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Optimisation of the Alkaline Peroxide Pretreatment for the Delignification of Rice Straw and its Applications

Meenoti M. Patel & Ranjan M. Bhatt

Department of Microbiology, Institute of Science, Bombay 400 032, India

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Abstract: Alkaline peroxide pretreatment for the delignification of rice straw was optimised by varying the concentrations of H_2O_2 and NaOH and changing the temperature and duration of the pretreatment. Changes in the lignin content, content of total carbohydrates and weight loss were measured during the pretreatments. Maximum delignification of 62% was obtained by pretreating rice straw at 50°C for 5 h with 1·5% (w/v) NaOH and 1% (v/v) H_2O_2. The preferential loss of hemicellulose and lignin from the straw resulted in an increase in the cellulose content of the insoluble residue after pretreatment from 47% (untreated) to 67·79% (treated).

The product of this treatment is characterised by having higher cellulose digestibility than untreated rice straw. It also has use as a carbohydrate source in ruminant feed since the in-vivo digestibility by the cow increased from 56·85% to 76·54% ($P < 0·001$). The treated rice straw could also be used for commercial process such as the generation of Single Cell Protein. Growth of *Sporotrichum pulverulentum* on treated rice straw gave a protein product of 24·41% as compared to 3·8% on untreated rice straw.

Key words: lignocellulose, rice straw, alkaline peroxide pretreatment, cellulose, ruminant feed, Single Cell Protein.

1 INTRODUCTION

Lignocellulosic materials, such as agricultural residues, are abundant renewable resources for bioconversion to sugars.[1] The fate and extent to which cellulose in lignocellulosic materials can be enzymatically saccharified is limited by two important factors:

(1) the close physical and chemical association between lignin and the cell-wall polysaccharides;
(2) the degree of crystallinity within the cellulose polymer itself.[2–5] Both hydrolysable and oxidative pretreatments have been used to dissolve the matrix components of lignocellulosics to accelerate the enzymatic hydrolysis.[6] Oxidative treatments are directed to principally affect the degradation of lignin, whereas hydrolytic agents are expected to cleave the lignin–carbohydrate linkages. Coupling of the hydrolysis and the oxidative pretreatment was shown to achieve a better pretreatment efficiency.[7]

Gould[8] has reported that relatively dilute alkaline solutions of hydrogen peroxide (H_2O_2) will remove about one-half of the lignin present in materials such as wheat straw, yielding a cellulose-rich insoluble residue that can be enzymatically converted to glucose. In this paper the alkaline peroxide pretreatment has been characterised with respect to the factors affecting delignification efficiency and the loss of total carbohydrates from rice straw during the pretreatment.

Alkaline hydrogen peroxide pretreatment increased the susceptibility of cellulose in agricultural residues to enzymatic and microbial degradation, suggesting that the treatment may be useful for improving the efficiency with which lignocellulosic materials are digested by ruminants.[9] This paper also deals with the susceptibility of the treated rice straw to cellulose, increase in the in-vivo digestibility in cow and the production of Single Cell Protein.

2 MATERIALS AND METHODS

2.1 Substrate

Rice straw obtained locally was ground in a mill and used. All weights and calculations were made on an oven-dried (80°C) basis. The composition (w/w) of the rice straw used was cellulose 47%, hemicellulose 21%, lignin 24% and ash 8%.

2.2 Alkaline peroxide pretreatment

In this study four pretreatment parameters: NaOH concentration, H_2O_2 concentration, temperature and treatment time, were optimised with respect to the delignification of rice straw. In all cases 2 g of substrate was placed in a 250 cm^3 flask containing 50 cm^3 glass-distilled water and the concentration of NaOH and H_2O_2 varied as indicated. The sample flasks were kept on a shaker at 240 rpm. In all pretreatments the solids:liquid ratio was 1:25. After the indicated period of time, the insoluble fraction was collected by filtration, washed repeatedly with distilled water until the filtrate was neutral and then oven-dried at 80°C.

NaOH concentration was varied from 0·05 to 3·0% (w/v) and H_2O_2 concentration was varied from 0·5 to 4·5% (v/v). Temperatures used for the pretreatments were room temperature (30°C), 40°C and 50°C, and treatment was carried out for 5 hours and 18 hours. The pretreatment either with H_2O_2 solution in the absence of NaOH or with NaOH solution in the absence of H_2O_2 was also done as control.

2.3 Chemical analysis

After pretreatment the material was washed and the treatment yield calculated as a percentage of the weight of the untreated rice straw. Total carbohydrates were estimated by the phenol–sulphuric acid method.[10] Cellulose contents were estimated as described by Updegraff.[11] The hemicellulose content was calculated as the difference between the total carbohydrates and cellulose. Lignin was estimated by the sulphuric acid hydrolysis method.[12] Ash content was determined as described in the AOAC manual.[13]

2.4 Enzymatic hydrolysis

The susceptibility of treated and untreated rice straw to digestion by cellulose was determined by incubating 0·5 g of dried residue in a solution containing 50 mM citrate buffer pH 4·8 and 10 IU FPA *Trichoderma viride* cellulase (EC 3.2.1.4) from Sigma Co. for 24 and 48 h at 37°C. Residual solids remaining after cellulose digestion were removed by filtration on a Whatman No. 1 filter paper. This filtrate was analysed for the presence of reducing sugars using the 3,5-dinitrosalicylic acid (DNSA) assay[14] and for total carbohydrates. The residue was dried in the oven at 80°C to constant weight to determine the percentage of dry weight solubilized. Enzymatic saccharification was determined as described by Tewari *et al.*[15]

2.5 In-vivo digestibility studies

The method of Van Keuren[16] was employed to study in-vivo forage digestibility. The treated and untreated samples were ground in a mill. Six replications of each sample were used for one experiment. The experiment was repeated twice. 4 g of the sample was placed in the nylon bag made of plain, woven nylon cloth. This tightly woven material was used to prevent the loss of very small feed particles. The bags measured 2 in × 4 in. The filled bags were placed in the rumen of a fistulated cow fed on hay, greens and concentrates. The nylon bags remained in the rumen for 48 h. Immediately upon removal the bags were cleaned of adhering ingesta by dipping in water. The bags were oven-dried for 48 h at 65°C after which they were removed and weighed. The dry matter digestibility was determined by weight difference.

2.6 Fermentation experiments

The cellulolytic mould *Sporotrichum pulverulentum* NCIM (National Collection of Industrial Micro-organisms) 1106 was obtained from the National Chemical Laboratory (Poona, India). The fungus was maintained by periodic subculture on potato dextrose agar slants. Shake flask experiments were carried out in 500 cm^3 Erlenmeyer flasks containing 100 cm^3 of medium and incubated at 30°C. The basal medium of Mandels and Weber[17] was used containing untreated and treated rice straw instead of cellulose. The autoclaved medium was inoculated with 10 cm^3 inoculum of spore suspension containing $7·5 \times 10^8$–8×10^8 spores cm^{-3}. Protein content of the fermented mash was estimated by the method of Herbert *et al.*[18]

3 RESULTS AND DISCUSSION

3.1 Changes in physical properties

As observed by Gould and Freer,[19] it was also noted here that the straw particles disintegrated into small, highly dispersed fibres, and the suspension acquired a more homogenous pulp-like consistency. Gould *et al.*[20] found that the alkaline hydrogen peroxide treatment increased the absorbency of wheat straw threefold from 6·8 to 21·9 g of water per gram of straw.

3.2 Effect of dilute NaOH solutions on delignification

Table 1 shows the effect of dilute alkaline peroxide solutions on the composition of rice straw. Increasing the

TABLE 1
Effect of Dilute Alkaline Peroxide[a] Solutions on the Composition of Rice Straw

NaOH concentration (% (w/v))	*Yield (%)*	*Residue composition*				
		Total carbohydrates g (% (w/w))	*Cellulose g (% (w/w))*	*Hemicellulose g (% (w/w))*	*Lignin g (% (w/w))*	*Ash g (% (w/w))*
Control	97	1·313	0·94	0·373	0·47	0·157
		(67·68)[b]	(48·45)	(19·23)	(24·22)	(8·09)
0·05	81·77	1·185	0·94	0·245	0·344	0·11
		(72·48)	(57·49)	(14·98)	(21·04)	(6·73)
0·1	79·81	1·156	0·94	0·216	0·335	0·1
		(72·43)	(58·9)	(13·53)	(21)	(6·27)
0·2	75·8	1·098	0·94	0·158	0·318	0·09
		(72·4)	(62·01)	(10·42)	(20·99)	(5·94)
0·3	70·03	1·014	0·94	0·074	0·294	0·084
		(72·38)	(67·09)	(5·28)	(20·98)	(5·85)
0·4	66·6	0·964	0·94	0·02	0·278	0·075
		(72·35)	(70·57)	(1·5)	(20·89)	(5·63)
0·5	65·61	0·95	0·94	0·01	0·274	0·07
		(72·3)	(71·65)	(0·762)	(20·86)	(5·33)

[a] 2 g of ground rice straw were incubated in 50 cm^3 of 1 % (v/v) H_2O_2 and varying concentrations of NaOH for 18 h at room temperature.
[b] % (w/w) of component is shown in parentheses.

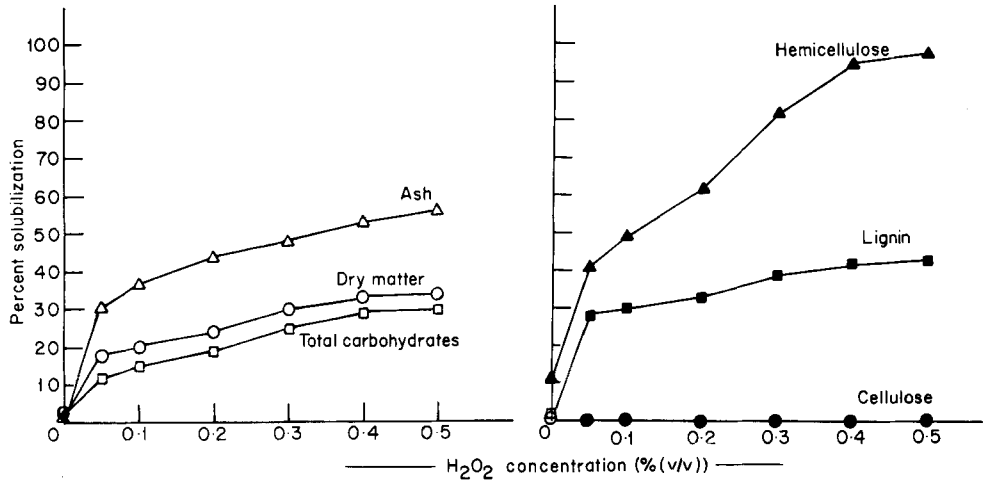

Fig. 1. Solubilisation of various components of rice straw after alkaline peroxide treatment using dilute solutions of NaOH. Other conditions: 1 % (v/v) H_2O_2, 18 h, room temperature; solids:liquid, 1:25.

concentration of NaOH from 0·05 % (w/v) to 0·5 % (w/v) at 1 % (v/v) H_2O_2, the yield of the residue decreased from 81·77 % to 65·61 %. This was due to the decrease in the lignin and hemicellulose content. Control sample treated only with 1 % H_2O_2 gave a yield of 97 %. Figure 1 shows the loss of various components of rice straw when treated with dilute alkaline peroxide solution. The levels of delignification obtained at 1 % (v/v) H_2O_2 and various concentrations of NaOH (0·05 % (w/v) to 0·5 % (w/v)) increased from 28·33 % to 42·98 %. Maximum delignification was achieved at 0·5 % NaOH (w/v). Hence further experiments were performed using higher concentrations of NaOH.

3.3 Effect of alkalinity on the delignification efficiency

Both the initial rate and the extent of delignification were substantially increased by raising the alkalinity of the solution with 1 % (v/v) initial H_2O_2 concentration.[7] The role of alkalinity for delignification in a hydrogen peroxide solution was studied by varying the initial NaOH concentration in solution. As shown in Table 2,

TABLE 2
Effect of Varying the Initial NaOH Concentration[a] on the Composition of Rice Straw

NaOH concentration (% (*w/v*))	*Yield* (%)	*Residue composition*				
		Total carbohydrates g (% (*w/w*))	*Cellulose* g (% (*w/w*))	*Hemicellulose* g (% (*w/w*))	*Lignin* g (% (*w/w*))	*Ash* g (% (*w/w*))
Control	97	1·313 (67·68)[b]	0·94 (48·45)	0·373 (19·23)	0·47 (24·22)	0·157 (8·09)
1·0	62·25	0·97 (77·91)	0·931 (74·78)	0·039 (3·13)	0·25 (20·08)	0·035 (2·81)
1·5	58·5	0·91 (77·8)	0·9 (76·92)	0·01 (0·856)	0·23 (19·66)	0·03 (2·56)
2·0	50·15	0·753 (75·07)	0·753 (75·07)	0 (0)	0·22 (21·93)	0·03 (2·99)
2·5	46·6	0·7 (75·06)	0·7 (75·11)	0 (0)	0·204 (21·89)	0·028 (3·00)
3·0	40·35	0·61 (75)	0·61 (75·59)	0 (0)	0·18 (22·3)	0·017 (2·11)

[a] 2 g of ground rice straw were incubated in 50 cm³ of 1% (v/v) H_2O_2 and varying concentrations of NaOH for 18 h at room temperature.
[b] % (w/w) of component in parentheses.

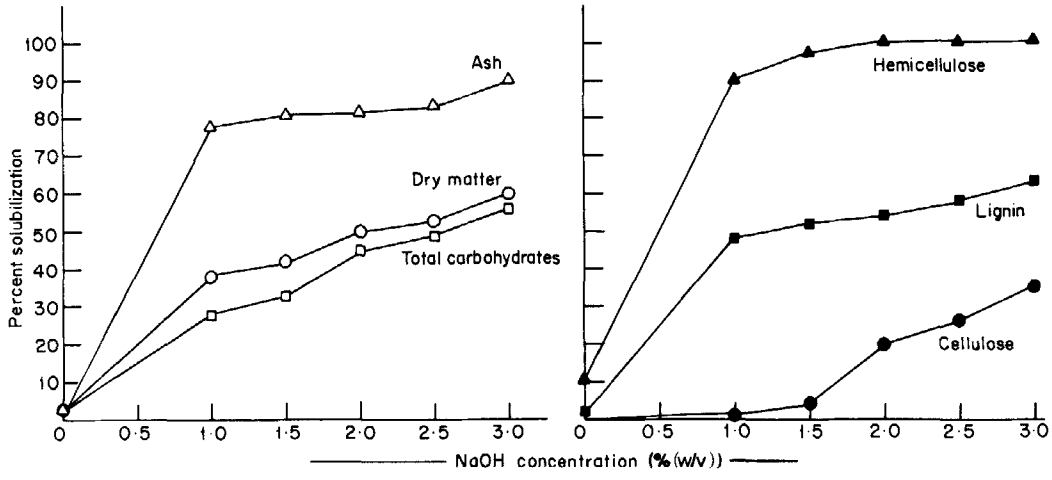

Fig. 2. Solubilisation of various components of rice straw after alkaline peroxide treatment as a function of NaOH concentration. Other conditions: 1% (v/v) H_2O_2, 18 h, room temperature; solids:liquid, 1:25.

the concentration of NaOH was varied from 1–3%. With the increase in the concentration of NaOH, there is a decrease in the weight of lignin and cellulose, although the insoluble residue becomes more enriched as the percentage of cellulose increases from 48·45% to 76·92%. However, as seen in Fig. 2 increasing the concentration from 1·5% to 2%, we found that the delignification efficiency increases only marginally from 52·1% to 54·18% whilst there is a dramatic increase in the loss of cellulose from 4·26% to 19·89%. Also, at 2% (w/v) NaOH and above, a total loss of the hemicellulose fraction was found.

Hence in the further experiments NaOH at a concentration of 1·5% (w/v) has been used.

3.4 Effect of the initial H_2O_2 concentration on the delignification of rice straw

Hydrogen peroxide is an effective pretreating agent for the improvement of enzymatic susceptibility of lignocellulosic subtrate. More than 90% of the cellulose present in wheat straw could be converted to glucose when the straw was treated with H_2O_2 at pH 11·5, compared to only about 30% conversion for straw treated at pH 11·5 in the absence of H_2O_2.[22] Table 3 shows the effect of varying the H_2O_2 concentration from 0·5% (v/v) to 4·5% (v/v) at 1·5% (w/v) NaOH. It can be seen that the yield of the insoluble residue decreased from 63·2% to 44·05%. Also, beyond 1% (v/v) H_2O_2

TABLE 3
Effect of Varying the H_2O_2 Concentration[a] on the Composition of Rice Straw

H_2O_2 concentration (% (v/v))	Yield (%)	Residue composition				
		Total carbohydrates g (% (w/w))	Cellulose g (% (w/w))	Hemicellulose g (% (w/w))	Lignin g (% (w/w))	Ash g (% (w/w))
Control	89·5	1·23 (68·72)[b]	0·94 (52·51)	0·29 (16·2)	0·42 (23·46)	0·14 (7·82)
0·5	63·2	0·987 (78·09)	0·93 (73·58)	0·057 (4·51)	0·245 (19·4)	0·031 (2·45)
1·0	58·95	0·920 (78·02)	0·908 (77·01)	0·012 (1·02)	0·228 (19·37)	00·03 (2·54)
1·5	57·07	0·879 (76·91)	0·878 (76·95)	0·001 (0·088)	0·234 (20·51)	0·026 (2·28)
2·0	56·07	0·861 (76·81)	0·861 (76·81)	0 (0)	0·239 (21·32)	0·021 (1·87)
2·5	55·94	0·849 (75·84)	0·849 (75·87)	0 (0)	0·246 (21·99)	0·019 (1·7)
3·0	52·55	0·794 (75·55)	0·794 (75·55)	0 (0)	0·247 (23·5)	0·017 (1·62)
3·5	50·9	0·758 (74·46)	0·758 (74·46)	0 (0)	0·255 (25·05)	0·015 (1·57)
4·0	48·05	0·692 (72·01)	0·692 (72·01)	0 (0)	0·024 (27·43)	0·015 (1·56)
4·5	44·05	0·60 (68·1)	0·60 (68·1)	0 (0)	0·264 (29·94)	0·013 (1·48)

[a] 2 g ground rice straw were incubated in 50 cm^3 of 1·5% (w/v) NaOH and varying concentrations of H_2O_2 for 18 h at room temperature.
[b] % (w/w) of component is shown in parentheses.

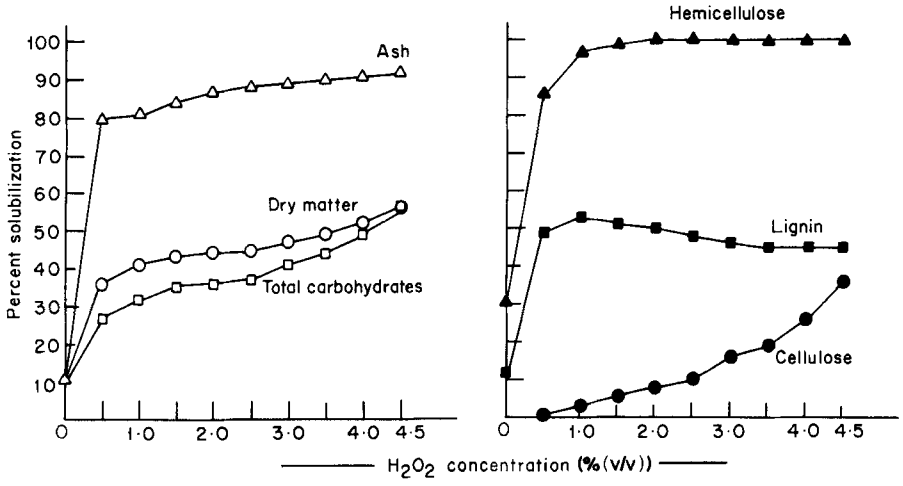

Fig. 3. Solubilisation of various components of rice straw after alkaline peroxide treatment as a function of H_2O_2 concentration. Other conditions: 1·5% (w/v) NaOH, 18 h, room temperature; solids:liquid, 1:25.

there was a total loss of the hemicellulose fraction. Also, the cellulose content in the residue decreased with increasing concentration of H_2O_2. Figure 3 shows the effect of the initial H_2O_2 concentration on the degree of delignification and loss of total carbohydrates in the presence of 1·5% (w/v) NaOH. Delignification increased to a maximum of 52% at 1% (v/v) H_2O_2. However, further increasing the concentrations of H_2O_2 had a marginally adverse effect on the delignification of the substrate with the delignification efficiency falling to 45% at 4·5% (v/v) H_2O_2. Hence a combination of 1·5% (w/v) NaOH with 1% (v/v) H_2O_2 appeared to be optimal for the delignification of rice straw.

Although this pretreatment ensures the removal of

TABLE 4
Effect of Alkaline H_2O_2[a] at Room Temperature on the Composition of Rice Straw

H_2O_2 concentration (% (v/v))	Yield (%)	Residue composition				
		Total carbohydrates g (% (w/w))	Cellulose g (% (w/w))	Hemicellulose g (% (w/w))	Lignin g (% (w/w))	Ash g (% (w/w))
Control	89·5	1·23 (68·72)[b]	0·94 (52·51)	0·29 (16·2)	0·42 (23·46)	0·14 (7·82)
0·5	81·9	1·19 (72·56)	0·94 (57·31)	0·25 (15·24)	0·37 (22·56)	0·11 (6·71)
1·0	81·4	1·183 (72·66)	0·94 (57·44)	0·243 (14·93)	0·345 (22·2)	0·09 (5·53)
1·5	80·14	1·17 (72·99)	0·94 (58·64)	0·23 (14·35)	0·336 (20·92)	0·08 (4·99)
2·0	79·91	1·16 (72·59)	0·94 (58·82)	0·22 (13·77)	0·322 (20·14)	0·076 (4·76)
2·5	79·06	1·155 (73·06)	0·94 (59·46)	0·215 (13·6)	0·317 (20·03)	0·074 (4·68)

[a] 2 g of ground rice straw were incubated in 50 cm³ of 1·5% (w/v) NaOH and varying concentrations of H_2O_2 for 5 h at room temperature.
[b] % (w/w) of component is shown in parentheses.

TABLE 5
Effect of Alkaline H_2O_2[a] at 40 °C on the Composition of Rice Straw

H_2O_2 concentration (% (v/v))	Yield (%)	Residue composition				
		Total carbohydrates g (% (w/w))	Cellulose g (% (w/w))	Hemicellulose g (% (w/w))	Lignin g (% (w/w))	Ash g (% (w/w))
Control	89·5	1·23 (68·72)[b]	0·94 (52·51)	0·29 (16·2)	0·42 (23·46)	0·14 (7·82)
0·5	77·48	1·168 (75·4)	0·94 (60·66)	0·228 (14·71)	0·302 (19·51)	0·095 (6·131)
1·0	73·07	1·1424 (78·17)	0·94 (64·32)	0·202 (13·82)	0·284 (19·43)	0·084 (5·75)
1·5	69·86	1·09 (78·01)	0·94 (62·68)	0·15 (10·7)	0·292 (20·88)	0·065 (4·65)
2·0	68·51	1·065 (77·73)	0·94 (68·6)	0·125 (9·12)	0·286 (20·85)	0·06 (4·39)
2·5	65·66	0·971 (73·95)	0·94 (71·59)	0·031 (2·36)	0·289 (22)	0·05 (3·81)

[a] 2 g of ground rice straw were incubated in 50 cm³ of 1·5% (w/v) NaOH and varying concentrations of H_2O_2 for 5 h at 40 °C.
[b] % (w/w) of component is shown in parentheses.

more than 50% of the lignin originally present in the substrate, a portion of the cellulose and a substantial portion of the hemicellulose are also lost.

3.5 Effect of temperature on the delignification efficiency of rice straw

The oxidative power of hydrogen peroxide is due to the formation of hydroxyl radical from the decomposition of hydrogen peroxide.[8] The degree of solubilisation of various components of corn stover in a dilute caustic slurry depends upon the degree of severity of the treatment, with time, caustic concentration and temperature being the three main parameters.[23] Wei and Cheng[7] found that the decomposition of H_2O_2 was almost complete within 5 h and hence a period of 5 h was used for studying the effect of hydrogen peroxide at three different temperatures: ambient temperature 30°C (Table

TABLE 6
Effect of Alkaline H_2O_2[a] at 50 °C on the Composition of Rice Straw

H_2O_2 concentration (% (v/v))	Yield (%)	Residue composition				
		Total carbohydrates g (% (w/w))	Cellulose g (% (w/w))	Hemicellulose g (% (w/w))	Lignin g (% (w/w))	Ash g (% (w/w))
Control	89·5	1·23 (68·72)[b]	0·94 (52·51)	0·29 (16·2)	0·42 (23·46)	0·14 (7·82)
0·5	71·53	1·144 (80)	0·94 (65·71)	0·2044 (14·29)	0·2 (14·0)	0·086 (6·02)
1·0	69·34	1·127 (81·27)	0·94 (67·79)	0·187 (13·49)	0·179 (12·91)	0·081 (5·82)
1·5	66·53	1·0644 (80)	0·94 (70·65)	0·1244 (9·4)	0·213 (16·02)	0·053 (3·98)
2·0	65·75	1·0278 (78·16)	0·94 (71·48)	0·088 (6·68)	0·234 (17·77)	0·05 (3·8)
2·5	63·7	0·9929 (77·94)	0·93 (73·00)	0·063 (4·94)	0·241 (18·92)	0·04 (3·14)
3·0	62·6	0·969 (77·4)	0·91 (72·68)	0·052 (4·15)	0·2514 (20·08)	0·032 (2·56)

[a] 2 g of ground rice straw were incubated in 50 cm^3 of 1·5% (w/v) NaOH and varying concentrations of H_2O_2 for 5 h at 50 °C.
[b] % (w/w) of component is shown in parentheses.

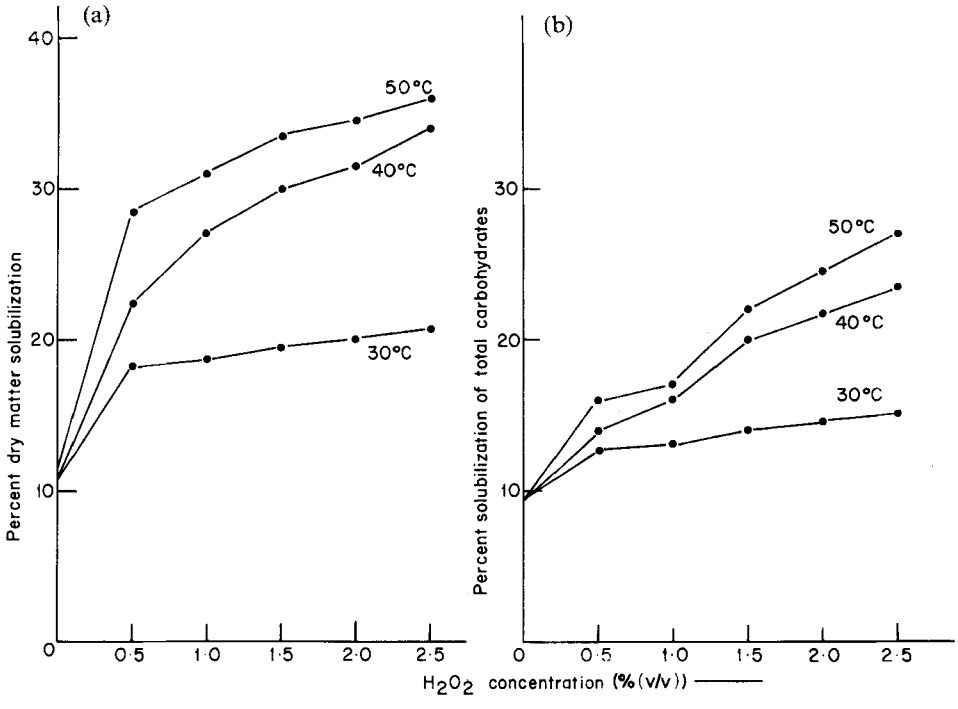

Fig. 4. Effect of temperature on the solubilisation of (a) dry matter and (b) total carbohydrates as a function of H_2O_2 concentration. Other conditions: 1·5% (w/v) NaOH, 5 h treatment; solids:liquid, 1:25.

4); 40°C (Table 5); and 50°C (Table 6). Figures 4 and 5 show the loss of various components of rice straw at 30°C, 40°C and 50°C. As shown in Table 4, the yield of the insoluble residue obtained after pretreatment was very high, ranging from 79% to 82%. However, as can be seen in Fig. 6, maximal delignification of 34% was obtained at 2·5% (v/v) H_2O_2 and 1·5% (w/v) NaOH, and the yield of the substrate obtained after pretreatment decreased as shown in Table 5. However, the delignification efficiency increased, reaching a maximum of 41% at 1% (v/v) H_2O_2 and 1·5% (w/v) NaOH as shown in Fig. 6. As the temperature was further increased

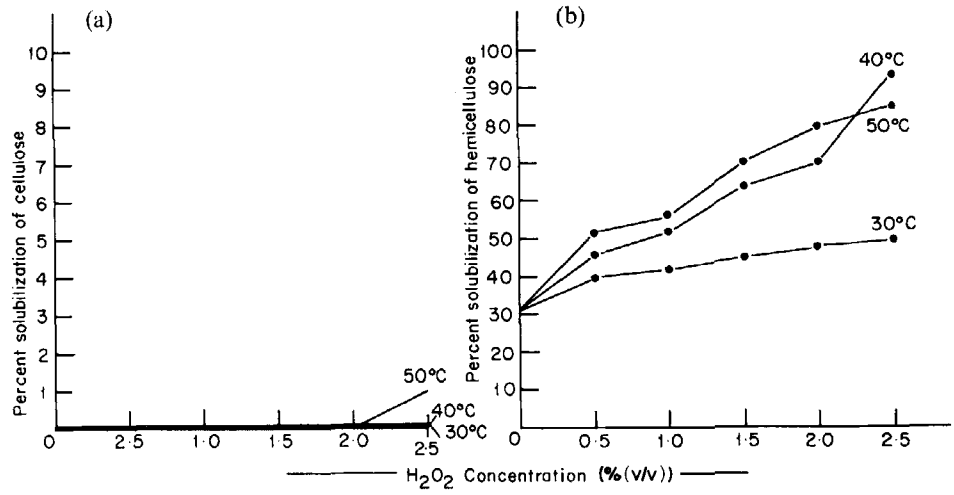

Fig. 5. Effect of temperature on the solubilisation of (a) cellulose and (b) hemicellulose as a function of H_2O_2 concentration. Other conditions: 1·5% (w/v) NaOH, 5 h treatment; solids:liquid, 1:25.

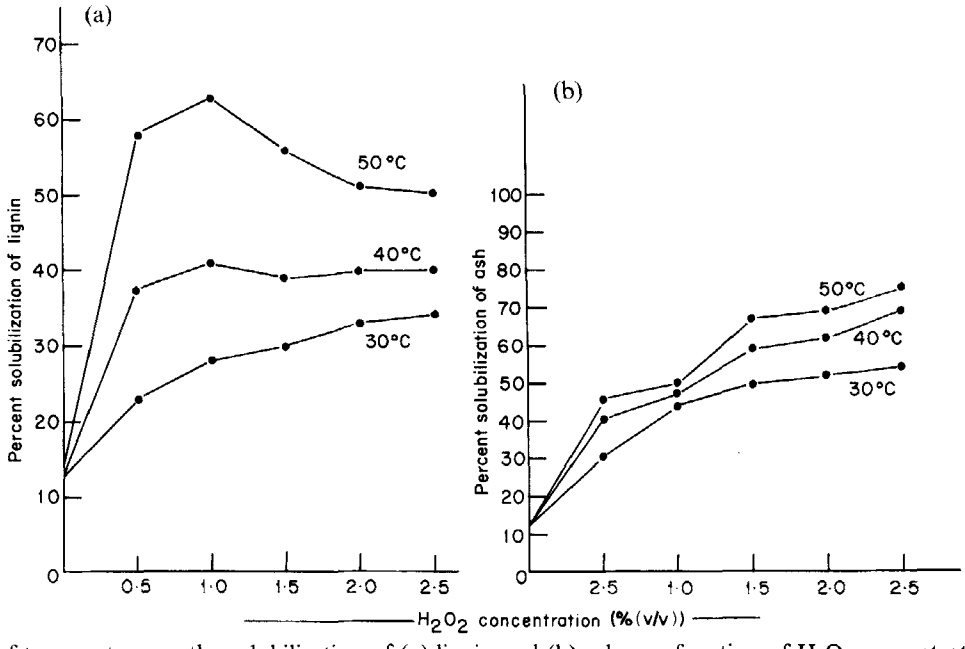

Fig. 6. Effect of temperature on the solubilisation of (a) lignin and (b) ash as a function of H_2O_2 concentration. Other conditions: 1·5% (w/v) NaOH, 5 h treatment; solids:liquid, 1:25.

to 50°C, more rapid rate of weight loss was found than that for rice straw pretreated at 40°C and 30°C. Figure 6 shows that the maximum delignification of 63% was obtained at 1% (v/v) H_2O_2 and 1·5% (v/v) NaOH. However no further increase in the degree of delignification was observed when the initial H_2O_2 concentration was further raised to 2·5% (v/v) in a system initially containing 1·5% (w/v) NaOH and 4% (w/v) straw. This pretreatment gave the highest delignification observed. The cellulose originally present in the straw was recovered in the insoluble residue after the treatment. As a result of the treatment, the straw residue was substantially enriched in cellulose over the starting material (68% cellulose versus 47% cellulose originally).

3.6 Enzymatic hydrolysis

Enzymatic hydrolysis is a natural and ideal method for the conversion of cellulosic materials to sugars which could be used as a source of food, fuel or chemicals.[24] Treatment of lignocellulosic materials with an alkaline hydrogen peroxide solution results in the disruption of the substrate morphological integrity and a dramatic increase in its susceptibility to hydrolysis by cellulolytic microorganisms and enzymes.[8] The effect of delignification on the dry matter solubilisation, saccharification and the release of reducing sugars and total carbohydrates was studied for 24 and 48 h. Treated rice straw showed greater solubilisation as compared to the untreated rice straw (Fig. 7a). The enzymatic hydrolysis of cellulose for

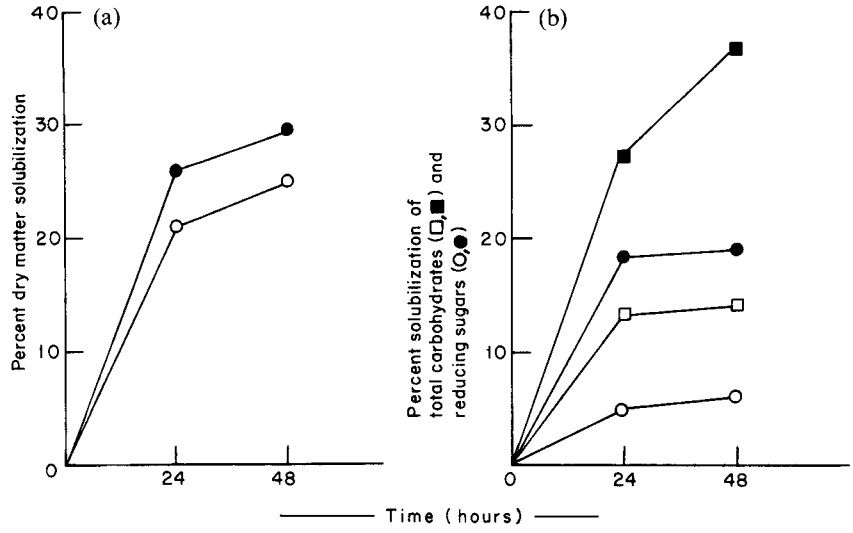

Fig. 7. (a) Dry matter solubilisation of rice straw after enzymatic hydrolysis with cellulase. Solubilisation of treated (●) and untreated (○) rice straw after 24 and 48 h is shown. (b) Release of reducing sugars (circles) and total carbohydrates (squares) after enzymatic hydrolysis with cellulase is shown for treated (●, ■) and untreated (○, □) rice straw after 24 and 48 h. Other conditions: 0·5 g of dried residue in 50 mM citrate buffer pH 4·8 and 10 IU FPA *T. viride* cellulase (EC 3.2.1.4) at 37°C.

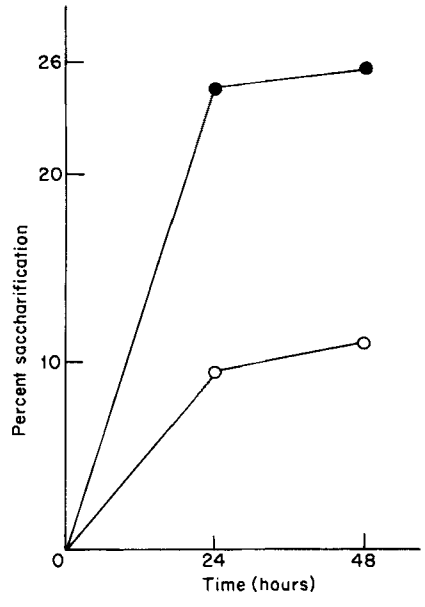

Fig. 8. Effect of cellulase treatment on the percentage saccharification of treated (●) and untreated (○) rice straw. Other conditions: 0·5 g of dried residue in 50 mM citrate buffer pH 4·8 and 10 IU FPA *T. viride* cellulase (EC 3.2.1.4) at 37°C.

TABLE 7
In-vivo Digestibility Studies

	Percentage digestibility (mean)	*Standard error of mean (SEM)*[a]
Untreated	56·85	2·94
Treated	76·54	4·88

[a] $t = 10{\cdot}84$, $P < 0{\cdot}001$; significant at 0·1% level.

sugar production has received a great deal of attention in recent years. Production of both reducing sugars and total carbohydrates was enhanced by treating rice straw at 50°C for 5 h with 1·5% (w/v) NaOH and 1% (v/v) H_2O_2 as shown in Fig. 7b. After 24 h, there was a 3·75-fold increase in the production of reducing sugars from 5 mg in the control to 18·82 mg in the treated rice straw. After 48 h of enzymatic hydrolysis, there was a 3·4-fold increase from 5·6 mg to 19·1 mg.

The production of total carbohydrates increased about twofold from 13·3 mg to 27·8 mg at 24 h with a 2·8-fold increase from 14·3 mg to 37 mg at 48 h.

The degree of saccharification increased 2·6-fold after 24 h and 2·4-fold after 48 h as shown in Fig. 8.

3.7 In-vivo digestibility studies

The inability of ruminant animals to digest lignocelluosic materials such as wheat straw and corn stover efficiently severely limits the extent to which these materials can be incorporated into feedstuffs.[25,26] Recent work showed that alkaline hydrogen peroxide treatment of wheat straw markedly increased wheat-straw structural carbohydrate digestibility by sheep and ruminants as a result of partial delignification of wheat straw.[27–30]

Rice straw is fed to cattle in India. Using the in-vivo nylon bag technique, the digestibility of this rice straw before and after treatment was studied. The results shown in Table 7 show the means of 12 independent samples. The digestibility increased from 57% to 77%, about a 1·4-fold increase. The results were highly significant at $P < 0{\cdot}001$. These data suggest that the barriers which limit digestibility of low-quality feedstuffs can be effectively removed by this alkaline peroxide treatment.

TABLE 8
Effect of Alkaline Peroxide Treatment on Protein Production by *S. pulverulentum*

Day of harvesting after inoculation	*Percentage biomass protein*
A. Untreated	
4th	2
5th	2·25
6th	3·0
7th	3·7
8th	3·7
9th	3·8
B. Treated	
4th	22·0
5th	22·5
6th	24·4
7th	23·8
8th	23·7
9th	23·7

CONCLUSIONS

From the results discussed above, it is clear that alkaline peroxide pretreatment of rice straw causes delignification of rice straw, yielding a cellulose-rich insoluble residue that can be enzymatically converted to glucose. For a 4% (w/v) straw concentration, an H_2O_2 concentration of 1% (v/v) an NaOH concentration of 1.5% (w/v) and treatment at 50°C for 5 h, a maximum delignification of 63% was obtained. This degree of delignification was found to increase the susceptibility of rice straw to enzymatic saccharification. Thus the alkaline hydrogen peroxide treatment has the potential for the utilisation of rice straw to produce useful products such as sugars, ethanol, and/or feedstock chemicals. Data on the in-vivo digestibility in cow suggest that barriers which limit digestibility of low-quality feedstuffs can be effectively removed by this treatment. The ability to feed highly digestible cellulosic materials to ruminants would eliminate dependence upon cereal grains and make available a nearly inexhaustible feed supply. It can further be concluded that alkaline peroxide-treated rice straw is a suitable substrate for SCP production. Since SCP is in short supply in developing countries, the commercial production of this product will have a tremendous impact on the national economy.

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