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Pretreatments to Enhance Chemical, Enzymatic, and Microbiological Attack of Cellulosic Materials

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It is now well established that the heterogeneous chemical reactions of cellulose are controlled largely by the high-order molecular packing of its crystalline regions, The greatly reduced accessibility imparted by such molecular order limits attack on the bulk of a cellulosic material to crystallite surfaces. Overall rates of conversion are thus only a fraction of those obtainable with the more open amorphous molecular structures associated with such carbohydrates as starch.

This difference in conversion rates is of special significance in a reaction such as acid hydrolysis where it has a far-reaching effect on sugar yield. Being a reactive molecule, glucose itself is destroyed under the conditions of its formation from a glucan polymer. Overall sugar yield is thereby governed by the relative rates of sugar formation and sugar destruction. In the case of starch, where the rate of hydrolysis greatly exceeds the rate of degradation, sugar yields approach the quantitative. With cellulose, on the other hand, the rate of glucose destruction is of the same order of magnitude as the rate of hydrolysis and maximum sugar yields under simple batch conditions are severely limited. This fact has been a major deterrent to a wood-based chemical industry.

In addition to the rate-limiting influence of cellulose "fine-structure," a further deterrent to substrate penetrability resides in that other major constituent of lignocellulosic material, lignin. This is especially evident when one attempts to utilize the carbohydrate values of these materials

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through enzymatic or microbiological pathways. For example, wood residues, containing some 70 to 80% total carbohydrates, would seem an ideal replacement for corn and other grain concentrates in fulfilling the cow's dietary energy requirements. However, numerous feeding trials and laboratory assays have shown that the carbohydrates of whole-wood residues are essentially unavailable for such use. Nor are they any more accessible to high-potency cellulolytic enzymes. Whether the inhibitory mechanism involves the existence of a lignin-carbohydrate chemical bond or whether the three-dimensional macromolecular lignin network functions as a protective barrier to the cell-wall carbohydrates has yet to be established.

Impending shortages of conventional foodstuffs for both man and his domestic livestock force us to contemplate the vast food-energy potential locked up in the millions of tons of currently unused lignocellulosic residues (some 80 million tons of wood residue alone). With all of our technological prowess, we should be able to devise some practical chemical or physical pretreatment to tap the carbohydrate values of these materials for a broad range of chemical, microbiological, or enzymatic conversions. The basic goal of such efforts would be to alter the fine structure of cellulose, and extensively disrupt or open up the lignin-carbohydrate complex. Changes in x-ray pattern, hydrolytic activity, or microbiological utilization provide means to monitor the extent of the induced modifications.

A survey of the literature reveals that the reactivity of cellulose and lignocellulose can indeed be modified by a wide variety of physical and chemical manipulations. While many of these yield only moderate changes, a few are capable of profound structural transformations and their practicality has been explored. Other possibilities are under investigation. This paper reviews the current status of these efforts with special emphasis on their application to wood residue. For convenience, the various pretreatments will be considered as either chemical or physical, as governed by their principal mode of action on a substrate.

CHEMICAL PRETREATMENTS

Swelling with Alkaline Agents

Sodium hydroxide

The treatment of a native cellulose with sodium hydroxide solutions of mercerizing strengths (above 20%) causes extensive swelling and

separation of structural elements, and leads to the formation of cellulose II with an x-ray pattern that differs considerably from the cellulose I pattern of native cellulose. Mercerized cellulose may undergo acid hydrolysis up to 40% faster than the parent cellulose [1]. The complete solution and regeneration of a cellulose by the viscose process brings about drastic alterations in its fine structure. A typical tire-cord rayon, for example, has the x-ray pattern of cellulose II, a degree of crystallinity of approximately 60%, and an acid hydrolysis rate up to six times that of the original cellulose [l]. Treatment of cellulose with liquid monoethylamine, followed by extraction with hexane or by evaporative procedures, yields a highly swollen product termed "decrystallized cellulose" [2, 3]. The material has a degree of crystallinity of approximately 30% and hydrolyzes about twice as fast as the starting cellulose. Wood celluloses are also more reactive toward acid hydrolysis than are native celluloses such as cotton or ramie. A summary of the influence that mercerization and viscose-process regeneration have on the hydrolytic response and other characteristics of some typical celluloses is presented in Table I. Hydrolysis rates are expressed in terms of half-life, i.e., time required for the disappearance of one-half of the resistant portion of the starting cellulose when exposed to constant-boiling hydrochloric acid. The resistant fraction is here defined in terms of the zero-time intercept of the linear semilogarithmic plot of residual cellulose as a function of time of hydrolysis [1]. The degree of polymerization data was obtained viscometrically.

As applied to lignocellulosic materials, alkali treatments have taken a more utilitarian approach, that of upgrading the nutritive value of forage and forest residues for ruminants. Of the many studies and reviews on the feeding of alkali-treated straw to ruminants, some of the more comprehensive are by Magnus [4], Godden [5], Ferguson [6,7], Dijkstra [8], Muller [9], Watson [10], Woodman and Evans [11], Hvidsten [12], Clawson et al. [13], and Guggolz et al. [14]. In general, soaking straw in about 1.5% sodium hydroxide for 24 hr increases its digestibility from an initial 30 to 50% up to 60 to 70%. The process patented by Beckmann in 1919 [15] was used extensively in Europe during both world wars and still finds occasional use in Norway.

One disadvantage of the Beckmann process is that considerable hemicellulose is solubilized, resulting in a loss of about 20% of the straw dry matter during washing operations. These washings are both an economic decrement and a disposal problem. To alleviate these problems, Wilson and Pigden developed a so-called "dry" process in

TABLE 1
Response of Various Celluloses to Hydrolysis with Constant-Boiling HCl

Cellulose	Hydrolysis data		Viscosity data	
	Resistant fraction	Half-life of resistant fraction	Initial DP	DP at half-life
	%	Min.		
Native				
Ramie	89	193	--	184
Cotton	89	188	1680	175
Wood Pulps				
Hemlock sulfite	90	88	1170	125
Southern pine pre-hydrolysis sulfate	88	94	975	140
Mercerized				
Ramie	73	114	--	88
Hemlock sulfite	76	91	1240	66
Regenerated				
Tire-cord rayon	66	30	425	31

which the straw is steeped in a minimum volume of 20% alkali which is then left in situ [16]. Feeding trials showed that treated wheat straw containing 2% residual sodium hydroxide was readily accepted by sheep when mixed with corn silage or ground alfalfa hay, or when neutralized with acetic acid. Alkali treatment can also significantly increase microbial growth as recently described by Han and Callihan [17]. Carbohydrate utilization of rice straw and sugarcane bagasse by *Cellulomonas* bacteria advanced from an initial 29% up to 73% through a 15-min digestion in 4% sodium hydroxide at 100°C.

Although alkali swelling of wood has not been used commercially to prepare feed, the relative effects of alkali on wheat straw and on poplar wood were studied by Wilson and Pigden [16]. Both materials were soaked in increasing amounts of sodium hydroxide up to 15% of the material weight, and the digestibilities of the products were estimated by in vitro rumen assay. Very similar relationships were obtained for straw

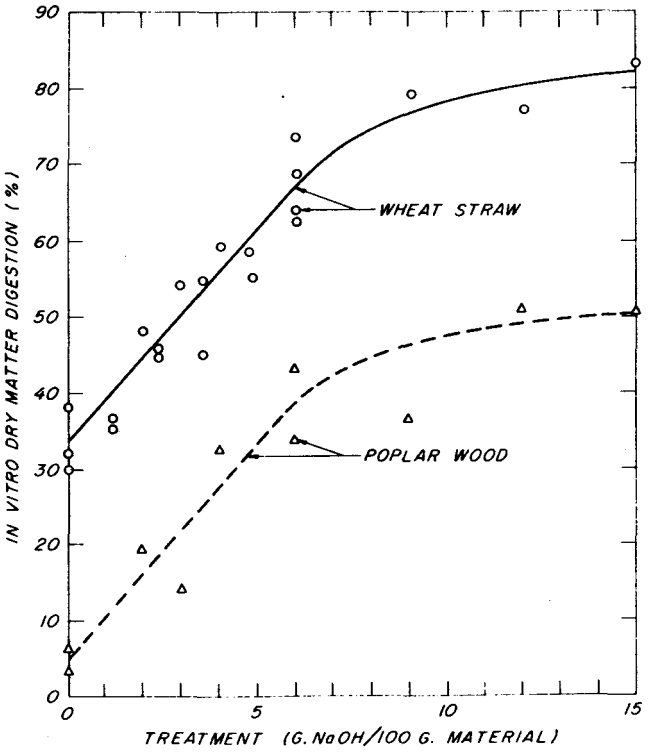

Fig. 1. Effect of NaOH pretreatment on the in vitro digestion of straw and poplar wood [16].

and wood (Fig. 1); however, the digestibility coefficient of the straw was always about 30 percentage points greater than that of the wood, the maximum digestibilities being about 80 and 50%. Thus, while the overall reaction mechanism of the alkali appears to be the same, the advantage of straw resides in the greater initial availability of its carbohydrates. A further demonstration of the effect of alkali on the digestion of wood was provided by Pew and Weyna [18]. By alternate swelling of 80-mesh spruce wood in cold 2 N sodium hydroxide followed by digestion with *Trichoderma viride* enzyme, 80% of the total carbohydrates were converted to sugars. Without the alkali treatment, the maximum attainable digestion was a few percent at most.

As part of its overall mission to make fuller use of our forest resources, the Forest Products Laboratory, in cooperation with the

University of Wisconsin and the Tennessee Valley Authority, has been taking a renewed look at ways and means for enhancing the availability of wood-residue carbohydrates for ruminant use. Treatment with alkaline swelling agents has been one approach. Treatment effectiveness is monitored by a Tilley and Terry in vitro rumen technique [19] augmented by animal feeding trials of the more promising products. Data are presented relative to the in vitro digestibility and lignin content of some typical hardwood species treated with 1% sodium hydroxide at a 20:1 solution-to-wood ratio (Table 11). All wood samples were ground through a 40-mesh screen in a Wiley mill.

No uniformity exists in the response of the various species to sodium hydroxide treatment. For example, while the digestibility of basswood increased from 5 to 56%, the digestibility of American elm increased from 9 to only 14%. Softwoods, as a group, exhibited digestibilities in the 1 to 5% range and were essentially unresponsive to alkali treatment. This difference in response appears to be related to the lignin content of the wood (Fig. 2). A similar relationship appears to apply to other lignocellulosic materials as evidenced by the common observation that, as forages mature, their lignin content increases and the digestibility of

TABLE II
Effect of NaOH Treatment on the in vitro Digestibility of Hardwoods[a]

Species	Lignin	Digestibility	
		Untreated	Treated
	%	%	%
Quaking aspen	20	33	55
Bigtooth aspen	20	31	49
Black ash	20	17	36
American basswood	20	5	55
Paper birch	21	8	38
Yellow birch	21	6	19
American elm	23	8	14
Silver maple	18	20	41
Sugar maple	23	6	28
Red oak	24	3	14
White oak	23	4	20

[a]Five grams wood treated 1 hr with 100 ml 1% NaOH, washed and dried.

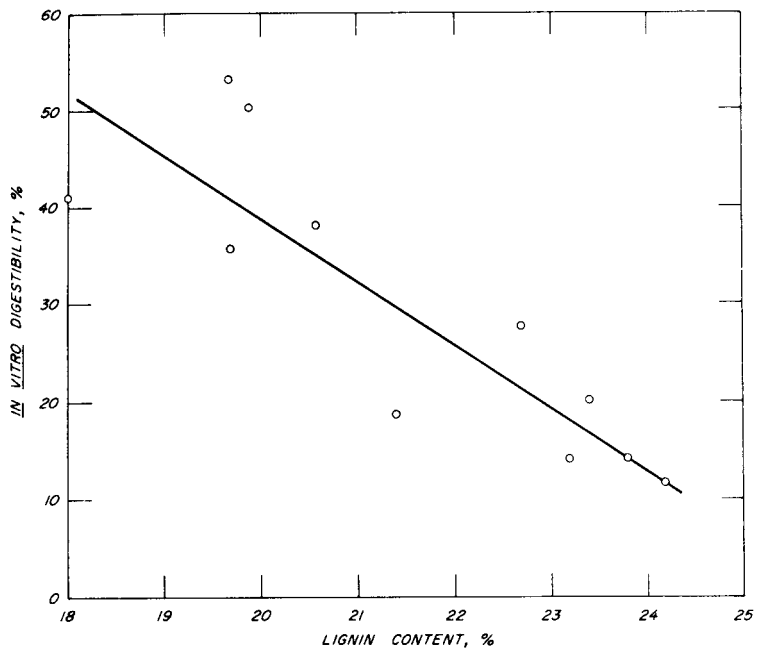

Fig. 2. Relationship between lignin content and in vitro digestion for NaOH pretreated hardwoods [20].

their cellulose and hemicellulose components by ruminants decreases.

To better define conditions for optimum processing, Feist et al. [20] and Millett et al. [21] investigated the influence of alkali concentration on the extent of in vitro digestion of aspen and red oak. The results (Fig 3) show that from 5 to 6 g of NaOH per 100 g of wood are necessary for maximum effect. This level of alkali is essentially equivalent to the combined acetyl and carboxyl contents of the woods. This has led Tarkow and Feist to postulate that the main consequence of alkali treatment is the saponification of intermolecular ester bonds, thus promoting the swelling of wood beyond water-swollen dimensions and favoring increased enzymatic and microbiological penetration into the cell-wall fine structure [22]. Wood-substance lost by water extraction at this level of treatment is about 5% for aspen and can be ascribed largely to the removal of saponified acetyl groups.

Verification of the in vitro results was obtained by Mellenberger et al. in a feeding trial with goats [23]. Hammer-milled aspen sawdust was

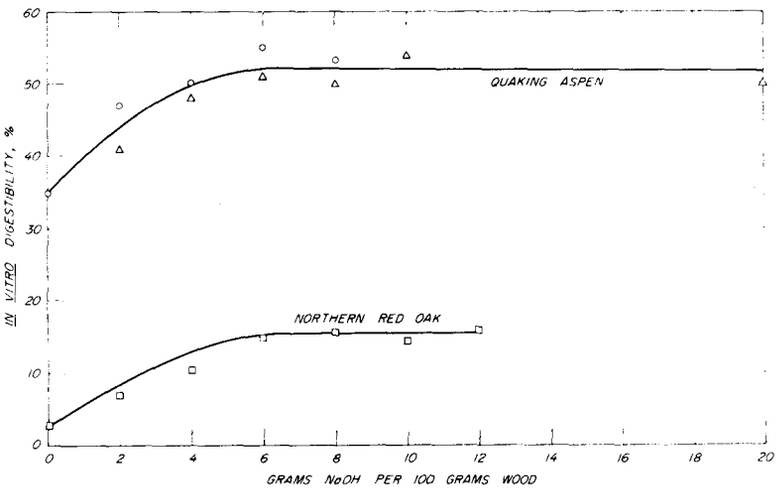

Fig. 3. Relationship between level of NaOH pretreatment and in vitro digestion for quaking aspen and northern red oak [20]. (Aspen: ○ = 2 hr in 0.5% NaOH, Δ = 1 hr in 1% NaOH; red oak: □ = 4 hr in 1% NaOH.)

treated with 0.5% NaOH at a 10:1 liquid-to-solid ratio for 2 hr at room temperature. After draining, washing once with water, and air drying, the product was incorporated into pelleted rations at levels of 0 to 60%. A similar set of rations was prepared from untreated sawdust. Overall ration digestibility, calculated from feed and feces analysis, is plotted as a function of aspen content (Fig. 4). Extrapolation of the curves to 100% aspen yielded dry-matter digestibilities of about 41% for untreated aspen and 52% for the alkali-treated aspen. Alkali treatments can thus increase ruminant utilization of aspen wood by a factor of approximately 25%, making it equivalent to a medium-quality hay as a source of dietary energy. Use of Wilson and Pigden's "dry process" would probably have increased digestibility by another 5 percentage units.

Ammonia

Another long-standing approach to upgrading the feeding value of lignocellulosic materials involves treatment with aqueous or gaseous ammonia. A number of patents have been issued on the process, the

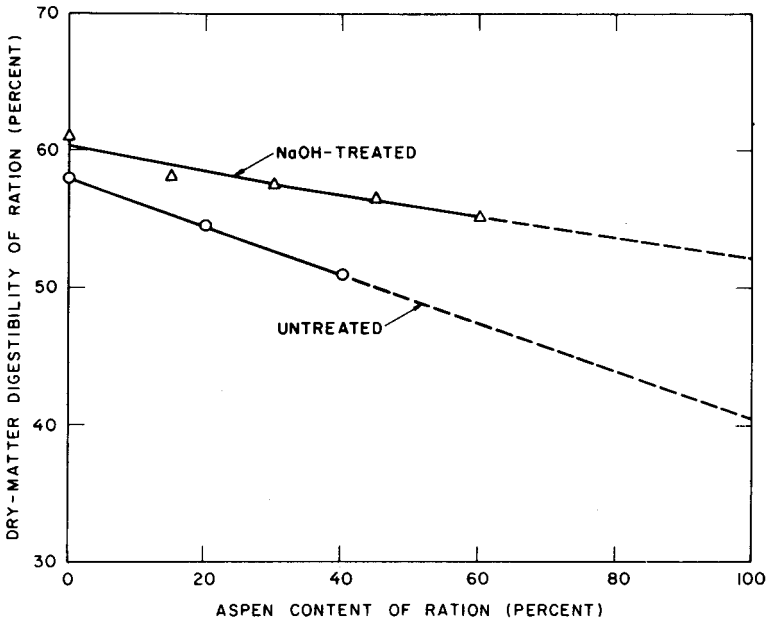

Fig. 4. In vivo dry-matter digestion of rations containing untreated and NaOH-treated aspen.

earliest being in 1905 to F. Lehmann in which the pressure treatment of straw with aqueous ammonia is described [24]. A later patent by Oehme and Köln-Rath discloses a two-stage process in which moist wood meal is first reacted with NH_3 at 130°C and then oxidized with air under 10 atm pressure [25]. The products contained from 5 to 8% nitrogen, which added to their nutritional value. More recently Waiss et al. applied a variety of ammonia treatments to rice straw [26]. Their optimum process involved a 30-day treatment of the dried straw in closed containers with 5% NH_3 and 30% water at room temperature. Product digestibility (in vitro) was about 62% and it contained about 1.3% nitrogen. In vivo digestion by sheep indicated a digestibility of about 56% compared to 50% for untreated straw when both were fed at a level of 65% of the total ration. Han and Callihan, using a similar treatment, showed that the utilization of rice straw by *Cellulomonas* bacteria could be increased from an initial 29% up to 57% [17].

That liquid ammonia exerts a strong swelling action on wood and cellulose and can effect a phase change in the crystal structure from

cellulose I to cellulose III has long been recognized [22, 27]. In an attempt to exploit these effects, a number of woods were exposed to anhydrous ammonia in both liquid and gaseous form and the results assayed by changes in in vitro digestibility [21]. Aspen appeared to be unique in the extent of its response to ammoniation, attaining a digestibility coefficient of about 50% as contrasted with Sitka spruce and red oak whose ammoniated-product digestibilities were only 2 and 10%, respectively.

In an extension of this work, some 800 lb of ammonia-treated aspen was prepared by subjecting air-dried aspen sawdust to a 2-hr pressure treatment (70 psi) with anhydrous NH_3 gas at room temperature. After blowdown and evaporative removal of excess NH_3, the product was blended into pelleted rations at levels of 0 to 50% and fed to goats, with ready acceptance over a 6-week feeding period. From extrapolation of the curve for ration digestibility versus wood content, an estimated digestibility of approximately 46% was obtained for the ammonia-treated product. This is about 6 percentage points lower than the in vivo digestibility of the NaOH-treated aspen but still sufficient to rank NH_3-treated aspen in the same range as hay as a potential source of energy. As an added benefit, the NH_3 treatment would provide some nitrogen. As with sodium hydroxide, Tarkow and Feist describe the pertinent action of ammonia treatment to the hydrolysis, ammonolysis in this case, of glucuronic acid ester crosslinks; thereby providing more ready access to structural carbohydrates by rumen bacteria and their associated enzymes [22]. Similarly, the wide response-range exhibited by the various wood species toward NH_3 treatment can be ascribed, in part at least, to the extent of lignification.

Delignification

With lignin being the major roadblock to widespread utilization of the carbohydrate content of our abundant lignocellulosic residues for ruminant and microbiological conversion, delignification would appear to provide a straightforward solution to our problems. That it can be was indicated by the more than 1.5 million tons of sulfate and sulfite wood pulps from pine, spruce, and fir consumed by cows and horses during World War II in the Scandinavian countries [28-32]. This was an emergency situation, however, and the use of high-quality wood pulps declined rapidly with the renewed availability of the more conventional feedstuffs. With the current cost for unbleached kraft pulps at $250 to $300 per ton and reportedly as high as $700 per ton for certain

dissolving pulps, there is also a strong economic deterrent to the use of high-grade wood pulps for ruminant feeding.

What are the prospects for less rigorous, and less capital-intensive, delignification procedures? Or of perhaps more immediate significance, what degree of delignification is actually required for a product having a reasonable level of carbohydrate utilization? A recent study by Baker sheds some light on the latter question [33]. Through judicious maneuvering of pulping variables, he was able to prepare a series of kraft pulps having a broad range of residual lignin contents whose digestibilities were then determined by in vitro rumen assay. Four wood species were included in his investigation: two hardwoods, paper birch and red oak; and two softwoods, red pine and Douglas fir.

As shown in Figure 5, taken from Baker's work, an appreciable difference exists in the delignification-digestibility response between hardwoods and softwoods. With the two hardwoods, digestibility increases rapidly with delignification and then approaches a digestibility plateau of about 90% as delignification approaches completion. With the two softwoods, there is a distinct lag phase, especially pronounced with Douglas fir, during which extensive delignification is accompanied by only minor increases in digestibility. Following this lag phase, digestibility rises rapidly and almost linearly with delignification up to the digestibility maximum.

As interpolated from these four curves, the extent of delignification necessary to obtain a product having an in vitro digestibility of 60%, that of a good-quality hay, is shown in Table III along with data on the lignin content of the original woods. In common with alkali treatment (Fig, 2), digestibility response strongly correlates with lignin content, response being measured in terms of the degree of pulping action needed to achieve a specified level of product utilization. Additional support for this lignin dependency was obtained by Saarinen et al. in an investigation of the in vivo digestibility of a series of birch and spruce pulps prepared by 10 different pulping techniques [34]. Recalculation of their data to fit Baker's format provided the results shown in Figure 6, which also includes Baker's curves for red pine and paper birch for comparison. In spite of the wide variation in delignification techniques employed by the two investigations, their results are quite comparable. This leads to the further conclusion that it is primarily the degree of delignification that governs pulp digestibility, not the method of pulping.

A similar relationship was encountered with respect to the growth of the fungus *Aspergillus fumigatus* on a variety of commercial pulps

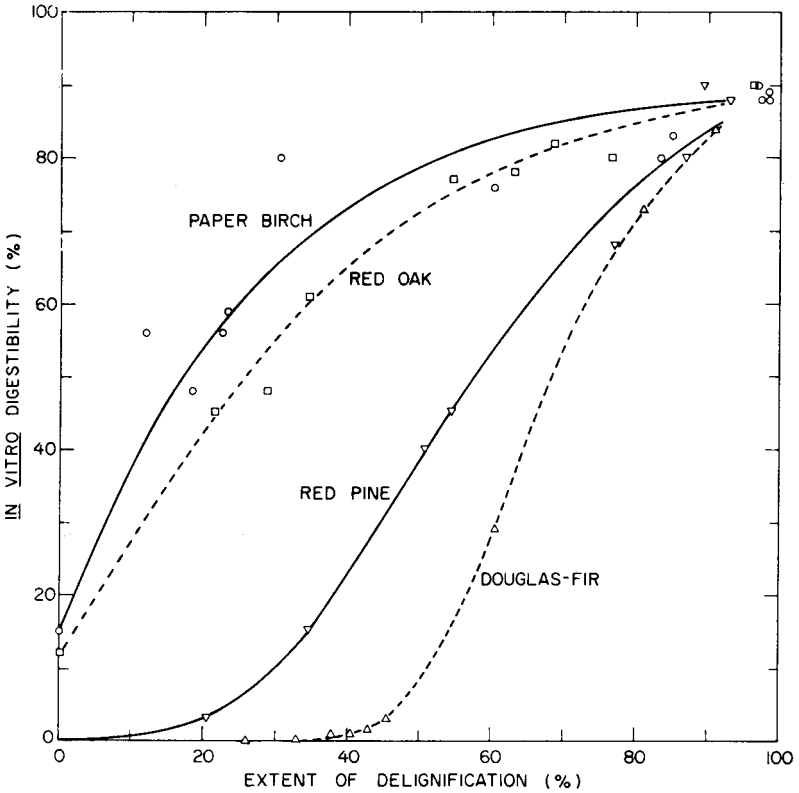

Fig. 5. Relationship between in vitro digestibility and extent of delignification for kraft pulps made from four wood species.

TABLE III
Degree of Delignification Required to Attain 60% in vitro Digestibility

Wood	Required delignification[a] %	Lignin in original wood %
White birch	25	20
Red oak	35	23
Red pine	65	27
Douglas-fir	73	32

[a]Based on original wood.

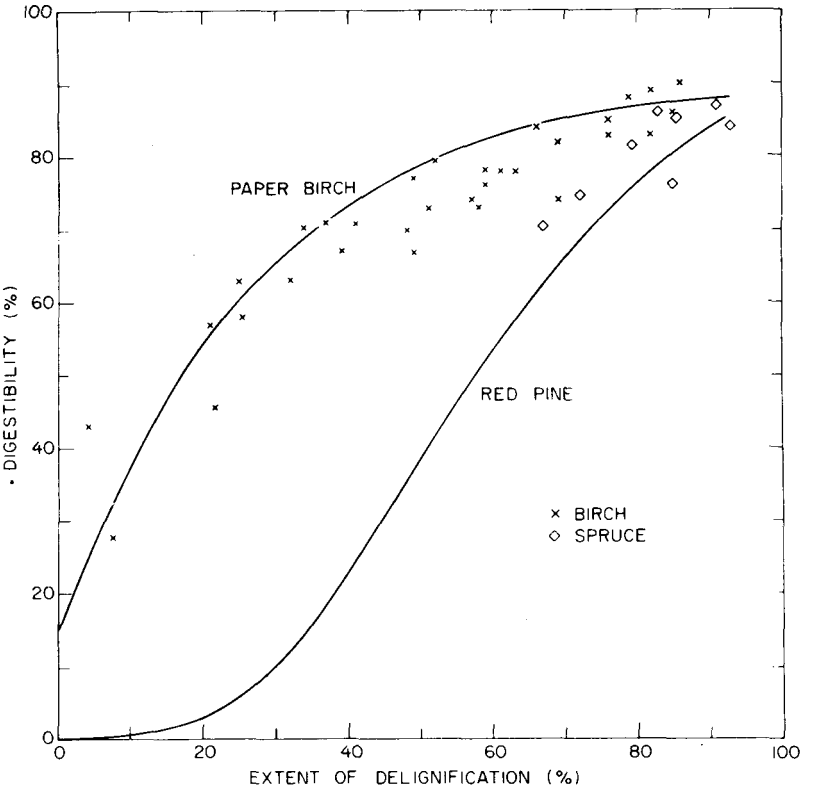

Fig. 6. Relationship between digestibility and extent of delignification for wood pulps. (Data points from Saarinen et al. [34]. Curves from Fig. 6.)

prepared under different conditions [35]. As determined by the protein content of the fungal mass, reasonable growth on hardwood could be obtained at lignin contents of 14% or less, whereas fungal growth on softwoods was restricted to pulps having less than 3% residual lignin.

Knowing that complete delignification is not required to prepare a nutritionally acceptable feedstuff, attention has turned to chemical pretreatments which can substantially disrupt the lignin-carbohydrate complex but may demand less capital than conventional pulping processes. Treatment with sodium chlorite has been one such approach. In acetic acid solution, sodium chlorite exerts a marked specificity for lignin solubilization and has long been a quantitative technique for the

preparation of "holocellulose," the total carbohydrate portion of a lignocellulose [36]. The high digestible yield of a birch wood holocellulose was described by Saarinen et al. [34].

Since chlorine dioxide is the active agent of the chlorite technique, Sullivan and Hershberger took the more direct approach of passing ClO_2 gas through a bed of dried and ground wheat straw [37]. They observed a marked improvement in the in vitro digestibility of the treated product. Impressive results with sodium chlorite alone have been reported by Stranks [38] and Goering and Van Soest [39] in their studies on poplar wood and forages. The latter workers have rather effectively negated the economic feasibility of the process, however, estimating $200 per ton for chemical cost alone. Palatability of the material was also a problem, due to the high residual sodium chloride content.

In another approach, Clarke and Dyer have taken a renewed look at a conventional pulping procedure, the ammonium bisulfite process [40]. By pulping shredded Douglas fir wood under conditions designed more for feed than pulp production, they obtained a sulfite pulp having a residual lignin content of 15% but with the dietary energy equivalence of a medium-quality hay when fed to steers at up to 70% of their total ration. No mention was made of the economics of the process.

All pulping procedures investigated thus far, however, depend upon the selective removal of lignin for their beneficial effects. There is yet another possibility for enhancing the availability of wood residue carbohydrates, that of disrupting the lignin-carbohydrate association in situ without the selective removal of either constituent. Under proper conditions, we have found that gaseous sulfur dioxide can effect just such a disruption and the treatment appears applicable to both hardwoods and softwoods.

For this work, wood residues in the form of sawdust were reacted for 2 hr (hardwoods) or 3 hr (softwoods) at 120°C with an initial SO_2 pressure at room temperature of 30 psi and a water-to-wood ratio of 3 (no free liquid). After Slowdown and a brief evacuation, the treated woods were neutralized to about pH 7 with sodium hydroxide and then air dried. Table IV presents analytical data and values for 48-hr enzyme digestion for both the original woods and the treated products [76]. A sample of alfalfa is included for comparison.

As expected, cellulase digestion of the original woods was minimal, from a high of 9% for aspen to essentially 0% for the two softwoods.

TABLE IV
Composition and Cellulase Digestion of Various Woods Before and After SO_2 Treatment

	Lignin %		Carbohydrate %		Digestibility %	
Species	Before	After	Before	After	Before	After
Quaking aspen	20	7	70	71	9	63
Yellow birch	23	9	66	67	4	65
Sweetgum	20	5	66	64	2	67
Red oak	26	8	62	60	1	60
Douglas-fir	30	24	65	63	0	46
Ponderosa pine	31	19	59	58	0	50
Alfalfa	17	--	51	--	25	--

Even with alfalfa, only about half of the available carbohydrate was convertible to sugars. Yields of the SO_2-treated products were 106 to 112% based on starting material, a result of the sulfonation and neutralization reactions. Although all of the lignin was retained in the products, Klason lignin analysis of the five treated hardwoods showed lignin values of only 5 to 9%. This suggested that the original lignin had been extensively depolymerized during SO_2 treatment and converted to soluble products, a fact subsequently confirmed by extraction with boiling water. Depolymerization was less extensive with the two softwoods, and the higher Klason values are reflected by a decreased digestibility. Enzymatic conversion of the hardwood carbohydrates was essentially quantitative, indicative of a complete disruption of the strong lignin-carbohydrate association in the original woods. The 60 to 65% digestibility of the treated hardwoods ranks these materials equivalent to high-grade hays for producing energy. The two softwood products would be equivalent to a low-quality hay, but might be upgraded through a better choice of processing conditions.

For information on palatability, possible toxic side effects, and in vivo nutritional value, a 300-lb batch of SO_2-treated material was prepared from red oak sawdust and fed to goats at levels of 0 to 50% of a pelleted roughage-type ration over an 8-week period. Average in vivo digestibilities for dry matter and carbohydrate as a function of wood content of the rations are plotted in Figure 7. Extrapolation of the curves to 100% SO_2-treated wood yielded values of about 52% for dry matter digestion and 60% for carbohydrate digestion. From the shallow slope of the curves, it appears that a vapor phase treatment with sulfur dioxide effectively converts red oak sawdust into a ruminant feedstuff having the dietary energy equivalence of a medium-quality hay. Neutralization of the treated product with ammonia rather than sodium hydroxide would augment its protein equivalence. Rough estimates of overall production costs indicate possible economic feasibility of the process but only at production rates in excess of 200 tons per day.

Delignified Residues

While direct production of "fodder" celluloses by commercial pulping processes now holds little economic appeal, feed potential exists in the fibrous residues generated by the pulp and paper industry and by the public at large. Each ton of wood pulp that is manufactured and processed into finished products results in about 80 lb of fibrous wastes commonly disposed of by burning or as landfill. On an annual basis, this

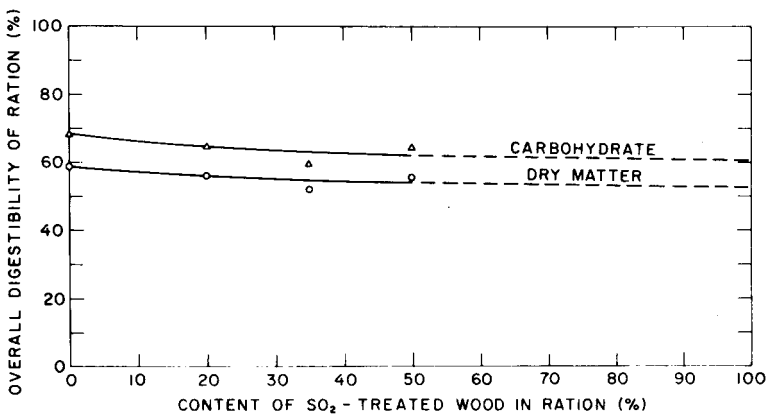

Fig. 7. In vivo dry-matter digestion of rations containing SO_2-treated red oak.

represents nearly 2 million tons of carbohydrate-containing material, most of which has received at least partial delignification. When one also recognizes that approximately 80% of the primary product of our nation's pulp mills (currently about 50 million tons of virgin fiber per year) ends up on trash heaps as postconsumer waste, we have a tremendous material resource that has already received substantial pretreatment to render its carbohydrate constituents susceptible to chemical, enzymatic, or microbiological conversion.

Research in this area has been in progress for a number of years, especially that concerned with the possible utilization of the various residues as sources of dietary energy for ruminants [41-47]. Tables V and VI present compositional and in vitro digestibility data for some typical pulp mill and combined pulp and paper mill residues [47]. The results of feeding trials (goats) with three of these residues is shown in

TABLE V
Composition and in vitro Digestion of Pulpmill Residues

Type of Residue	Lignin %	Carbohydrate %	Ash %	Digestibility %
Groundwood fines				
Aspen	21	73	1	37
Southern pine	31	59	1	0
Spruce	31	60	1	0
Screen rejects				
Aspen sulfite	19	77	2	66
Mixed hardwood, sulfite	24	65	14	54
Mixed hardwood, kraft	25	74	9	44
Chemical pulp fines				
Mixed hardwood, kraft (bleached)	<1	109	1	95
Aspen sulfite (parenchyma cells)	20	73	2	73
Southern pine, kraft (unbleached)	28	68	4	46

TABLE VI
Composition and in vitro Digestion of Combined Pulp and Papermill Sludges

Type of Residue	Lignin	Carbohydrate	Ash	Digestibility
	%	%	%	%
Groundwood mill				
Mixed species + mixed chemical pulps	50	41	38	24
Southern pine + mixed hardwood kraft	24	60	15	19
Semichemical pulpmill				
Aspen	20	71	2	57
Aspen + mixed hardwoods	55	29	13	6
Chemical pulpmill				
Deinked waste paper, tissue	23	71	22	72
Milk carton stock	28	67	25	65
Mixed chemical pulps. tissue	17	76	13	60
Aspen and spruce sulfite	45	46	45	35

Figure 8. Extrapolation of the curves to 100% residue yields digestibility values of about 80% for the mixed hardwood kraft fines, 65% for the aspen sulfite screen rejects, and 50% for the aspen sulfite parenchyma fines, indicative of a substantial utilization of available carbohydrate by the ruminant animals. Additional feeding studies are in progress.

Steaming

Yet another approach toward upgrading the digestibility of lignocellulosic residues is steaming. While generally involving no added chemical, the cleavage of acetyl groups early in the process provides an acidic medium conducive to hydrolytic action. Steaming has been applied to both straw [26, 48-53] and wood [54-57], the most recent application to wood being that of Bender and co-workers [56, 57]. These latter workers showed that steam treatment of aspen chips for about 2 hr at 100-115 psi (160-170°C) gave a product that was readily

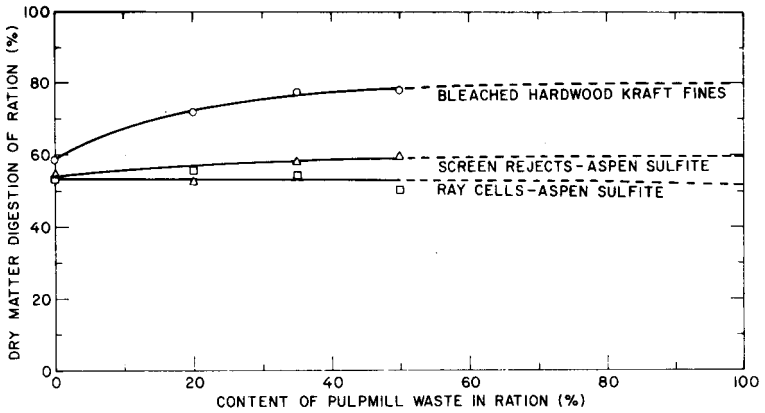

Fig. 8. In vivo dry-matter digestion of rations containing typical pulp-mill residues.

accepted by sheep at up to 60% of the total ration and provided normal weight gains and carcass yields. They estimated overall processing costs at about $5 per ton for a plant processing 4,000 tons of material per year. Steam treatment (as with a number of the pretreatments discussed earlier) shows a strong species response; hardwoods exhibit a much greater improvement in digestibility than softwoods, with aspen being the outstanding hardwood with respect to the magnitude of its response. Currently, the only wood-carbohydrate feed additive in commercial production is derived from a steaming operation. Masonex, a wood-sugar molasses, is obtained by concentration of the wash liquors generated during the manufacture of hardboard [58]. The high-temperature steaming used to effect fiber separation solubilizes from 5 to 10% of the wood carbohydrates along with other whole-wood constituents, and these solubles must be removed prior to board formation. This is another example of by-product recovery in which the costs of manufacturing the salable by-product are shared with another product.

PHYSICAL PRETREATMENTS

Grinding

The subdivision of wood to a very small particle size yields a product remarkably susceptible to hydrolytic, bacterial, and enzymatic attack.

Thus, Virtanen et al. [59, 60] demonstrated that thermophilic bacteria could utilize 75% of the polysaccharides of birch and 53% of the polysaccharides of pine if the wood was in the form of fine sander dust. Vibratory ball milling has provided an even more effective means of size reduction. Pew and Weyna obtained essentially complete cellulase digestion of milled spruce and aspen compared to less than 10% digestion for the initial 60-80 mesh sawdust [18]. Vibratory ball milling also enhanced cellulose digestibility of wood and forages by rumen bacteria [61, 62] and provided nearly quantitative yields of wood sugars when the grinding was performed in the presence of either gaseous hydrogen chloride [63] or small amounts of concentrated sulfuric acid [64].

In our own efforts to improve ruminant utilization of wood residues, we have found that, while vibratory ball milling is indeed an effective pretreatment, the milling response is quite species selective [21]. As an example, Figure 9 shows the in vitro rumen fluid digestibilities of five hardwoods as a function of the time of milling. With 140 min of milling, all woods more or less attained a digestibility plateau. However, the plateau is widely different for the different species, ranging from about 80% for aspen and sweetgum to only 20% for red alder. Softwoods are even less responsive than red alder; five different

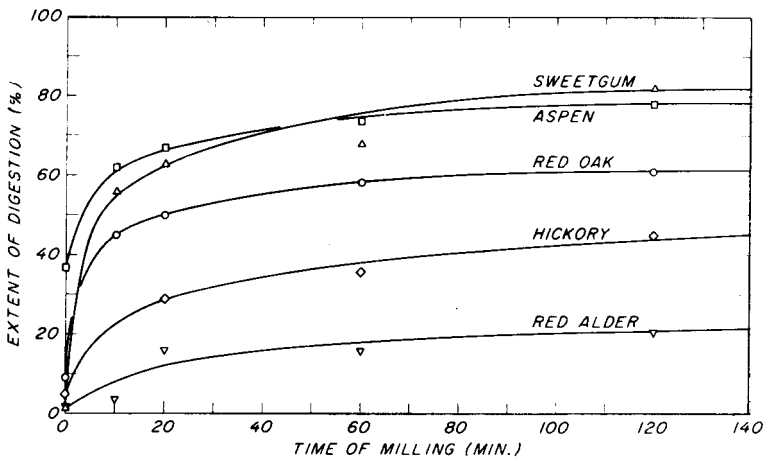

Fig. 9. Relationship between in vitro digestibility and time of vibratory ball milling.

softwoods show a maximum digestion of only 18% after 120 min of vibratory milling. Thus, this selective species response severely limits the broad application of the milling technique. Time and energy costs impose further restrictions.

Irradiation

The technique of irradiating wood or straw by gamma rays or by high-velocity electrons substantially improves digestibility of these materials by rumen organisms [21, 65, 66]. However, a strong species specificity appears again (Table VII). The digestion of aspen carbohydrate is essentially quantitative after an electron dosage of 10^8 rad while spruce is only 14% digestible at this dosage.

Electron irradiation also increased both rate of hydrolysis and maximum yield of sugar when applied as a pretreatment prior to the simple batch hydrolysis of cellulosic materials [67]. Action was minor up to dosage levels of 10^6 rad but then increased rapidly with dosage; at 5×10^8 rad the substrate became essentially water soluble but had lost nearly 45% of its original carbohydrate values. Optimum effectiveness was attained at about 10^8 rad, at which dosage sugar yield was nearly three times that of the control and the rate of hydrolysis was increased

TABLE VII
Effect of Electron Irradiation on the in vitro Digestion of Aspen and Spruce

	Digestibility	
Electron Dosage rads	Aspen %	Spruce %
0	55	3
10^6	52	3
10^7	59	5
5×10^7	70	8
10^8	78	14

seventeenfold. The degree of polymerization of the irradiated cellulose had decreased to about 40, an indication of extensive bond breakage, but carbohydrate destruction was only about 14%. Bond breakage was therefore mostly beneficial. However, with irradiation costs estimated at well over $100 per ton for both gamma and electron beam equipment at dosage levels of 10^8 rad, the technique would appear to have little commercial interest.

Another radiative pretreatment showing promise for inducing deep-seated structural alterations is that of photodegradation. As set forth in the patent disclosure [68], the process involves exposure of polysaccharides to high-intensity ultraviolet light (3650 Å) in the presence of a dilute solution of sodium nitrite which functions as a photosensitizer. Recent application of the technique was made by Rogers et al. in their work on the production of fungal protein by *Aspergillus fumigatus* [69]. They reported that a 24-hr irradiation pretreatment gave up to a fourfold increase in the rate of biodegradation of a variety of cellulosic substrates.

Miscellaneous Physical Methods

High temperature

As first proposed by Sharkov in 1956 [70, 71], modifying cellulose fine structure by simple thermal exposure represents an appealing approach to improving reactivity. Sharkov claimed that a 3-hr heating of cellulose at 200°C in nonpolar liquid, such as kerosene, or in dry air or nitrogen, gave a product having a greatly enhanced rate of acid hydrolysis. However, a later reassessment of thermal pretreatment, while confirming 200°C as being about the optimum temperature, indicated that the maximum increase in rate of dilute acid hydrolysis was only about 35% with an increase of about 27% in maximum sugar yield [72]. Considering that a 32-hr treating period was required to obtain even these modest benefits, it is unlikely that simple thermal pretreatment has much to offer as a commercial venture.

Low temperature

At the other end of the temperature scale, the freezing of cellulosic materials in water suspension at -75°C has been reported to reduce both strength and degree of polymerization and to increase reactivity as measured by dye adsorption [73]. The effect was augmented by

repeated freezing and thawing cycles. Again, the energy requirements would appear too great to make this a viable pretreatment procedure.

Pressure

Sharkov and Levanova [74] have shown that compression of a cotton hydrocellulose for 30 min at room temperature approximately doubled the quantity of material dissolved during ethanolysis as the pressure was increased from 0 to 8000 kg/cm^2. Repeated compression of spruce sulfite pulp sheets between calendar rolls increased the solubility during ethanolysis from 12 to 54%. Substantial improvement in cellulose saccharification by pressure milling with low ratios of 75% sulfuric acid has also been reported [75]. This approach has received extensive attention in the Russian literature during the past 20 years. In part, the milling action may be ascribed as contributing to better distribution of acid throughout the cellulosic mass but milling, especially vibratory ball milling, can markedly enhance cellulose reactivity through reducing particle size.

SUMMARY AND CONCLUSIONS

With few exceptions, the carbohydrates of whole-wood residues are essentially immune to attack by cellulolytic enzymes or by ruminal microorganisms. This resistance apparently stems from the close physical and chemical association between cellulose and lignin augmented by the crystalline nature of cellulose itself. Whether or not the carbohydrate energy values contained in these materials can be utilized thus depends upon our ability to alter or open up the lignin-carbohydrate complex. Even a native cellulose would exhibit a more desirable chemical reactivity following modification of its fine structure. From the practical standpoint, these alterations must be low cost (economical) as well as effective; and therein lies our challenge.

Of the many chemical and physical pretreatments explored thus far, delignification is by far the most universally applicable. Purified wood pulps of all species provide an excellent source of dietary energy for ruminant animals. These materials, however, enjoy a much more valuable end-use for their fiber or chemical conversion qualities. On the other hand, partial delignification holds some promise; removal of only one third of the lignin content of hardwoods and about two thirds of that

of softwoods appears necessary to provide a product equivalent to hay in ruminant digestibility. Processing cost should be appreciably less than for full chemical pulping. Gas-phase treatment with sulfur dioxide, leaving the lignin in situ, is especially intriguing in this respect.

Then there is the broad array of pulp- and paper-making residues, which have already received varying degrees of delignification but which no longer have fiber values. Many of these show excellent potential as energy feeds for ruminants and should serve well as substrates for enzymatic or microbiological conversions. Problems would be largely those associated with collecting and transporting such residues to processing centers.

Of the remaining physical and chemical pretreatments covered in this survey, all were effective to varying degrees but exhibited a strong species preference that severely limits their broad applicability. Hardwoods were generally much more responsive to pretreatment than softwoods, but even hardwoods exhibited a broad range of responsiveness (aspen being outstanding in its degree of susceptibility). Treatment with aqueous sodium hydroxide at a level of 5 to 6 g of NaOH per 100 g of wood raised the in vitro digestibility of a number of hardwoods into the range of hay (45-60%). Anhydrous ammonia was about equally effective. Steaming at 160-170°C yielded an aspen product that was readily accepted by sheep. Vibratory ball milling was very effective with aspen, sweetgum, and red oak but translation of laboratory results into an economical commercial operation appears doubtful. High energy radiation is a simple way to increase digestibility and reactivity of lignocelluloses, but application is dependent on reduced irradiation cost.

The Forest Products Laboratory is maintained at Madison, Wisc., in cooperation with the University of Wisconsin.

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