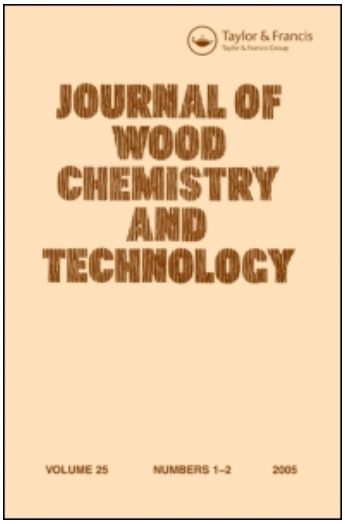

Journal of Wood Chemistry and Technology
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597282

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To cite this Article Clark, T. A. and Mackie, K. L.(1987) 'Steam Explosion of the Softwood *Pinus Radiata* with Sulphur Dioxide Addition. I. Process Optimisation', Journal of Wood Chemistry and Technology, 7: 3, 373 — 403
To link to this Article: DOI: 10.1080/02773818708085275
URL: http://dx.doi.org/10.1080/02773818708085275

JOURNAL OF WOOD CHEMISTRY AND TECHNOLOGY, 7(3), 373-403 (1987)

STEAM EXPLOSION OF THE SOFTWOOD *PINUS RADIATA* WITH SULPHUR DIOXIDE ADDITION. I. PROCESS OPTIMISATION

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ABSTRACT

An experimental optimisation procedure has been used to investigate steam explosion of *Pinus radiata*. Enzymatic digestibility of this softwood is enhanced by steam explosion and addition of sulphur dioxide greatly improves effectiveness of the process. The effects of the variables time, temperature, and sulphur dioxide level have been described by empirical models. The response parameters modelled are: dry-matter yield; water-insoluble fibre yield; water- soluble sugar yield; enzymatic-hydrolysis sugar yield; and total sugar yield. Near optimal total sugar yields were obtained with the conditions: 3 minutes; 215°C; and 2.55% sulphur dioxide. Under these conditions the steam-exploded fibre was 82% digestible (cellulose to glucose) by the cellulase enzymes used, and a total sugar yield of 57 g/100 g o.d. wood was obtained. This study has shown that softwoods, of which *Pinus radiata* is typical, can be made as amenable to hydrolysis by cellulase enzymes as hardwoods, which have previously been considered the only viable substrates for such processes.

INTRODUCTION

Steam explosion is recognised as one of the most cost effective pretreatments for hardwoods and agricultural residues prior to enzymatic saccharification[1-3]. As a pretreatment for

softwoods, however, steam explosion has been considered ineffective, although for reasons which have not been made clear[4,5].

The softwood, *Pinus radiata* is the predominant commercial forest species in New Zealand and large quantities of forest and mill residues (sawdust, shavings and chips) will become available over the next 10 to 20 years for use in fuel or chemical production processes[6]. Research into improving the effectiveness of steam explosion on this softwood species was initiated to assist utilisation of such residues.

The treatment of both softwoods and hardwoods with high levels of gaseous sulphur dioxide at 120°C was shown by Millet *et al.*[5] to significantly improve enzymic digestibility of the woody substrates. Subsequently, research at General Electric[7,8], involving addition of low levels of sulphur dioxide during true steam explosion, confirmed the beneficial effects of sulphur dioxide addition. However, whereas Millet *et al.* attributed these effects to delignification, the latter group showed that the improved enzymatic digestion of poplar wood resulted from a more complete removal of hemicelluloses. The addition of sulphuric acid or sulphur dioxide during steam explosion of the hardwood Populus tremuloides (aspen)[9] has also been shown to be beneficial in terms of hemicellulose removal and improved enzymatic digestibility.

Recent work involving prehydrolysis of woody substrates with and without the addition of acid catalysts has shown that improved carbohydrate survival and better hemicellulose removal are obtained when the treatment pH is low, around pH 2-3. Without catalyst addition, the pH attained during autohydrolysis is around pH 3-4, and, at this pH, degradation of carbohydrate

by hydroxyl ion is significant[10]. Mineral acid catalysts, such as sulphuric acid, may be used[9], or sulphur dioxide[11] may be added to achieve the improved prehydrolysis. Wayman[11] demonstrated that softwoods were equally amenable to prehydrolysis as hardwoods when using sulphur dioxide catalysis. Recently Grohmann *et al*[12] have shown that the improvements in cellulose enzymic digestibility, obtained following dilute acid prehydrolysis of both aspen wood and wheat straw, were related to the extent of xylan removal during prehydrolysis.

The most detailed work on sulphur dioxide addition to softwoods during steam pretreatment is that of Mamers and Menz[13]. Using the Siropulper procedure (which is a combination of prehydrolysis and explosive depressurisation) they showed that addition of sulphur dioxide significantly enhanced enzymic digestibility of the pretreated softwood *P. radiata*. At sulphur dioxide levels of 4.7 to 18.7% of o.d. wood, water-soaked chips gave pulps with enzymic digestibilities (percentage weight loss of solids) ranging from 34 to 53%. Under the same conditions of treatment, but with no sulphur dioxide addition, the pulp digestibility was only 2.5%. The suitability of sulphur-dioxide pretreated Siropulper substrates (both softwood and hardwood) for enzymic hydrolysis and the production of cellulolytic enzymes has been reported recently by Dekker[14]. He found cellulose to glucose conversion yields of 60% under the test conditions used, whereas without sulphur dioxide addition only 27% conversion was obtained.

The benefits of adding sulphur dioxide to softwoods prior to prehydrolysis or steam explosion have thus been clearly demonstrated. In such pretreatments there are three main process variables: temperature; time; and sulphur dioxide level.

However effects of these variables on the properties of the derived products have not been investigated in sufficient detail to allow selection of optimal processing conditions. If a commercial process is to be considered, the significance of each variable will be important. In particular, for reasons of cost, the lowest practical level of sulphur dioxide must be sought. This paper examines effects of the above three variables on chemical composition and enzymatic digestibility of steam exploded *P. radiata* and recommends processing conditions.

EXPERIMENTAL

Wood Supply

Pinus radiata logs from a stand of 18-year-old trees were chipped in a whole-tree chipper following debarking. A sample of chips (approximately 400 kg wet weight) was collected over a period of 8 h and mixed thoroughly. The chips were then screened using a Williams rotary screen classifier. Those chips passing through a 22-mm screen but retained on a 16-mm screen were combined and the bulked chips again thoroughly mixed. The moisture content of the chips was then determined (42.96% dry content) before bagging and sealing in 300 o.d. gram equivalent lots. These bags were then immediately frozen at -20°C and subsequently thawed as required. The wood composition is shown in Table 1.

The Experimental Procedure

The experimental procedure used in this study is shown schematically in Figure 1. Following impregnation with gaseous SO_2, the wood (300 g O.D. lots) was steam exploded under various conditions of time and temperature. The steam-exploded products (SEW) were then water washed to remove water-soluble components (WS), and the resultant insoluble fibre (WI) was enzymatically hydrolysed to determine its enzymatic

TABLE 1.
Composition of *Pinus radiata* (all Figures % o.d. Original Wood)

Ash			0.31
Extractives			2.21
Klason lignin			26.16
Acid soluble lignin			nil
Carbohydrate			
		($+H_2O$)	
Glucan	43.31	(48.12)	
Mannan	10.71	(11.90)	
Galactan	2.89	(3.21)	
Xylan	5.27	(5.99)	
Arabinan	1.63	(1.85)	
Total anhydro polymer			63.81
Unaccounted (4-OMe-Glucuronic anhydride, O-acetyl, analytical error)			7.51
			100.00

digestibility. Twenty individual steam-explosion experiments were performed, with operating conditions selected according to a statistical design (see Experimental Design).

Sulphur Dioxide Impregnation

Bagged chip lots (300 g o.d.) were thawed overnight and the bag plus chips weighed. Anhydrous SO_2 gas was then added to the bag via a plastic tube, for a period varying from 20 s to 30 min, depending on the level of impregnation required. The bag was opened briefly to allow gaseous SO_2 to disperse and then tightly sealed to minimise the gas space in the bag. The increase in weight of the bag and chips was recorded as percent SO_2 on oven-dry chips. The impregnated chip samples were then steam exploded within 10 to 20 min from the time SO_2 addition was complete.

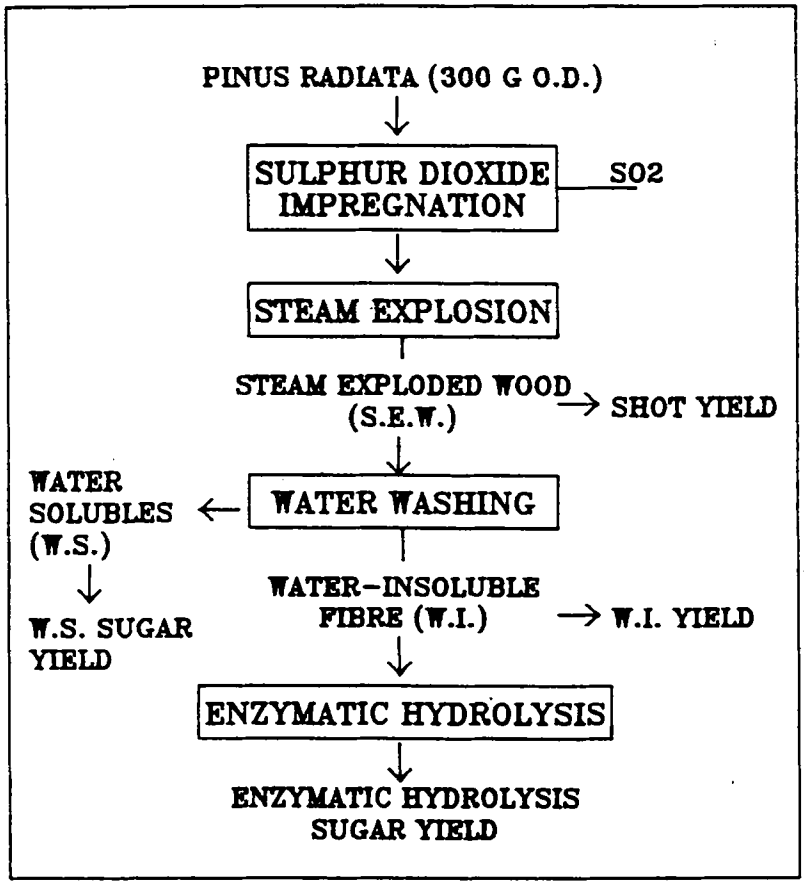


Figure 1. The experimental procedure.

Steam Explosion Gun Operation

The design and operation of the steam-explosion equipment described below is based upon that at Forintek Canada Corporation, Ottawa, Canada, and has been published elsewhere[9,15].

A 3-litre, 316 stainless steel gun fitted with a 75-mm wide bore ball valve at each end was used throughout this study. Saturated steam was supplied to the gun via two 12.5-mm side ports (one near the top and one near the bottom) at pressures varying from 1.3 to 3.2 MPa. Steam pressure and steam temperature were recorded in the steam main immediately adjacent to the gun. The lower 75-mm ball valve was activated by an air actuator (opening time approximately 1 s) but the upper ball valve was manually opened or shut. The receiving cyclone was approximately 50 litres and designed to allow quantitative recovery of solid and liquid products from the gun. The cyclone, which was water-jacketed at approximately 15°C, was open to the atmosphere and hence volatile components were not retained.

During operation, the gun assembly (which was heavily insulated) was preheated by repeatedly allowing steam into the gun and then discharging the condensate. The top ball valve was then opened and the SO_2-impregnated chips emptied directly into the gun. After closing the upper ball valve, steam was admitted to the gun - zero time was taken from this point (pressure and temperature in the steam main, remained effectively constant). At the end of the desired treatment time the bottom air-actuated ball valve was opened and simultaneously the steam entry lines were closed. The contents of the gun were discharged by the force of expanding steam and were recovered in the receiving cyclone. The heat up times for chips of different geometries and moisture contents, in steam-explosion equipment of the same design, have been described in detail elsewhere[15].

For chips of the size used in this study, approximately 30-50 seconds would be required to bring the centre temperature to within 10°C of the steam temperature.

Removal of the lid from the receiving cyclone facilitated recovery of all the solid and liquid material produced from the chips. A minimum quantity of washing water was used during recovery. Hold up of solids in the gun assembly was negligible.

Determination of Shot Yield, Y_{shot}

Due to the low dry matter content (5 to 15%) of the steam-exploded wood (SEW), accurate yield data could not be obtained in the usual manner, i.e., sampling the exploded material and determining the oven-dry content. An alternative procedure was developed whereby the substrates were filtered onto a 70-um expanded polypropylene filter and the weight of filtrate and filter cake accurately determined. The exploded substrate was then regenerated for oven-dry estimation at 105°C by accurately recombining samples of the filter cake and filtrate in the correct proportions. Due to the gradual release of volatile components from the steam-exploded products, oven drying was, in all cases, for a period of 2 h. All substrates were therefore air dried or, where necessary, freeze dried before being placed in the oven. The shot yield, Y_{shot}, was expressed as the oven-dry weight of SEW per 100 g of original o.d. wood.

Determination of Water-Insoluble Fibre Yield, Y_{WI}

All steam-exploded substrates were stirred at room temperature using an overhead stirrer for 1 h at 5% (oven-dry) consistency in water and then filtered onto a 70-um expanded polypropylene filter. The water- insoluble fibre was then washed again under the same conditions, giving two products:

(1) water-insoluble fibre (WI); and (2) water-soluble material (WS) from the combined filtrates (see Figure 1). The dry-weight yield of WI was determined after 2 h oven drying (at 105°C) of air-dried samples. The parameter Y_{WI} was calculated as the oven-dry weight of WI per 100 g of original o.d. wood. Until used, the WI and WS samples were frozen at -20°C.

Determination of Enzymatic Digestibility and Enzymatic Sugar Yield, Y^{S}_{EH}

Enzymatic hydrolysis of the water-insoluble fibre (WI) was performed using the commercially available Novo Celluclast 1.5 l (NC 3000 84-10) cellulase preparation, supplemented with exogenous β-glucosidase in the form of Novozym 188 (DCN 001 84-6). Both enzyme preparations were obtained from Novo Industri A/S, Denmark and had the following activities: Celluclast, 69.9 FPU/g and 1.8 Cellobiase Units/g; Novozym, 0 FPU/g and 673 Cellobiase Units/g. Enzyme assays were performed according to IUPAC recommendations[16]. Digestions were performed in 150-ml conical flasks with a total liquid volume of approximately 100 ml. Digests comprised Celluclast (40 FPU), Novozym (50 Cellobiase Units), and pretreated substrate (2 g o.d. equivalent) in 0.05 M citrate buffer (pH 4.8), containing either sodium azide (0.01%) or thimerosal (0.001%) as preservative, and were mixed to give a slurry density of 2% (wt. dry fibre/wt. liquid). Incubation was at 50°C in a shaking waterbath, operating at 150 rpm. Samples were withdrawn at 0, 6, 24, 48 and 72 h and the supernatants, which were recovered by centrifugation, were heated in a boiling water bath for 5 min prior to resin treatment (see analytical methods) and high performance liquid chromatography (hplc) analysis. As an internal control between batches of digests, Sigmacell 50 was digested in duplicate as above, but at a slurry density of 1.2% in order that FPU/gm

cellulose was in a similar range to that for the pretreated samples.

The WI digestibility is reported as the yield of glucose after 72 h of enzymatic hydrolysis, expressed as a percentage of the potential glucose in the fibre. The yield of enzymatically released sugars, Y^S_{EH}, is the total weight of all sugars released at 72 h, expressed per 100 g of original o.d. wood.

Determination of Water-Soluble Sugar Yield, Y^S_{WS}

Monomeric sugars present in the WS were determined by hplc. All WS samples were post-hydrolysed, using 4% H_2SO_4 (see below), to convert any oligomeric sugars to monomers. The yield of water-soluble sugars, Y^S_{WS}, is expressed as grams per 100 g of original o.d. wood.

Determination of Total Sugar Yield, Y^S_T

Total sugar yield is calculated as the sum of the enzymatic sugar yield, Y^S_{EH}, and the water-soluble sugar yield, Y^S_{WS}, and is expressed as grams total sugar per 100 g of original o.d. wood.

Analytical Methods

Ash - was determined using Tappi Standard T211 om-80.

Extractives - exhaustive extraction with methanol was used to prepare extractive-free material. The Klason lignin and carbohydrate analyses of *Pinus radiata* wood were performed on extractive-free samples.

Klason lignin - was determined using a procedure[17] analagous to Tappi Standard T223-OS-71. Acid-soluble lignin was estimated on the Klason lignin filtrates using an extinction coefficient of 110 $g.l^{-1}.cm^{-1}$.

Carbohydrate determinations - samples were initially hydrolysed with 72% H_2SO_4 and subsequently a secondary hydrolysis was performed using 4% H_2SO_4, as for the Klason lignin determination[17]. After filtering off insoluble Klason lignin the filtrates were prepared for hplc analysis as described below. Soluble carbohydrates (from WS samples) were post-hydrolysed using 4% H_2SO_4, as above, in order to convert all oligosaccharides to monosaccharides. In calculating carbohydrate compositions, hydrolysis loss factors have been applied to the results. These factors were determined by subjecting individual sugars to the hydrolysis procedure and measuring losses. The hydrolysis loss factors used are glucose (1.053), xylose (1.155), galactose (1.045), arabinose (1.082), and mannose (1.092).

Liquid chromatography - monosaccharides were quantitatively determined using dual Biorad HPX 87P columns with water at 0.4 ml per min as the eluant. A Hewlett Packard 1037A refractive index detector operating at a sensitivity of 1/4 was used for detection. The analytical columns were protected by two precolumns; one a strong cation and the other a weak anion (Biorad Cation -H^+ and Anion -OH^- cartridges). The analytical columns (only) were held at 85°C and the feed water thermo- statically controlled at 65°C. Samples (30 ml) containing H_2SO_4 were first adjusted to pH 4.5 to 5.0 by stirring with solid $Ba(OH)_2$ for 30 min and then filtering. All samples were then passed through a mixed-bed ion exchange column containing equal proportions of Amberlite IR 120 (H^+) 100-200 mesh and Merck MP7080 (OH^-) 100-200 mesh. The colourless samples were adjusted to approximately 1 mg per ml total carbohydrate and filtered through a 0.45-um filter prior to injection (25 ul). Erythritol, added to samples prior to treatment, was used as an internal standard for all samples

except those originating from enzymatic digests. In the latter case glycerol, present as a stabiliser in the enzyme preparation Celluclast, was used as the internal standard.

Experimental Design

The effects of the three variables, time, temperature, and SO_2 level, were investigated using a technique from response-surface methodology, the central composite design, which was first described by Box and Wilson[18]. The design, shown in Table 2, consists of a 20-run experiment, carried out in three orthogonal blocks. Six repeats of the centre-point conditions (3 min, 215°C, 2.55% SO_2) were included, two in each block. Time (min), temperature (°C) and SO_2 (% w/w) were coded and, where necessary, linearised by the following expressions:

$$T = \frac{\log_e (\text{time})}{\log_e (3)} - 1$$

$$\theta = \frac{\text{temperature} - 215}{20}$$

$$S = \frac{\log_e (\% SO_2)}{\log_e (2.55)} - 1$$

Statistical analysis of the results was performed by multiple linear regression, fitting equation (1) to the experimental data.

$$Y = \beta_0 + \beta_1 T + \beta_2 \theta + \beta_3 S + \beta_{12} T.\theta + \beta_{13} T.S + \beta_{23} \theta.S + \beta_{123} T.\theta.S + \beta_{11} T^2 + \beta_{22} \theta^2 + \beta_{33} S^2 + \beta_{112} T^2.\theta + \beta_{221} \theta^2.T + \beta_{113} T^2.S + \beta_{1122} T^2 \theta^2 \quad \ldots (1)$$

TABLE 2.
The Experimental Design

Variable	Coded levels				
	-1.633	-1	0	1	1.633
Time (min)	0.5	1	3	9	18
Temperature (°C)	182	195	215	235	248
Sulphur dioxide (% w/w)	0.55	1	2.55	6.50	11.8

Run no.	Coded variable levels			Block No.
	T	θ	S	
1	-1	-1	-1	2
2	1	-1	-1	1
3	-1	1	-1	1
4	1	1	-1	2
5	-1	-1	1	1
6	1	-1	1	2
7	-1	1	1	2
8	1	1	1	1
9	-1.633	0	0	3
10	1.633	0	0	3
11	0	-1.633	0	3
12	0	1.633	0	3
13	0	0	-1.633	3
14	0	0	1.633	3
15	0	0	0	1
16	0	0	0	1
17	0	0	0	2
18	0	0	0	2
19	0	0	0	3
20	0	0	0	3

Terms which were statistically insignificant (at the 95% confidence level) were eliminated from equation (1) to give an empirical model for each dependent variable in terms of time, temperature, and SO_2 level. The coefficient of determination, R^2, and the lack-of-fit test ratio, F_{lof}, are reported in Table 3 to indicate the adequacy of fit for each empirical model. Pure error variance was calculated from the results for the six repeats of the centre-point conditions[19]. The ratio, F_{lof}, is compared with the appropriate value from the F-distribution, at the 95% confidence level.

Selected response parameters were thus modelled to provide mathematical descriptions of the response surfaces formed by the interactions of time, temperature, and SO_2 impregnation level. Table 3 gives the model coefficients and statistical data for the response parameters shot yield, WI fibre yield, WS sugar yield, enzymatic-hydrolysis sugar yield, and total sugar yield. In all but one case the models provide an excellent description of the experimental data, with R^2 values greater than 95% and F_{lof} values less than 5 (cf. F(10, 5, 0.95) = 4.74). The exception is the model for water-soluble sugar yield which, despite having an R^2 value of 95%, exhibits significant lack of fit. This is largely due to the exceptionally low standard error for the centrepoint replicates (see later in Table 5). Examination of the coefficients in each model indicates that all three main variables produce quite complex interactions, yielding response surfaces with a high degree of curvature.

RESULTS

The purpose of this study was to optimise the steam-explosion process in terms of obtaining the maximum total sugar yield (Y_T^S)

TABLE 3.
Fitted Coefficients in Equation 1 for Empirical Models of the Parameters
Y_{shot}, Y_{WI}, Y^S_{WS}, Y^S_{EH}, and Y^S_T

	Y_{shot}	Y_{WI}	Y^S_{WS}	Y^S_{EH}	Y^S_T
β_0	90.5	59.8	28.4	28.3	57.0
β_1	-6.67	-8.19			
β_2	-7.56	-9.77			
β_3	-1.34	-4.11	2.16	1.83	3.99
β_{12}	-3.96	-2.15	-4.06	-7.74	-11.8
β_{13}		2.50	-2.67		-2.65
β_{23}	-1.61			-1.18	
β_{123}		3.26	-2.37	-2.30	-4.68
β_{11}	-0.84		-2.40	-8.03	-10.3
β_{22}	-1.44		-2.05	-9.20	-11.1
β_{33}				-1.69	-2.04
β_{112}				1.69	1.72
β_{221}			-1.16		-1.82
β_{113}					
β_{1122}		5.35	-2.64	3.27	
R^2	98.2	97.0	95.0	99.4	99.3
F_{lof}	2.16	4.19	21.1	0.59	3.73

from *Pinus radiata*. To do this, standard response-surface investigative techniques were employed. These involved fitting empirical models to the experimental data and the subsequent identification of optimum conditions of time, temperature, and SO_2 impregnation level.

Tables 4 and 5 present the basic analytical data for the 20 run experiment, namely, in Table 4, shot yield, water-insoluble fibre yield, fibre composition, fibre enzymatic digestibility and, in Table 5, the water-soluble sugar yields. Six repeats of the centre-point conditions (3 minutes, 215°C, 2.55% SO_2) were

TABLE 4.
Analytical and Digestibility Data for Water Washed Insoluble Fibre

Run no.[a]	Shot yield (% OD wood)	WI yield (% OD wood)	WI Composition (% WI)						Digestibility of WI (%)[h]
			KL[b]	ASL[c]	Total[d] carb	Glc[e]	Xyl[f]	Man[g]	
1	98.5	81.5	34.0	0.42	66.8	57.2	3.9	5.6	4.4
2	92.2	72.4	38.3	0.70	65.8	64.0	0.9	0.9	25.5
3	91.9	77.6	36.2	0.47	64.5	58.7	2.0	3.8	41.6
4	73.0	46.8	80.1	1.95	16.7	16.7	-	-	96.0
5	99.3	77.0	35.2	0.43	67.0	60.4	2.7	3.9	6.8
6	93.6	64.8	44.0	0.82	59.4	59.4	-	-	59.2
7	89.5	54.3	45.2	1.14	55.6	55.6	-	-	84.3
8	64.7	46.6	72.6	1.32	24.4	24.4	-	-	35.6
9	98.9	77.0	34.4	0.36	66.0	58.8	2.9	4.2	12.8
10	78.6	46.8	82.9	1.80	14.3	14.3	-	-	101.3
11	98.3	75.3	36.3	0.38	65.4	60.1	2.4	2.8	7.3
12	76.0	38.7	90.0	2.49	7.0	7.0	-	-	105.1
13	94.5	66.0	39.3	0.72	59.9	59.9	-	-	51.0
14	88.6	54.3	48.7	1.24	52.0	52.0	-	-	94.2
15	91.1	62.3	42.5	0.84	58.4	58.4	-	-	72.9
16	88.9	58.0	44.5	0.88	55.7	55.7	-	-	83.4
17	90.5	60.1	44.9	0.98	56.4	56.4	-	-	79.8
18	88.6	59.0	44.2	0.95	59.1	59.1	-	-	82.4
19	90.6	58.9	44.5	0.92	56.2	56.2	-	-	86.3
20	91.6	61.1	45.9	1.18	55.2	55.2	-	-	87.4
[i]mean	90.2	59.9	44.4	0.96	56.8	56.8	-	-	82.0
SE (%)	1.33	2.62	2.50	12.4	2.78	2.78	-	-	6.39

[a]See experimental for time, temperature and SO_2 level corresponding to run number.
[b]KL = Klason lignin, [c]ASL = acid-soluble lignin, [d]Total carb = total carbohydrate corrected by hydrolysis loss factors but not converted to anhydropolymers. [e]Glc = Glucose, [f]Xyl = xylose, [g]Man = Mannose,
[h]Digestibility is defined as the yield of glucose at 72 h as a % of the theoretical glucose obtainable from the WI, [i]mean and standard error for centrepoint repeats.

TABLE 5.
Water-Soluble Sugar Yields (All Figures % OD Wood Starting Material)

Run no.[a]	Total soluble[b] sugar yield (Y_{WS})	Glc[c]	Xyl[d]	Gal[e]	Ara[f]	Man[g]
1	16.3	2.27	3.26	1.88	1.31	7.55
2	22.6	4.66	4.73	2.27	1.30	9.68
3	18.9	4.61	3.86	1.99	1.08	7.35
4	18.5	14.7	0.59	0.88	0.27	2.11
5	20.5	2.87	4.23	2.48	1.54	9.40
6	25.7	9.02	4.10	2.54	1.29	8.79
7	34.2	15.5	4.51	3.05	1.62	9.55
8	13.7	7.49	1.37	0.97	0.65	3.24
9	19.2	2.90	4.02	2.33	1.29	8.68
10	24.8	18.8	0.77	1.25	0.77	3.19
11	21.2	3.05	4.52	2.40	1.47	9.72
12	24.7	18.0	1.16	1.07	0.72	3.76
13	23.8	6.11	4.59	2.19	1.28	9.65
14	30.5	15.5	3.50	2.58	1.01	7.85
15	28.7	10.7	4.42	3.10	1.29	9.18
16	29.0	11.7	5.09	2.77	1.21	8.16
17	29.5	12.2	4.24	3.12	1.22	8.72
18	29.0	12.0	4.04	2.90	1.30	8.70
19	28.1	11.7	3.94	2.81	1.20	8.45
20	28.7	12.8	3.82	2.74	1.08	8.32
Mean[h]	28.8	11.9	4.26	2.91	1.22	8.58
SE (%)	1.6	5.8	10.8	5.7	6.5	4.2

[a] See Experimental for time, temperature and SO_2 level corresponding to run number. [b] Samples post-hydrolysed and hydrolysis loss factors applied. Carbohydrate expressed as free reducing sugars. [c] Glc = Glucose, [d]Xyl = xylose, [e]Gal = galactose, [f]Ara = arabinose, [g]Man = mannose, [h]mean and standard error for centrepoint repeats.

performed (see Experimental) and, for convenience, this block of data has been grouped together (run numbers 15-20). These replicates provide an estimate of pure error variance for the experiment and enable lack-of-fit tests to be performed to determine the adequacy of fit between experimental data and the fitted models. The standard error for the six centre-point runs is given in Tables 4 and 5, for each determination, and indicates that acceptable reproducibility was obtained in the experiment.

Dry Matter Yields, Y_{shot} and Y_{WI}

The shot yield (Table 4) is the total yield of oven-dry material recovered from the steam exploder. Yields from 64.7 to 99.3% were obtained. The difference from 100% reflects the quantity of volatile wood decomposition products formed during the process and lost either during gun operation or during dry-weight determinations on SEW. This loss of wood substance during steam explosion is considered to result from both pyrolysis and acid-catalysed carbohydrate decomposition[20]. The former can result in CO_2 and H_2O as volatile products while the latter may yield furfural and formic acid. Physical losses in the steam- explosion equipment were minimal and do not account for the loss of wood substance upon explosion.

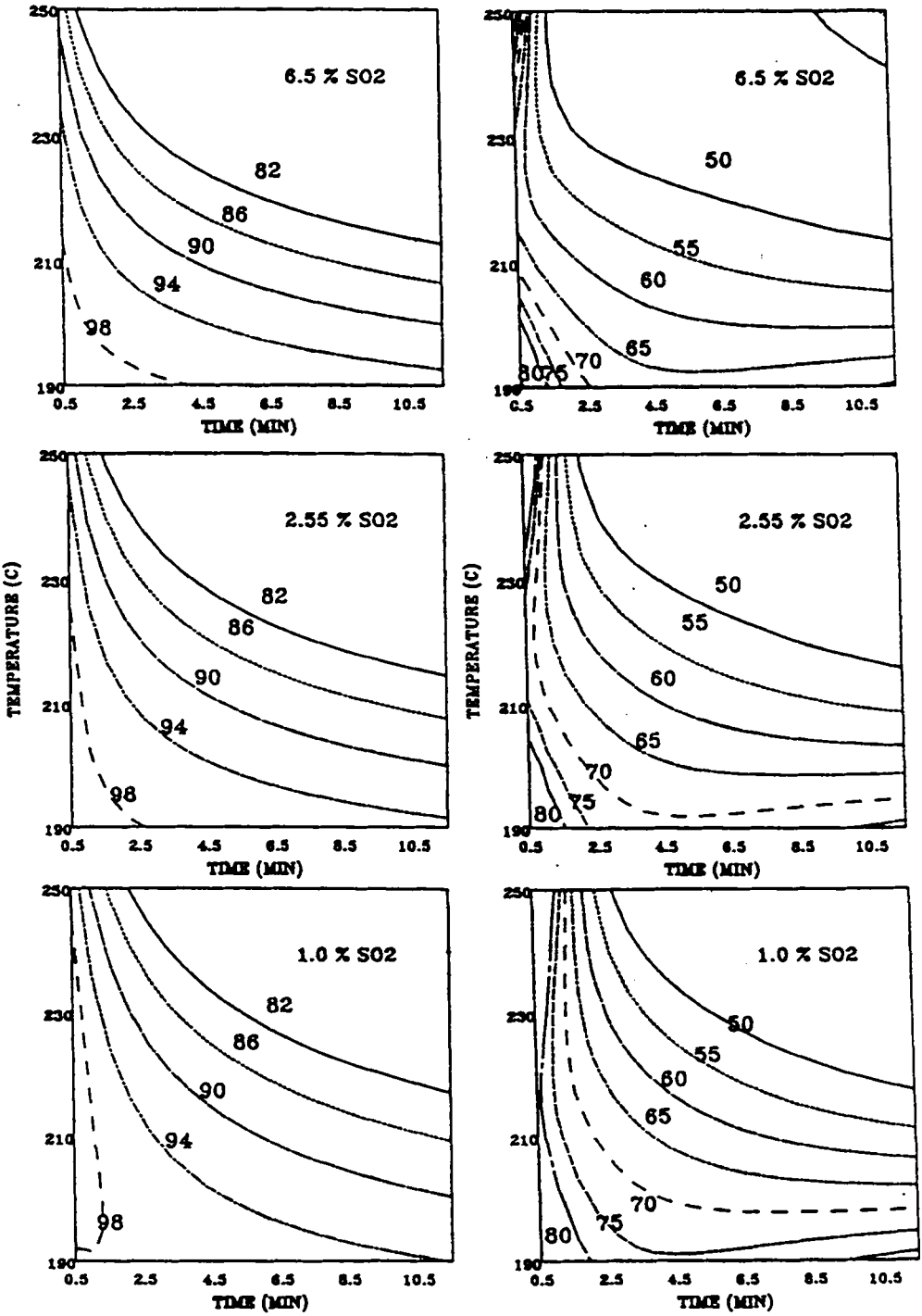
The water-insoluble fibre yield was always less than the shot yield (Table 4) and the difference reflects the extent of solubilisation of wood substance during the explosion process. Yields varied from 38.7 to 81.5%. When Klason lignin content and carbohydrate composition of the WI (Table 4), are compared with those for untreated *Pinus radiata* (Table 1), it is clear that the hemicellulose component of the wood is being solubilised. Under the most severe experimental conditions used

(runs 4, 8, 10 and 12) the WI yields were 46.8%, 46.6%, 46.8% and 38.7% respectively. For each of these runs, Klason lignin contents exceeded 70%, and correspondingly low total carbohydrate contents were obtained. Substantial cellulose solubilisation was, therefore, also occurring. With the exception of runs 4, 8, 10 and 12, yields of Klason lignin in the WI (g/100 g o.d. original wood) were between 24.5 and 28.5%. As the Klason lignin content of *P. radiata* is 26.2% (Table 1), it can be concluded that the lignin component of the wood was not significantly solubilised. The severely processed runs 4, 8, 10 and 12 returned Klason lignin yields from 33.8 to 38.8%, suggesting the production of condensed carbohydrate decomposition compounds which analyse as Klason lignin. This phenomenon has been recognised by others[21] working on steam explosion of aspenwood and the material has been referred to as "pseudo lignin".

The response surfaces for Y_{shot} and Y_{WI} (see Table 3 for equations) are shown in Figure 2 where contours of constant yield are plotted against temperature and time, for different SO_2 levels. The contours for both yield parameters are characteristically hyperbolic, showing that time and temperature are inversely related for a given yield. For both Y_{shot} and Y_{WI}, increasing either time or temperature decreases yield. Increasing the concentration of sulphur dioxide also has a negative effect, but this is comparatively small. As discussed earlier, Y_{WI} is always less than Y_{shot} for a given set of conditions, the difference reflecting the amount of water solubles formed.

Enzymatic Digestibility of the Water-Insoluble Fibre

Only minor amounts of sugars other than glucose were present in all digestions, the proportion being highest for runs 1, 2,



3, 5, 9, and 11. The WI fibres for these runs still contained small amounts of the hemicellulose sugars xylose and mannose (Table 4). As shown in Figure 3, digestibility correlates very closely with the total carbohydrate content of the WI. As the proportion of carbohydrate in the WI decreased, its digestibility increased. The corollary to this observation, is that high Klason lignin contents in the WI do not, *per se* result in poor enzyme digestibilities. The four runs conducted under the most severe conditions (Runs 4, 8, 10 and 12), gave correspondingly low WI carbohydrate contents, and three of these showed complete digestibility. Run 8, however, which represents the outlier in Figure 3, gave only 35.6% digestibility. Apparently, the conditions in Run 8 (9 min., 235°C, 6.50% SO_2) resulted either in poor enzyme access to the small amount of residual cellulose, or alternatively, formation of a water-insoluble enzyme inhibitor.

Typical digestion profiles are shown in Figure 4. Digestions were performed at a constant enzyme level of 20 FPU/g of oven-dry WI. Since the carbohydrate content of the WI varied from 7.03% to 67.02%, FPU of cellulase/g cellulose is clearly not constant. For this reason, initial rates of digestion cannot be meaningfully compared. However, it is valid to compare the 72-h hydrolysis yields since in all digestions only slight increases in sugar yield occurred between 24 and 72 h (Figure 2), indicating that all digestions had proceeded to completion. The yields of total sugars after 72 h hydrolysis, expressed as g/100 g of original o.d. wood are tabulated in Table 6.

Figure 2. Dry matter yield contour diagrams for three levels of SO_2-impregnation, 1.0, 2.55, and 6.5%. Diagrams on left hand side describe shot yield (Y_{shot}) and on right hand side WI yield (Y_{WI}). Numbers beside each contour line refer to respective yield levels, expressed as g/100 g original o.d. wood.

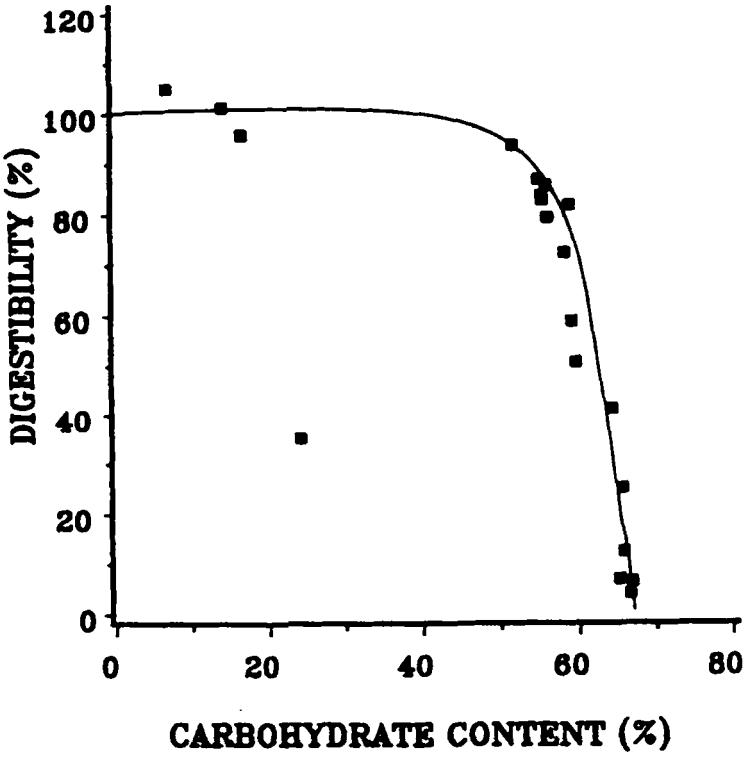


Figure 3. Relationship between WI fibre enzymatic digestibility and carbohydrate content.

Carbohydrate Hydrolysis During the Steaming Process

Table 5 gives the analytical data for the water-soluble sugars, obtained by water washing of the steam-exploded products. The yields of individual sugars are expressed as g/100 g of original o.d. wood. The WS samples were post-hydrolysed to ensure that all sugars were present as monomers. Analysis of these samples prior to post-hydrolysis gave significantly lower yields of monomers indicating the presence of residual oligomeric carbohydrate or possibly the formation of sugar reversion products.

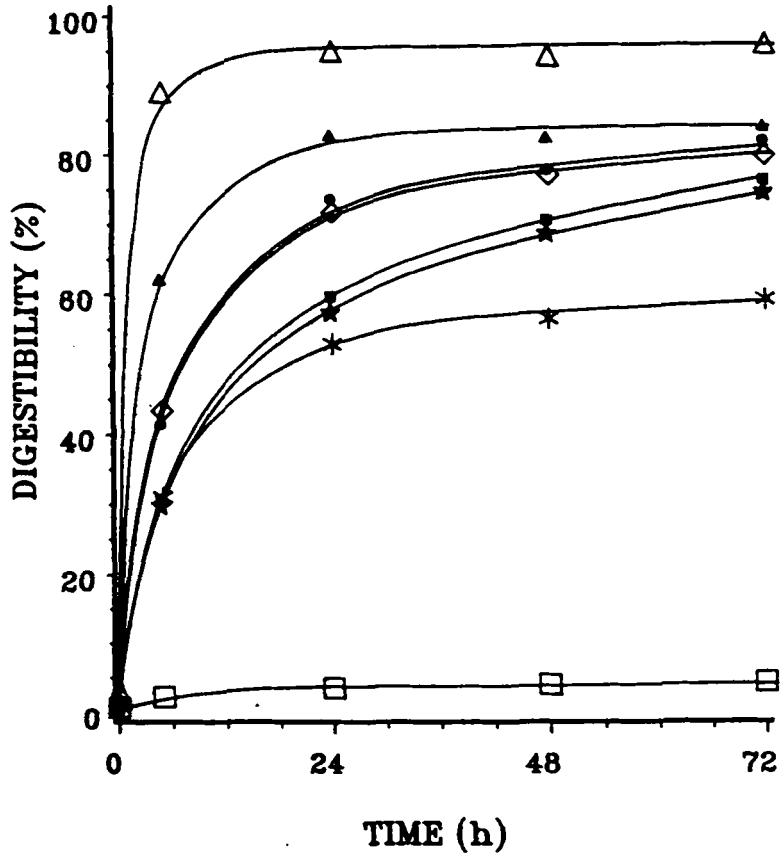


Figure 4. Enzymatic digestion profiles for the digestion of WI fibre from selected experimental runs. Run 1 (□), run 4 (△), run 6 (*), run 7 (▲), run 17 (●), run 18 (◇), control Sigmacell 50 (■, ★).

The yield of total sugars in the WS is presented in Table 6 along with the yield of sugars enzymatically released from the WI. In comparing these two yields it is clear that a greater quantity of sugar was solubilised during steam explosion than was subsequently obtained by enzymatic hydrolysis of the WI fibre. This was true under almost all the experimental conditions.

TABLE 6.
Enzymatic Hydrolysis, Water-Soluble, and Total Sugar Yields
(All Figures % o.d. Original Wood)

Run	Y^S_{EH}	Y^S_{WS}	Y^S_T
1	3.2	16.3	19.5
2	12.7	22.6	35.3
3	19.8	18.9	38.7
4	7.6	18.5	26.1
5	4.5	20.5	25.0
6	23.3	25.7	49.0
7	25.6	34.2	59.8
8	4.2	13.7	18.0
9	6.8	19.2	26.0
10	6.9	24.8	31.7
11	4.5	21.2	25.7
12	2.9	24.7	27.6
13	20.7	23.8	44.5
14	26.8	30.5	57.3
15	27.0	28.7	55.7
16	27.1	29.0	56.1
17	27.4	29.5	56.9
18	29.0	29.0	58.0
19	28.8	28.1	56.9
20	30.1	28.7	58.9
Mean[a]	28.2	28.8	57.1
SE (%)	4.5	1.6	2.1

[a]mean and standard error for centrepoint repeats.

Optimal Conditions

Inspection of the total sugar yield data in Table 6 suggests that the central conditions of the design experiment lie fortuitously close to the true optimum. However, by examining the response surface, predicted by the total sugar yield model (Table 3), the effects of time, temperature, and SO_2 level can be more clearly seen. Figure 5 shows three-dimensional plots of total sugar yield versus time and temperature for SO_2

impregnation levels of 1.0, 2.55, and 6.5%. Also shown are contour plots, where contours of constant yield are presented on axes of time versus temperature. Only those yields greater than 50% have been plotted in order to more clearly show the optimal region. Increasing SO_2 level from 1.0% to 2.55% greatly increases the maximum yield predicted, but has little effect on the optimal treatment temperature and time which remain close to 215°C and 3 minutes. The diagonal orientation of the response surfaces indicates that similar yields can be obtained along a range of times and temperatures, from high temperatures with short times, to lower temperatures with longer times. This relationship is most marked on changing from an SO_2 level of 2.55% to a level of 6.5%, which results in the predicted maximum moving from 215°C, 3 minutes to the more extreme conditions of the experiment, ca. 245°C, 0.5 minutes. While predictions of yield under such extreme conditions may not be very reliable, it can be concluded that time and temperature are inversely related, in a manner dependent upon SO_2 level.

DISCUSSION

The maximum total sugar yields achieved in this experiment, (57 to 60%) represent 80 to 84% of the sugars potentially available in *Pinus radiata* (Table 1). The residual carbohydrate in the undigestible portion of the WI accounts for a further 5 to 8% of the potential sugar. The remaining 10 to 12% is unaccounted for and, therefore, must have degraded during the steam explosion process. The percentage survival of each sugar following steam explosion under centre-point conditions (3 min, 215°C, 2.55% SO_2) is shown in Table 7.

The high percentage survival of glucan (94.3%) and its subsequent, efficient hydrolysis to glucose results in a total-

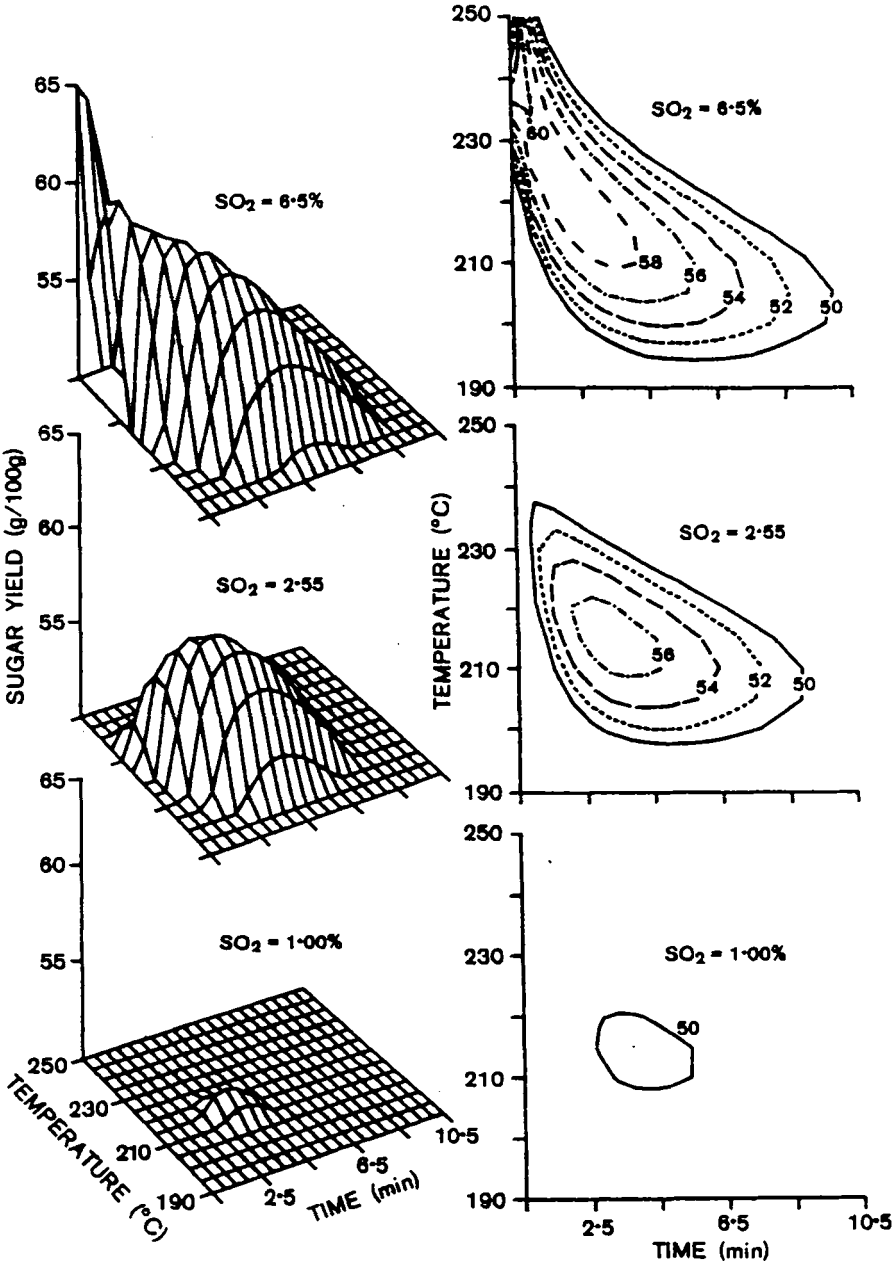


TABLE 7.
Percentage Survival of the Wood Sugars Following Steam Explosion (3 min. at 215°C and 2.55% SO_2)

Sugar	Survival (%)
Glucose	94.3
Xylose	71.8
Galactose	90.3
Arabinose	64.9
Mannose	72.3
Total	87.9

sugar hydrolysate with a high proportion of glucose. By contrast, glucose survival in dilute acid-hydrolysis processes is normally only around 64%; consequently such hydrolysates have a lower proportion of glucose[22]. The sugar composition of the combined WS and EH streams is shown in Table 8, again for the centrepoint treatment.

The very high proportion of hexose sugars is a favourable feature, highlighting the advantages of (i) using softwoods in hydrolysis processes, and (ii) using steam explosion and cellulase enzymes to hydrolyse the cellulose component. The hexose sugars are readily fermented to ethanol in high yield, using the traditional ethanolic fermentation, whereas pentoses such as xylose are only fermentable by some yeasts, as yet, with poor efficiency[23].

Figure 5. The total sugar yield response surface. Y_{TS}^{S} plotted against temperature and time, for three levels of SO_2-impregnation, 1.0, 2.55, and 6.5%. For each SO_2 level, yields greater than 50 g/100 g are plotted both as three-dimensional diagrams (at left) and as corresponding contour diagrams (at right).

TABLE 8.
Percentage Sugar Composition of the Combined Water-Soluble and Enzymatic Hydrolysates

		% composition	
Hexoses:	Glucose	69.6	
	Mannose	15.2	
	Galactose	5.4	
		Total	90.2
Pentoses:	Xylose	7.6	
	Arabinose	2.1	
		Total	9.7

The wide spread of operating conditions examined in this experiment precluded exact determination of optimal conditions. However, the regions of the response surface enclosing such conditions have been clearly identified. In particular, processing times of approximately 1 min, with temperatures greater than 220°C, at least at high SO_2 levels, appear to be superior to the centrepoint conditions of 3 min. and 215°C. Figure 6 shows predicted total sugar yield plotted against SO_2 level for the centrepoint time and temperature conditions. Under these conditions, the effect of increasing SO_2 level on yield reaches a plateau above about 3%. However, at levels between 0.5 and 3% the effect of SO_2 is highly significant.

The WI fibre produced in this study was substantially more digestible than has been reported by other workers investigating steam pretreatment of *Pinus radiata*[13,14,24]. Dekker[14] reported cellulose to glucose (72 h) digestibilities of approximately 60% for pulps obtained by processing at 200°C for

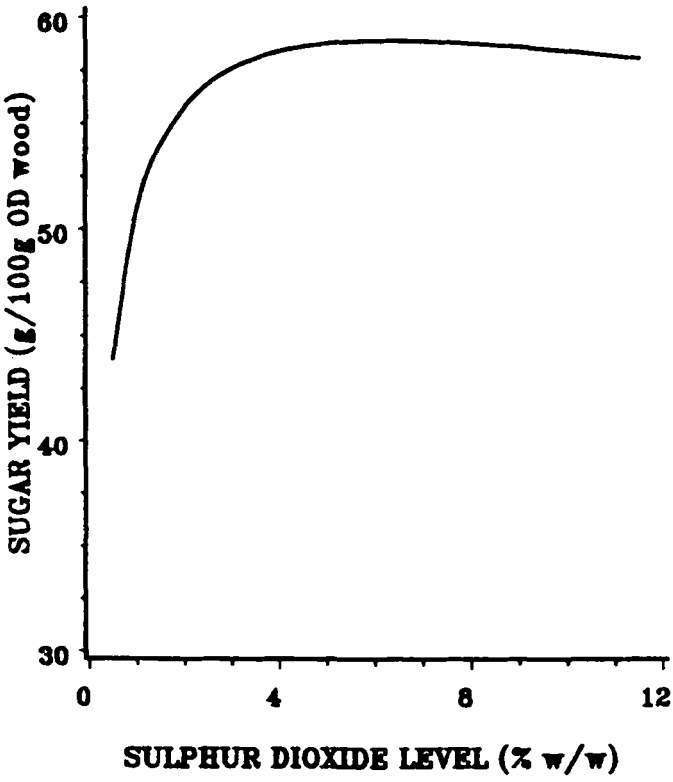


Figure 6. Predicted total sugar yield versus SO_2-impregnation level for constant conditions of time and temperature (Time = 3 min., Temperature = 215°C).

10 min with 4% SO_2-impregnation. Although data on the water-soluble sugars were not reported, these conditions are certainly non-optimal for maximising total sugar yield.

By using an experimental optimisation procedure to investigate the steam explosion of *Pinus radiata* it can now be claimed that softwoods, of which *P. radiata* is typical, can be made as amenable to hydrolysis by cellulase enzymes as hardwoods, which have previously been considered the only viable substrates for such processes. The only difference in treatment

required is the selection of more severe conditions of time and temperature for softwoods and the use of SO_2 as an acid catalyst. Whether SO_2 acts solely as an acid catalyst or actively sulphonates lignin is being currently investigated, as are the properties of the steam-explosion lignins obtained from the process.

ACKNOWLEDGEMENTS

The skilled technical assistance of P.H. Dare, K.F. Deverell and D. Steward is gratefully acknowledged.

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