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Screening of white-rot fungi on (^{14}C)lignin-labelled and (^{14}C)whole-labelled wheat straw

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Summary. 74 Basidiomycetes have been tested for ligninolytic capability on (^{14}C)lignin-labelled wheat straw. Fifteen strains were selected and tested more accurately for ligninolytic activity and the capacity to degrade wheat straw. The asymptote, inflexion point and degradation rate were determined using a model approach. The fungi exhibited very different responses with respect to lignin biodegradation: high asymptote for *Pleurotus ostreatus* (77%), low inflexion points for *Sporotrichum pulverulentum* Nov. (6.1 days) and *Pycnoporus* spp. (2.7 to 4.7 days) with high and slow degradation rates, respectively (0.91% and 0.45% of $^{14}CO_2$ release/day). Degradation values for (^{14}C)whole-labelled wheat straw exhibited less variation. Finally, the strains *Pleurotus ostreatus, Dichomitus squalens* and *Bjerkandera adusta* showed the highest selectivity of lignin removal.

Introduction

The white-rot fungi (class of Basidiomycetes) are the most efficient of all known lignin degraders. Their ligninolytic capabilities for upgrading lignocellulosic materials as alternatives to non-biological treatments have been explored (Kirk and Moore 1972; Zadrazil 1977). However, almost all efforts at microbial delignification have focussed on *Phanerochaete chrysosporium* (syn. = *Sporotrichum pulverulentum*) even though some other white-rot fungi have recently been investigated (Reid and Seifert 1982; Hatakka and Uusi-Rauva 1983).

Most lignin biodegradation studies have been conducted using wood or synthetic lignins as substrates (Reid and Seifert 1982; Kirk et al. 1975), while degradation studies using *Graminae* lignins are relatively few. Even though, Monocot lignins possess some particular structural and chemical characteristics such as the presence of significant amounts of phenolic acids (Hartley 1972; Higuchi et al. 1967) and their high solubility in sodium hydroxide (Beckmann et al. 1923), which may influence biodegradation patterns.

In the first part of this study, we have examined the ability of 74 Basidiomycetes to degrade (^{14}C)lignin-labelled wheat straw to $^{14}CO_2$. Subsequently, the 15 strains with relatively high ligninolytic activity have been defined in more detail in terms of lignin and total degradation capacities. Degradation kinetics for the different components studied have been compared in terms of asymptote, degradation rate, inflexion point time and degradation yield using a mathematical model.

Materials and methods

Preparation of (^{14}C)lignin-labelled wheat straw and (^{14}C)whole-labelled wheat straw. (^{14}C)lignin-labelled wheat straw was prepared by feeding 4625 KBq of L-(U-^{14}C)-phenylalanine per wheat stem (*Triticum aestivum* var. Champlein) harvested at the blooming period; 15–20 wheat plants were cut under the last node and the radioactive precursor administrated according to the stem infusion method (Alibert and Boudet 1979). After 72 h of photosynthesis (24 h of photoperiod), the internodes (stems + leaves) were harvested, finely ground and extracted to remove water and organic-soluble compounds (Crawford and Crawford 1976). A proteinase digestion was carried out to eliminate radioactive proteins, as described previously (Odier et al. 1981). This treatment released 10% of the total radioactivity administered.

(^{14}C)whole-labelled wheat straw was kindly provided by Dr. Pinto and prepared according to Pinto (1981).

Specific radioactivities of samples were determined by combustion to $^{14}CO_2$ using an Oxymat Intertechnique apparatus followed by scintillation counting. Klason lignin and sol-

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uble radioactivity were determined according to the Jarrige method (Jarrige 1961).

The phenolic acid content was measured after saponification (2 h, 35 °C) of 50 mg of (^{14}C)lignin-labelled wheat straw with 2 N NaOH under argon. The mixture was filtered and two aliquots of 1 ml of the soluble fraction taken to estimate total ^{14}C solubilization. The alkali extract was then neutralized to pH 7.0 with 6 N HCl. After extraction with 40 ml diethyl ether (3 times), the solution was acidified to pH 2.0 and again extracted with ether. The ethered extracts of neutral and acidic fractions were evaporated to dryness and the residue resuspended in 1 ml methanol for ^{14}C determination by scintillation counting.

Fungi and inocula. The fungi used in this study are reported in Fig. 1. They have been separated into three groups, according to mean optimum growth temperature, determined by incubating each fungus on malt extract agar plates (in triplicate) at 25°, 30°, and 37 °C and recording the time required for the mycelium to extend a distance of 25 mm.

Sporotrichum pulverulentum Nov. ATCC 32629 was kindly provided by Prof. K. E. Eriksson (STFI, Sweden). *Pycnoporus cinnabarinus* (Jacq. ex. Fr.)Karst. strain 115 was obtained by courtesy of Dr. A. Hatakka (Univ. of Helsinki, Finland). Strains Cuba 11 and Nancon are two unidentified fungi provided by Dr. R. Contreras (CENIC, Cuba). The strains of *Fomes annosus* were donated by Dr. C. Delatour (CNRF, France) and *Armillaria* species were obtained from Dr. Jacques Félix (Université Pierre et Marie Curie, France).

Test cultures were inoculated with two circles of agar (6 mm diameter) taken 2 mm from the edge of a fungal culture grown on a 2% malt agar plate.

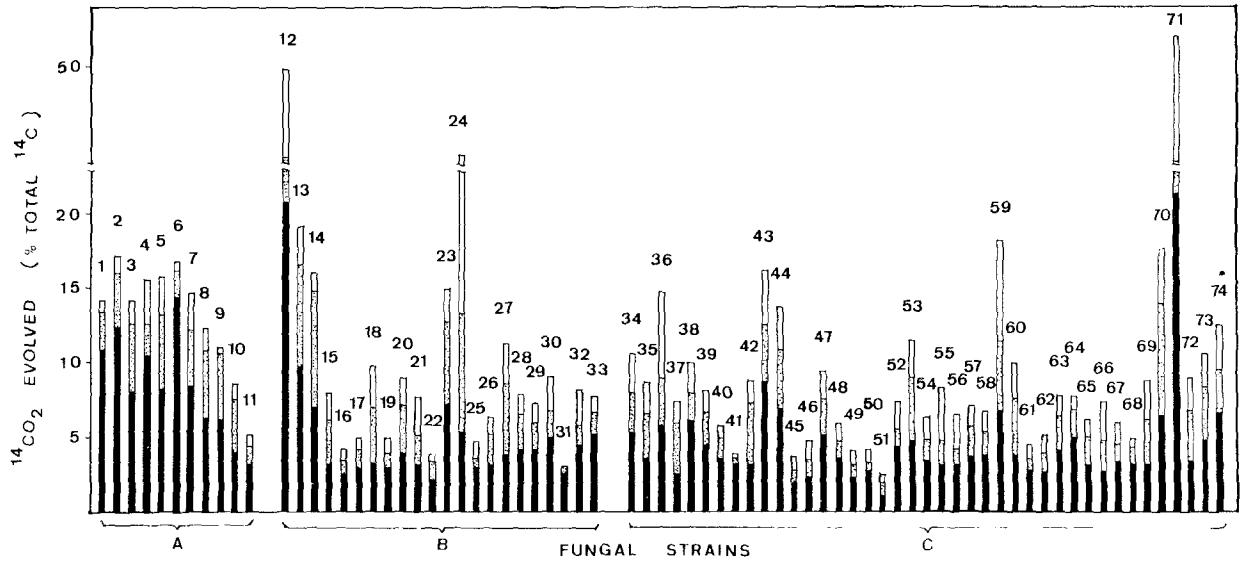

Fig. 1. Evolution of $^{14}CO_2$ after 3 (■), 6 (▨) and 10 (□) weeks of cultivation of 74 white-rot fungi on (^{14}C)lignin-labelled wheat straw (for cultivation methods, see Materials and methods).

Group A (37 °C): 1. *Sporotrichum pulverulentum* Nov. ATCC 326.29; 2—3. Unidentified strains Nancon and Cuba 11; 4. *Dichomitus squalens* (Karst) Reid CBS 432.34; 5—6. *Pycnoporus cinnabarinus* (Jacq ex Fr) Karst strains 115 and CBS 311.33; 7—8. *P. sanguineus* (L ex Fr) Murr. strains DFP 8732 and CBS 357.63; 9. *P. coccineus* (Fr) Bond. and Sing. CBS 355.63; 10. *Poria cinnerascens* Bres S 130; 11. *Phellinus contiguus* (Pers ex Fr) Pat. CBS 335.49.

Group B (30 °C): 12. *Vararia effuscata* S 408; 13. *Cyathus stercoreus* (Schw) de Toni NRRL 6473; 14. *Bjerkandera adusta* (Wild ex Fr) Karst CBS 595.79; 15—18. *Poria subvermispora* Pilàt strains CBS 347.63, DAOM 21398, DAOM 31816 and DAOM 31817; 19—20. *P. subacida* (Peck) Sacc Strains FPPL 104 and CBS 374.52; 21. *P. lindblajii* (Berck) Cooke CBS 290.71; 22. *P. vincta* (Berck) Cooke FRI 1041; 23. *Polyporus versicolor* (L ex Fr) Fr. CBS 100.29; 24. *P. resinosus* Fr. CBS 325.29; 25. *P. betulinus* (Bull ex Fr) Karst CBS 378.51; 26. *P. abietinus* Dicks ex Fr Donk CBS 324.29; 27. *P. berkeleyi* Fr. CBS 312.36; 28. *Trametes hirsuta* (Nulf ex Fr) CBS 128.14; 29. *T. pini* (Thore ex Fr) Karst CBS 210.36; 30. *Fomes ulmarius* (Son ex Fr) Gill CBS 186.60; 31. *F. durissimus* (Bolt ex Fr) Kum. FRI 316; 32. *F. robustus* Karst. DFP 9327; 33. *Stereum frustulatum* Fr. DFP 120.47.

Group C (25 °C): 34. *Peniophora gigantea* (Fr) Mas. CBS 262.33; 35—37. *Phlebia radiata* Fr. strains DAOM 229.63, DAOM 523.09 and CBS 287.73; 38. *Armillaria mellea* (Fr ex Vahl) Karst.; 39. *A. astoyae* Romagn.; 40. *A. bulbosa* (Barba) Romagn.; 41. *A. mellea* var lutea Secretan.; 42. *Ganoderma applanatum* (Pers ex Wallr) Pat. CBS 250.61; 43. *Mammaria echinobotryoïdes* Ces. CBS 545.69; 44. *Psilocybe mexicana* (Fr) Quil. CBS 609.79; 45. *Merulius lacrymans* (Wulfen ex Fr) CBS 217.29; 46. *Peniophora cremea* Bres. CBS 109.20; 47—48. *Agaricus bisporus* (Lange) Sing. var. avellaneus CBS 204.49 and var. albidus CBS 136.42; 49. *Mycena galopus* (Pers ex Fr) Kum. CBS 500.79; 50. *M. lactea* (Pers ex Fr) Kum. CBS 234.47; 51. *M. sanguinolenta* (Alb and Schw ex Fr) Kum. CBS 518.79; 52. *Collybia dryophila* (Bull ex Fr) Kum. CBS 177.48; 53. *Marasmius peronatus* (Bolt ex Fr) Fr. CBS 223.37; 54. *M. scorodinius* (Fr) Fr. CBS 251.48; 55. *Hypholoma fasciculare* (Hudson ex Fr) Kum. CBS 177.48; 56. *H. capnoïdes* Fr. CBS 568.79; 57. *Clytocybe cerussata* (Fr) Quél. CBS 124.46; 58. *C. odora* (Bull) Fr. CBS 128.46; 59. *Lentinus edodes* (Berk) Sing. CBS 454.59; 60. *Polyporus frondosus* Dicks ex Fr CBS 317.29; 61. *P. pergamenus* (Fr) Bond. and Sing. CBS 324.29; 62. *Lenzites betulina* (L ex Fr) CBS 222.33; 63. *Pholiota mutabilis* (Schaeffer ex Fr) Kum. CBS 444.79; 64. *P. adiposa* (Fr) Kum. CBS 279.29; 65—69. *Fomes annosus* (Fr) Cooke strains 4, 38, 45, 43 and 78; 70. *Pleurotus ostreatus* (Jacq. ex Fr) Kum. CBS 342.69; 71. *Pl. cornucopiae* (Paul ex Fr) Rol. CBS 383.80; 72. *Panellus serotinus* (Pers ex Fr) Kühner CBS 581.79; 73—74. *Stropharia ferrii* Bres. CBS 789.73 and CBS 410.76.

Study of growth conditions. Growth parameters (optimal temperature and pH) of the fifteen fungi selected for further study after screening for ligninolytic ability, were determined on 5% ball-milled wheat straw broth agar. Growth rates were determined as described by Boddy (1983).

Optimum pH values were determined over the range 4.0 to 6.5. Plates were incubated at 25°, 30° or 37°C, according to the previously determined mean optimal temperature of each strain.

Media and cultivation methods. Liquid media contained (per liter): KH_2PO_4, 0.6 g; K_2HPO_4, 0.4 g; $MgSO_4 \cdot 7H_2O$, 0.5 g; $CaCl_2 \cdot 2H_2O$, 74 mg; ferric citrate, 12 mg; $ZnSO_4 \cdot 7H_2O$, 6.6 mg; $MnSO_4$, 5.0 mg; $CoCl_2 \cdot 6H_2O$, 1.0 mg; $CuSO_4 \cdot 5H_2O$, 1.0 mg; thiamine hydrochloride, 0.1 mg. 2,2'-dimethylsuccinate (10 mM) was used as a buffer; 1.2 mM nitrogen as asparagine (46.6 mg) and NH_4NO_3 (23.3 mg) were added to the cultures.

Screening experiments were conducted in duplicate, using 10 ml static liquid cultures in 125 ml conical flasks incubated at the gross optimum growth temperature of each fungal strain (25°, 30° or 37°C). Amounts of 15 mg of 40-mesh ground wheat straw (*Triticum aestivum* var. Champlein) and 5 mg of (^{14}C)lignin-labelled wheat straw (630 Bq) were added to each flask and autoclaved for 20 min at 120°C with 5 ml of distilled water. Thereafter, 5 ml of a double strength concentrated filter-sterilised mineral medium, adjusted to pH 5.0, was added. After inoculation, the flasks were tightly closed with sterile rubber stoppers equipped with suspended 3-ml glass tubes containing 1 ml of 2 N NaOH, to absorb released CO_2 (Haider and Trojanowsky 1975). The $^{14}CO_2$ produced was collected and quantified as described by Ander et al. (1980). The yield of ^{14}C trapping was quantitative as tested with $Na_2{}^{14}CO_3$. Collection tubes for $^{14}CO_2$ were changed after 3, 6 and 10 weeks of incubation.

To assess ligninolytic and total wheat straw degradation abilities, four replicate cultures containing 80 mg of ground wheat straw and 7—10 mg of (^{14}C)lignin-labelled (1200 Bq) or (^{14}C)whole-labelled (2500 Bq) wheat straw were used for each strain tested. Nitrogen was omitted. $^{14}CO_2$ collection tubes were replaced after 1, 2, 4, 6, 8, 11 and 15 weeks of incubation. The 15 week-old cultures were filtered through 0.45 μ membrane filters (Millipore) and 1 ml of the filtrate assayed for soluble radioactivity ^{14}C.

Mathematical modelisation. The Von Berthalanfy model has been applied to the kinetics of lignin and wheat straw degradation by the 15 fungi selected for further study in order to allow comparison between the strains. This model has the following mathematical expression:

$$f(t) = (\theta_1 + \theta_2 \theta_3^t)^{\theta_4}$$

The constraint $f(0) = 0$ has been introduced in order to impose an inflection point in the curve. Indeed, in some cases, the small number of observations at the beginning of degradation did not allow for a sigmoid curve unless this constraint was imposed.

These considerations necessitated the use of the modified Von Berthalanfy model

$$f(t) = (\theta_1 - \theta_1 \theta_2^t)^{\theta_3}.$$

The parameters θ_1, θ_2, θ_3 were estimated by the mean square method. The computations were made with the software UWHAUS (Bachacou et al. 1981) which uses the iterative procedure of Gauss-Marquardt (1963). It was determined experimentally that a choice of initial values for θ_1, θ_2 and θ_3, within the range (3—10), (0—1) and (1—2), respectively, gave the best results. Convergence was generally obtained within twenty iterations.

The fit between predicted and observed values is given by the determination coefficient R^2. The closer R^2 is to 1, the better the fit. Confidence intervals for the predicted value of $f(t)$ have been constructed from the residual variance. Asymptotic confidence intervals were also obtained from the asymptotic variances of the estimates.

However, θ_1, θ_2, θ_3 have no biological meaning. Therefore, the three parameters that fully characterize the curve, that is, the asymptote a, the time required to reach the inflexion point, b, and the degradation rate at this time $c = f'(b)$, have been derived from θ_1, θ_2, θ_3 by the following formulae:

$$a = \theta_1{}^{\theta_3}$$

$$b = -\log\theta_3 / \log\theta_2$$

$$c = -\theta_1{}^{\theta_3} \log\theta_2 (1 - 1/\theta_3)^{\theta_3 - 1}.$$

For each model, the asymptotic variances of a, b and c were calculated using the linearization technique (Kendall and Stuart 1977).

Results and discussion

The characteristics of the (^{14}C)labelled lignocelluloses are given in Table 1. A significant amount of

Table 1. Distribution of ^{14}C in (^{14}C)lignin-labelled wheat straw and (^{14}C) whole-labelled wheat straw

	% of total radioactivity				
	Specific radioactivity Bq/mg	Solubles[a]	Acid-soluble ^{14}C after		Klason lignin[d]
			5% H_2SO_4	72% H_2SO_4[c]	
(^{14}C)Lignin-labelled wheat straw	125.3	—	27.2	3.0	68.1
(^{14}C)Whole-labelled wheat straw[b]	251.7	17.6 (12.2)	32.1 (23.8)	32.8 (36.3)	20.1 (14.6)

[a] solubles = (100-extraction 24 h with ethanol-toluene 1:1, 8 h with ethanol 100% and 72 h with water)
[b] values in parentheses indicate centesimal composition
[c] hydrolysis of the 5% H_2SO_4 residues
[d] determined after combustion with Oxymat Intertechnique

Table 2. Growth characteristics and kinetic parameters associated with (^{14}C)whole-labelled wheat straw mineralization in static liquid cultures by white-rot fungi (for explanation see *Material and methods*)

Fungus	Temp. (°C)	pH	Growth rate (mm/day)	Asymptote (% $^{14}CO_2$)	Inflexion point (days)	Degradation[a,b] rate (% $^{14}CO_2$/day)
Pleurotus ostreatus	29	5.7	7.5	65.5	11.5	0.93
Pleurotus cornucopiae	23	5.6	6.1	53.2	7.7	1.11
Strain Cuba 11	38	4.6	28.6	57.4	4.3	2.54
Strain Nancon	37	4.5	27.7	59.8	4.8	2.65
Sporotrichum pulverulentum	39	4.6	28.6	52.1	4.4	1.63
Cyathus stercoreus	32	5.2	10.0	61.9	9.3	1.23
Dichomitus squalens	34	4.6	14.1	41.4	8.3	0.98
Bjerkandera adusta	30	4.9	17.0	50.4	7.5	1.32
Vararia effuscata	28	4.8	14.1	61.3	6.5	1.06
Pycnoporus cinnabarinus	36	4.6	12.6	46.6	3.3	1.20
Pycnoporus cinnabarinus 115	36	4.6	12.4	48.3	4.0	1.16
Pycnoporus sanguineus DFP 8732	38	4.5	12.5	43.7	4.2	1.16
Lentinus edodes	28	NT	3.7	55.0	28.9	0.58
Polyporus resinosus	25	NT	4.3	36.7	7.0	0.88
Phlebia radiata DAOM 53209	26	5.0	11.7	39.8	9.1	1.00
Gleophyllum trabeum	30	NT	8.5	38.9	5.3	1.03

[a] At inflexion point
[b] Standard deviation ranged between 2% and 6% of the mean

(^{14}C)lignin-labelled wheat straw was solubilized by 5% sulphuric acid hydrolysis. The presence of acid-soluble lignin has already been described for woods (Crawford et al. 1977; Lai and Sarkanen 1971). However, very little radioactivity was solubilized after hydrolysis with 72% H_2SO_4, suggesting that ^{14}C-lignin was mainly associated with 5% H_2SO_4 soluble components, that is, hemicelluloses. Acid hydrolysis followed by thin layer electrophoresis to separate aromatic amino acids as described earlier (Odier et al. 1981), showed that no radioactive proteins were present in this substrate. Phenolic acids represented a significant fraction of the ^{14}C-lignin material (17.6% of total ^{14}C). Furthermore, although 58.4% of the ^{14}C was solubilized after alkali treatment, neutral phenols constituted only 2.3% of this material. Distribution of ^{14}C in (^{14}C)whole-labelled wheat straw in non-structural components (organic and water-solubles), structural polysaccharides and Klason lignin, grossly reflects chemical composition (Table 1). Water-soluble components are known to represent a significant proportion of straws (Cowling and Kirk 1976). Therefore this material was used unextracted.

When grown on ^{14}C(lignin)-labelled wheat straw, the majority of the fungal strains tested released 8—14% of the label as $^{14}CO_2$ after 10 weeks incubation (Fig. 1). Up to 52% $^{14}CO_2$ was released by the most active of the 15 ligninolytic strains selected for further study (Table 2). In most cases, evolution of $^{14}CO_2$ was almost complete after 6 weeks cultivation. Differences were observed in total $^{14}CO_2$ production among different strains of the same species, for example *Phlebia radiata* and *Fomes annosus*. Several of the more active lignin degrading strains had an optimum growth temperature of 37° C.

The lignin degrading capacity may be even higher than that shown, since the optimal temperature for growth may not always be the same as the optimal temperature for lignin degradation. Indeed, Hatakka and Uusi-Rauva (1983) have reported increased lignin degradation when various thermotolerant fungi were cultivated at temperatures below the optimum growth temperature.

Similarly, the pH routinely adopted for growth of the fungal strains (pH 5.0) might not, in some cases, have allowed maximum expression of ligninolytic activity. For example, the optimum pH for lignin degradation by *Phanerochaete chrysosporium* is 4.0—4.5 (Kirk et al. 1978). However, preliminary experiments suggested that pH 5.0 would support reasonable growth of all the fungal strains examined. To some extent, this appears to have been confirmed by the optimum pH values for growth of the 15 fungi selected for further study. These ranged between 4.5 and 5.7 (Table 2).

For comparison purposes, a model has been applied to the kinetics of lignin and wheat straw degradation of each fungus. Typical results are il-

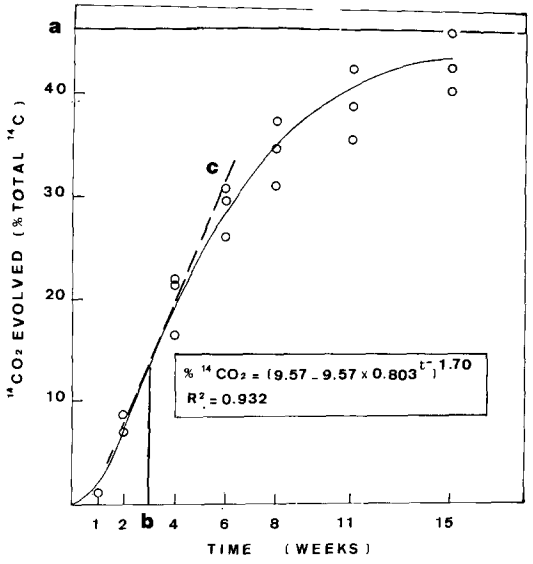

Fig. 2. Example of modified Berthalanfy model describing the kinetics of (^{14}C)lignin-labelled wheat straw degradation with *Cyathus stercoreus* in static liquid cultures. Each culture contained 10 mg of (^{14}C)lignin-labelled wheat straw and 80 mg of wheat straw in 10 ml of medium. Values represent 3—4 individual cultures of each fungus tested

lustrated in Fig. 2. The model applied proved to be adequate, as shown by the low residual standard error ($\sigma = 2.4$). The high value of the coefficient of determination R^2 (0.932) confirms the good fit. Determination of kinetic parameters showed that 15 week-old cultures have not yet reached the asymptote *a* (46.5% of total ^{14}C). A large standard deviation would be obtained (Table 3) if the asymptote was reached some considerable time after the final measurement was made. The inflexion point time *b* (16.9 days) gives the time necessary to reach *c*, the maximal rate of $^{14}CO_2$ release per day (0.78).

The 15 more active lignin-degrading strains are presented in Table 2 together with their respective growth conditions. *Gleophyllum trabeum*, a brown-rot fungus, was also included as a reference. Several of the fungi selected were thermotolerant. The two unidentified strains, Nancon and Cuba 11, and *Sporotrichum pulverulentum* had very similar characteristics and the fastest growth rates. *Lentinus edodes* and *Pleurotus cornucopiae* were found to have the slowest growth rates. Table 2 also shows the various kinetic parameters associated with the mineralization of (^{14}C)whole-labelled wheat straw. With the exception of strains Nancon, Cuba 11 and *S. pulverulentum* which exhibited very high degradation rates, and *L. edodes* which showed a very slow rate of mineralization, almost no differences were observed among the fungal cultures. The coefficients of determination R^2 ranged between 0.92 and 0.99. Nevertheless, the times required to reach maximal mineralization rates correlated well with times re-

Table 3. Kinetic parameters associated with (^{14}C)lignin-labelled wheat straw degradation in static liquid cultures by white-rot fungi (for explanation see *Materials and methods*)

Fungus	Asymptote[a] (% $^{14}CO_2$)	Inflexion point (days)	Degradation[b,c] rate (% $^{14}CO_2$/day)	R^2
Pleurotus ostreatus	77.3± 4.5 (1.2)	24.6±1.0	0.80	0.98
Pleurotus cornucopiae	38.2± 1.5 (0.7)	17.5±0.6	0.95	0.91
Strain Cuba 11	32.2± 1.1 (0.6)	5.2±1.5	1.21	0.89
Strain Nancon	33.3± 0.9 (0.6)	4.1±1.2	1.18	0.96
Sporotrichum pulverulentum	39.9± 1.4 (0.8)	6.1±1.9	0.91	0.78
Cyathus stercoreus	46.5± 1.1 (0.8)	16.9±1.1	0.78	0.93
Dichomitus squalens	39.0± 8.6 (0.9)	11.4±6.7	0.53	0.90
Bjerkandera adusta	57.3± 2.8 (1.1)	12.5±4.3	0.49	0.97
Vararia effuscata	47.0± 2.8 (0.8)	8.8±1.0	0.61	0.99
Pycnoporus cinnabarinus	34.4± 3.9 (0.8)	4.0±3.2	0.53	0.97
Pycnoporus cinnabarinus 115	28.2±11.9 (0.6)	4.7±5.8	0.46	0.96
Pycnoporus sanguineus DFP 8732	19.3± 1.6 (0.4)	2.7±2.8	0.47	0.82
Lentinus edodes	68.4±18.9 (0.2)	50.1±5.9	0.40	0.98
Polyporus resinosus	26.0± 3.1 (0.7)	18.2±1.8	0.37	0.99
Phlebia radiata DAOM 53209	35.8± 3.9 (0.9)	20.4±2.9	0.39	0.96
Gleophyllum trabeum	16.9± 2.1 (0.4)	6.3±7.6	0.31	0.92

[a] Values in parenthesis represent specificity for lignin = $\left(\frac{\text{total } ^{14}CO_2 \text{ from } ^{14}\text{C-lignin}}{\text{total } ^{14}CO_2 \text{ from } ^{14}\text{C-wheat}}\right)$

[b] At inflexion point

[c] Standard deviation ranged between 2% and 6% of the mean

quired to reach ^{14}C-lignin inflexion points (Table 3), although the slower the fungal mineralization rate, the wider the time difference to reach inflexion points.

All the fungi produced similar quantities of ^{14}C water-soluble compounds (25.1 ± 4.8%) after 15 weeks of culture on (^{14}C)whole-labelled wheat straw. *Gleophyllum trabeum* degraded (^{14}C)whole-labelled wheat straw to $^{14}CO_2$ only at moderate rates which may be explained by the low pH (3.1) of these cultures. Indeed, brown-rot fungi are known to accumulate organic acids such as oxalic acid which can reduce the pH of the cultures to values as low as pH 2.0 (Koenigs, 1972). The final pH of the other fungal cultures ranged from 5.3 to 5.8.

The fungi exhibited very different responses with respect to lignin biodegradation (Table 3). Some strains converted large amounts of ^{14}C-lignin to $^{14}CO_2$, although the rate of conversion varied markedly and could be low in the case of *L. edodes,* moderate in cultures of *Bjerkandera adusta* or high with *Pleurotus ostreatus.* However, relatively long incubation times were required to reach maximal degradation rates. Other fungi were able to convert low (*Pycnoporus* spp.) or moderate (strains Nancon, Cuba 11 and *S. pulverulentum*) quantities of ^{14}C-lignin to $^{14}CO_2$, although in these cases lignin biodegradation rates soon decreased as shown by the short time required to reach the inflexion point. However, the maximal rate of $^{14}CO_2$ release could be very high, as for strains Nancon, Cuba 11 and *S. pulverulentum* or moderate as for *Pycnoporus* spp. *G. trabeum* degraded approximately 17% of the ^{14}C-lignin to $^{14}CO_2$, although $^{14}CO_2$ evolution was very slow. Furthermore, only 18.1% of water-soluble radioactivity was found in 15 week-old cultures, as compared with cultures of white-rot fungi which contained much higher amounts of ^{14}C-water-soluble compounds (e.g. 49% for *D. squalens* and *P. cinnabarinus,* 35.6% for *S. pulverulentum,* 42.3% for *C. stercoreus*).

Total $^{14}CO_2$ production from ^{14}C-lignin during this experiment was 2—4 times higher than observed in the general screening. The differences in the C/N ratio could explain this result (Reid, 1979). Indeed, in the first experiment the C/N ratio was rather low (C/N = 36) while in the second one, where no nitrogen was added to the cultures, the C/N ratio was approximately 80; and, in this case, the $^{14}CO_2$ produced was much higher.

Under the conditions used here, total production of $^{14}CO_2$ from (^{14}C)lignin-labeled wheat straw are quite high with respect to the total $^{14}CO_2$ evolved from other lignin-labelled lignocelluloses (see for example Hatakka and Uusi-Rauva, 1983) suggesting that grass lignins are more easily degraded than wood lignins by white-rot fungi.

Finally, the selectivity towards lignin shown by the different fungi was estimated by dividing total $^{14}CO_2$ evolved from (^{14}C)lignin-labelled by total $^{14}CO_2$ evolved from (^{14}C)whole-labelled wheat straw (Table 3). It is evident that some strains such as *P. ostreatus* and *B. adusta* are highly specific lignin degraders, and that other strains which include *Vararia effuscata* and *C. stercoreus* exhibit significant but lower specificity. Finally, a third group of strains exemplified by strain Nancon or *Pycnoporus sanguineus* appear to be non-specific.

On the basis of the different criteria adopted, 8 fungi (*P. cinnabarinus, P. ostreatus, S. pulverulentum,* strain Nancon, *C. stercoreus, D. squalens, V. effuscata* and *B. adusta*) have been selected for further study under semi-solid conditions.

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Note added in proof: Strains Cuba 11 and Nancon have been identified as *Phanerochaete chrysosporium* strains.

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