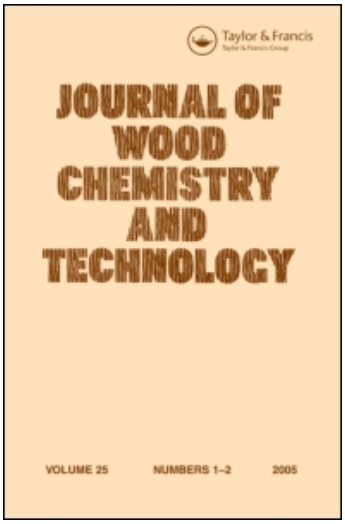

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Steam Explosion of the Softwood *Pinus Radiata* with Sulphur Dioxide Addition. II. Process Characterisation

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STEAM EXPLOSION OF THE SOFTWOOD PINUS RADIATA WITH SULPHUR DIOXIDE ADDITION.

II . PROCESS CHARACTERISATION

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ABSTRACT

The enzymatic digestibility of the softwood Pinus radiata is substantially enhanced by SO_2-catalysed steam explosion. The factors controlling the effectiveness of this pretreatment have been studied. Under conditions of constant time (3 minutes) and temperature (215°C) the effects of SO_2-impregnation levels from 0-11.8% were examined. Up to about 3% SO_2 there is a pronounced effect on the enzymatic digestibility of the pretreated substrate and a rapid increase in the yield of water soluble sugars. At 2.55% SO_2, a total sugar yield of 57.4 g/100 g oven-dry wood was obtained. SO_2 levels above 3% had no beneficial effects in terms of increased sugar yields. Equivalent total sugar yields could be obtained by shortening the treatment time to 1 minute and increasing the temperature to 235°C (still at 2.55% SO_2). Percentage solubilisation of wood glucan correlates strongly with the enzymatic digestibility of the pretreated substrate and also with the surface area accessible to a 51A° molecule. This suggests that carbohydrate solubilisation and cell wall accessibility are closely related. The major, beneficial effect of SO_2 during steam explosion of P. radiata is to promote hemicellulose and cellulose hydrolysis while limiting excessive carbohydrate degradation. However, sugar reversion products were observed under near-optimal treatment conditions, this being an undesirable consequence of SO_2 catalysis. Evidence is presented suggesting a very low level of lignin sulphonation and consequently very little soluble lignin is produced. The almost total retention of lignin in the pretreated substrate appears to present little hindrance to the enzymatic digestion of its cellulose component.

INTRODUCTION

We have recently reported the influence of the parameters time, temperature, and SO_2-impregnation level upon the effectiveness of steam explosion, as a pretreatment for enhancing the enzymatic digestibility of the softwood Pinus radiata[1]. This work stemmed from the knowledge that SO_2 addition during steam explosion of hardwoods improved enzymatic digestibility and also enhanced overall carbohydrate survival [2,3,4]. Similar benefits could be obtained with softwoods, as demonstrated by Mamers and Menz[5] and Dekker[6] using the Siropulper procedure. In recent work, addition of SO_2 during the pretreatment of pine and spruce chips, using a large Stake II reactor has yielded a readily digestible fibre with high overall sugar recoveries[7,8]. Sudo et al.[9] have also recently studied the catalysed steam explosion of larchwood using various organic acids and inorganic salts, and they were able to obtain high enzymatic digestibilities of the pulps produced.

Our previous paper investigated the significance of the variables time, temperature, and SO_2-impregnation level during the steam explosion of Pinus radiata[1]. In contrast to earlier reports on the use of SO_2 addition during the steam pretreatment of softwoods, this work described response surfaces for the important process parameters, dry matter yield, water-insoluble fibre yield, water soluble sugar yield, enzymatic-hydrolysis sugar yield, and most importantly the overall sugar yield so that a process optimisation could be performed.

The current paper continues this work by examining, in more detail, certain critical regions of the response surface. The effects of SO_2-impregnation level are examined in greater detail under near-optimal conditions of time and temperature. Also the high-temperature, short-time region is investigated, at moderate levels of SO_2 addition, to determine if any advantages can be gained by operating in this region.

The total sugar yield is a key parameter in the measurement of pretreatment effectiveness[1]. This is intrinsically linked with carbohydrate solubilisation and survival, and the enzymatic digestibility of the steam exploded fibre. The inter-relationship of these factors and the nature of the water-soluble oligomeric sugars are examined in this paper.

Finally, the mode of action of SO_2 during steam explosion is investigated. SO_2 may act simply as an acidic catalyst of carbohydrate hydrolysis[10], but it may also actively sulphonate the lignin, forming lignosulphonic acids as in the acid sulphite pulping of wood[11]. Thus, the chemistry of SO_2 action may influence the nature and potential uses of the steam explosion lignins, and the presence of water soluble lignosulphonic acids may affect the fermentability of sugar solutions.

EXPERIMENTAL

Steam Explosion Substrate and Methodology

The Pinus radiata substrate used in this work was the same as described previously[1]. Its composition on an original oven dry (O.D.) wood basis was ash, 0.31%; extractives, 2.21%; Klason lignin, 26.16%; acid soluble lignin, nil; glucan, 43.31%; xylan, 5.27%; galactan, 2.89%; arabinan, 1.63%; and mannan, 10.71% (7.51% unaccounted (4-OMe-glucuronic anhydride and O-acetyl))[1]. Techniques for SO_2-impregnation, steam explosion gun design and operation, and experimental methods have also been described[1].

The basic methodology for each steam explosion run involved impregnation of the wet chips (300 g oven dry equivalent) with SO_2 to a predetermined level (percent dry weight basis). The impregnated chips were then heated with saturated steam at a known temperature for a controlled time, after which the chips were explosively discharged from the apparatus. The steam exploded wood was then water washed to yield a water-soluble (WS) fraction and a water-

insoluble (WI) fraction. The water-insoluble fibre was enzymatically hydrolysed to determine its enzymatic digestibility, which is defined as the yield of glucose after 72 h of hydrolysis, expressed as a percentage of the potential glucose in the fibre[1]. All reported yields are expressed as g/100 g original O.D. wood and have the following definitions[1]:

shot yield (Y_{shot}) is the dry-matter yield of steam exploded wood recovered from the gun

water-insoluble fibre yield (Y_{WI}) is the dry -matter yield of water-washed fibre

enzymatic sugar yield (Y^{S}_{EH}) is the yield of sugars released from the WI fibre after 72 h of enzymatic hydrolysis

water-soluble sugar yield (Y^{S}_{WS}) is the yield of sugars in the water-soluble fraction

total sugar yield (Y^{S}_{T}) is calculated as the sum of enzymatic sugar yield and water-soluble sugar yield

Methods for the determination of these yield parameters have been fully described[1].

The standard steam explosion conditions referred to in this paper, were those which had been shown to give a near-optimal total sugar yield[1], namely time, 3.0 minutes; temperature, 215°C; and SO_2 level, 2.55% w/w. Variations on these standard conditions were as follows:

(i) runs 1-12 used the standard conditions of time and temperature, but SO_2 level was varied from 0-11.8%, and

(ii) runs 13-16 used the standard SO_2 level but time was set at 1.0 minute, and temperature was varied from 225-240°C.

Previously unreported results from the earlier optimisation study are also given in this paper[1]. This study was conducted as a 20 run, central composite design experiment employing a range of times,

temperatures and SO_2-impregnation levels and has been fully described[1].

Analytical Methods

Methods for the determination of Klason lignin and carbohydrate composition were as previously described[1]. Other methods were as follows:

Elemental sulphur determinations - the sulphur content of WI samples was determined by X-ray spectroscopy[12] after pressing the ground material into discs of about 10 g weight. The sulphur content of WS solutions was determined by ICAP emission spectroscopy, after sample digestion in nitric/perchloric acids[13]. Results were corrected for background sulphur by subtracting the WI and WS sulphur contents, obtained after steam explosion under the standard conditions, but without SO_2 addition (runs 1 and 2).

Determination of Sulphate and Sulphite - these ions were determined by ion chromatography on a Waters I.C.-PAK anion exchange column at 30°C, eluted with 1.3 mM potassium gluconate/$Na_2B_4O_7$ at 1.2 ml.min^{-1}. The ions SO_4^{2-} and SO_3^{2-}, which co-chromatograph on this system, were detected using a Waters 4230 conductivity detector.

Determination of strong acid groups in water solubles - the conductimetric titration procedure of Katz et al.[14] was used, which locates two end-points, corresponding to the titration of weak and strong acid groups. The sample was first treated with a strong cation exchanger (IR 120, H^+ form) and then evaporated to dryness (in vacuo) and washed 3 times with water, with further evaporation between each washing. This ensured that all acids were in the free acid form and only the non-volatile acids remained.

Dissolved lignin in WS solutions - this was estimated by measuring the absorption at 205 nm as described by Beatson et al.[15].

GPC analysis of water soluble oligomers - Samples of water solubles were concentrated by freeze-drying, taken up in water and extracted with diethyl ether. The aqueous fraction was applied to an anion exchange resin column (Dowex 1X8, OAc^- form, 1.5 cm x 25 cm). The neutral sugars were eluted with water. The acidic fraction was then eluted with 5M acetic acid. The neutral sugars were analysed by GPC (Biogel P-2, 2 x 1.6 cm x 100 cm columns, temperature 55°C, eluted with water at 0.5 ml. min^{-1}). The column was calibrated with blue dextran, raffinose, cellobiose, and glucose.

Identification of Disaccharides - Separation of the disaccharide fraction (isolated by G.P.C.) was achieved using an h.p.l.c. amino bonded silica column (Econosphere NH_2, 5μ, 150 mm) eluted with 80:20 acetonitrile-water. Individual peaks, detected by refractive index, were collected and subsequently methylated, hydrolysed and converted into their corresponding alditol acetates. Qualitative analysis of the partially methylated alditol acetates was performed by g.c. and g.c.-m.s. on an SP 2340 capillary column fitted to a Hewlett packard 5985 g.c.-m.s.

RESULTS AND DISCUSSION

Effects of SO_2 Impregnation Level

In a steam explosion process the economics may be influenced by the level of SO_2 which is used. Since, the central composite design experiment showed that SO_2 level had a major effect on total sugar yield[1], a series of experiments was performed to examine this effect in detail. Using the near-optimal processing conditions of time (3 min) and temperature (215°C), the SO_2-impregnation level was varied between 0 and 11.8% (w/w).

The results are presented in Table 1, showing shot yield and WI yield as well as the composition and digestibility of the WI fibres produced. The duplicated runs at zero SO_2-impregnation level (runs 1

TABLE 1

Effect of SO_2 Level on Shot Yield, WI Yield, WI Composition, and WI Enzymatic Digestibility (time = 3 min., temperature 215°C)

Run No.	SO_2 (% OD wood)	Shot yield (% OD wood)	WI yield (% OD wood)	WI composition (% WI)						Digestibility of WI (%)[g]
				KL[a]	ASL[b]	Total carb[c]	Glc[d]	Xyl[e]	Man[f]	
1	0.0	96.8	76.8	34.8	0.42	66.1	57.7	3.23	5.24	6.8
2	0.0	102.0	79.4	33.6	0.32	67.1	57.0	3.62	6.57	5.6
3	0.33	99.8	70.7	37.9	0.48	65.6	61.0	1.31	3.41	26.1
4	0.55	94.5	66.1	39.3	0.72	59.9	59.9	-	-	51.0
5	0.77	97.5	67.0	39.5	0.96	59.2	59.2	-	-	53.6
6	1.03	92.7	63.1	40.2	1.02	59.5	59.5	-	-	54.7
7	1.47	92.5	64.4	42.5	0.63	57.6	57.6	-	-	66.9
8	2.10	93.1	59.9	44.9	0.72	57.7	57.7			78.6
9[h]	2.55	90.0 (1.4)	59.4 (2.0)	44.8 (1.5)	0.98 (11.9)	56.5 (2.7)	56.5 (2.7)	-	-	83.8 (3.6)
10	5.03	88.6	54.5	46.9	nd	55.6	55.6	-	-	86.5
11	8.03	89.3	53.6	48.9	nd	53.1	53.1	-	-	89.4
12	11.76	88.6	54.3	48.7	1.24	52.0	52.0	-	-	94.2

[a] KL = Klason lignin. [b] ASL = acid soluble lignin. [c] Total carb = total carbohydrate corrected for hydrolysis loss factors but not converted to anhydropolymers. [d] Glc = glucose, [e] Xyl =xylose, [f] Man - mannose, [g] Digestibility is defined as the yield of glucose at 72 h as a % of the theoretical glucose obtainable from the WI, [h] averaged results from 5 replicate treatments (standard errors (%) in parentheses)

and 2) gave somewhat variable shot yields, due to imprecisions in this determination. These resulted from the presence of colloidal material, which caused filtration difficulties (see reference 1 for method). As shown in Table 1, at SO_2 levels greater than 0.33%, all the hemicellulosic sugars had been removed from the WI fibre, so that only cellulose and lignin remained. As the SO_2 level was further increased, increasing cellulose solubilisation occurred so that at 11.8% SO_2 the WI fibre contained only 59% of the original P. radiata glucan. The WI fibre from run 3 (0.33% SO_2) contained only 15.5 and 20.3% of the original xylan and mannan, respectively, but its digestibility was only 26.1%. Apparently, the continued increase in digestibility is related to the further removal of cellulosic glucan from the WI fibre. The yields of Klason lignin in the WI (g/100 g o.d. original wood) were unaffected by the level of SO_2 impregnation with an average for runs 1-12, of 26.4 g/100 g (s = 0.58). This is the same level as in the original wood (26.2% Klason lignin[1]) which suggests that no significant solubilisation of lignin had occurred although, as is later discussed, carbohydrate degradation products can analyse as Klason lignin.

Enzymatic digestibility and yield of the WI fibre are plotted against SO_2 level in Figure 1. Clearly, the higher the SO_2 level, the more digestible was the fibre. However as shown in Figure 2, the yield of sugars enzymatically released from the fibre is not maximised at the highest SO_2 level, but at approximately 2-3% SO_2. This is because the WI yield decreased with SO_2 level, while digestibility increased, resulting in a maximum point on the enzymatic sugar yield versus SO_2 curve.

The data for the water soluble sugars are presented in Table 2. Free monomeric sugars were quantified in the water solubles before and after a post-hydrolysis step[1]. The differences between the two sets of data give the amount of soluble, oligomeric (DP 2 and greater) sugars which were present. The water-soluble sugar yield is also plotted versus SO_2 level in Figure 2, along with the total sugar

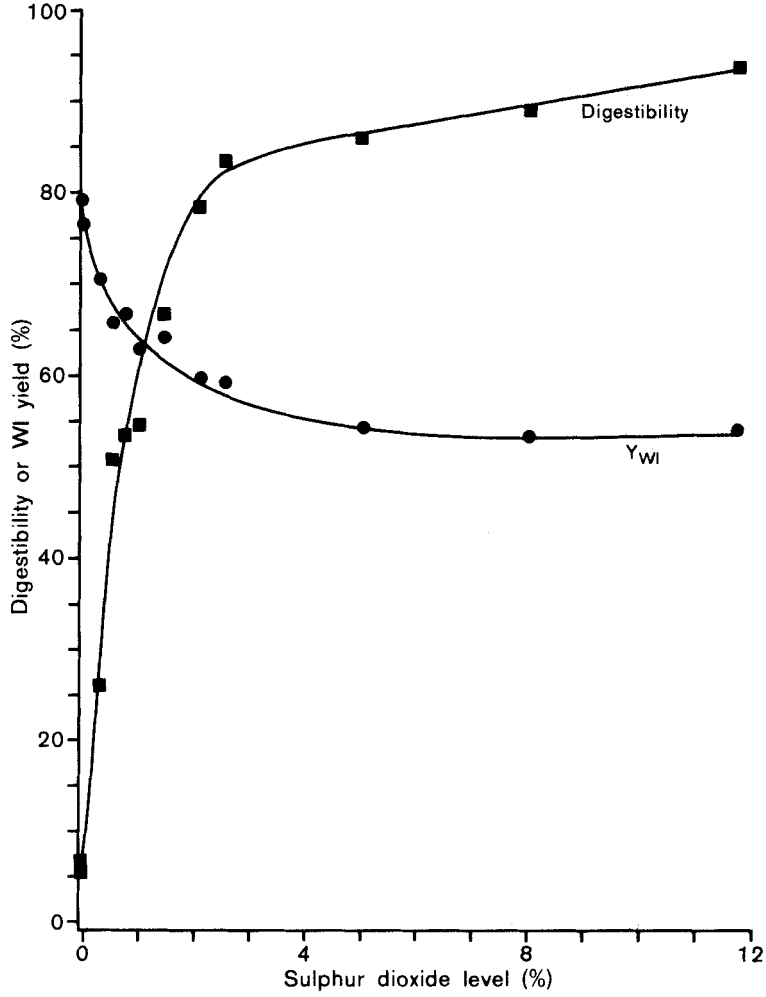

Figure 1. Effect of sulphur dioxide impregnation level on water insoluble fibre yield (Y_{WI}) and water insoluble fibre digestibility. Steam explosion conditions: time, 3 min.; temperature, 215°C.

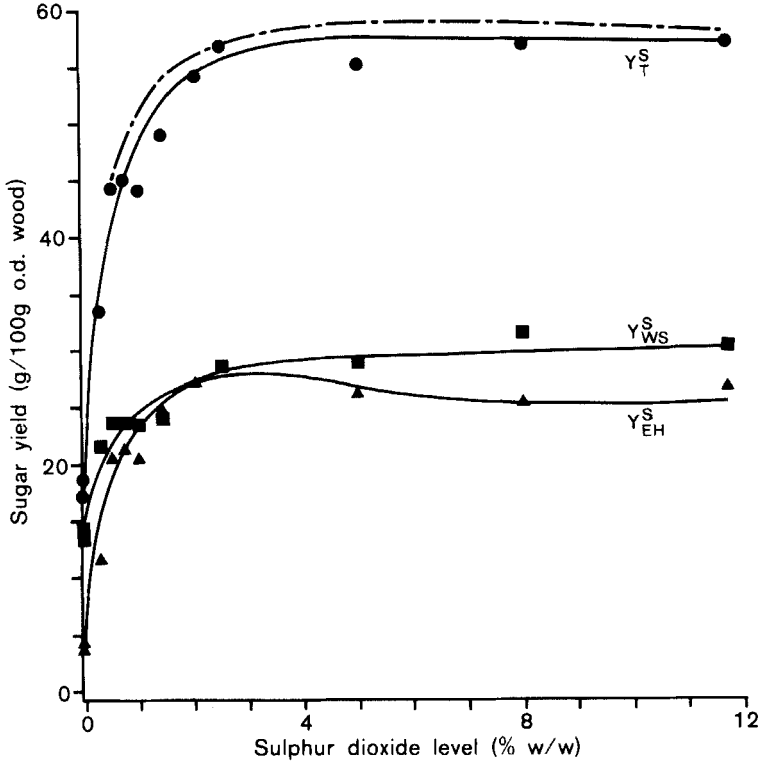

Figure 2. Relationship between sulphur dioxide impregnation level and total sugar yield (Y^S_T), water-soluble sugar yield (Y^S_{WS}), and enzymatic sugar yield (Y^S_{EH}). The dashed line is the predicted total sugar yield from the response surface model[1]. Steam explosion conditions: time, 3 min.; temperature 215°C.

yield, derived by summing the water-soluble and enzymatic sugar yields. Between 0 and 2.55% SO_2 the yield of soluble sugars rapidly increased. From 2.55 to 11.8% SO_2, however, only a small additional increase occurred.

The overall measure of pretreatment effectiveness, total sugar yield, was virtually constant at SO_2 levels greater than 2.55% (Table 3,

TABLE 2

Effect of SO_2 Level on the Yields of Water Soluble Sugars[a] (time = 3 min, , temperature = 215°C)

Run No.	SO_2 Level (% w/w)	Glc[b]		Xyl[c]		Gal[d]		Ara[e]		Man[f]		Total Sugars (Y^a_{WS})	
		(all data as g/100 g original O.D. wood)											
1	0.0	2.01	(0.15)	3.03	(0.83)	1.78	(0.45)	0.85	(0.75)	6.81	(0.39)	14.5	(2.6)
2	0.0	1.80	(0.12)	2.87	(0.75)	1.70	(0.36)	0.81	(0.71)	6.29	(0.32)	13.5	(2.3)
3	0.33	3.99	(2.55)	4.58	(3.90)	2.49	(1.99)	1.22	(1.16)	9.54	(4.30)	21.8	(13.9)
4	0.55	6.11	(4.79)	4.59	(4.22)	2.19	(1.76)	1.28	(1.18)	9.65	(6.07)	23.8	(18.0)
5	0.77	6.36	(5.13)	4.50	(4.20)	2.46	(2.33)	1.21	(1.26)	9.28	(6.01)	23.8	(18.9)
6	1.03	7.50	(6.04)	4.16	(3.87)	2.29	(2.06)	1.18	(1.17)	8.55	(5.93)	23.7	(19.1)
7	1.47	8.29	(6.53)	4.03	(3.72)	2.42	(2.40)	1.17	(1.12)	8.51	(5.82)	24.4	(19.6)
8	2.10	11.4	(8.99)	3.71	(3.43)	2.34	(2.25)	1.20	(1.11)	8.66	(6.10)	27.3	(21.9)
9[g]	2.55	12.1	(9.47)	4.23	(3.00)	2.87	(1.61)	1.20	(1.03)	8.47	(5.81)	28.9	(20.9)
		3.4	*(3.3)*	*12.0*	*(6.2)*	*5.3*	*(5.4)*	*6.6*	*(2.5)*	*2.9*	*(1.8)*	*1.8*	*(1.8)*
10	5.03	14.1	(11.8)	3.51	(3.09)	2.21	(1.46)	1.19	(1.20)	8.09	(6.08)	29.1	(23.7)
11	8.03	16.5	(11.9)	3.54	(2.89)	2.48	(1.58)	1.12	(1.12)	8.09	(5.33)	31.8	(22.8)
12	11.76	15.5	(12.2)	3.50	(2.55)	2.58	(1.51)	1.01	(0.88)	7.35	(5.23)	30.5	(22.4)

[a] all samples were post-hydrolysed and hydrolysis loss factors applied (figures in parentheses give monomeric sugar yields prior to post-hydrolysis), [b] Glc = glucose, [c] Xyl = xylose, [d] Gal = galactose, [e] Ara = arabinose, [f] Man = mannose, [g] averaged results from 5 replicate treatments with standard errors (%) in italics below.

TABLE 3

Effect of SO_2 Level on Water Soluble Sugar Yield(Y^S_{WS}), Enzymatic Sugar Yield (Y^S_{EH}), and Total Sugar Yield (Y^S_T), Following Steam Explosion of P. radiata at 3 min, 215°C

SO_2 Level % (w/w)	(Y^S_{WS})	(Y^S_{EH})	(Y^S_T)
	(all data as g/100 g original O.D. wood)		
0.0	14.48	4.37	18.85
0.0	13.47	3.85	17.32
0.33	21.81	11.80	38.61
0.55	23.82	20.67	44.49
0.77	23.81	21.41	45.22
1.03	23.69	20.63	44.32
1.47	24.42	24.78	49.20
2.10	27.27	27.16	54.43
2.55[a]	28.86 (1.8)	28.48 (4.3)	57.36 (1.9)
5.03	29.08	26.31	55.39
8.03	31.71	25.53	57.24
11.76	30.50	26.84	57.34

a averaged results from 5 replicate treatments (standard errors (%) in parentheses).

Figure 2) but below this it was strongly dependant on SO_2 level. Therefore, there is no advantage in using SO_2-impregnation levels greater than about 3%. As shown in Figure 2, the total sugar yield versus SO_2 curve, predicted by the response surface model[1], is in close agreement with the experimental data, providing further support for the accuracy of this model.

High-Temperature, Short-Time Conditions

Our previous study indicated that there could be some processing advantage by operating at high SO_2-impregnation levels (i.e., 6%)[1]. The advantage is that shorter steaming times can be used (although at higher temperatures) to achieve total sugar yields equal to, or greater

than those attainable under the "standard" conditions of time and temperature (3 min., 215°C). Such high SO_2 levels, however, may be undesirable for economic reasons. In contrast, at more acceptable SO_2 levels (ca. 2-3%) the response surface model predicts that short-time, higher temperature conditions will result in total sugar yields substantially lower than at the standard conditions[1]. The confidence in such predictions, at processing times of one minute or less, is however poor because the number of experiments performed at this treatment time was limited in the initial optimisation experiment[1].

In order to establish whether any real advantage exists using short time treatment conditions, at moderate SO_2-impregnation levels (i.e., 2.55%), four experiments were undertaken with a treatment time of 1 minute (runs 13-16). Temperature was varied from 225-240°C. The results are presented in Tables 4 and 5 for the water insoluble and water soluble fractions, respectively.

As shown in Figure 3, WI digestibility increased with temperature. However, because WI yield (Table 4) was decreasing, the yield of enzymatically released sugars reached a maximum at 229°C and then decreased. Increased temperatures also resulted in greater yields of water soluble sugars (Table 5, Figure 3). The maximum yield of total sugars (Figure 3) was about 59 g/100 g original O.D. wood and was obtained at approximately 235°C. This yield is approximately the same (within experimental error) as that obtainable at the standard conditions of 215°C, 3 minutes. Clearly, short processing times, such as 1 minute, can be used even at SO_2 levels as low as 2.5%, with no loss in achievable sugar yield.

It is also of interest that the point of maximum total sugar yield does not correspond to the point of highest WI digestibility, and the maximum yields of enzymatic and water soluble sugars are located at different points. This further supports the earlier findings that maximum sugar yield results from an optimal combination of the yields of sugars solubilised during the pretreatment and those released from

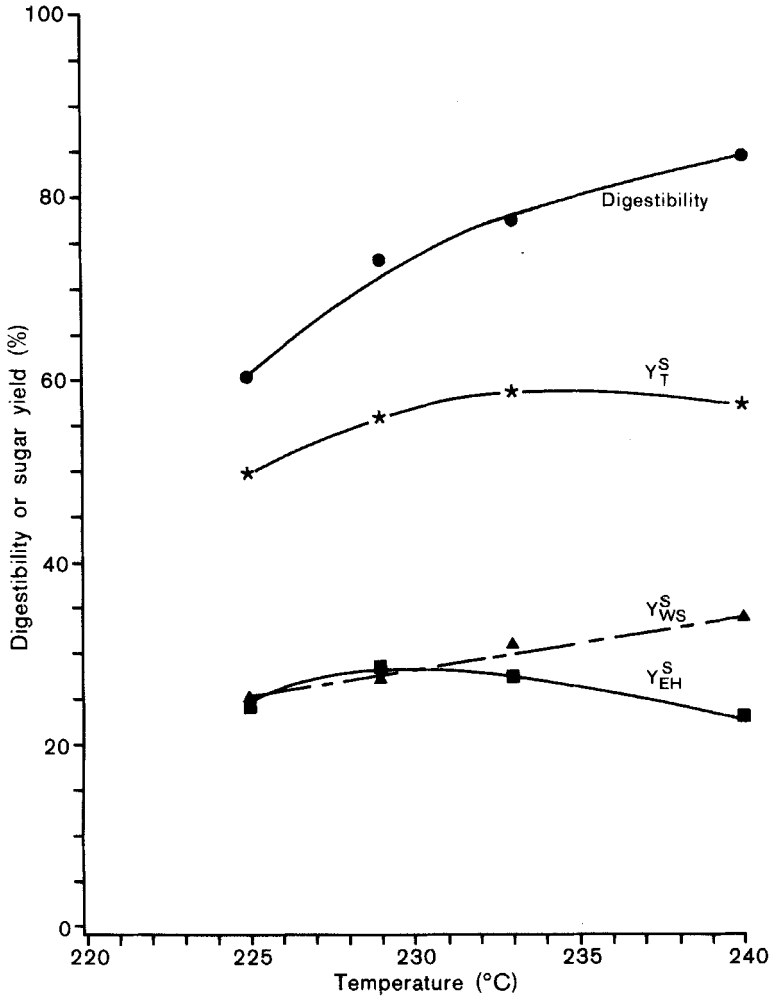

Figure 3. Relationship between temperature and digestibility of WI fibre, yield of total sugars (Y_T^S), yield of water soluble sugars (Y_{WS}^S), and yield of enzymatic sugars (Y_{EH}^S). Steam explosion conditions: time, 1 min.; SO_2 level, 2.55%.

TABLE 4

Analytical and Digestibility Data for the Water Insoluble (WI) Fibre From P. radiata Treated for 1 minute at Various Temperatures (SO_2-impregnation Level 2.55%)

Run No.	Temp. (°C)	Shot[a] Yield	WI[a] Yield	WI Composition		Digest-[c] (%)	Y^s_{EH} (g/100 g)
				Klason[b] Lignin	Glucose[b]		
13	225	93.81	65.79	40.49	62.16	60.5	24.74
14	229	92.43	64.00	41.24	60.86	73.3	28.54
15	233	88.42	59.57	43.13	59.64	77.7	27.64
16	240	85.56	51.94	48.05	53.04	84.8	23.37

a expressed as % original O.D. wood b expressed as % of WI

c digestibility defined as yield of enzymatically released glucose from WI, at 72 h, as percentage of potential glucose in WI.

TABLE 5

Analytical Data for the Water Soluble (WS) Fraction From P. radiata Treated for 1 minute at Various Temperatures (SO_2-impregnation level = 2.55%)

Run	Temperature (°C)	Glc	Xyl	Gal	Ara	Man	Total yield of soluble sugars (Y^s_{ws})
		(all figures as g/100 g of original O.D. wood)					
13	225	7.62	4.63	2.85	1.24	8.79	25.13
14	229	8.73	4.80	3.14	1.59	9.06	27.32
15	233	12.34	5.06	3.07	1.31	9.40	31.18
16	240	17.04	4.15	3.19	1.34	8.46	34.18

All samples post hydrolysed and hydrolysis loss factors applied.

the WI by enzymatic hydrolysis. This point is not necessarily coincident with the point of maximum WI digestibility.

Carbohydrate Solubilisation, WI Digestibility, and Sugar Survival

Figure 4 shows fibre digestibility plotted against the percentage of original glucan which was solubilised during the steam explosion process. Data plotted are for runs 1-12, runs 13-16, and also includes the data from the central composite design experiment, reported previously[1]. All the data conform to approximately the same curve, regardless of processing conditions, and this suggests a strong relationship between the removal of cell wall glucan and the susceptibility to enzymatic hydrolysis. Since only 5% of radiata glucan is present as the glucose in glucomannan, it is clear from Figure 4 that approximately 25-30% solubilisation of cellulose is required to produce a fibre of 80-90% digestibility. This is in marked contrast to steam exploded or acid pretreated hardwoods, such as aspenwood, for which 80% digestibility can be obtained under conditions where only 90% of the xylan is hydrolysed[16,17] (with greater than 95% cellulose retention). This basic difference between pretreated softwoods and hardwoods has also been described by Grethlein[18,19].

We have previously shown a correlation between enzymatic digestibility of the WI fibre and its surface area accessible to a 51A° molecule (a similar size to cellulase enzymes)[20]. When this data is plotted as accessible surface area (51A°) versus percent solubilisation of both the original glucan and total carbohydrate (Figure 5) two things are apparent:

(i) there is a direct relationship between removal of cell wall carbohydrate and increasing accessibility, and

(ii) the initial removal of hemicellulose sugars (without any cellulose hydrolysis) causes an increase in accessible surface area (up to 100 m^2/g) but, by reference to Figure 4, no significant increase in digestibility is obtained until cellulose solubilisation occurs.

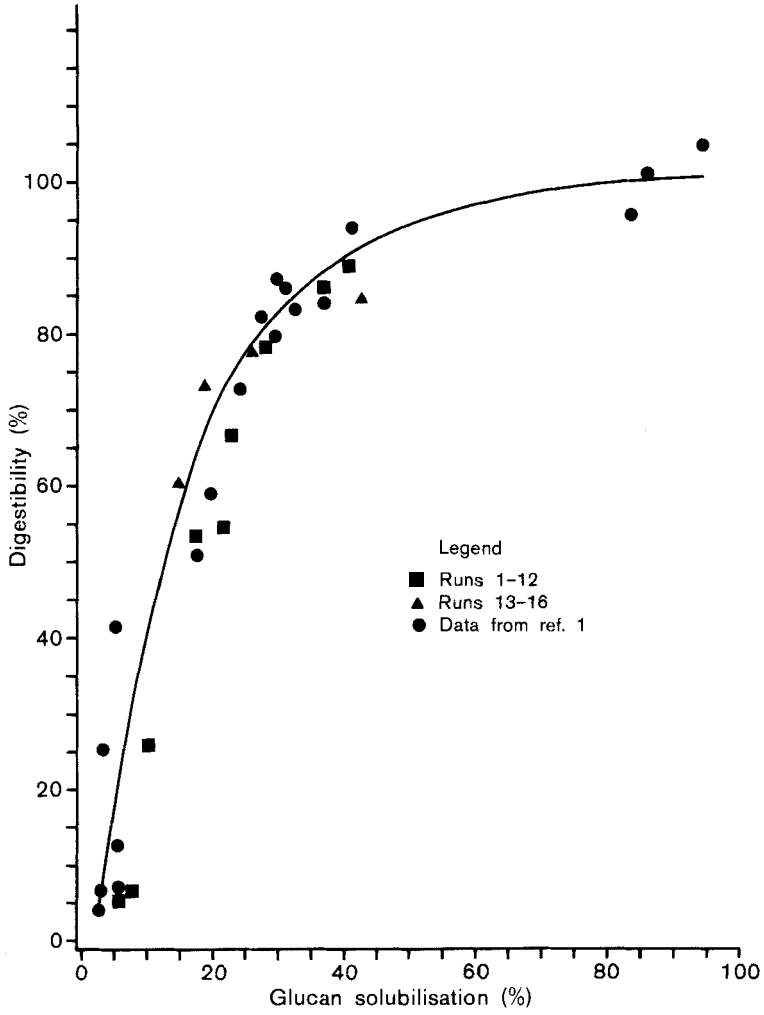

Figure 4. Relationship between water insoluble fibre digestibility and the extent of original wood glucan solubilised during steam-explosion under a variety of times, temperatures, and SO_2 levels (see text for details).

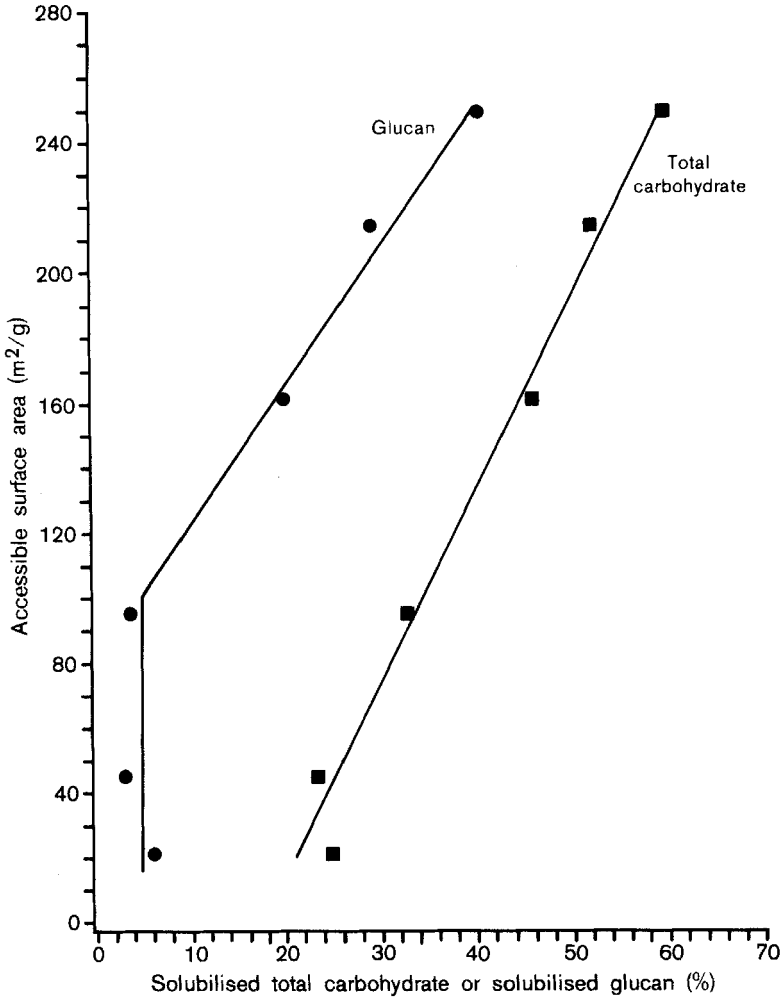

Figure 5. Relationship between accessible surface area and solubilised total carbohydrate or solubilised glucan. (Data replotted from Wong et al.[20])

These results therefore support a growing body of evidence which suggests that enzymatic digestibility of pretreated lignocellulosics is critically dependent on the physical accessibility of the enzymes within the cell wall and that carbohydrate solubilisation is a major factor by which accessibility is developed[17,18,19,20,21]. Other factors such as cellulose crystallinity or lignin content in steam exploded substrates do not appear to be important. Hemmingson[22] has shown that the crystallinity of the cellulose component of P. radiata actually increases following steam explosion and, in agreement with our own findings[1,20], has shown that the physical presence of steam-explosion lignin is no hindrance to enzymatic digestibility although its physical distribution may be important[23,24]. Similar findings have been reported for steam exploded hardwood substrates[25,26].

As has been described, successful steam pretreatment of radiata pine requires that complete hemicellulose removal and partial cellulose removal occur, in order to produce a WI fibre of high enzymatic digestibility. It therefore follows that total sugar yield will be maximised if the solubilised sugars are not excessively degraded. Therefore, carbohydrate survival during the steam explosion of P. radiata is of major importance in determining total sugar yield. Table 6 compares individual and overall carbohydrate survival following steam explosion under the standard conditions (run 9) and the optimum, high temperature, short time conditions (run 15). Although both process conditions gave approximately the same total sugar yield the survival of carbohydrates under the 1 min., 233°C conditions was significantly higher. The reason total sugar yield was not proportionally higher at 233°C, 1 min, is that WI digestibility was only 77.7% compared with 83.8% under the standard conditions. Greater carbohydrate survival should be achieved under higher-temperature conditions as the activation energies for acid-catalysed cellulose and hemicellulose hydrolysis are greater than those for the sugar degradation reactions[27]. These considerations would suggest that even higher total sugar yields could be obtained in a two-stage pretreatment, in which the first stage is designed to achieve hydrolysis

TABLE 6

Percent Survival[a] of Individual Carbohydrates Following Steam Explosion Under Two Different Process Conditions

Sugar	3 min., 215°C, 2.55% SO_2 (run 9)	1 min., 233°C, 2.55% SO_2 (run 15)
Glucose	94.3	99.5
Xylose	71.8	84.5
Galactose	90.3	95.6
Arabinose	64.9	70.8
Mannose	72.3	79.0
Total	87.9	93.8

a Percentage survival defined as monomeric sugar in WS + potential monomeric sugar in WI, as a percentage of potential monomeric sugar in original wood.

of the hemicelluloses and easily hydrolysed cellulose component, with high sugar survival being the prime concern. The second stage would then further treat the insoluble cellulosic residue for maximum enzymatic sugar yield.

Water-Soluble Oligomers

WS samples were post-hydrolysed to ensure that all sugars were present as monomers. Analysis of these samples by h.p.l.c. prior to post-hydrolysis gave significantly lower yields of monomers (Table 2), indicating the presence of residual oligomeric carbohydrate or possibly the formation of dimeric sugar reversion products[27]. The presence of the latter products would significantly affect sugar yields from enzymatic hydrolysis of the WS fraction, since the [1,6]-linked glycosides typical of reversion are not substrates for (1,4)-β-glucosidase enzymes in cellulase preparations.

At the standard steam explosion conditions 27% of the WS sugars were present as oligomers of DP ≥2 (Table 2). This percentage of oligomers varied with processing conditions and SO_2-impregnation level[1]. With no SO_2 addition, but standard time and temperature (runs 1 and 2), approximately 80% of the WS sugars were non-monomeric (Table 2). The effect of SO_2 level on the yields of monomeric, oligomeric, and total WS sugars is shown in Figure 6, for the experimental runs 1-12. From 0 to 0.5% SO_2, the yield of oligomers rapidly declined (as the yield of monomers increased), but above 2% SO_2 the yield gradually increased again. This increase was unexpected as the hydrolysis of soluble oligomeric carbohydrate should, in general, be much faster than the release of these products from the wood substrate.

The oligomeric composition of the WS fraction was examined by analysis of the neutral WS sugars by gel permeation chromatography, as shown in Figure 7. Without SO_2 addition (run 1, Fig. 7(a)) oligomers up to at least DP 10 were present (oligomers of DP >10 elute with the void volume). The effect of SO_2 was to markedly reduce the DP of the oligomers and greatly increase the proportion of monomeric sugars (run 9, Fig. 7(b)). Under the more severe treatment conditions of 3 min., 248°C, 2.55% SO_2 (reference 1, run 12, Figure 7(c)), the proportion of oligomeric WS sugars was apparently greater than under the standard conditions (Fig. 7(b)).

As the disaccharides were the single major oligomer group, they were further examined. Sugar compositions for the disaccharide fractions were determined for the three WS samples shown in Figure 7, and are given in Table 7.

Without SO_2 addition, the disaccharide fraction was composed of sugars in proportions similar to those present in the main radiata pine hemicelluloses, namely arabinoxylan and galactoglucomannan. Under the standard treatment conditions almost 50% of the hydrolysate of the disaccharide fraction consisted of glucose, indicating the

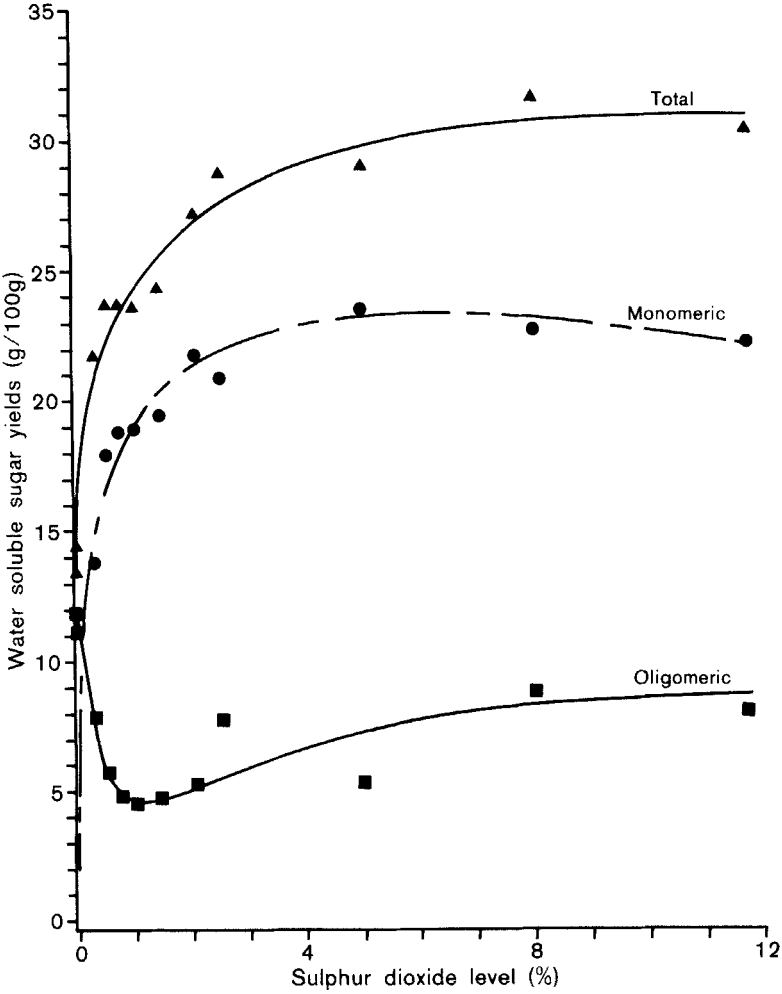

Figure 6. The influence of sulphur dioxide impregnation level upon the yield of total, monomeric and oligomeric water-soluble sugars. Steam explosion conditions; time, 3 min.; temperature, 215°C.

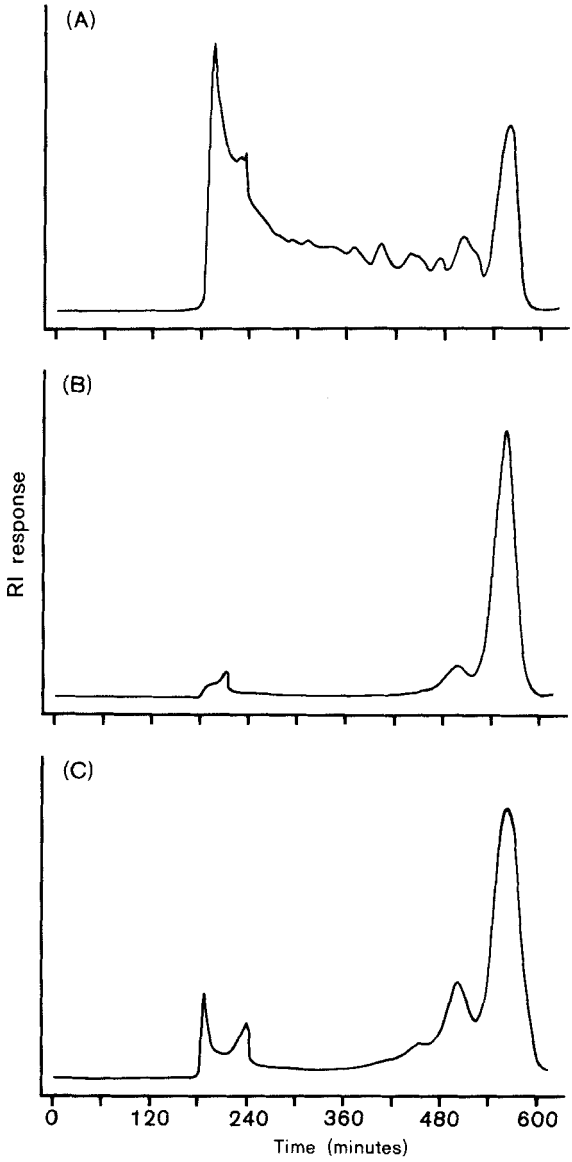

Figure 7. Gel permeation chromatograms of neutral water-soluble carbohydrates produced under three different steam explosion conditions (A) no SO_2 addition, 3 min., 215°C (B) 2.55% SO_2, 3 min., 215°C (C) 2.55% SO_2, 3 min., 248°C..

TABLE 7

Analysis of Individual Sugars Comprising the Disaccharides Present in the Neutral, Water-Soluble Fraction

Treatment Conditions	% Carbohydrate Glu	Xyl	Gal	Ara	Man
3 min, 215°C, 0% SO_2	9.7	41.7	12.8	4.9	30.9
3 min, 215°C, 2.55% SO_2	47.1	10.9	10.3	5.1	26.5
3 min, 248°C, 2.55% SO_2	67.6	6.7	7.7	5.5	12.5

substantial presence of cellulose-derived dimers. At the increased treatment temperature of 248°C, the proportion of glucose in the hydrolysate of the disaccharides increased to almost 70%. Therefore, the greater proportion of oligomers observed under these conditions (as compared to the standard conditions) results from the greater extent of cellulose hydrolysis which has occurred.

The individual disaccharides present in the WS neutral sugars, produced under the standard treatment conditions, were separated by hplc and identified by methylation analysis and gas chromatography/mass spectrometry. The identities of the main disaccharides are given in Table 8. Significantly, the [1,6]-linked sugars, characteristic of reversion reactions, were present. While the yields of these undesirable hydrolysis products have not been determined, nor the effects of steam explosion conditions on their formation, their presence indicates that sugar reversion is an additional factor which should be considered when selecting optimal steam explosion conditions.

TABLE 8

Disaccharides Isolated From the Water Soluble Neutral Carbohydrate Fraction

Identity[a]	Source
$Xyl^1 - {}^4Xyl$	Xylan backbone
$Glc^1 - {}^4Glc$	Glucan backbone
$Man^1 - {}^4Man$	(Gluco) mannan backbone
$Glc^1 - {}^6Glc$	)
$Glc^1 - {}^6Man$	) acid reversion)

[a] configuration of glycosidic linkage not determined unequivocally and hence not reported. Identity established by g.c. retention time and mass spectra of partially methylated alditol acetates.

Mode of Action of SO_2

The value in using SO_2 in the steam explosion pretreatment of radiata pine has been clearly shown. However, the mode of action of SO_2 is somewhat less clear. It may act simply as an acid catalyst, lowering the pH of the treatment and so preventing degradation of carbohydrates via basic catalysis[28]. Carbohydrate degradation is thus restricted to acid catalysed mechanisms, the rates of which are substantially slower than those of basic catalysis. Due to the lower pH, SO_2 also enhances the hydrolysis and solubilisation of the hemicelluloses (and cellulose). In summary, the balance between carbohydrate hydrolysis and degradation is pushed in favour of the hydrolytic reactions as pH is lowered. As well as the above, SO_2 may also actively sulphonate the lignin forming lignosulphonic acids, as occurs during acid-sulphite pulping of wood[11,29].

Under the standard steam-explosion conditions, (300 g O.D. wood treated) the distribution of applied sulphur (3.825 g) was 0.15 g S in the WI and 1.26 g S in the WS, with 2.41 g (63%) lost during the

process. Extensive water (or 0.1 N hydrochloric acid) washing of the WI fraction reduced the sulphur content by only 15%, to 0.13 g S. Based on the WI lignin content, this equates to sulphonation of 0.9% of lignin units (when lignin is considered as phenyl propane units). Under conditions typical of acid sulphite pulping (high SO_2 levels, pH 1-2, 120-140°C for 2-3 hours) extensive sulphonation and depolymerisation of lignin occurs[11,29,30]. Clearly, this is not the case under the conditions of SO_2-catalysed steam explosion. Our findings are, however, in contrast to those of Mamers and Menz[5] and Hemmingson[31], who found no sulphur incorporation when treating P. radiata by the Siropulper procedure, at SO_2 levels of 4-6%. (It should be noted however, that the Siropulper procedure and the explosion technique used in this work are significantly different.)

Using the conductimetric titration procedure of Katz et al.[14], the WS fraction described above was found to contain 43 meq of strong acid groups. This is in close agreement with the value of 39 meq, obtained by assuming the sulphur detected in the WS (1.26 g S) was present as strong acids. The content of sulphate plus sulphite was determined via ion chromatography as 0.35 g S (11 meq), therefore both approaches suggest that about 30 meq of sulphonic acid groups may be present in the WS fraction. Such a level of lignosulphonic acids represents 5.5 g of soluble lignin from the original 300 g of O.D. wood. This is equal to 1.8 g/100 g original O.D. wood, or 7% of the original lignin. Figure 8 shows WS lignin content (measured spectrophotometrically) versus SO_2-impregnation level for runs 1-12. Soluble lignin increased substantially with increasing SO_2 levels. From 0 to 2.55% SO_2, an increase of approximately 1.4 g was observed, which is in reasonably close agreement with the apparent content of soluble sulphonated lignin as determined above.

An explanation for the very low amount of lignin sulphonation observed can be found in Rydholm[11]. Pulping with SO_2-solutions, in the absence of a base such as Ca^{2+}, is normally only possible at low temperature and with a very high total SO_2 content. Under the conditions of SO_2-catalysed steam explosion the concentration of the

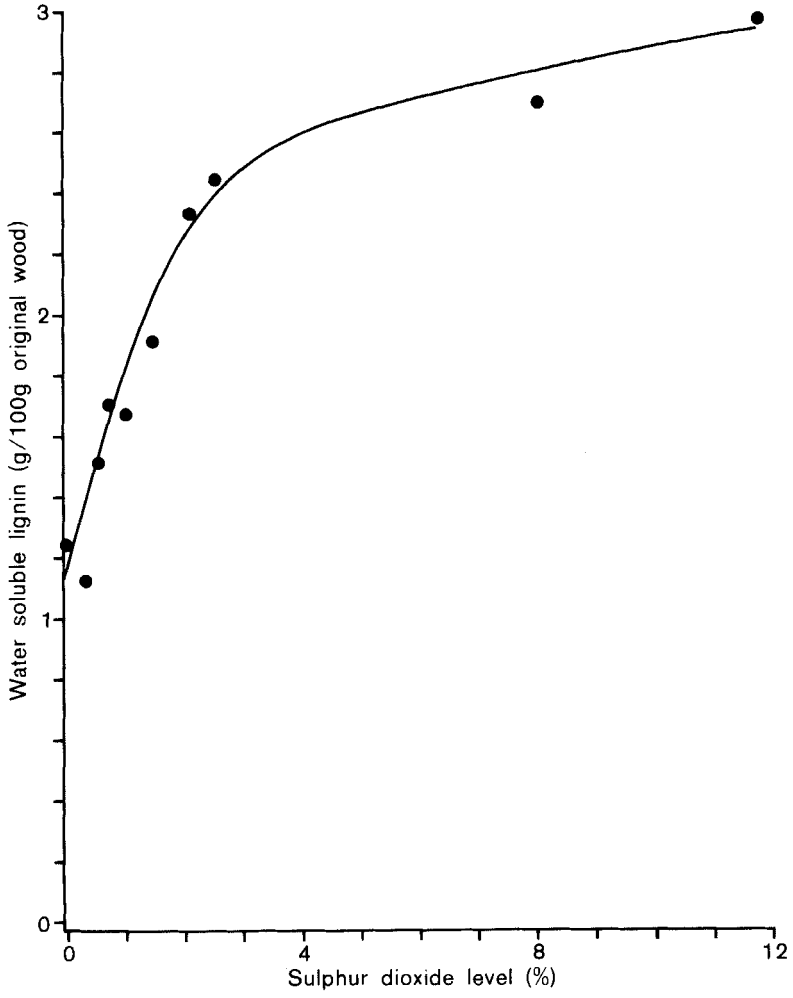

Figure 8. Influence of sulphur dioxide impregnation level upon the yield of water soluble lignin. Steam explosion conditions: time, 3 min.; temperature, 215°C.

sulphonating agent, the bisulphite ion, would be extremely low due to the high temperature and high acidity of the process (both factors working against the dissociation of sulphurous acid). Consequently, little sulphonation of lignin should be expected. In fact, conditions of high acidity and low bisulphite ion concentration favour lignin condensation over sulphonation[11].

Mass balances on Klason lignin, before and after the steam explosion process, suggest negligible lignin solubilisation. However, as has been observed previously[1], carbohydrate degradation products may analyse as Klason lignin. Therefore, the small amount of lignin solubilisation determined by the above results is not inconsistent with the Klason lignin analyses. By comparison, when the hardwood Populus robusta was treated under the standard steam explosion conditions[32], significant Klason lignin solubilisation was observed (3.44 g Klason lignin/100 g O.D. original wood) and dissolved WS lignin content was measured as 4.75 g/100 g O.D. wood; substantially higher than for the radiata substrate.

Hemmingson has examined the lignins obtained from P. radiata, after steam-explosion by the Iotech process, the Siropulper process, and the process used in this work[31,33,34]. Under the standard treatment conditions, (2.55% SO_2, 3 min, 215°C) 30% of the WI lignin (Klason plus acid soluble) was extractable in acetone[34]. Elemental analysis showed the presence of 0.44% sulphur in the acetone-soluble lignin which equates to 0.12 g S/300 g original O.D. wood. In other words, 96% of the sulphur in the WI fibre (0.13 g) was bound to extractable lignin. This strongly suggests that lignin sulphonation may be a desirable reaction with regard to obtaining a high yield of extractable lignin as suggested by Mackie et al.[2].

Steam explosion with SO_2 addition promotes extensive cleavage of the major (β-O-4) lignin interunit linkage with the formation of free phenolic hydroxy groups at C-4[23]. Extraction with alkali or acetone leaves a residual lignin significantly more etherified at C-4 than the

total lignin implying the extraction is selective for cleaved lignin fragments. However, the extent of β-ether cleavage in the residual lignin is still substantial (55-60%), so this lignin is not unreacted, native lignin, but rather, a result of repolymerisation reactions which successfully compete with cleavage of ether linkages and depolymerisation reactions[23].

Compared with acidic sulphite pulping, in which lignin sulphonation is the dominant reaction[29], the major effect of SO_2 on lignin, in steam explosion, is acid-catalysed hydrolytic reactions with the release of free phenolic hydroxy-groups and subsequent lignin condensation[31].

Steam explosion of the hardwood Eucalyptus regnans, without SO_2 addition, yielded lignin which was 60% extractable in acetone[33,34]. Structural changes in both the extracted and residual lignins were again dominated by β-ether cleavage, however, the greater acetone-extractability of the lignin suggests less repolymerisation, possibly due to the presence of more highly substituted, syringyl units in hardwood lignin. Mackie et al., studied the SO_2-catalysed steam explosion of aspenwood and found the lignin to be 55% extractable in 90% ethanol[2]. They also obtained a small amount of sulphur incorporation into the WI fibre and evidence suggesting that half the water soluble lignin was sulphonated. Sudo et al. have also observed a difference between the solvent extractability of steam-exploded hardwood and softwood lignins[9]. They explained this, not only in terms of the more readily condensing guaiacyl moiety, but also because of the higher content of β-aryl ether bonds in hardwood lignin and its greater resistance to hydrolysis.

CONCLUSIONS

Up to approximately 3.0% SO_2 there is a very pronounced effect of SO_2 impregnation (when time and temperature are held constant). Enzymatic digestibility of the water insoluble fibre and the total sugar

yield are substantially improved over the situation where no SO_2 impregnation is employed. Above 3% SO_2 there are no benefits in higher levels of impregnation. While a near-optimal, total sugar yield (57.4 g/100 g O.D. wood) can be obtained under the standard conditions used in this study (3 min., 215°C, 2.55% SO_2), approximately the same yield can also be obtained with higher temperature, shorter time conditions (1 min., 235°C, 2.55% SO_2).

In order to produce a WI fibre of 80-90% digestibility, complete hemicellulose solubilisation and approximately 25-30% cellulose solubilisation is required for P. radiata. The percentage removal of cell wall carbohydrate shows a direct relationship with WI fibre digestibility and cell wall accessible surface area. Accessibility, we believe, is the most important factor governing enzymatic digestibility of pretreated biomass substrates.

The major, beneficial effect of SO_2 action, during steam explosion of P. radiata, is to lower the treatment pH and so promote the hydrolysis and solubilisation of the hemicelluloses and cellulose, while preventing carbohydrate degradation via basic catalysis. However, the observed presence of [1,6]-linked disaccharides, characteristic of sugar reversion, shows that some undesirable consequences can result from SO_2-catalysis.

As well as catalysing carbohydrate hydrolysis, SO_2 also catalyses lignin depolymerisation, although only a small amount of soluble lignin is obtained. This is because substantial repolymerisation of the lignin occurs during the steam treatment. Nevertheless, the presence of this lignin appears to offer little hindrance to enzymatic digestion of the cellulose component, presumably because its physical distribution has been altered by the steaming process. Only a very small amount of lignin sulphonation occurs under the steam explosion conditions used in this study.

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REFERENCES

1. T.A. Clark and K.L. Mackie, J. Wood Chem. Technol., 7(3), 373 (1987)

2. K.L. Mackie, H.H. Brownell, K.L. West and J.N. Saddler, J. Wood Chem. Technol., 5(3), 405 (1985)

3. R.E. Brooks, T.M. Su, J.M. Brennan, J. Frick and M. Lynch. Final report to United States Department of Energy. Contract No. EG-77-C-02-4147 by General Electric Company 1979.

4. T.M. Su, R.J. Lamed, J. Lobos, M. Brennan, J.F. Smith, D. Tabor and R. Brooks. Final report to United States Dept of Energy. Subcontract No. XR-9-8271-1 by General Electric Company, 1980.

5. H. Mamers and D.J. Menz, Appita, 37, 644 (1984).

6. R.H. Dekker, Biocatalysis, 1, 63 (1987).

7. M. Wayman, S. Parekh, E. Chornet, and R.P. Overend, Biotechnol. Lett., 8, 749 (1986).

8. M. Wayman, C. Seagrave, and S. Parekh Process Biochemistry, 22, 55 (1987).

9. K. Sudo, K. Shimizu, T. Ishii, T. Fuji, and S. Nagasawa, Holzforschung, 40, 339 (1986).

10. M. Wayman, A.Tallevi and B. Winsborrow, Biomass, 6, 183 (1984).

11. S.A. Rydholm, Pulping Processes, Interscience Publishers, 1965.

12. K. Norrish and J.T. Hutton, X-Ray Spectrom, 6(1), 6, (1977).

13. I.J. Callander, T.A. Clark, P.N. McFarlane, and K.L. Mackie, Environ. Technol. Lett., 7, 325, (1986).

14. S. Katz, R.P. Beatson, and A.M. Scallan, Svensk Papperstidning, 6, R48 (1984).

15. R.P. Beatson, C. Heitner and D. Atack, J. Wood Chem. Technol., 4(4), 439 (1984).

16. H.H. Brownell and J.N. Saddler, Biotechnol. Bioeng. 29, 228 (1987).

17. K. Grohmann, R Torget, and M. Himmel Biotechnol. Bioeng. Symp. No. 15, 59 (1985).

18 H.E. Grethlein, Bio/Technology, 3, 155 (1985).

19. H.E. Grethlein, D.C. Allen and A.O. Converse, Biotechnol. Bioeng., 26, 1498 (1984).

20. K.K.Y. Wong, K.F. Deverell, K.L. Mackie, T.A. Clark and L.A. Donaldson, Biotechnol. Bioeng., 31, 447 (1988).

21. P.J. Weimer and W.M. Weston Biotechnol. Bioeng., 27, 1540 (1985).

22 J.A. Hemmingson and R.H. Newman J. Wood Chem. Technol., 5(2), 159 (1985)

23. J.A. Hemmingson and R.F.H. Dekker, J. Wood Chem. Technol., 7(2), 229 (1987).

24. L.A. Donaldson, K.K.Y. Wong, and K.L. Mackie, Wood Research, submitted, (1987).

25. M. Tanahashi, S. Takada, T. Aoki, T. Goto, T. Higuchi and S Hanai, Wood Research, 69, 36, (1983).

26. J. Puls, K. Poutanen, H-U. Körner, and L. Viikari, Appl. Microbiol. Biotechnol., 22, 416 (1985).

27. J.F. Harris et al. "Two-Stage, Dilute Sulfuric Acid Hydrolysis of Wood: An investigation of fundamentals", General Technical Report FPL-45, U.S. Dept. of Agriculture, Forest Service (1985).

28. E.L. Springer and J.F. Harris, Sven. Papperstidn., 85, R152 (1982).

29. G. Gellerstedt and J. Gierer, Sven. Papperstidn., 74, 117 (1971).

30. M.A. Millet, A.J. Baker and L.D. Satter, Biotechnol. Bioeng. Symp. 6, 125 (1975).

31. J.A. Hemmingson, J. Wood Chem. Technol., 6, 113 (1986).

32. T.A. Clark and K.L. Mackie, in proceedings of Biotechnology in the Pulp and Paper Industry, Stockholm, June 16-19, 1986, Swedish Forest Products Research Laboratory, p.101.

33. J.A. Hemmingson, J. Wood Chem. Technol., 5, 513 (1985).

34. J.A. Hemmingson, J. Wood Chem. Technol., 7, 527 (1987).