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Pretreatment of Wheat Straw by White-Rot Fungi for Enzymic Saccharification of Cellulose

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Summary. Of 19 white-rot fungi tested, *Pleurotus ostreatus, Pleurotus* sp. 535, *Pycnoporus cinnabarinus* 115 and *Ischnoderma benzoinum* 108 increased the susceptibility of straw to enzymic saccharification, thus indicating that these organisms degraded or modified the lignin component. After pretreatment cultivation with *Pycnoporus cinnabarinus* 115, as much as 54.6% of the residue was converted to reducing sugars in the enzymic saccharification process. *Phanerochaete sordida* 37, *Phlebia radiata* 79 and two unidentified fungi also gave better results than *Polyporus versicolor,* a non-selective reference fungus. After 5 weeks pretreatment with *Pleurotus ostreatus,* 35% of the original straw was convertable to reducing sugars, 74% of which was glucose; compared with this, only 12% of the untreated control straw was convertable to reducing sugars, 42% of which was glucose. After an alkali pretreatment (2% NaOH, 0.4 g NaOH/g straw, 10 min at 115° C) enzymic saccharification converted 41% of the straw to reducing sugars, of which only 50% was glucose. In the best cases the efficiency of biological pretreatment was comparable with that of alkali treatment, but resulted in a higher proportion of glucose in the hydrolysates. Pretreatment by the fungi *Phanerochaete sordida* 37 and *Pycnoporus cinnabarinus* 115 in an oxygen atmosphere reduced the treatment time by approximately 1 week. However, the economic feasibility of a non-optimized biological pretreatment process is still poor due to the long cultivation times required.

Introduction

Many problems associated with the production of cellulolytic enzymes, fermentation of cellulose-derived sugars to ethanol and single-cell protein production from lignocellulosic materials have largely been solved or will be solved in the near future (Flickinger 1980; Bisaria and Ghose 1981; Fiechter 1981). However, pretreatment of lignocellulosic materials is still considered to be the rate-limiting step in an economically feasible process for enzymic hydrolysis of cellulose. Hydrolysis of native wood or straw by cellulolytic enzymes is slow and inefficient, mainly because the lignin in plant cell walls forms a barrier against enzymic attack (Fan et al. 1982). Therefore, an ideal pretreatment would reduce the lignin content, reduce crystallinity of the cellulose and increase surface area. Fan et al. (1982) have comprehensively reviewed recent developments in this area. Biological pretreatment techniques have not been developed as intensively as physical and chemical methods, and if the capacity of microorganisms is to be utilized more fully, a better understanding of microbial lignin degradation is necessary (cf. Crawford 1981). Such knowledge would allow methods of improving the rate and selectivity of lignin degradation, for example by genetic manipulation, to be developed.

The most promising organisms for biological pretreatment of lignocellulose are the white-rot fungi, which belong to the class *Basidiomycetes.* These organisms can degrade all wood components, although the relative enzyme activities and the order of attack vary between different species (Eriksson and Vallander 1980). In many other potential applications of bio-ligninolytic systems, for example in biopulping and conversion of lignocellulose to more digestible feedstock, the most useful fungi would be those which selectively remove lignin (Kirk and Chang 1981). Various means of achieving this aim include: (1) to use naturally occurring white-rot fungi which preferentially attack lignin (Kawase 1962; Kirk and Moore 1972; Ander and Eriksson 1977; Setliff and Eudy 1980); (2) to use cellulase-less mutants of

efficient lignin degraders (Ander and Eriksson 1975; Eriksson and Vallander 1980; Eriksson et al. unpublished results); and (3) to repress enzymes which degrade wood carbohydrates (Yang et al. 1980). This study adopts the first approach.

The aim was to determine if it is possible to pretreat a representative lignocellulose, wheat straw, with white-rot fungi so as to increase susceptibility of the residue to enzymic hydrolysis by *Trichoderma reesei* cellulase. Fungal treatments, combinations of fungal and alkali treatment and the effect of oxygen atmosphere in fungal treatment were studied.

Materials and Methods

Fungi and Inocula. The fungi used in this study are presented in Table 1. *Pleurotus ostreatus, Pycnoporus cinnabarinus* A-360, *Sporotrichum pulverulentum* and *Polyporus versicolor* were obtained through the courtesy of Prof. Karl-Erik Eriksson (see Hatakka and Uusi-Rauva 1983). The other fungi were selected from the collection of wood-rotting fungi at the Department of Microbiology, University of Helsinki, mainly on the basis of their relatively low cellulolytic activity. Most of them were isolated, characterized and tentatively identified by Hatakka and Pirhonen (unpublished results).

Preparation of spore *(S. pulverulentum)* and mycelial (other fungi) inocula have been described by Hatakka and Uusi-Rauva (1983). A propagule suspension of 1 ml was used per cultivation flask containing 1 g of straw.

Media. The basic medium contained per liter of distilled water: KH_2PO_4, 1.6 g; K_2HPO_4. 0.44 g; $MgSO_4 \cdot 7\,H_2O$, 0.1 g; $CaCl_2$, 0.02 g; L-asparagine, 0.1 g; NH_4NO_3, 0.05 g; thiamine · HCl, 5 mg; ferric citrate · $3\,H_2O$, 0.2 mg; $ZnSO_4 \cdot 7\,H_2O$, 0.2 mg; $MnSO_4 \cdot 4\,H_2O$, 1 mg; $CoCl_2 \cdot 6\,H_2O$, 0.2 mg; $CuSO_4 \cdot 5\,H_2O$, 0.02 mg. The pH of the medium was adjusted with 1 M H_3PO_4 to 4.6 before autoclaving. Air-dry straw from autumn wheat *Triticum aestivum* variety "Vakka" was used in most experiments. Variety "Aura" was used in experiments in which the effect of oxygen atmosphere was studied. Straw was chopped into approximately 1-cm pieces; 1 g straw was added to a 250-ml conical flask and supplemented with 10 ml of basic medium. The medium was autoclaved for 20 min at 121° C and inoculated as described earlier. To prevent drying, the cotton-plugged cultivation flasks were placed in loosely closed polyethylene bags, one flask in each bag. Fungi were incubated at 28° C for 5 weeks. Samples, sometimes four but usually five replicate cultures, were harvested 7, 14, 21, 28, and 35 days after inoculation.

Prior to hydrolysis the straw residue with fungal mycelium was autoclaved for 10 min at 115° C to inactivate the fungus. The residue was then washed with distilled water in a Büchner funnel on Whatman no. 4 filter paper using 200 ml water per 1 g original straw. For dry weight determinations five additional replicate flasks were used and handled as described. Dry weights were determined after drying the residues at 105° C to constant weight.

Hydrolysis of Straw Residue with Trichoderma reesei cellulase. *Trichoderma reesei* cellulase preparation (protein concentration 53%) was obtained in powder form from the Biotechnical Laboratory, Technical Research Center of Finland, Espoo, Finland. Cellulase powder was suspended in 0.05 M sodium acetate buffer (pH 4.8, 0.0025% merthiolate, Thiomersal, BDH Chemicals, Poole, England) at a concentration of 3 mg ml^{-1} to give 1.0 FPU ml^{-1} (for FPU units, see Bailey and Nevalainen 1981). The suspension was centrifuged to remove any undissolved residue.

From each culture flask the residual straw plus mycelium was transferred to a test tube (21 mm × 200 mm); 10 FPU per 1 g original chopped straw in 20 ml 0.05 M sodium acetate buffer, pH 4.8, was added. Tubes were closed with polyethylene film and placed in a 40° C water bath. The mixtures were magnetically stirred. Controls without the fungal pretreatment were handled in the same way as the pretreated samples.

Samples from the hydrolysis mixture were taken 4, 24, 48, and 72 h after starting, heated for 3 min at 100° C to halt the reaction, cooled in an ice bath, diluted with distilled water and centrifuged to remove the protein precipitate. Supernatants were collected and glucose and reducing sugars determined.

Alkali Treatment of Straw. After 1, 2, or 3 weeks fungal growth, 20 ml of 2% (w/v) sodium hydroxide (p.a.) solution was added to a growth flask originally containing 1 g straw and the mixture autoclaved for 10 min at 115° C. Controls without fungus were treated in the same way. After cooling, the residue was washed with 200 ml of distilled water per 1 g of original straw and dry weights determined using five replicate flasks. Five other replicates were hydrolysed with *T. reesei* cellulase.

Cultivation in an Oxygen Atmosphere. Culture flasks were closed with rubber stoppers equipped with two glass tubes fitted with silicone rubber tubing. The inlet glass tube extended 1–2 cm above the culture medium and the outlet tube < 1 cm into the flask. Flasks were flushed thrice weekly with filter-sterilized oxygen for 10 min and sealed to maintain the oxygen atmosphere.

Analytical Methods. The cellulolytic activities of the fungi from our own collection were screened by the tube test of Rautela and Cowling (1966), which is based on the size of the clearance zones produced in test tubes containing phosphoric acid swollen cellulose. Cellulose was prepared by the method of Walseth (1952) from Whatman no. 1 filter paper powder and from Solka Floc (Brown Co., Berlin, New Hampshire, USA).

Glucose was determined using the glucose oxidase method (GOD Perid Test, Boehringer-Mannheim, Federal Republic of Germany) and reducing sugars by the dinitrosalicylic acid method (Fisher and Stein 1961) using glucose as standard. After hydrolysis (72 h), the residue was washed with distilled water (200 ml) on Whatman no. 4 filter paper and dried to constant weight at 105° C.

The percentage conversion was calculated as follows:

$$\text{Conversion} = \frac{\text{sugars in the hydrolysate after 72 h (g)}}{\text{dry weight of the washed residue after growth (g)}} \times 100\%$$

Results

Fungal Pretreatment of Straw

Chopped straw was not dried prior to hydrolysis. After autoclaving and standing for 1–5 weeks at 28° C (control samples), 6.040 ± 0.002% (30 replicates) of the substrate became water-soluble (Table 1). After enzymic hydrolysis, the degree of conversion of these control samples was 13.80 ±

Table 1. White-rot fungi used in pretreatment experiments, their cellulolytic activities and dry weight losses in solid state fermentation of wheat straw

Fungus	Abbreviation	Clearance zones (mm)		Loss of dry weight after 4 weeks, (%)
		Whatman no. 1	Solka Floc	
Pleurotus ostreatus (Jacq. ex Fr.) Kummer IBP No. 53	Pl.o.	NT	NT	23.6[c]
Pleurotus sp. 535	Pl.535	NT	NT	NT
Pycnoporus cinnabarinus (Jacq. ex Fr.) Karst. A-360	P.c.A-360	NT	NT	NT
Pycnoporus cinnabarinus (Jacq. ex Fr.) Karst. 115[a]	P.c.115	+	13	35.6[c]
Sporotrichum pulverulentum Novobranova 127-1 (ATCC 32629)	S.p.	NT	NT	NT
Phanerochaete sordida (Karst.) Erikss. & Ryv. 37[b]	P.s.37	+	7	42.3
Phlebia radiata Fr. 79[a]	Ph.r.79	23	57	45.2
Fomes fomentarius (L. ex Fr.) Kickx 40	F.f.40	12	26	33.3
Fomes fomentarius (L. ex Fr.) Kickx 80	F.f.80	25	27	NT
Ischnoderma benzoinum (Wahlenb. ex Fr.) Karst. 108[a]	I.b.108	21	27	20.2[c]
Polyporus versicolor L. ex Fr. A-361	P.v.	NT	NT	30.8
Polyporus zonatus Nees ex Fr. 43	P.z.43	7	24	27.0
Polyporus zonatus Nees ex Fr. 81	P.z.81	6	16	28.2
Polyporus brumalis (Pers. ex Fr.) Fr. 71	P.b.71	10	18	38.5
Fomes igniarius (L. ex Fr.) Gill. 46	F.i.46	8	19	51.8
Lenzites betulina L. ex Fr. 84	L.b.84	13	19	35.6
Not identified, strain 35	35	8	18	20.4
Not identified, strain 41	41	5	15	28.4
Not identified, strain 59	59	5	19	26.8
Control (no fungus)				6.040 ± 0.002[d]

Clearance zones were measured after 5 weeks cultivation
NT = not determined
[a] Identification was confirmed at Centraalbureau voor Schimmelcultures, Baarn, The Netherlands
[b] Identified by K. K. Nakasone and H. Burdsall Jr., Center for Forest Mycology Research, USDA Forest Products Laboratory, Madison, Wisconsin, USA
[c] Means of two separate experiments
[d] Means of 30 replicates ± SD

1.33% to reducing sugars and 6.03 ± 0.97% to glucose (32 replicates, based on the dry weight of washed straw). Drying and milling of the straw gave slightly better yields. However, these treatments were routinely omitted because of their high cost. When fungal delignification occurred the straw usually became structureless during the subsequent enzymatic hydrolysis and a rather fine suspension was formed.

The effect of the biological pretreatment could be evaluated in two ways: (1) by comparing the yields of sugars calculated on the basis of original dry weight of washed or unwashed straw, i.e., yield of sugars from straw, and (2) by comparing the sugar yields on the basis of dry weight of straw residue after fungal growth, i.e., conversion of straw residue to sugars. The first method was used for evaluation of the efficiency of pretreatment by various fungi because it takes into account the dry weight losses during the fungal cultivation. These losses may be considerable, as shown in Table 1, varying between 20% and 52% after 4 weeks cultivation.

Attack on the wood polysaccharides by the cellulolytic and hemicellulolytic enzymes of the fungi was thought to be the main reason for dry weight losses. However, no direct relationship was observed between the cellulolytic activity as determined by the method of Rautela and Cowling (1966) (Table 1) and dry weight losses. Hemicellulolytic activities were not determined.

In Table 2 the fungi are listed in order of best subsequent yields of sugars from straw after 72 h hydrolysis. *Polyporus versicolor,* which degrades wood carbohydrates and lignin at the same rate (Cowling 1961), was used as a reference fungus. Fungi which gave better yields than *P. versicolor,* i.e., yields of reducing sugars better than 20% (0.2 g sugar per gram straw) and yields of glucose better than 10% (0.1 g glucose/g straw), were considered potentially useful for pretreatment purposes, i.e., to have a positive pretreatment effect. The fungi *Fomes fomentarius* 40, *F. fomentarius* 80, *Polyporus zonatus* 43, *P. zonatus* 81, *Fomes igniarius* 46, *Polyporus brumalis* 71, *Lenzites betulina* 84, *Sporotrichum pulverulentum, Pycnoporus cinnabarinus* A-360, and an unidentified strain 59 did not exhibit any positive pretreatment effect. The most promising fungi were the two *Pleurotus* spp. and *P. cinnabarinus* 115. Pretreatment of straw by these and some other fungi was studied in more detail.

Table 2. Yields of reducing sugars and glucose after enzymic hydrolysis of fungal-treated straw

Fungus	Growth time, days	Yield g/100 g original dry straw		Glucose % of reducing sugars
		Reducing sugars	Glucose	
Control (uninoculated straw)	–	12.2 ± 1.3[a]	5.1 ± 0.9[a]	42
Pleurotus ostreatus	7	15.4	7.3	47
	14	23.4	12.0	51
	21	20.3[b]	11.4[b]	56
	28	27.4[c]	15.7[c]	57
	35	35.2	26.0	74
Pleurotus sp. 535	7	13.2	6.3	48
	14	23.3	11.9	51
	21	26.0	14.4	55
	28	32.6	18.0	55
Pycnoporus cinnabarinus 115	21	25.8[b]	14.0[b]	54
	28	27.1 ± 2.6[d]	15.5 ± 1.6[d]	57
	35	32.0	26.4	83
Ischnoderma benzoinum 108	21	16.3[b]	8.0[b]	49
	28	24.4[c]	11.8[c]	48
	35	35.9	23.9	67
Phanerochaete sordida 37	21	17.4	7.2	41
	28	21.0	9.1	43
	35	20.3	9.8	48
Phlebia radiata 79	21	20.5	11.2	55
	28	17.6	10.9	62
	35	17.5	13.2	75
Strain 35	21	19.7	7.6	39
	28	21.8	8.8	40
	35	21.5	9.2	43
Strain 41	21	21.2	7.8	37
	28	20.6	9.1	44
	35	19.8	9.2	46
Polyporus versicolor	21	17.4	8.5	49
	28	16.7	8.8	53
	35	14.8[e]	8.5[e]	57

Hydrolysis time 72 h
Values are means of four or five replicates
[a] Means ± SD of 16 separate experiments
[b] Means of two separate experiments
[c] Means of three separate experiments
[d] Means ± SD of four separate experiments
[e] Hydrolysis time 48 h

Figure 1 shows the results of hydrolysis after 20, 28, and 35 days cultivation with several of the potentially most promising fungi. The best results were obtained after cultivation of *P. cinnabarinus* 115 and *Pleurotus ostreatus* (Fig. 1 and Table 3): yields of reducing sugars amounted to between 335 and 369 g/kg washed straw and of glucose to between 273 and 275 g/kg straw. *Ischnoderma benzoinum* 108 grew very slowly but gave good results after 35 days with about 50% of the substrate being converted to reducing sugars following hydrolysis. Treatment with *P. cinnabarinus* 115 resulted in a relatively large amount of glucose (83% of reducing sugars) in hydrolysates (Table 3). This fungus apparently used carbohydrates other than cellulose for growth and lignin degradation.

Figure 1 also shows the total amount of solubilized material following hydrolysis, i.e., the material passing through filter paper during washing. The composition of this material was not determined.

The concentrations of reducing sugars and glucose produced on hydrolysis increased with increasing pretreatment times (Figs. 1 and 2A, B). Treatment with both *Pleurotus* fungi produced sugar

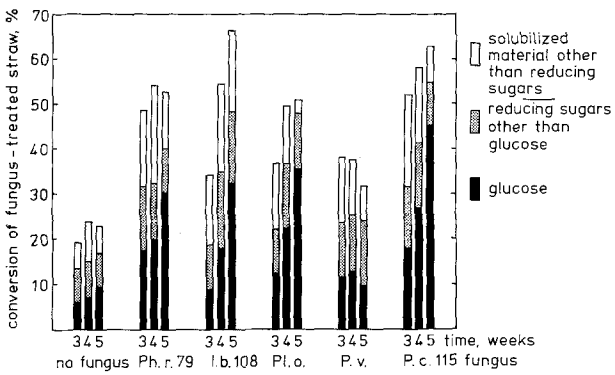

Fig. 1. Yields of glucose, reducing sugars other than glucose and water-soluble material from fungus-pretreated straw after hydrolysis by *Trichoderma reesei* cellulase. Percentage yields are based on the dry weight of straw after fungal growth and washing with water. Growth times of the fungi were 3, 4, and 5 weeks at 28° C. For abbreviations of the fungi see Table 1

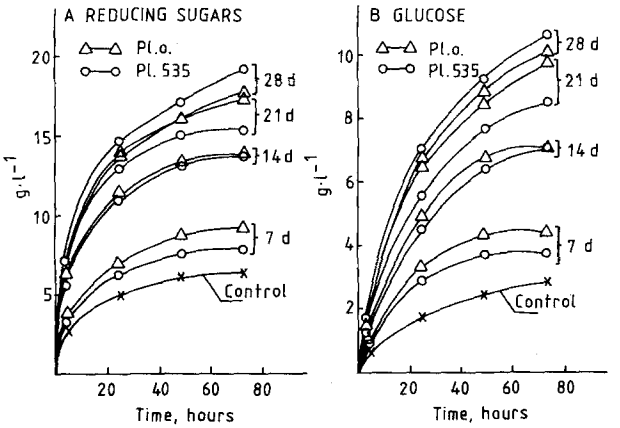

Fig. 2A–B. Hydrolysis of fungus-treated wheat straw by *Trichoderma reesei* cellulase: Concentration of sugars in hydrolysates (**A** = reducing sugars; **B** = glucose). △———△ *Pleurotus ostreatus;* ○———○ *Pleurotus* sp. 535. Growth times of the fungi were 1, 2, 3, and 4 weeks at 28° C. Uninoculated controls were kept for 2 weeks at 28° C. For conditions of hydrolysis and other details, see Materials and methods

Table 3. Yields and concentrations of sugars from straw after hydrolysis by *Trichoderma reesei* cellulase following pretreatment with fungi and/or alkali

Fungus	Growth time, days	NaOH treatment g NaOH/ g straw	Reducing sugars[a]		Glucose[a]	
			Yield, g/100 g straw	Concentration ($g \cdot l^{-1}$)	Yield, g/100 g straw	Concentration ($g \cdot l^{-1}$)
No fungus	–	–	16.9[b]	9.5 ± 1.2[b]	9.3[b]	5.2 ± 0.5[b]
No fungus	–	0.4	40.7[c]	22.2 ± 1.9[c]	20.5[c]	11.1 ± 2.1[c]
Ischnoderma benzoinum 108	35	–	37.6	21.2 ± 2.3	25.0	14.0 ± 1.6
Pleurotus ostreatus	35	–	36.9	20.7 ± 1.1	27.3	15.3 ± 0.7
Pycnoporus cinnabarinus 115	35	–	33.5	18.8 ± 0.7	27.7	15.5 ± 1.0
Ischnoderma benzoinum 108	14	0.4	38.8	20.9 ± 0.2	19.0	10.2 ± 1.4
Pleurotus ostreatus	14	0.4	30.8	16.6 ± 0.3	9.0	4.9 ± 0.2
Pycnoporus cinnabarinus 115	14	0.4	34.3	18.4 ± 0.3	18.3	9.9 ± 0.6

[a] Values are means of five replicates (*I. benzoinum* 108 and *P. cinnabarinus* 115 with NaOH) or four replicates (others) ± SD
[b] Uninoculated straw was kept at 28° C for 35 days
[c] Means of two separate experiments, five replicates in each

concentrations approximately three times higher than those obtained from controls without fungal treatment. After pretreatment (28 days cultivation time) with *P. cinnabarinus* 115 the concentrations of reducing sugars and glucose were 1.5% ($15 g \cdot l^{-1}$) and 1% ($10 g \cdot l^{-1}$), respectively. Somewhat lower values were obtained with *Ischnoderma benzoinum* 108 and *Phanerochaete sordida* 37.

After fungal cultivation the straw residues were washed and autoclaved for 10 min at 115° C in order to inactivate the fungus. The washing and mild heat treatment did not markedly improve the hydrolysis results after pretreatment with *P. cinnabarinus* 115 and *P. ostreatus*, but slightly better results were obtained with *Ischnoderma benzoinum* 108 (data not shown).

Combined Fungal and Alkali Treatment

A rather drastic alkali pretreatment was applied, in which straw was heated for 10 min at 115° C with 2% (w/v) sodium hydroxide (0.4 g NaOH/g straw). The straw was then thoroughly washed with distilled water, which at least partially removed xylan and other hemicellulose. The residue was hydrolysed with *Trichoderma reesei* cellulase and was readily converted to reducing sugars (67.4%) and glucose

(33.4%) in a 72 h hydrolysis. Concentrations of sugars in the hydrolysates were 22.2 ± 1.9 g · l^{-1} reducing sugars and 11.1 ± 2.1 g · l^{-1} glucose (10 replicates) (Table 3).

Combining fungal and alkali pretreatments did not prove to be more efficient than either treatment alone (Table 3). In combined treatment experiments fungi were cultivated for only 1, 2, or 3 weeks, after which the straw residues were treated with alkali solution. Most probably the strong alkali treatment totally masked the effect of the fungi. Milder alkali treatments were not studied. The advantage of fungal treatment compared with alkali treatment alone was that it resulted in mixtures containing a higher proportion of glucose.

Effect of Atmosphere

The effect of oxygen atmosphere on fungal pretreatment was studied using *P. sordida* 37 and *P. cinnabarinus* 115, which in ^{14}C-lignin degradation studies were shown to exhibit different responses to oxygen (Hatakka and Uusi-Rauva 1983). Oxygen atmosphere shortened the time needed to obtain a positive pretreatment effect by approximately 1 week. For example, after 14 days growth of *P. sordida* 37 in an oxygen atmosphere the concentration of reducing sugars after hydrolysis was 8.8 g · l^{-1}, but only 5.4 g · l^{-1} when the fungal pretreatment was carried out in a normal atmosphere (Fig. 3B). The same phenomenon was observed with *P. cinnabarinus* 115, but the effect levelled out after 21 days of fungal pretreatment (Fig. 3A).

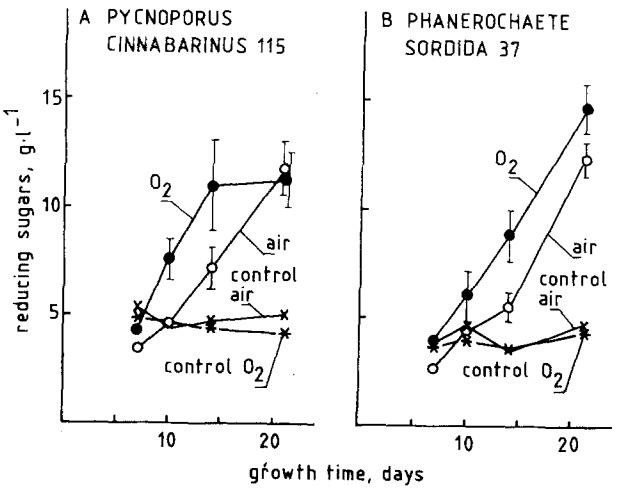

Fig. 3A–B. Concentration of reducing sugars following hydrolysis of straw residue by *Trichoderma reesei* cellulase after growth of *Pycnoporus cinnabarinus* 115 (**A**) and *Phanerochaete sordida* 37 (**B**) in 100% oxygen and in an air atmosphere. Hydrolysis time 72 h. Points represent means of four replicate cultures and bars represent standard deviations. ●———● 100% O_2; ○———○ air; *———* uninoculated control in 100% O_2; ×———× uninoculated control in air

Discussion

The rate and extent of lignin degradation by white-rot fungi is strongly oxygen-dependent (Kirk et al. 1978; Reid and Seifert 1982; Hatakka and Uusi-Rauva 1983; Leisola et al. 1983). Poor results obtained when milled straw was used as a substrate in fungal cultivations (data not shown) probably reflected poor oxygen accessibility. When cultures were periodically flushed with oxygen the treatment time could be shortened by about 1 week with the two fungi tested (Figs. 3A–B). The efficiency of treatment was also enhanced in the case of *Phanerochaete sordida* 37 when an oxygen atmosphere replaced air. Thus, an oxygen atmosphere had more effect on the treatment with *P. sordida* 37 compared with *Pycnoporus cinnabarinus* 115, which may reflect their differing abilities to use the available oxygen for lignin degradation. *P. sordida* 37 degraded only 1.5% of the ^{14}C-lignin of poplar wood to $^{14}CO_2$ in air after 27 days, whereas *P. cinnabarinus* 115 degraded 14.4% after 27 days under the same conditions (Hatakka and Uusi-Rauva 1983).

It is known that lignin degradation by white-rot fungi requires a more readily metabolized carbon co-substrate (Ander and Eriksson 1975; Hiroi and Eriksson 1976; Kirk et al. 1976). Thus, the cellulolytic and hemicellulolytic activities of the fungus play an important role in lignin degradation when the fungus is grown on a lignocellulosic substrate such as straw or wood. A weak cellulolytic activity, as assayed by the Rautela-Cowling test, did not necessarily mean that the fungus was suitable for the pretreatment of straw. On the other hand, relatively high cellulolytic activity as found in *Phlebia radiata* 79 and *Ischnoderma benzoinum* 108, did not rule out good pretreatment results. However, selective removal of lignin may occur at the expense of wood or straw carbohydrates other than cellulose. Results with *Pycnoporus cinnabarinus* 115 indicated a preference for xylan in straw, since after pretreatment with this fungus a high proportion of glucose was obtained in hydrolysates.

The sugar yields following enzymic hydrolysis were lower if after fungal growth washing and autoclaving were omitted. The cellulolytic enzymes from white-rot fungi apparently did not contribute to the subsequent saccharification of the straw residue by *Trichoderma reesei* cellulase. Water-soluble lignin degradation products (Reid et al. 1982; Hatakka and Uusi-Rauva 1982; 1983) may have repressed the

action of cellulolytic and hemicellulolytic enzymes (Váradi 1972).

The selectivity of attack may also be dependent on the wood species (Kirk and Moore 1972). For example, *Pleurotus ostreatus* was apparently suitable for pretreatment of straw, although it did not preferentially remove lignin from hardwood (birch) (Kirk and Moore 1972) of softwood (pine) (Ander and Eriksson 1977). However, the fungus efficiently degraded lignin in hardwood determined by measuring evolved $^{14}CO_2$ from ^{14}C-lignin of poplar wood (Hatakka and Uusi-Rauva 1983). In our experiments on straw pretreatment both *Pleurotus* spp. increased the sugar yields from straw on subsequent enzymic hydrolysis. In earlier experiments *P. ostreatus* (Kaneshiro 1977) and two other *Pleurotus* spp. (Zadražil 1977; 1980) were shown to improve in vitro rumen digestibility of straw. Detroy et al. (1980) also enhanced the extent to which straw was hydrolysed by cellulase by pretreating with *P. ostreatus* for 50 days and obtained 275 g glucose/kg fungus-treated wheat straw. In the work described here about 350 g glucose/kg fungus-treated straw was obtained (Fig. 1) following enzymic hydrolysis after 35 days cultivation of *P. ostreatus*.

It must be remembered that the efficiency of saccharification depends heavily on the efficiency of the cellulase enzyme and not only on the pretreatment. In this study only relative values are presented to evaluate fungal treatment with respect to untreated and alkali-treated straw. When the results of this investigation are compared with recent studies on the enzymic saccharification of wheat straw carbohydrates treated by various chemical means, it is evident that similar efficiencies may also be achieved by biological pretreatment. Fan et al. (1981) compared various chemical and physical treatments of wheat straw and found that treatment with alkali (2 h at 129° C, 2.57 atm, 1% NaOH, 0.1 g NaOH/g straw) was the most promising method for large-scale applications. The yield of reducing sugars from straw was 341 g/kg straw. In our alkali treatment the yield of reducing sugars was 407 g/kg straw (original, washed) and the yield of glucose 205 g/kg straw (Table 3). This indicates that the efficiency of our combined alkali treatment and enzymic hydrolysis method, i.e., our reference treatment, was adequate in comparison. Our fungal pretreatment was almost as efficient as the alkali treatment (Table 2): *P. ostreatus* treatment (5 weeks growth) resulted in 352 g reducing sugars/kg straw and 260 g glucose/kg straw after hydrolysis.

The most promising fungi for straw pretreatment were the two *Pleurotus* spp. tested and *P. cinnabarinus* 115. *P. radiata* 79 may have potential for softwood treatment, since it readily degraded guaiacyl-type synthetic lignin (^{14}C-(RING)-DHP) (Hatakka et al. 1983). *Ischnoderma benzoinum* 108 grew slowly, especially at temperatures above 25° C, which makes it biotechnologically less suitable. After fungal pretreatment the hydrolysates often contained relatively high amounts of glucose, when compared, for example, with the results of alkali pretreatment experiments. It has been stressed, for example by Saddler et al. (1982), that any system which can convert the greater part of the cellulose to glucose is desirable.

On the basis of these results, the economic feasibility of fungal pretreatment appears poor, mainly due to the long treatment times required. The best results were obtained after the longest growth time adopted, which was 5 weeks. However, rapid progress in lignin biodegradation (Crawford 1981) resulting from optimization of cultivation conditions (cf. Kirk et al. 1978) has, in recent years, made it possible to greatly accelerate lignin degradation even in natural substrates (for example, see Reid and Seifert 1982; Hatakka and Uusi-Rauva 1983; Hatakka et al. 1983). Therefore, it may be possible in the near future to improve the selectivity of attack on lignin by certain white-rot fungi by choosing suitable conditions which stimulate lignin degradation while at the same time repressing degradation of polysaccharides.

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