is too low during the mixing of $(CH_3CO)_2O$ and H_2O_2 complete mixing of reactants may occur before any appreciable hydrolysis and perhydrolysis have taken place; at which point the reaction may proceed with explosive and uncontrollable speed. A reaction mixture temperature range between 60 and 70° C will produce maximum concentrations of CH_3O_3H.

3. Stir reaction solution for approximately 16 hours, and allow to stand for an additional 24 hours to complete the reaction.

II. Analysis for peroxyacetic acid concentration. The ceric sulfate-sodium thiosulfate titration method of Greenspan and MacKellar (1948) was used to determine the peroxyacetic acid concentration and the amount of hydrogen peroxide present.

A. Chemicals

1. Peroxyacetic acid solution
2. 5 percent sulfuric acid (H_2SO_4)

3. Ceric sulfate (ammonium tetrasulfatocerate) 0.1 N in 0.05 N sulfuric acid
4. Ferroin indicator (o-phenanthroline-ferrous complex)
5. 10 percent potassium iodide solution
6. 0.1 N sodium thiosulfate solution
7. Starch indicator

B. Procedure

1. To a 500 ml Erlenmeyer flask, add 100 ml of 5 percent H_2SO_4 and sufficient cracked ice to maintain a temperature range between 0 and 10 C.
2. Add to the flask 5 ml of diluted peroxyacetic acid solution (5 ml CH_3CO_3H diluted to 250 ml).
3. Add 3 drops of ferroin indicator and titrate with 0.1 N ceric sulfate solution to the disappearance of the pink color.
4. Then add 10 ml of 10 percent KI solution and titrate the liberated iodine with 0.1 N sodium thiosulfate. Starch indicator is added near the end point of thiosulfate titration.

C. Calculations

$$\% H_2O_2 = \frac{\text{ml of ceric sulfate} \times N \times 1.7}{\text{dilution factor}}$$

$$\% CH_3CO_3H = \frac{\text{ml of thiosulfate} \times N \times 3.8}{\text{dilution factor}}$$

$$\text{Dilution factor} = \frac{\text{ml } CH_3CO_3H \text{ aliquote}}{\text{volume of flask}} \times \text{ml of diluted } CH_3CO_3H \text{ titrated}$$

Procedure for Treatment of Substrates with Peroxyacetic Acid

In preparation for treatment, duplicate 20 g samples of bark or sawdust dry matter were weighed into 400 ml griffin beakers. Peroxyacetic acid solutions of appropriate concentrations were then added to the beakers. Following the addition of peroxyacetic acid the beakers were covered with 3 mill polyethylene sheets held tightly in place by rubber bands to contain vapors.

Upon termination of treatment the contents of the beakers were filtered through 100 mesh silk bolting cloth in a Buchner funnel with the aid of suction. The residue was washed with one liter of 50 C water to remove unreacted acid and all soluble components. The filtered samples were transferred to evaporating dishes and dried *in vacuo* for 24 hours at 38-40 C and 700 mm Hg. After drying, the treated substrates were stored in tightly capped glass bottles.

In Vitro Fermentation Assay

The following modification of the Van Soest (1966) procedure for the *in vitro* digestion of cell walls was used to evaluate the untreated and peroxyacetic acid treated samples:

> Samples were ground to pass a 0.4 mm screen in a Weber laboratory hammermill; dried *in vacuo* (700 mm Hg) for four hours at 38 to 40 C, and weighed (0.5 g) in duplicate into 100 ml polypropylene centrifuge tubes. Each group of 25 tubes included duplicates of a "standard" forage sample of known digestibility, duplicate samples of a purified wood cellulose,

Solka Floc (Brown Co., Berlin, N.H.), and one reagent blank containing only rumen innoculum and buffer. Just prior to collection of the rumen innoculum the samples were wetted by the addition of 3 ml of water to each tube. The tubes were placed under 700 mm Hg vacuum for 10 to 15 minutes before random placement in a 39 C waterbath.

The donor cow for the innoculum was a mature Holstein steer maintained on a diet of mixed species grass hay. Rumen ingesta was obtained via a ruminal fistula 4 to 5 hours post-feeding. The ingesta obtained from the animal was squeezed through three layers of cheesecloth into a prewarmed insulated flask. Following transport to the laboratory the fluid obtained from the squeezings was filtered through three layers of cheesecloth into a 6 liter boiling flask equipped with a magnetic stirrer and CO_2-inlet tube. By means of a calibrated dispensing syringe 15 ml of rumen innoculum and 35 ml of McDougalls buffer (1948) was added to each tube. Following addition of the innoculum-buffer solution each tube was gassed with CO_2 and sealed with a rubber stopper equipped with a gas release check valve. During the fermentation stage the tubes were swirled three times daily to resuspend the contents.

After 48 hours of fermentation the contents of the tubes were transferred to a beaker for cell-wall determination by the neutral detergent fiber procedure of Van Soest (1967).

Calculations

Percent *in vitro* dry matter disappearance was calculated as:

$$S_w - (R_w - B_w)\ (100)/S_w,$$

where S_w = weight of initial substrate dry matter; R_w = weight of residue dry matter; B_w = weight of reagent blank dry matter.

Calculation for percent *in vitro* cell walls digestion was:

$$S_{cw} - (R_{cw} - B_{cw})\ (100)/S_{cw},$$

where S_{cw} = cell walls weight of initial substrate; R_{cw} = weight of residue cell walls; B_{cw} = cell walls weight of reagent blank.

Cell-Wall Constitutents

The neutral-detergent procedure of Van Soest and Wine (1967) was used to estimate cell-wall components and cellular contents.

Acid Detergent Fiber and Acid Detergent Lignin

To estimate the sum of cellulose and lignin the acid-detergent fiber (ADF) procedure of Van Soest (1963) was used. The residue remaining from the ADF procedure was used for the determination of acid insoluble lignin by the method of Fonnesbeck and Harris (1970).

Determination of Cellulose, Moisture, and Nitrogen

The cellulose content of the untreated ponderosa bark and sawdust samples was determined according to the widely used method of Crampton and Maynard (1938). Moisture and nitrogen analyses were conducted by A.O.A.C. (1960) methods.

RESULTS AND DISCUSSION

A series of treatments, involving the application of peroxyacetic acid to ponderosa pine (Pinus ponderosa) bark and sawdust samples, were conducted to study the chemical's effectiveness as a delignifying agent and the effects of lignin removal on in vitro rumen digestibility.

Preliminary Examination of the Substrates Used in Subsequent Peroxyacetic Acid Treatment Study

The ponderosa pine sawdust and ground bark substrates used in the peroxyacetic acid treatment study were first analyzed for neutral-detergent fiber (NDF), acid-detergent fiber (ADF), Crampton and Maynard Cellulose (CMC), nitrogen (N) and ash. The results of these determinations are presented in Table 2.

TABLE 2. COMPOSITION OF SUBSTRATES TREATED WITH PEROXYACETIC ACID

	Ponderosa Sawdust	Ponderosa Bark
	%	%
Neutral Detergent Fiber	92.8	74.4
Acid Detergent Fiber	79.5	64.5
Acid Detergent Lignin	27.4	33.7
Crampton and Maynard Cellulose	49.1	26.2
Nitrogen	0.2	0.3
Ash	0.2	3.8

Effects of Peroxyacetic Treatment on Fiber Component Fractions

Treatment conditions, results of analyses for neutral-detergent fiber, acid-detergent fiber, and acid-detergent lignin are presented in Table 3.

Least squares analysis of variance revealed a highly significant ($P<.01$) treatment effect on the component fractions of NDF, ADF and ADL. In general as the amount of lignin removed increased the values for these fractions decreased.

The general trends of changes in NDF and ADF values of sawdust and bark with increasing delignification are depicted in Figures 4 and 5. The greatest changes in the NDF and ADF fractions resulting from peroxyacetic acid delignification is in the chemical composition of these fractions. Since the acid-detergent fiber represents essentially the sum of cellulose and lignin (Colburn and Evans, 1965) the ADF in the completely delignified residues is composed almost exclusively of cellulose. Neutral-detergent fiber, which is comprised of hemicellulose plus acid-detergent fiber (Van Soest, 1967), also increases in cellulose content as lignin is removed.

Yields of residues resulting from the treatment of the bark samples were determined in order to investigate the selectivity of peroxyacetic acid as a delignifying agent. If the action of the reagent was to remove only lignin and leave the carbohydrates intact the yields would follow the line marked "theoretical" as seen in Figure 6. It can be observed that the yields are somewhat less than

TABLE 3. PREPARATION, COMPOSITION AND IN VITRO DIGESTIBILITIES OF PEROXYACETIC ACID TREATED SUBSTRATES

CH_3CO_3H Concentration	Grams of CH_3CO_3H Per 100 g Substrate	Yield	Time	Temperature	NDF[a]	ADF[b]	ADL[c]	Delignification	IVDMD[d]	IVCWD[e]
%		%	Hr	°C	%	%	%	%	%	%
					Sawdust					
-----	---	100	---	--	92.8	79.5	27.4	0	7.3	0.2
2.50	20	---	168	25	88.6	75.9	17.6	35.8	12.6	1.3
3.75	30	---	168	25	85.7	73.2	14.3	47.9	26.9	14.7
5.00	40	---	168	25	82.6	71.1	8.7	68.2	48.6	37.7
6.25	50	---	168	25	79.9	69.5	5.8	78.8	61.5	48.2
7.50	60	---	168	25	78.5	67.2	3.9	85.8	68.3	59.6
8.75	70	---	168	25	78.3	65.8	1.5	94.5	72.5	64.9
20.0	200	---	1	75	73.0	60.8	0.2	99.4	81.1	69.0
20.0	200	---	2	75	79.0	68.1	0.3	99.1	77.4	71.4
20.0	200	---	3	75	76.5	67.3	0.1	99.5	80.3	74.2
10.0	100	---	1	75	77.8	66.2	0.3	99.1	80.5	75.0
10.0	100	---	2	75	80.4	69.9	0.1	99.5	81.8	77.3
10.0	100	---	3	75	83.3	73.2	0.2	99.4	81.0	77.2
5.0	50	---	1	75	77.3	65.7	4.7	82.7	67.9	58.5
5.0	50	---	2	75	77.0	64.7	1.9	93.2	75.1	67.6
5.0	50	---	3	75	75.9	64.9	0.9	96.7	78.0	70.7
2.5	25	---	1	75	90.7	76.3	13.5	50.8	19.1	10.8
2.5	25	---	2	75	89.6	76.0	11.2	59.0	30.3	22.2
2.5	25	---	3	75	89.1	76.4	9.6	64.9	38.7	31.2
2.5	25	---	1	85	88.8	76.2	14.0	49.0	28.4	14.1
2.5	25	---	2	85	89.7	75.8	13.0	52.6	38.2	27.0
2.5	25	---	3	85	88.6	76.1	13.2	51.9	37.8	25.6
2.0	20	---	1	85	87.1	76.4	16.3	40.7	15.5	2.9
2.0	20	---	2	85	86.1	76.7	15.7	42.8	18.9	5.9
2.0	20	---	3	85	85.9	77.2	15.6	43.2	19.0	5.9
					Bark					
0	0	100	---	--	74.4	64.5	33.7	0	30.0	5.9
4.0	20	85.8	168	25	67.3	60.8	24.8	26.3	46.6	20.6
6.0	30	80.5	168	25	68.1	57.9	20.5	39.3	53.2	31.3
8.0	40	75.8	168	25	69.4	56.8	16.4	51.3	56.5	37.3
10.0	50	70.7	168	25	69.8	55.6	13.8	59.1	60.0	42.7
12.0	60	67.2	168	25	68.3	52.9	8.6	74.6	66.9	51.5
14.0	70	65.2	168	25	66.1	51.1	5.7	83.2	73.3	59.6

[a]NDF = neutral-detergent fiber

[b]ADF = acid-detergent fiber

[c]ADL = acid-detergent lignin

[d]IVDMD = *in vitro* dry matter disappearance

[e]IVCWD = *in vitro* cell-wall digestibility

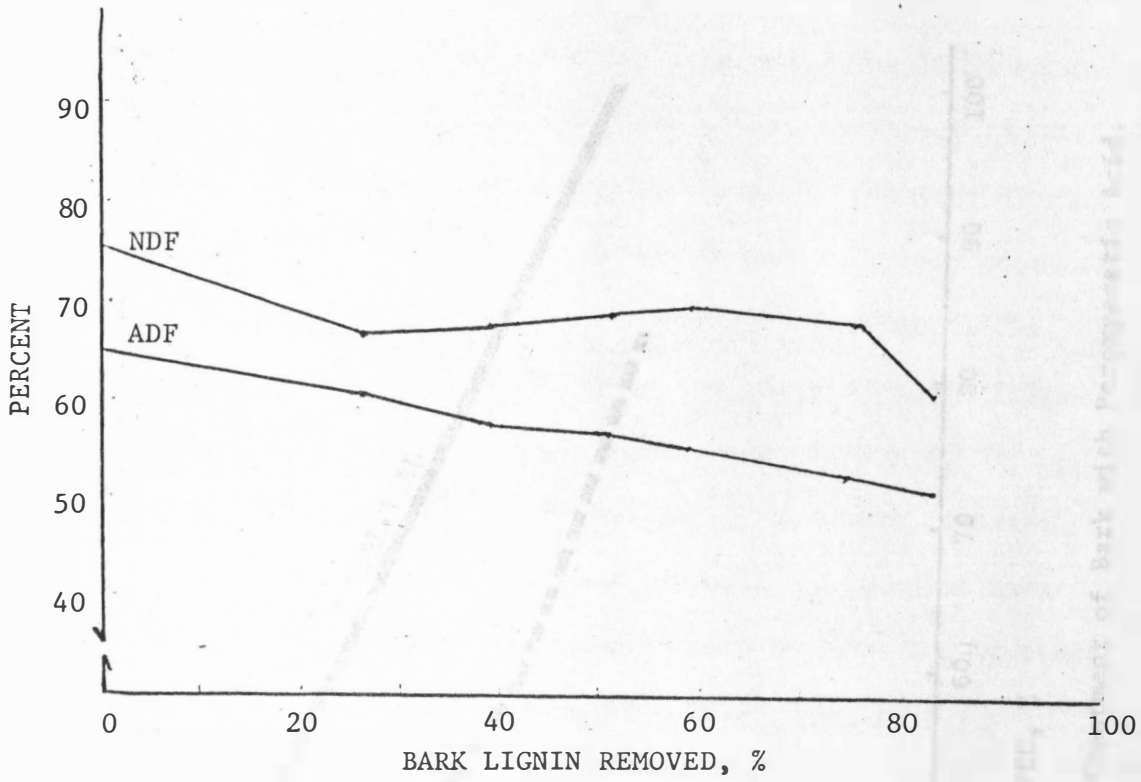


Figure 4. Effect of Peroxyacetic Acid Delignification on the NDF and ADF Components of Bark.

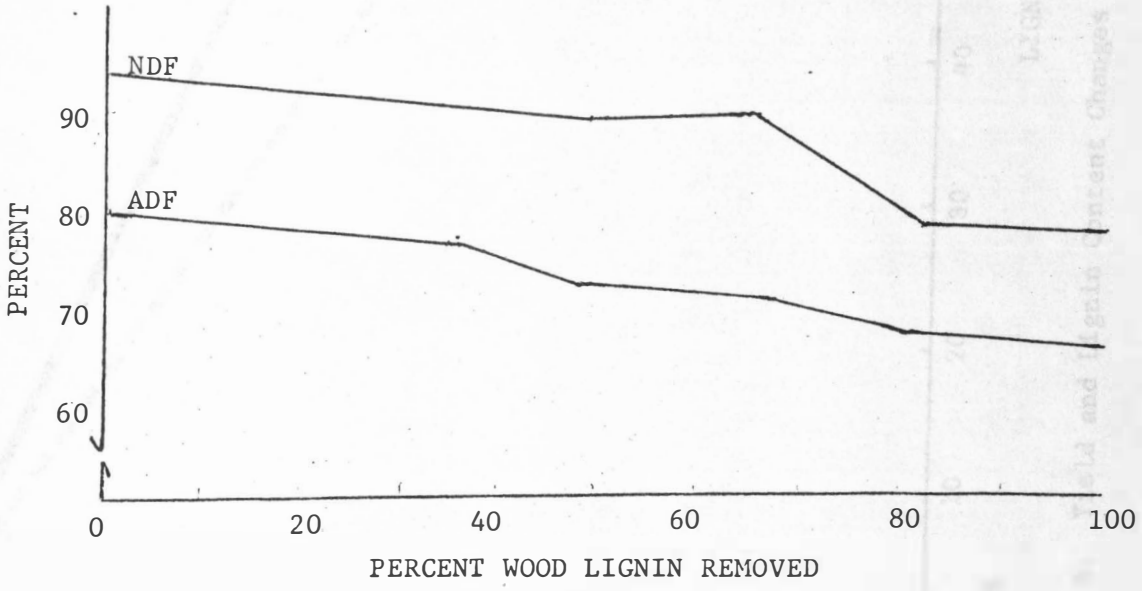


Figure 5. Effect of Peroxyacetic Acid Delignification on the NDF and ADF Components of Wood

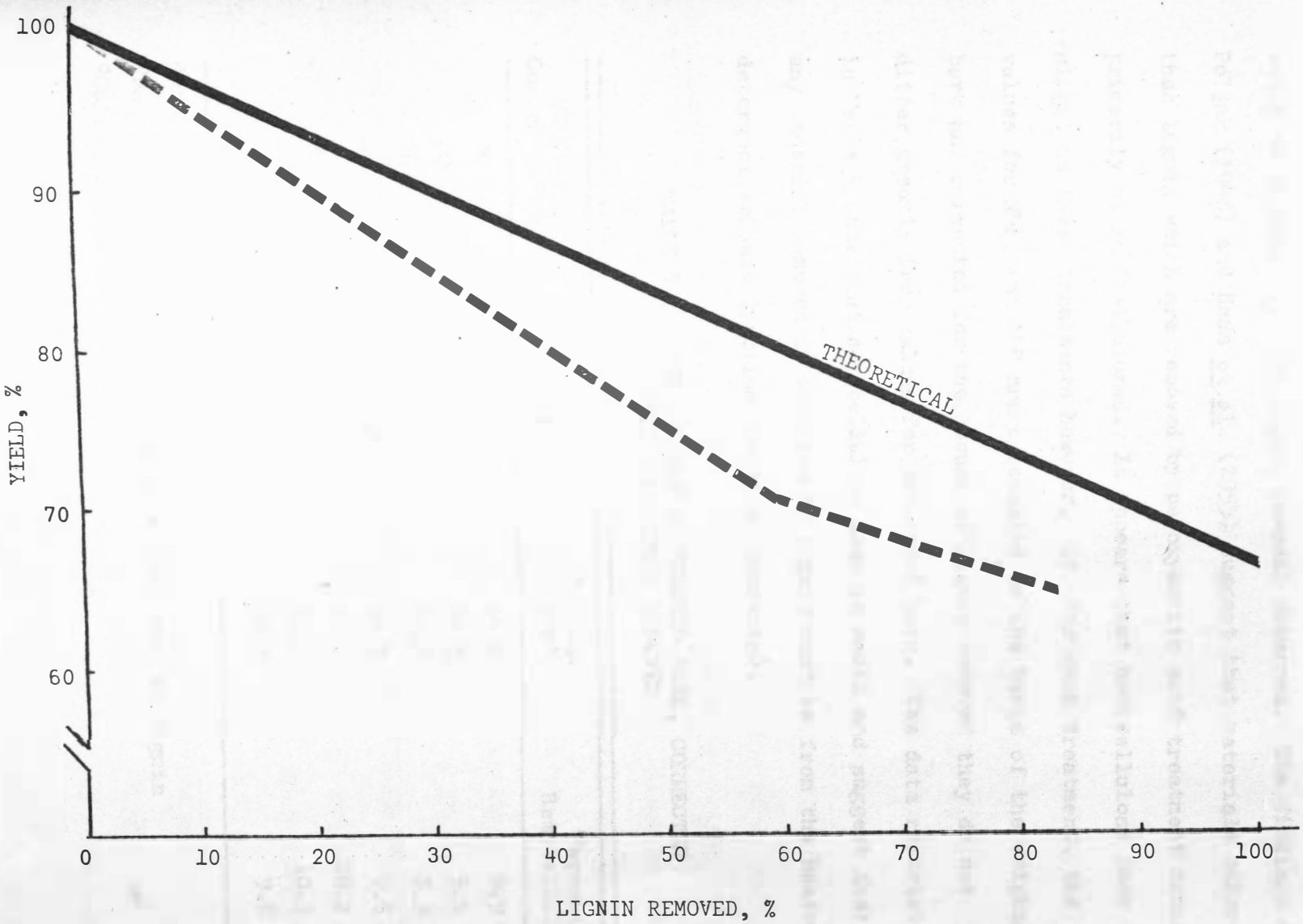


Figure 6. Yield and Lignin Content Changes During Treatment of Bark with Peroxyacetic Acid.

would be obtained if only lignin removal occurred. The findings of Poljak (1948) and Haas et al. (1955) suggest that materials other than lignin which are removed by peroxyacetic acid treatment consist primarily of hemicelluloses. It appears that hemicellulose loss is slight in these treatments however. If, for each treatment, the values for NDF, and ADF are calculated on the basis of the original bark and corrected for the amount of lignin removed they do not differ greatly from values for untreated bark. The data reported in Table 4 show that hemicellulose loss is small and suggest that any material removed in addition to lignin must be from the neutral-detergent soluble fraction (cellular contents).

TABLE 4. FIBER CONTENT OF TREATED BARK, CORRECTED FOR YIELD AND LIGNIN REMOVED

CH_3CO_3H Concentration[a]	Percent NDF[b]	Percent ADF[b]	Percent Hemicellulose[c]
0	74.4	64.5	9.9
20	70.1	64.5	5.6
30	72.0	63.8	8.2
40	73.9	64.3	9.6
50	73.3	63.2	10.1
60	73.8	63.5	10.3
70	73.1	63.3	9.8

[a]Grams per 100 g of bark

[b]Based on original bark and adjusted for loss of lignin

[c]Calculated as NDF-ADF

Comparison of the results obtained by treating sawdust with 50 percent its weight in peroxyacetic acid at 75 C and 25 C shows that increasing temperature increased the rate and extent of delignification (96.7 percent for 3 hours at 75 C versus 59.1 percent for 7 days at 25 C). This same effect was noted in treatments at lower concentrations as well. When the sawdust was treated with 20 percent its weight in peroxy acid for 2 and 3 hours at 85 C about 43 percent of the lignin was removed as compared to 26 percent lignin removal when treated at the same level for 7 days at 25 C.

The possibility exists that the benefits of increased temperature could be obtained without the application of external heat. When peroxyacetic acid was applied to sawdust or bark at 25 C the reaction was observed to be mildly exothermic. Therefore, if large amounts of material were treated under conditions that minimize heat loss to the external environment internal temperatures might be increased enough to enhance the rate and extent of reaction.

Lignin was found to be effectively removed by the peroxyacetic acid treatment. With appropriate conditions essentially total delignification of sawdust was achieved. The maximum amount of lignin removed from the bark was about 83 percent. The 5.7 percent residual lignin is not likely to be reduced further except by very severe treatment; as Browning (1967) cited work showing that delignification of bark by the acid chlorite method cannot reduce the lignin content below 5 percent without severe losses of carbohydrates occurring.

It is apparent from Table 3 that the chemical requirements for lignin removal from softwoods are rather high. To obtain 50 percent lignin removal from sawdust 25 to 30 grams of actual peroxyacetic acid per 100 g of substrate were required. A comparable level of delignification of bark (about 50 percent) required somewhat more chemical (40 g per 100 g of bark). Complete lignin removal from sawdust was obtained at acid concentrations of 200 and 100 percent of wood weight. These strengths are clearly much greater than required, however, as peroxyacetic acid applied at 50 percent of wood weight effected 97 percent lignin removal when treated for 3 hours at 75 C.

The economic feasibility for complete delignification of softwoods to form feedstuffs is questionable. Cost estimates obtained by Schroeder (1973) indicate that peroxyacetic acid could be produced for about 10 cents per pound on a 100 percent actual chemical basis. Assuming that the chemical requirement for complete degradation of softwood lignin equals 50% of the wood dry matter, one ton of dry wood could be delignified for about 100 dollars in chemical cost. Other costs, such as depreciation on treatment facilities, labor, and raw material charge would be in addition to this. It is possible that factors such as technological improvements in peroxyacetic acid manufacture, recovery of unconsumed chemical, or utilization of lignin degradation products could reduce expenses considerably.

In Vitro Digestibility of Peroxyacetic Acid Treated Substrates

The *in vitro* digestion followed by determination of undigested cell-wall material permitted analysis for both cell-wall digestion and dry matter disappearance.

The greatest difference between duplicate determinations was 2.7 percentage points for dry matter disappearance and 2.9 percentage points for the cell-wall digestion. Average difference was 0.7 and 0.8 percentage points for the two analyses respectively. A standard brome hay sample of known digestibility averaged 79.1 percent dry matter disappearance and 66.4 percent cell-wall digestion. Solka Floc, a purified wood cellulose, was also used as a standard for the *in vitro* assay. Since this material is composed entirely of a cell wall component the values for cell-wall and dry matter digestion were equal (73.8 percent). For all samples, dry matter disappearance exceeds the value for cell-wall digestion. These differences result because dry matter disappearance includes the digestion of cell contents as well as cell-wall digestion. Non-cell-wall has been found to have a true *in vivo* digestion of at least 98 percent on all forage diets; while cell-wall material is digested to a lesser extent (Jarriage, 1965). Therefore, unless the material in question is composed entirely of cell-wall constituents its dry matter digestion can be expected to exceed that of cell-walls.

Regression equations, correlations, and standard errors for the relationship of lignin removed to the dry matter and cell-walls digestibility of sawdust and bark are presented in Table 5.

TABLE 5. REGRESSIONS AND CORRELATIONS BETWEEN LIGNIN REMOVAL AND DIGESTIBILITIES

Combination	Regression Equation	Correlation Coefficient	Standard Error
Lignin removed X dry matter digestion of sawdust	$Y = 7.01\ e^{.0254X}$	+0.97	---
Lignin removed X cell-wall digestion of sawdust	$Y = -43.04 + 1.18X$	+0.99	3.6
Lignin removed X dry matter digestion of bark	$Y = 31.77 + 0.49X$	+0.99	1.5
Lignin removed X cell-wall digestion of bark	$Y = 5.14 + 0.64X$	+0.99	1.0

For the sawdust samples, the regression of dry matter digestibility on the extent of delignification was found to be curvilinear, while the regression of sawdust cell-wall digestibility on delignification was found to be linear. These relationships are shown on Figure 7.

This figure shows that approximately 84 percent of the lignin must be removed to obtain 60 percent dry matter digestibity of ponderosa sawdust. This result is not in close agreement with the work of Baker (1973) who found that 60 to 70 percent delignification of softwoods was sufficient to obtain 60 percent *in vitro* dry matter digestibility. This discrepancy is explainable by the differences in fermentation times used. Baker used the *in vitro* procedure of

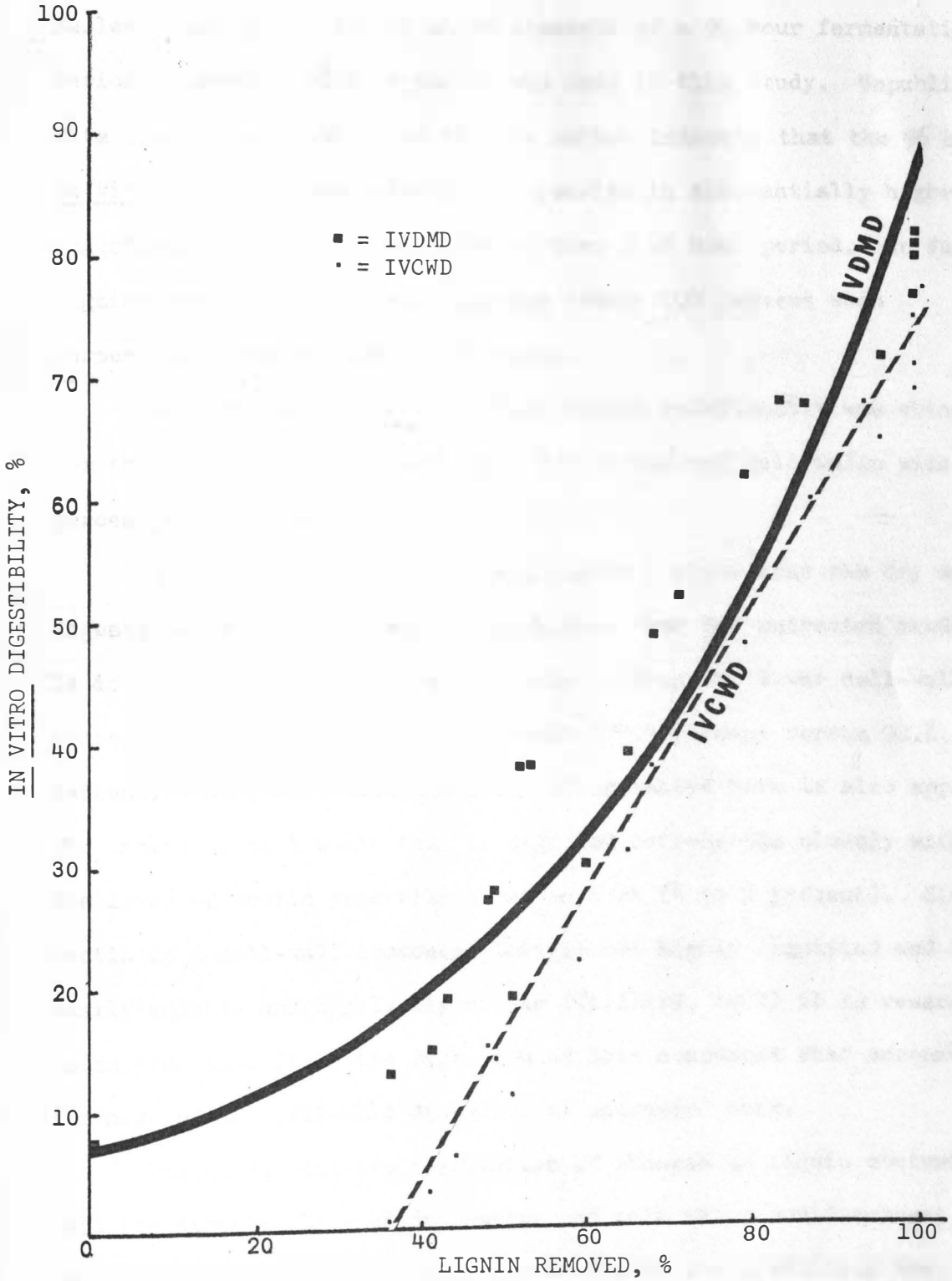


Figure 7. Cell-Wall and Dry Matter Digestibilities of Ponderosa Sawdust Compared with Extent of Delignification.

Mellenberger *et al.* (1970) which consists of a 96 hour fermentation period, whereas a 48 hour period was used in this study. Unpublished data from a study conducted by this author indicate that the 96 hour *in vitro* rumen fermentation period results in substantially higher digestibilities than those obtained from a 48 hour period. In fact, lignin-free wood dry matter digested nearly 100 percent when fermentation was extended to 96 hours.

As shown on Figure 8, a close linear relationship was obtained for the digestibility of both bark dry matter and cell walls with percent delignification.

A comparison of Figure 7 and Figure 8 shows that the dry matter digestibility of untreated bark is higher than for untreated sawdust. It is likely that this difference results from the lower cell-walls content of bark as compared to sawdust (74.4 percent versus 92.8 percent). Some cell wall digestion of untreated bark is also apparent. The amount of cell walls that is digested corresponds closely with the level of pectin generally found in bark (4 to 5 percent). Since pectin is a cell-wall component that is not highly lignified and is easily soluble and highly digestible (Gaillard, 1962) it is reasonable to suggest that it is the digestion of this component that accounts for much of the cell-wall digestion of untreated bark.

The high, positive correlation of changes in lignin content with the digestibility of dry matter and cell walls would suggest that delignification regression can be useful for predicting the digestibility of chemically modified wood materials. The results of

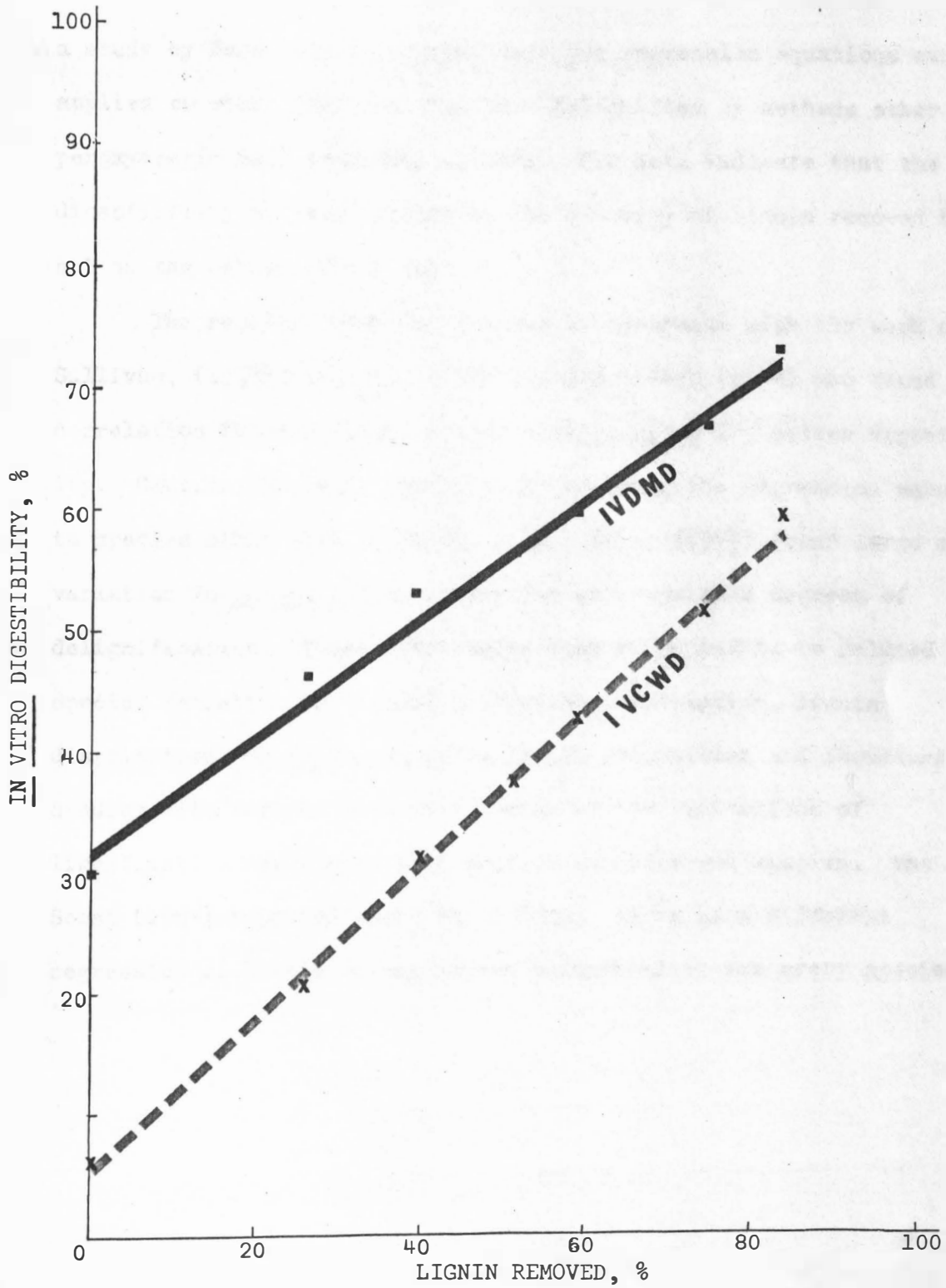


Figure 8. Cell-Wall and Dry Matter Digestibilities of Ponderosa Bark Compared with Extent of Delignification.

a study by Baker (1973) suggest that the regression equations can be applied to wood fiber that has been delignified by methods other than peroxyacetic acid treatment as well. His data indicate that the digestibility of wood depends on the quantity of lignin removed but not on the method of removal.

The results reported here are in agreement with the work of Sullivan, (1955); Kamstra, (1958); and Schrempp (1974) who found good correlation between lignin content and in vitro dry matter digestibility. Caution should be exercised in applying the regression equations to species other than ponderosa pine. Baker (1973) found large species variation in in vitro digestibilities at comparable degrees of delignification. These differences were suggested to be related to species variation in lignin-carbohydrate association, lignin distribution, or to variation in lignin composition and structure. Studies with forages have also indicated the limitations of lignification regression when applied to different species. Van Soest (1964) reported that, for forages, there is a different regression of lignin on dry matter digestibility for every species.

SUMMARY AND CONCLUSIONS

There is an abundance of available wood residues that could potentially serve as an energy source for ruminants. Without some form of treatment, however, the carbohydrates of wood remain essentially unavailable for absorption and metabolism. Numerous methods of treatment have been tested, and some encouraging results have been reported, especially for the hardwood species. At this time, however, there are no economically feasible methods that produce good results for treating softwoods. These factors prompted this investigation on the efficacy of peroxyacetic acid to remove lignin and allow subsequent availability of wood carbohydrate for nutrient utilization.

In regard to the lignin removal capability of peroxyacetic acid, it was found that ponderosa pine sawdust and bark could be effectively delignified by this chemical. Chemical action was found to be highly selective as components other than lignin were largely unaffected.

It was found that the extent of delignification is highly correlated with the *in vitro* rumen digestibility of cell walls and total dry matter of ponderosa sawdust and bark. The use of lignification regression as for predicting *in vitro* digestion was also discussed.

Of particular interest is the data obtained on bark since very little information is currently available concerning its

evaluation as a potential feed source. Even though bark represents a substantial portion of the unused wood residues its economic utilization has been extremely limited. Other residue materials such as sawdust and wood chips are being utilized in ever increasing amounts in manufactured wood products and it is conceivable that bark may one day be the primary wood material available for animal feed use.

Since this is the first reported evaluation of peroxyacetic acid treated wood fiber as a feedstuff much work remains to be done to establish the technological and economic feasibility of this process. The most critical area remaining to be investigated is the *in vivo* response to feeding peroxyacetic acid modified wood materials. It is anticipated that, once delignified, wood carbohydrates are capable of providing energy in amounts comparable to that provided by low to medium quality hay. A decomposition product of peroxyacetic acid that may increase the energy value of treated products is acetic acid. This short-chain fatty acid is a normal product of rumen fermentation and is utilized for energy. The presence of acetic acid in treated residues should, therefore, enhance the feasibility of a peroxyacetic acid treatment process.

LITERATURE CITED

Anthony, W. B. and J. P. Cunningham, Jr. 1968. Hardwood sawdust in all concentrate rations for cattle. J. Anim. Sci. 27:1159 (Abstr).

Anthony, W. B., J. P. Cunningham, Jr. and R. R. Harris. 1968. Hardwood sawdust as feed for ruminants. Advances in Chem. Ser. 95:279. American Chemical Society, Washington, D. C.

A.O.A.C. 1960. Official Methods of Analysis (9th Ed.). Association of Official Agriculture Chemists. Washington, D. C.

Archibald, J. G. 1926. The composition, digestibility and feeding value of hydrolyzed sawdust. J. Dairy Sci. 9:257.

Baker, A. J. 1973. Effect of lignin on the in vitro digestibility of wood pulp. J. Anim. Sci. 35:768.

Beckmann, E. 1922. Conversion of grain straw and lupins into feeds of high nutritive value. Chem. Abstr. 16:765.

Bender, F., D. P. Heaney and A. Bowden. 1970. Potential of steamed wood as a feed for ruminants. Forest Prod. J. 20:36.

Blaxter, K. L. 1962. The Energy Metabolism of Ruminants. Hutchinson Scientific and Technical, London, England.

Brownell, H. H. 1971. Stability of the lignin-carbohydrate complex. Tappi, 54:66.

Browning, B. C. 1967. Methods of Wood Chemistry. Vol. I. Interscience Publishers, New York, New York.

Choate, G. A. and J. S. Spencer, Jr. 1969. Forests in South Dakota. (U.S.D.A. Forest Serv. Resource Bull. INT-8).

Clarke, S. D., S. Iqbal Shah and I. A. Dyer. 1971. Chemically degraded wood as an energy source for fattening cattle. J. Anim. Sci. 33:279 (Abstr.).

Clarke, S. D. and I. A. Dyer. 1973. Chemically degraded wood in finishing beef cattle rations. J. Anim. Sci. 37:1022.

Cody, R. E., J. L. Morrill and C. M. Hibbs. 1968. Evaluation of health and performance of bovines fed wood fiber as a roughage source or intake regulator. J. Dairy Sci. 51:952 (Abstr.).

Cody, R. E., Jr., J. L. Morrill and C. M. Hibbs. 1972. Effect of dietary screened sawdust on health, feed intake, and performance of the bovine. J. Anim. Sci. 35:460.

Colburn, M. W. and J. L. Evans. 1965. Chemical composition of the acid-detergent fiber fraction of orchard grass, alfalfa and mixed forages. J. Dairy Sci. 48:1557.

Cowling, E. B. and W. Brown. 1969. Structural features of cellulosic materials in relation to enzymatic hydrolysis. Adv. Chem. Series. 95:152.

Crampton, E. W. and L. A. Maynard. 1938. The relation of cellulose and lignin to the nutrient value of animal feeds. J. Nutr. 15:383.

Cullison, A. E. and C. S. Ward. 1965. Noncellulosic wood carbohydrates in steer rations. J. Anim. Sci. 24:877 (Abstr.).

Dinius, D. A. and B. R. Baumgardt. 1970. Regulation of food intake in ruminants. 6. Influence of caloric density of pelleted rations. J. Dairy Sci. 53:311.

Ellis, C. and R. Durham. 1968. Maintaining cattle on ground mesquite wood. J. Anim. Sci. 27:1132 (Abstr.).

El-Sabban, F. F., T. A. Long and B. R. Baumgardt. 1971. Utilization of oak sawdust as a roughage substitute in beef cattle finishing rations. J. Anim. Sci. 32:749.

Enzmann, J. W., R. D. Goodrich and J. C. Meiske. 1969. Chemical composition and nutritive value of poplar bark. J. Anim. Sci. 29:653.

Erlinger, L. L. 1974. The potential of a hydrolyzed wood residue as a source of nitrogen, roughage, and energy in ruminant rations. Ph. D. Thesis. University of Nebraska.

Feist, W. C., A. J. Baker and H. Tarkow. 1970. Alkali requirements for improving digestibility of hardwoods by rumen microorganisms. J. Anim. Sci. 30:832.

Fonnesbeck, P. V. and L. E. Harris. 1970. Determination of plant cell walls in feeds. Proc. Amer. Soc. Animal Sci., Western Sec. 21:153.

Gaillard, B. D. E. 1962. The relationship between cell-wall constituents of roughages and the digestibility of the organic matter. J. Agr. Sci. 59:369.

Gilbert, R. A., N. S. Hale, D. M. Kinsman and W. A. Cowan. 1973. Sawdust versus hay in a complete lamb ration. J. Anim. Sci. 37:367 (Abstr.).

Grantham, J. B. and T. H. Ellis. 1974. Potentials of wood for producing energy. J. Forestry. 72:554.

Greenspan, F. G. and D. G. MacKeller. 1948. Analysis of aliphatic per acids. Anal. Chem. 20:1061.

Haas, H., W. Schoch and V. Strole. 1955. Preparation of holocellulose by the use of peracetic acid. Das Papier 9:469. Chem. Abst. 50:2164.

Heaney, D. P. and F. Bender. 1970. The feeding value of steamed aspen for sheep. For. Prod. J. 20:98.

Homb, T. 1949. The connection between the content of lignin and digestibility. Nutr. Abst. and Rev. 19:470.

Huffman, J. G., W. D. Kitts and C. R. Krishnamurt. 1971. Effects of alkali treatment and gamma irradiation on the chemical composition and in vitro rumen digestibility of certain species of wood. Can. J. Anim. Sci. 51:457.

Hvidstein, H. 1949. Experiments with cellulose for ruminants. Nutr. Abstr. and Rev. 19:470.

Ishikawa, J., K. Okubo and T. Oki. 1966. J. Jap. Tappi. 20:485 (1966); Chem. Abstr. 65:15652.

Jarrige, R. 1965. The composition of sheep feces and its relation to forage digestibility. Proc. IX Intern, Grassland Congr., Sao Paulo, Brazil. Paper 389.

Kamstra, L. D., A. L. Moxon and O. G. Bently. 1958. The effect of stage of maturity and lignification on the digestion of cellulose in forage plants by rumen microorganisms in vitro. J. Anim. Sci. 17:199.

Kellner, O. 1913. The Scientific Feeding of Animals. The MacMillan Company. New York.

Kinsman, D. M., D. A. Grant, A. R. Cobb and L. A. Peterson. 1969. Growth and carcass performance of sawdust-fed lambs. J. Anim. Sci. 28:875 (Abstr.).

Kitts, W. D., C. R. Kristhnamurtu, J. A. Shelford and J. G. Huffman. 1969. Use of wood and woody by-products as a source of energy in beef cattle rations. Adv. in Chem. Ser. 95:279.

Lai, Yuan-Zong and K. V. Sarkanen. 1968. Delignification by peracetic acid. II. Comparative study on softwood and hardwood lignins. Tappi. 51:449.

Leipold, B. 1961. Chemical composition and physical properties of wood fibers. I. Preparation of holocellulose fibers from loblolly pinewood. Tappi. 44:230.

Mageli, O. L. and C. S. Sheppard. 1967. Peroxides and peroxy compounds. Encyclopedia of Chemical Technology. 14:795.

Marion, P. T., C. E. Fisher and E. D. Robison. 1959. Ground mesquite wood as a roughage for yearling steers. J. Anim. Sci. 18:1174 (Abstr.).

McDougall, E. I. 1948. Studies on ruminant saliva. I. The composition and output of sheep's saliva. Biochem. J. 43:99.

Mellenberger, R. W., L. D. Satter, M. A. Millett and A. J. Baker. 1970. An in vitro technique for estimating digestibility of treated and untreated wood. J. Anim. Sci. 30:1005.

Mellenberger, R. W., L. D. Satter, M. A. Millett and A. J. Baker. 1971. Digestion of aspen, alkali-treated aspen, and aspen bark by goats. J. Anim. Sci. 32:756.

Mertens, D. R. and P. J. Van Soest. 1971. Paper composition and estimated nutritive value. J. Anim. Sci. 34:293 (Abstr.).

Mertens, D. R., F. A. Martz, J. R. Campbell and P. J. Van Soest. 1971. Relation of Chemical composition and inoculum to in vitro digestion of selected waste papers. J. Dairy Sci. 54:778 (Abstr.).

Meyer, J. H., R. Kroman and W. N. Garrett. 1964. Digestion (Influence of roughage preparation) in Physiology and Digestion in the Ruminant. Butterworths, Washington, D. C.

Millett, M. A., A. J. Baker, W. C. Feist, R. W. Mellenberger and L. D. Satter. 1970. Modifying wood to increase its in vivo digestibility. J. Anim. Sci. 31:781.

Millett, M. A., A. J. Baker, L. D. Satter, J. N. McGovern and L. A. Dinus. 1973. Pulp and papermaking residues as feed stuffs for ruminants. J. Anim. Sci. 37:599.

Moore, L. A. 1964. Symposium on forage utilization: Nutritive value of forage as affected by physical form. J. Anim. Sci. 23:230.

Nordfelt, S. 1951. Problems in animal nutrition. The use of wood pulp for feeding farm animals. Proc. 11th Intern. Congr. Pure Applied Chem., London. 3:391. Chem. Abstr. 45:8673.

Pfander, W. H., S. E. Grebing, G. Hejny, and W. Tyree. 1969. The value of wood-derived products in ruminant nutrition. Adv. Chem. Ser. 95:298.

Poljak, A. 1948. Wood decomposition with peracetic acid. Angew. Chemie. 60:45. Chem. Abstr. 42:7981.

Saarinen, P., W. Jensen and J. Alojarui. 1959. Digestibility of high yield chemical pulp and its evaluation. Acta Agral. Fennica. 94:41; Chem. Abstr. 53:10748.

Sakai, K., K. Kuroda, and S. Kishimoto. 1972. Delignification in peracid bleaching. VI. Reaction of the β-aryl ether type model compounds with peracetic acid. Tappi. 55:1702.

Sarkanen, K. V. and J. Suzuki. 1965. Delignification by peracetic acid. Studies on oxidative delignification mechanisms. Tappi. 48:459.

Sarkanen, K. V., Hou-Min Chang and G. G. Allan. 1967. Species variation in lignins. II. Conifer lignins. Tappi. 50:583.

Sarkanen, K. V., Hou-Min Chang and G. G. Allan. 1967. Species variation in lignins. III. Hardwood lignins. Tappi. 50:587.

Satter, L. D., A. J. Baker and M. A. Millett. 1970. Aspen sawdust as a partial roughage substitute in a high-concentrate dairy ration. J. Dairy Sci. 53:1455.

Satter, L. D., R. L. Lang, A. J. Baker and M. A. Millett. 1973. Value of aspen sawdust as a roughage replacement in high-concentrate dairy rations. J. Dairy Sci. 56:1291.

Schrempp, R. E. 1974. Storage quality measurements of two large hay packages. M.S. Thesis, South Dakota State University.

Schroeder, H. A. 1973. Peracetic acid pretreatment pulping processes. Unpublished. Colorado State University.

Schroeder, H. A. and R. C. France. 1974. Preparation and analysis of peracetic acid. Unpublished. Colorado State University.

Sherrard, E. C. and G. W. Blanco. 1921. The preparation and analysis of a cattle food consisting of hydrolyzed sawdust. J. Ind. Eng. Chem. 13:61.

Slyter, A. L. and L. D. Kamstra. 1973. Pine sawdust as a roughage replacement in gestating beef heifer rations. J. Range Manage. 26:303.

Slyter, A. L. and L. D. Kamstra. 1974. Utilization of pine sawdust as a roughage substitute in beef finishing rations. J. Anim. Sci. 38:693.

Sullivan, J. T. 1955. Cellulose and lignin in forage grasses and their digestion coefficients. J. Anim. Sci. 14:710.

Swern, D. 1970a. Organic peroxy acids—preparation, properties and structure. Organic Peroxides. Vol. I:313. D. Swern, Ed., Wiley-Interscience, New York.

Swern, D. 1970b. Methods of preparation and analysis of organic peroxy acids. Organic Peroxides. Vol. I:475. D. Swern, Ed., Wiley-Interscience, New York.

Swern, D. 1970c. Organic peroxy acids as oxidizing agents. Epoxidation. Organic Peroxides. Vol. II:355. D. Swern, Ed., Wiley-Interscience, New York.

Tarkow, H. and W. C. Feist. 1969. A mechanism for improving digestibility of lignocellulosic materials with dilute alkali and liquid ammonia. Advances in Chem. Ser. 95:197.

Tomlin, D. C., R. R. Johnson and B. A. Dehority. 1965. Relationship of lignification to in vitro cellulose digestibility of grasses and legumes. J. Anim. Sci. 24:161.

Tsoumis, G. 1968. Wood as a Raw Material. Pergamon Press, London.

Van Soest, P. J. 1963. Use of detergents in the analysis of fiberous feeds. II. A rapid method for the determination of fiber and lignin. J. Assoc. Official Agr. Chem. 46:829.

Van Soest, P. J. 1964. Symposium on nutrition and forage and pastures. New chemical procedures for evaluating forages. J. Anim. Sci. 23:838.

Van Soest, P. J. 1966. Non-nutritive residues: a system of analysis for the replacement of crude fiber. J. Assoc. Official Agr. Chem. 49:546.

Van Soest, P. J. 1969. Composition, maturity, and the nutritive value for forages. Advances in Chem Ser. 95:262.

Van Soest, P. J., R. H. Wine and L. A. Moore. 1966. Estimation of the true digestibility of forages by the in vitro digestion of cell walls. Proc. IX Int. Grassland Congr., Paper 20.

Van Soest, P. J. 1967. Development of a comprehensive system of feed analysis and its application to forages. J. Anim. Sci. 26:119.

Van Soest, P. J. and R. H. Wine. 1967. Use of detergents in the analysis of fiberous feeds. IV. Determination of plant cell-wall constituents. J. Assoc. Official Agr. Chem. 50:50.

Vara, M., A. Bacigalupo and J. Tellez. 1968. Use of cottonwood in fattening cattle. J. Anim. Sci. 27:1137 (Abstr.).

Wenzl, H. F. J. 1970. The Chemical Technology of Wood. Academic Press, New York.

Wilson, R. K. and W. J. Pigden. 1964. Effect of a sodium hydroxide treatment on the utilization of a wheat straw and poplar (Populus alba) wood by rumen micro-organisms. Can. J. Anim. Sci. 44:122.

Woodman, H. E. and J. Stewart. 1932. The mechanism of cellulose digestion in the ruminant organism. III. The action of cellulose-splitting bacteria on the fibre of certain typical feeding stuffs. J. Agr. Sci. 22:527.