

STUDIES ON SACCHARIFICATION OF CONIFER WOOD-WASTE: I.
EFFECT OF VARIED CONCENTRATION OF H_2SO_4 AND PERIOD OF HEATING
ON INITIAL HYDROLYSIS AND SACCHARIFICATION PROCESS

by

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Summary. *Saccharification of saw-dust of deodar (Cedrus deodara) and kail (Pinus wallichiana) was conducted under varied conditions of temperature, period of heating, and acid concentration. The hydrolysed products from pre-, main-, and post-hydrolysis were pentoses, oligo saccharides and mono-saccharides, respectively. The glucose content ranged from 2.63 to 25.29% in the saw-dust of deodar and 2.15 to 25.17% in kail. The treatment of saw-dust with 5-70% H_2SO_4 dissolved and hydrolysed 16.60-54.93% and 13.01-52.98% of original material of deodar and kail, respectively. Higher acid concentration and prolonged period of heating increased the amount of hydrolysed and dissolved material in hydrolyzate. The chemical conversion of wood was not only affected by the experimental conditions but also by the nature of wood material.*

Wood saccharification is the acid hydrolysis of cellulosic constituents to produce simple sugars. The development of wood saccharification process has been studied for long from the view point of recovering of acid, finding of acid resistant materials, devising of equipment, and discovering of a simple way of refining sugar. Several plants, using concentrated and dilute sulfuric acid, were built in Italy, Germany, Korea, Switzerland, U.S.A., U.S.S.R., Japan etc. (15). These plants produced alcohol from dilute sugar solution obtained by hydrolysis. After the second world war, all these plants were closed with the exception of those in the U.S.S.R. The main reasons for closing these plants were the low economic returns of the process and the absence of sustained market for the principal products. Recent studies have helped in restoring production in Japan and other countries (15).

The production of sugar from wood and its further fermentation is influenced by several conditions, such as method of treatment, size of particles, acid strength, liquid-to-solid ratio, reaction time and the temperature and pressure maintained during the reaction (13, 16, 17, 18.) In general, high temperature and increased period of heating, together with higher acid concentration, increase rate of hydrolysis. However, the reverse polymerization occurs simultaneously under these conditions. For obtaining higher yield of sugar, it is essential to suppress side reactions. Various combinations of con-

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centration of acids and reaction temperature processes have been studied to make the whole operation industrially economical.

According to the estimate of various agencies, an enormous quantity of lumber-mill waste and residue is obtained from wood industries (15,19). This waste is useless as structural or pulp material but can be employed as a raw-material for wood-based chemical industries. The importance of wood hydrolysis was stressed by the Chairman F. A. O. Tokyo Meeting on Wood Chemistry (1960), who said "Although wood hydrolysis did exist as an industry in Europe for several years, it is now primarily in the laboratory-development and pilot-plant stages, except in U.S.S.R. In spite of its present status, a chemical industry, based on wood, will soon be making a substantial contribution to the world economy". Studies on saccharification have been carried out during the past few years (7,15,16,20). At present, fourteen wood saccharification plants are in operation throughout the U.S.S.R. (15). The main products are yeast, alcohol and furfural. Since Pakistan is poor in forest wealth, every effort should be made to effectively utilise all wood material. This research project was undertaken to explore the possibilities of utilising coniferous wood-waste as a raw material for the production of sugar and ethyl alcohol.

Material and Methods. The saw-dust samples of deodar and kail were sieved and the material of 12 to 60 mesh particle size was used for the laboratory studies. Various preliminary constants like moisture content, density, specific gravity and ash content of experimental wood species were determined by following standard methods (2).

The extraneous components of deodar and kail were determined in cold and hot water, ether, ethyl alcohol and alcohol benzene mixture (1:2) by the TAPPI Standard Methods(1).

Hydrolysis of wood was carried out both qualitatively and quantitatively. For pre-hydrolysis, saw-dust was treated with 50 parts of 5% H_2SO_4 at about $100^\circ C$ for 2 hours. The residue was repeatedly washed with water, and dried. The filtrate was neutralised with lime, and the neutral solution was concentrated and qualitatively tested for furfural and pentoses.

The pre-hydrolysed and dried saw-dust was subjected to main hydrolysis, and was submerged in H_2SO_4 ranging from 5-70% concentration at atmospheric pressure. The hydrolysis was carried out with 10-50 parts of acid to one part of saw-dust by weight at various temperatures and for different reaction times. At lower concentrations of acid, the temperature was maintained at about $100^\circ C$, whereas at higher concentrations the hydrolysis was performed at room temperature to avoid decomposition of sugar formed. The reaction time was varied from 15 minutes to 8 hours. The saw-dust was filtered after main hydrolysis. The concentration of H_2SO_4 of combined filtrate was maintained between 5-10%. The reaction mixture was diluted to 5% for converting various

poly-saccharides to mono-saccharides. The sugar solution was neutralised with commercial lime, and CaSO_4 was filtered off. The pH of sugar solution was adjusted to 2.5 in order to remove excess calcium. Various intermediate products such as methanol, acetone, etc. were removed by distillation. Sugar solution was concentrated to 50%, and activated carbon was added to decolorise the solution and to remove the organic polymers. The suspended impurities were removed by repeated filtration. Filterates were tested for sugars. Optical activity of sugar solution was determined. Sugar solution was further diluted to a known volume, and glucose was estimated by Pavy's method (3).

Chir wood samples, infected with wood-rotting fungus *Lenziter sepioria*, were hydrolysed with 65% H_2SO_4 at room temperature in order to determine the effect of fungal decay on the yield of sugar. The extent of decay was ascertained by treatment with 1% NaOH -2 gms of air-dried saw-dust was normally treated with 5-70% H_2SO_4 at 100°C with occasional stirring in order to determine the dissolving and hydrolysing effects of H_2SO_4 . The mixture was cooled and diluted to 300 ml. and refluxed for 2 hours. The undissolved material was filtered, repeatedly washed with water, dried and weighed.

Several procedures were followed for alcoholic fermentation (11). 250 ml of wood sugar solution was treated with 5 gms. of yeast. K_2HPO_4 and NH_4 were added as nutrients in some cases. Fermentation was carried out from 2 to 16 days.

Results and Discussions. Preliminary determinations of moisture content, density and specific gravity and ash content of kail and deodar sample woods are presented in Table 1.

Table 1

Some Physical Characteristics of Kail and Deodar Wood Samples

Determination	Wood species	
	deodar	kail
Moisture	10.22%	7.20%
Density	0.5606	0.4645
Specific gravity	0.4984	0.4263
Ash content	0.50%	0.36%

The extraneous components in wood included various organic compounds, and their contents in wood samples are given in Table 2.

Table 2.

Various Extractives in kail and deodar wood samples

Solvent	Percent Extractives	
	deodar	kail
Cold water	2.34	3.13
Hot water	3.14	3.39
Ether solvent	3.43	4.04
Ethyl alcohol (95%) v	4.67	4.18
Alcohol-Benzene (1:2)	4.89	5.32

The extractives were, in general, coloured and comprised waxes, fats, resins, tannins, colouring matter and similar other soluble constituents. These components are partially responsible for contaminating sugar solution and causing complication in the purification and fermentation of solution, and also in restricting the phased crystallization of sugar components.

Wood Hydrolysis.

Wood saccharification, being a complicated process, is processed through hydrolysis with different acids, *i. e.*, H_2SO_4 or HCl , using these as acid catalysts for this reaction. The amount and nature of sugars obtainable from wood depend upon the polysaccharides in wood (4). Cellulose is the main polysaccharide of interest and it is hydrolysed with much difficulty to yield hexose sugars, preferably glucose. The hemi-celluloses constitute roughly one-third of total carbohydrates in wood. These are hydrolysed much more easily than cellulose (15). The resistance of cellulose to hydrolysis is due to its partially crystalline nature which causes hinderance in the penetration of chemicals. Such a reaction is overcome by treatment over prolonged period to effect complete hydrolysis (15,20). The sugars are simultaneously decomposed under severe conditions of hydrolysis. Nevertheless, the utilization of carbohydrate fraction of wood as a starting material for the manufacture of sugar remained a complicated problem (4,5,8.)

Pre-hydrolysis : The process of extracting hemi-cellulose and removing pentoses from wood is called pre-hydrolysis. The rate of de-

composition of hemi-cellulose is about one hundred times faster than that of cellulose (4,15,20). The hemi-celluloses are amorphous and are hydrolysed easily than cellulose. The main-hemi-cellulose components of wood are glucan, galactan, mannans, xylan and araban (9). The proportion of these components differ in different wood species, and the most significant differences being between soft-woods and hard woods. Various sugars, chiefly derived from hemi-cellulose, contaminate the saccharified solution of wood. In order to obtain higher yield of glucose, it is essential to remove as much pentoses as possible prior to undertaking main hydrolysis. Thus, one of the purposes of prehydrolysis is to minimise the impurities in the sugar solution. The process of prehydrolysis was tried both with stean digestion at about 100° C for 4 hours, and with 3-5% H_2SO_4 at 100° C for 2 hours. Prehydrolysis with acid solution was more effective in restricting the contamination of mother liquor with sugars other than glucose. The pre-hydrolysis product was qualitatively tested, since sugar separated on crystallization. These gave positive tests for pentoses and furfural.

Main hydrolysis : The crystalline structure of cellulose is very strong and cannot be hydrolysed easily (14). However, the treatment of cellulose with concentrated H_2SO_4 results in cleavage of molecule and its conversion into water-soluble glucose polymers. The rate of hydrolysis depend upon the concentration of acid, temperature, pressure and heating time (20). The rate of hydrolysis increases considerably at high temperature. A lower reaction temperature is preferred, but it slows down the reaction rate. For obtaining a high yield of glucose, it is necessary to suppress the side reaction. The concentration of acid also affects the rate of hydrolysis. The main components of hydrolysis appear to be a mixture of various polysaccharides. These could possibly be converted into mono-saccharides by post-hydrolysis.

Post-hydrolysis. Wood cellulose gives water-soluble polysaccharides through hydrolysis with concentrated acid at low temperature. Under such conditions, the reaction is not carried further to mono-saccharides stage. The post-hydrolysis under suitable conditions converts the glucose polymers into mono-saccharides. It is commercially desirable to treat the hydrolyzate with dilute acid at low temperature in order to produce higher yield of mono-saccharides.

The saccharified solution was neutralised with lime and its glucose content was estimated. The results are presented in Table 3.

Table 3

Percentage of Glucose formed from the Hydrolysis of saw-dust of *Cedrus deodara* and *Pinus wallichiana*.

S. No.	Hydrolysing conditions		Glucose % age	
	H ₂ SO ₄ (%)	Time (hours)	Deodar	Kail
1.	5	8	2.63	2.15
2.	10	2	3.09	2.72
3.	10	3	7.69	4.12
4.	10	4	9.27	6.97
5.	10	6	10.09	8.02
6.	10	8	10.71	9.12
7.	15	1	3.47	2.91
8.	15	2	4.81	3.80
9.	15	3	9.55	5.07
10.	15	4	9.82	7.92
11.	15	6	10.19	9.03
12.	15	8	11.10	10.07
13.	20	1	5.97	3.83
14.	20	2	10.45	7.25
15.	20	4	11.35	9.91
16.	20	8	12.92	9.37
17.	25	1	7.34	6.41
18.	25	2	10.98	9.18
19.	25	4	11.86	8.99
20.	25	8	13.20	10.39
21.	30	1	8.34	7.44

22.	30	2	11.15	9.32
23.	30	4	12.79	10.73
24.	30	8	13.90	11.80
25.	35	1	8.89	7.83
26.	35	2	12.10	9.93
27.	35	4	13.47	11.22
28.	35	8	9.83	7.22
29.	40	1/4	10.03	9.05
30.	40	1/2	11.16	8.51
31.	40	1	12.09	9.58
32.	45	no heating	10.72	9.30
33.	50	„	11.60	10.75
34.	55	„	12.18	11.69
35.	60	„	15.71	14.70
36.	65	„	20.30	19.03
37.	70	„	25.29	25.17

The wood cellulose in pre-hydrolysis remained almost unaffected, and hydrolysed products were largely pentoses. The whole mechanism of wood hydrolysis is a complicated process, and it is difficult to precisely predict the proportion of principal products of mainhydrolysis.

The saccharification of saw-dust of deodar and kail was studied by treatment with various concentrations of H_2SO_4 , reaction time and temperature. Yield of sugar improved when saw-dust was treated with acid 5 times the weight of wood. A large quantity of carbohydrates remained unsaccharified on treatment with lower concentration of H_2SO_4 . Besides, various by-products formed were acetic acid, methyl alcohol, acetone, aliphatic aldehydes, furfural, terpenes and aromatic hydrocarbons, which were tested qualitatively. Extraction with hot water before pre-hydrolysis helped removal of impurities contaminating the wood sugar solution but practically did not influence the yield of sugar product. The proportion of glucose in the hydrolysed product varied from 2.15 to 25.29 %

Since cellulose is the sugar-forming component, and the yield of sugar is proportional to the cellulose content of raw-material, glucose content of cellulose from sound and decayed wood samples was determined. The pre-hydrolysis and post-hydrolysis of such samples was carried out with 5% H_2SO_4 at 100° C; the main-hydrolysis was performed with 65% acid at room temperature. The glucose formed was as follows:—

Glucose % age

sound wood	21.32
partially decayed	17.43
advanced decayed	14.22

The decayed sample was infected with *Lenzites sepiaria*. One percent NaOH solubles of sound, partially decayed and advanced decayed chir wood samples were 14.45%, 27.12% and 40.46%, respectively. This showed the extent of damage and degree of decay. It is also apparent from the low sugar content of these samples.

Lignin of woody material can be isolated by saccharifying the carbohydrates, cellulose, and wood polyoses. Strong H_2SO_4 has been used as a solvent for carbohydrates (10,12). The hydrolysing effect of various concentrations of H_2SO_4 on Saw-dust of kail and deodar was determined and the data are presented in Table 4.

Table 4.
Acid Concentration and Dissolution and Hydrolysis of
Saw-Dust of Deodar and Kail.

Sl. No.	% H_2SO_4	Loss in weight of saw-dust		% residue	
		deodar	kail	deodar	kail
1.	5	16.60	13.01	83.40	86.99
2.	10	19.99	16.98	80.10	83.02
3.	15	24.98	20.11	75.02	79.89
4.	20	32.32	23.73	71.68	76.27
5.	25	30.54	26.07	69.46	73.93
6.	30	33.32	30.88	66.68	99.12
7.	35	35.60	33.64	64.60	66.36
8.	40	38.39	36.97	61.68	63.03
9.	45	39.89	39.12	60.11	30.88
10.	50	43.05	52.89	56.95	57.11
11.	55	45.42	44.51	55.58	55.49
12.	60	49.00	47.20	51.00	52.80
13.	65	51.12	50.02	48.88	49.98
14.	70	34.93	52.98	45.07	47.02

Various concentrations of H_2SO_4 showed a marked difference in converting polysaccharides into soluble products. At high concentrations, though more wood substance was dissolved, some of it might not have been converted into sugars. With 5-70% H_2SO_4 treatment of saw-dust of deodar and kail, dissolved and hydrolysed material ranged from 16.60 to 54.93 % and 13.01 to 52.98% respectively. Since the success of wood saccharification process also largely depends upon the profitable use of lignin, various experiments on the commercial use of lignin as soil-mulch, as a carrier for fertilizer, as a source of humus and for the production of various chemicals have given promising results (15,19).

Fermentation of wood molasses. Fermentation of wood sugar was carried out with yeast in dilute solution. The treatment period was varied from 2 to 16 days. Although several attempts were made, no satisfactory economical product was obtained on account of inhibition of fermentation by undesirable hydrolysed by-products. High concentration of yeast and continuous stirring slightly improved the fermentation reaction. The frequent use of activated charcoal to decolourise and absorb the colour products also improved this process.

CONCLUSION

Various extractives of wood which were coloured and comprised of waxes, fats, resins, tannins and colouring matter caused complication in fermentation and crystallization of sugars. Removal of these extraneous components, improved the hydrolysis of end products. Graded hydrolysis of wood could be made to yield specific mono-di- or polysaccharides. A mild hydrolysis caused selective action on hemi-celluloses present in wood which were mainly converted to pentoses. The wood cellulose has to be treated under drastic conditions for further conversion to carbohydrates. Not only the experimental conditions but also the nature of wood influenced the yield of sugars in the saccharification process. Studies on sound and decayed chirwood samples showed that the yield of sugar was proportional to the cellulose content of raw-material.

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