

Potential applications of bio-ligninolytic systems

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Summary. Members of various fungal taxa and actinomycetes have been shown to degrade lignin at least partially. The white-rot wood-decomposing basidiomycetes completely metabolize the complex polymer, exhibit the highest reported rates, and are the most studied. Evidence indicates that their degradation of lignin involves oxidative, non-specific reactions, but the nature of the catalysts and the reactive species remain undefined, the catalysts have not been separated from living cells. Culture conditions optimal for lignin metabolism by white-rot fungi have been described, and several potential applications of whole ligninolytic cultures have been explored preliminarily: (a) partial delignification for the production of cellulosic products (bio-mechanical pulping, bio-bleaching); (b) conversion of lignocellulosics (improving ruminant digestibility, cultivating edible mushrooms) into feed and food; and (c) treatment of lignin-derived wastes (decolonizing, removing BOD, COD). The possibility to biomodify by-product lignins to yield valuable polymeric or low molecular weight chemicals has not been approached experimentally, but is another area of potential application. Improved waste treatment processes might well be the first intentional application of bio-ligninolytic systems.

Introduction

Microbial processing is based on the catalysis of reactions by whole cells or by isolated enzymes. It has several potential advantages over conventional chemical processing for many applications, such as: (a) greater substrate and reaction specificity, (b) lower energy requirements, (c) lower pollution generation, (d) higher yields of desired products, and (e) opportunities for transformations not feasible with chemical reagents. Until just a few years ago, applications of microbial technology in lignocellulose utilization were limited to the production of ethanol and microbial protein by yeast fermentation of spent liquor from sulphite pulping of wood, retting of flax and hemp, production of edible mushrooms, and various waste treatment processes. More recent research and development has resulted in several new uses, including the bacterial treatment of stored logs to increase permeability to preservatives,¹ the Ritter process for bacterial treatment to increase storage life and to depith bagasse,² the use of mycelial fungi for producing fodder protein from spent sulphite liquor,³ and

various improved waste treatment processes, e.g. the UNOX process.⁴ Very recent research has been directed at exploring several other potential applications of the expanding repertoire of microbial technology in lignocellulose utilization. These include schemes for enzymatic or acid-catalysed hydrolysis followed by fermentation to a variety of chemicals;^{5,6} direct fermentation of waste cellulose to microbial protein or chemicals;^{7,8} biological pulping of bast fibres with pectinolytic bacteria;⁹ treatment of chemical woodpulping liquors to de foam,¹⁰ decolonize,^{10,11} and deodorize;¹² direct conversion to food¹³ and feed;¹⁴ fungal delignification;^{15,16} and improved waste treatments.¹⁷

Some of these exploratory processes are first attempts to apply the potentially very useful lignin-degrading system of certain microorganisms. This paper will review and attempt to evaluate those potential uses. Attempts at present to apply bio-ligninolytic systems are empirical, because the chemistry and biochemistry of lignin biodegradation are only beginning to be described. Fundamental research on this complex problem has reached its highest level in very recent years, and current progress is rapid. To provide some background, this paper will briefly outline recent developments. Several comprehensive reviews can be consulted for detailed coverage.¹⁸⁻²¹

Background

Nature of lignin

The structure, distribution, and other aspects of lignin have been reviewed and summarized in several contexts recently^{18,22-24} including twice in this journal.^{20,25} Sarkanen and Ludwig²⁶ have provide a comprehensive treatise. For the present review, it is worth reiterating that (a) lignin is the most abundant biopolymer next to cellulose and contains 1.5 times the carbon content of cellulose, (b) it is a complex macromolecule synthesized by the dehydrogenative radical polymerization of p-hydroxycinnamyl alcohols, (c) it contains several different interunit linkages, many of which are non-hydrolysable, and (d) it physically protects the polysaccharides in lignocellulosics and must be disrupted for enzyme accessibility to the polysaccharides.

Microbiology of lignin degradation

A flurry of activity in the past six to seven years has begun to clarify the microbiology of lignin degradation,¹⁹ which until recently was a problematical research area because of the lack of reliable assays for biodegradation. Recent progress has been made possible mainly by the use of assays based on ¹⁴C-labeled lignins. Among other things, recent studies have shown that biodegradation of the lignin polymer is apparently a strictly aerobic process.²⁷ This finding contradicts earlier reports, which were based on inadequate assays. Resistance to anaerobic metabolism apparently resides in the polymeric nature of lignin because anaerobic degradation of low molecular weight aromatics does occur,²⁸ including those derived from lignin (J. G. Zeikus and T. K. Kirk, unpublished results).

In other studies, increasingly strong evidence has been presented that certain soil bacteria, particularly actinomycetes (*Nocardia*, *Streptomyces*) can cause at least limited degradation of lignin.²⁰ Ability of common moulds and soil fungi such as *Aspergillus* and *Fusarium* to degrade kraft lignin²⁹ and synthetic lignin^{30,31} has also been reported. It has been known for some time that soft-rot wood-decay fungi slowly degrade lignin in wood,³² but Haider³³ has recently shown that these Ascomycetes and Fungi Imperfecti are actually relatively efficient degraders of lignin under some conditions. These results suggest that various groups of ligninolytic microorganisms might eventually become available for biotechnological exploitation.

These recent microbiological studies have also added to our knowledge of lignin metabolism by the white-rot wood-decay fungi, which have long been known to decompose lignin. The rates of lignin degradation by these fungi have not been approached with other microbes, although optimization studies with the other groups have been minimal. At present, only the white-rot fungi, almost all of which are Basidiomycetes, have been studied from the applications standpoint, and this paper is limited to them. Most investigations have, in fact, involved a single species, *Phanerochaete chrysosporium* Burds. (= *Sporotrichum pulverulentum*, *S. pruinatum*, *Chrysosporium lignorum*, and *Peniophora* 'G'),³⁴ in the family Corticiaceae.

Lignin degradation by the white-rot fungi

Research with *P. chrysosporium* has shown that the culture parameters that influence the degradation of isolated lignin (lignin → CO₂) are simple although critical.^{35,36} The growth medium, containing the usual minerals, trace metals, and thiamin, must also contain a growth substrate (lignin will not support growth), and a growth-limiting amount of nutrient nitrogen. Good aeration and pH control are important. Recent research (unpublished) has disclosed marked variation among strains.

Physiological studies have revealed unusual features: growth and lignin degradation occur separately in batch cultures, the ligninolytic system [or essential component(s)] being associated with secondary metabolism^{19,37} (also termed 'maintenance metabolism,' or the 'idiophase'). Recent research has shown that the onset of lignin degradation (lignin → CO₂) can be triggered by limiting cultures for carbohydrate or sulphur in the presence of excess nutrient nitrogen, but that maximum sustained rates are obtained in nitrogen-limited cultures.³⁸ The ligninolytic system (lignin → CO₂) is not induced by its substrate; maximum activity is found in cultures grown with or without lignin.³⁷

Recent evidence suggests that one or more key components of the system might actually be induced by molecular oxygen.³⁰

The structural features of lignin dictate some unusual constraints on the biodegradative system, most notably a requirement that the catalysts be non-specific (or of unusual complexity) and extracellular, and that hydrolytic enzymes can be of only secondary importance.³⁶

Study of degraded polymeric lignin isolated from wood partially decayed by white-rot fungi has disclosed heavy oxidation with formation of numerous carboxyl groups. Direct evidence indicates C_α-C_β side chain cleavage and C_α-oxidation, and compelling, but indirect, evidence points to loss of aromatic methoxyl groups and cleavage of aromatic rings in the polymer.^{19,39} (Haider and coworkers⁴⁰ and Chua *et al.*⁴¹ have recently provided direct evidence for oxidative ring cleavage in the polymer using ¹³C-n.m.r. spectroscopic techniques.) Low molecular weight products extracted from white-rotted wood include numerous aromatic acids, the formation of which clearly involves C-C cleavage in the side chains.^{42,43} Other products, tentatively identified by mass spectrometry, contain aliphatic fragments from cleaved rings attached to intact aromatic nuclei.⁴³ These various results, considered in detail, suggest that white-rot fungi degrade lignin by a few fundamental reactions which lead to a variety of intermediate structures.¹⁹ From the applications standpoint, a key question at present is whether, as suspected, the C-C cleavage in side chains is of central importance in lignin polymer fragmentation, and if so, what the mechanism of cleavage is.

The catalysts responsible for lignin polymer degradation have not yet been identified, but recent research is providing strong indications of their probable nature.³⁶ The catalysts have not been separated from living cells. Their oxidative mode of action is apparent from studies of the degraded polymer¹⁹ and of model compound degradation in cultures.^{44,45} Non-specificity has been apparent merely from a consideration of the heterogeneous nature of lignins^{19,36} and from the fact that after heavy modification in chemical pulping and bleaching reactions lignin is still readily degraded.⁴⁶ Elucidation of the fates of lignin substructure model compounds in ligninolytic cultures has now provided strong evidence for non-specificity: degradation intermediates containing new asymmetric carbons are optically inactive,⁴⁴ and multiple 'pathways' exist for metabolism of certain substructure model compounds⁴⁵ (T. K. Kirk, C.-L. Chen and H.-m. Chang, unpublished results).

These very recent findings provide the first evidence for the possible involvement of key non-enzyme mediated reactions.³⁶ Evidence that oxygen induces one or more components of the ligninolytic system is in accord with this view, as is the observation that the presence of lignin does not increase the titre of the system that oxidizes it to CO₂.³⁷ So the speculations by Hall²⁵ and Zeikus⁴⁷ that lignin may be degraded by various 'active oxygen' species might well be correct in principle. Peracid oxidations and fungal oxidation share many features, but the nature of the biological oxidizing species and how it (or they) arises are not known. The key to progress is to have specific reactions identified which can be used to develop assays for the oxidizing agent. Recent research has provided these,^{36,44,45} and progress in the biochemistry of lignin degradation is expected to follow rapid. Specific assays will also permit an old question to the answers whether lignin-degrading

catalysts diffuse away from the hyphae, as microscopic studies of wood decay indicate,⁴⁸ or whether they are closely associated with them, as chemical investigations suggest.⁴⁹ This is important from the standpoint of practical applications.

The rapidly increasing knowledge of the chemistry and biochemistry will facilitate research and development of several potential applications of bio-ligninolytic systems in lignocellulose conversion.

Potential applications of bio-ligninolytic systems

Potential applications of ligninolytic systems can be divided into four classes: (a) manufacture of cellulosic products, (b) conversion of lignocellulose into feed and food, (c) conversion of by-product lignins to useful products, and (d) treatment of lignin-derived wastes.

Cellulosic products

Biomechanical pulping of wood chips. The idea of biological delignification of wood was inherent in the original concept of 'white-rot,' which was first thought to involve only lignin degradation. It was subsequently shown that white-rot fungi actually destroy all of the wood components. Indeed, evidence indicates that white-rot fungi do not use lignin as growth substrate?⁷⁷ so that completely selective removal of lignin would seem to be impossible. Even so, some white-rot fungi remove lignin more selectively than others,^{15,51,52} particularly in the early period of decay. This fact, plus the knowledge that these fungi, in contrast to the closely related brown-rot fungi, do not cause a rapid depolymerization of cellulose as they decay wood^{53,54} has resulted in research into 'biological pulping.'

Partial delignification without cellulose loss is possible. Eriksson and coworkers have developed mutants of *Sporotrichum pulverulentum* (= *P. chrysosporium*) and other fungi which no longer degrade cellulose; degradation of wood is limited to lignin and hemicelluloses (Figure 1)^{21,55} A United States patent has been obtained for the use of such mutants as a 'method for producing cellulose pulp.'⁵⁶ Other research showed that addition of glucose to alder thermomechanical pulp (TMP) led to selective removal of up to 29% of the lignin (Table 1).⁵⁷ These results indicate that much of the lignin in lignocellulosics is accessible to the degradative catalysts of white-rot fungi without cellulose depletion being necessary.

Because of the time involved, removal of a substantial amount of the lignin has not been seriously considered. Rather, the properties of wood after partial bio-delignification have been examined with particular attention to the energy requirements for subsequent mechanical pulping, and the properties of the resulting 'biomechanical' pulps.^{16,21,56}

Preliminary studies²¹ with PFI mill* refining showed that

Table 1 Effect of glucose on degradation of alder TMP by *Phanerochaete chrysosporium* in two weeks⁵⁶

Glucose (%, dry pulp basis)	Loss of component (% of original amount)		
	Lignin	Carbohydrates	Total weight
0	31	23	24
7	34	24	25
35	29	0	6
70	25	0	0

* The PFI mill is a laboratory refiner used to 'beat' (fibrillate) pulps, wood chips, etc.¹⁹

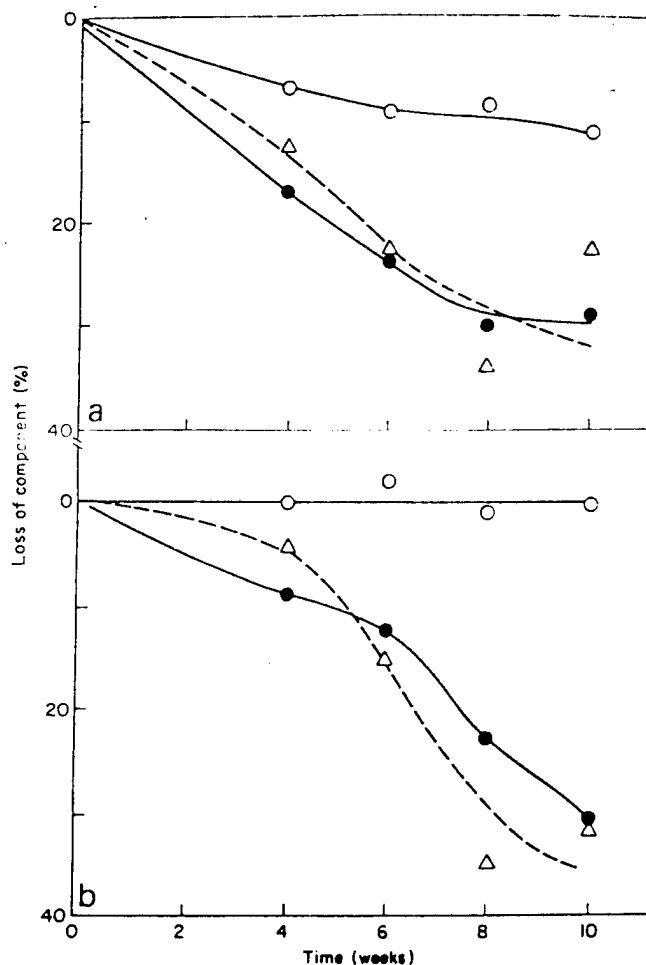


Figure 1 Depletion of components from birch wood by (a) wild type and (b) a cellulase-less mutant of *Sporotrichum pulverulentum* (= *Phanerochaete chrysosporium*)⁵⁵ ○, Glucan; △, xylan; ●, lignin. [Reproduced from Ander, P. and Eriksson, K. -E. *Sven. Papperstidn.* 1975, 78, 641-642 by permission of AB Svensk Papperstidning[©]]

removal of even small amounts of lignin (2.1% of the original) from pine chips resulted in disproportionate energy savings. At similar extents of refining (freeness of 100 ml) pulps from fungus-treated wood showed 20 to 30% improvements in breaking length, burst factor and tear factor.

However, although subsequent studies⁵⁸ confirmed a saving of 20% in the refining energy demand as a result of the biological pretreatment, the strength properties of the resulting mechanical pulp were lower than those of the untreated pulp at a given degree of refining. Thus, fungal pretreatment did not appear to offer any energy savings if the pulps were refined to the same strength level (Figure 2). Pretreatment with a cellulase-less mutant gave essentially identical results as pretreatment with the wild-type fungus. A decrease in brightness as a result of fungal pretreatment was also observed.⁵⁸ The causes for the discrepancy between the preliminary and the latter studies have not been clarified. Clearly, further studies are needed to explore the potential of biomechanical pulping.

Assuming that research can overcome the strength loss problem observed by Samuelson *et al.*,⁵⁸ projected major technical problems with biomechanical pulping would appear to be three-fold: (a) in scaling up with the required careful control of humidity, aeration, and temperature; (b) in preventing contamination by unwanted microorganisms; and (c) in the slowness of degradation of the lignin in softwoods compared with hardwoods. Of course, the energy savings and/or pulp property improvements must

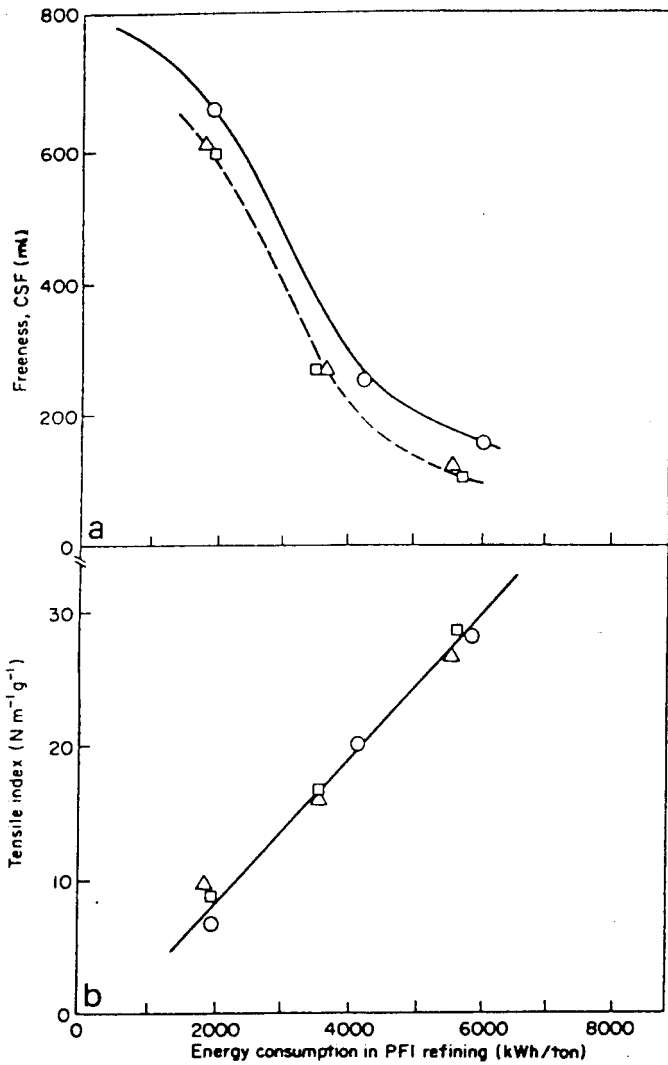


Figure 2 Effect of pretreatment with wild type and a cellulase-less mutant of the ligninolytic fungus *Phlebia radiata* on (a) Canadian Standard Freeness (CSF; a measure of fibrillation) and (b) tensile index of paper, as functions of refining energy consumption for pine chips.⁵⁸ ○, Control; △, fungus-treated, wild type; □, fungus-treated, mutant

offset the expenses connected with fungal treatment. These expenses are likely to be primarily in (a) production of the fungal inoculum, (b) sterilization of the wood chips if required, and (c) environmental control during fungal treatment. Ander and Eriksson have suggested large silos as possible treatment chambers,²¹ with introduction of wood chips and inoculum at the top and removal of treated chips at the bottom after a suitable residence time (Figure 3). For hardwoods, residence times of 1 week or less are probably adequate. Silos large enough to accommodate this residence time can be constructed (170 000 tons of pulp per year per silo).²¹ Meaningful economic analyses require pilot-scale studies, which have not yet been conducted. In fact, the research effort on practical bio-delignification of wood has been quite limited. Further study would seem to be warranted.

Biomechanical pulping of coarse thermomechanical pulps. A modification of biomechanical pulping involves partial delignification of coarse thermomechanical pulp (TMP) prior to 'post refining' for final pulp production. The coarse TMP would be expected to be freer of unwanted microbes than would the chips, and it can be produced with a relatively small proportion of the total energy needed for final quality pulp.

When coarse TMP was treated with a white-rot fungus to a weight loss of ~2%, a considerable decrease in energy consumption was observed in the post-refining.⁵⁸ However, this decrease in energy consumption was accompanied by loss of strength properties, especially when wild-type rather than a cellulase-less mutant was employed (Figure 4). Thus, it appears important to prevent carbohydrate degradation during the fungal treatment. In another study, this was achieved by addition of glucose to the culture

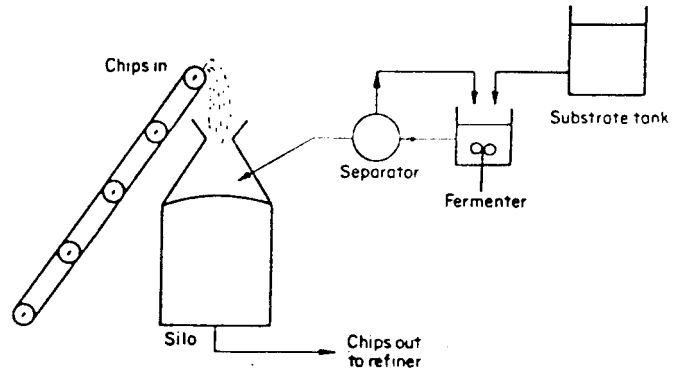


Figure 3 Silo arrangement for treating wood chips with delignifying fungi, suggested by Ander and Eriksson.²¹ [Reproduced from Ander, P. and Eriksson, K.- E. *Progr. Ind. Microbiol.* 1978, 14, 1-58 by permission of Elsevier Scientific Publishing Co.©]

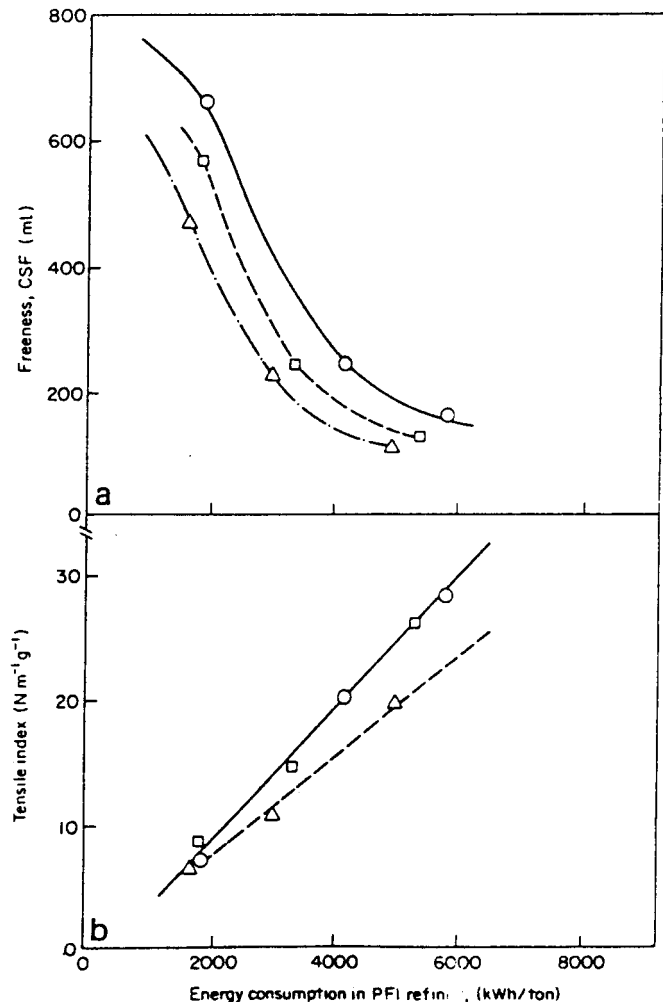


Figure 4 Effect of pretreatment with wild type and a cellulase-less mutant of *Phlebia radiata* on (a) Canadian Standard Freeness (CSF; a measure of fibre fibrillation) and (b) tensile index of paper, as functions of refining energy consumption for pine thermomechanical pulp.⁵⁸ ○, Control; △, fungus-treated, wild type; □, fungus-treated, mutant

medium (S. S. Bar-Lev, H.-m. Chang and T. K. Kirk, unpublished results). Coarse TMP from red alder was partially delignified by *P. chrysosporium* under optimized conditions.⁵⁷ The energy requirements for subsequent post-refining, and the strength properties of the resulting pulp were evaluated both with and without alkali swelling. (Hardwood TMP is generally very weak but alkali-swelling of coarse pulp before post-refining greatly improves the strength properties and decreases the energy requirement.)⁶⁰ As shown in Figure 5, both the energy requirements, as deduced from PFI refining, and the strength properties were greatly improved in both cases. On the other hand, fungal treatment of the same coarse TMP without the addition of glucose resulted in decreased energy consumption but also caused a decrease in strength properties. These results suggest that the decrease in energy consumption and the development of strength properties may be independent of each other and that the loss in strength properties can be avoided. Furthermore, comparing our results with glucose addition and those of Samuelson *et al.*⁵⁸ with the cellulase-less mutant, it becomes apparent that the mutant used in the latter either was not completely cellulase-free or contained other systems capable of degrading carbohydrate. More studies, especially for controlling carbohydrate degradation, are needed in order to reveal the potential of this biotechnical approach.

The fungal degradation rate of lignin appeared to be faster in coarse TMP than in wood chips. Lignin degradation rates of >3% per day, averaged over a 2-week period, have been observed; this, however, is preceded by a 3- to 4-day lag period for primary growth of the fungus in the pulp. As with wood chip treatment, technical problems with fungal

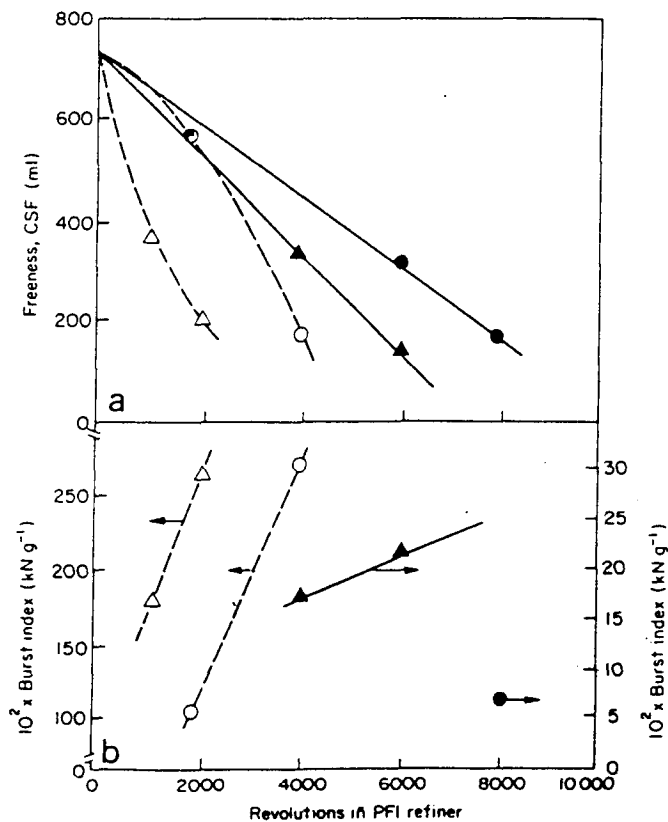


Figure 5 Effect of pretreatment with the ligninolytic fungus *Phanerochaete chrysosporium* on (a) Canadian Standard Freeness (CSF; a measure of fibre fibrillation) and (b) burst index, as functions of revolutions in PFI-refining (Bar-Lev, S. S., Chang, H. m. and Kirk, T. K., unpublished results). Normal beating: ●, control; ▲, fungus-treated. Alkali swollen: ○, control; △, fungus-treated

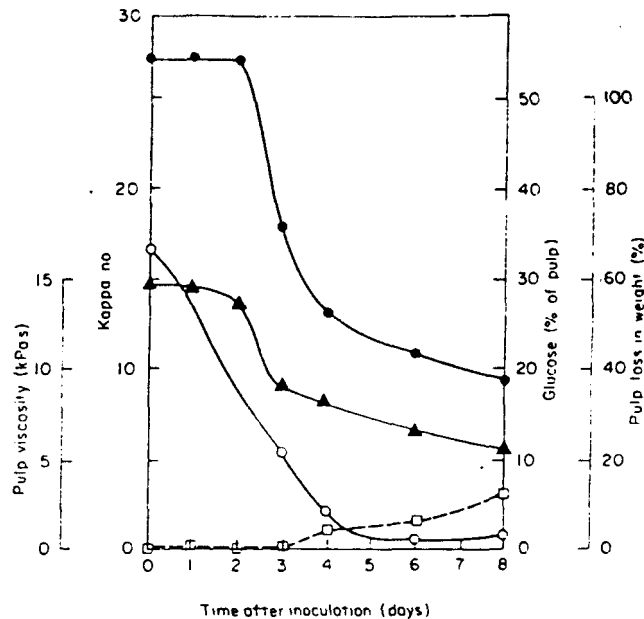


Figure 6 Changes in kappa number (\cong lignin \times 6), viscosity and weight of kraft pulp during incubation with *Phanerochaete chrysosporium*. Decrease in kappa number is proportional to bleaching. Glucose was added to retard cellulose degradation.⁶¹ ●, Kappa number; ▲, viscosity; □, weight loss; ○, glucose. [Reproduced from Kirk, T. K. and Yang, H. H. *Biotechnol. Lett.* 1979, 1, 347-352 by permission of Science and Technology Letters⁶¹]

treatment of TMP include maintenance of correct environmental conditions on a large scale and the slowness of lignin degradation in conifer TMP⁵⁷ Here, the time required even for hardwoods might be more serious than in chip treatment, unless a 'low technology' treatment chamber could be devised for the TMPs.

Other possible bio-delignification processes include treatment of round-wood, which is unlikely to be sufficiently controllable, and variations such as the treatment of ordinary mechanical pulps, and the use of chemical treatment in conjunction with fungal treatment.

Bio-delignification as part of kraft pulp bleaching. 'Bio-bleaching' has received even less research attention than the pulping applications of ligninolytic fungi mentioned above, although it too has been shown in the laboratory to be experimentally feasible. The idea stemmed from studies with a 'laboratory kraft lignin' which was prepared from "C-labelled synthetic lignin, and which was shown to be metabolized more rapidly than the original lignin by *P. chrysosporium*.⁴⁶ This suggested that the residual lignin in kraft pulp might be removed by white-rot fungi, which subsequent experiments have shown to be the case⁶¹ (Figure 6). Removal of the lignin (i.e. kappa number decrease) from kraft pulp of pine followed a triphasic pattern: (a) no lignin removal during establishment of the fungus in the pulp, (b) rapid delignification, and (c) a slower phase of delignification. The 'rapid' phase could perhaps be attractive if it could be isolated. Problems with 'bio-bleaching,' however, appear substantial: (a) the time required, particularly for the establishment phase: (b) the need carefully to control environmental conditions on a large scale: (c) the need completely to suppress cellulose degradation while providing a growth substrate (Figure 6); and (d) the fact that a significant part of the lignin is removed at an impractically slow rate. Cellulase-less mutants would be expected to help solve problem (c).

Conversion of lignocellulosics into feed and food

Limited research has been conducted recently on the Microbial production of feeds from various lignocellulosics. The idea, however, is not new. In 1929 Knoche *et al.*⁶² described "palo podrido, which is decayed wood used as animal feed in southern Chile (see also Kuhlwein).⁶³ It is known that the digestibility of intact lignocellulosics varies inversely with the lignin content, and laboratory studies have shown that lowering the lignin content of wood with white-rot fungi increases the polysaccharidase and rumen fluid digestibilities.⁶⁴ Recent studies have aimed at feed production by controlled cultivation of microorganisms. Detroy and coworkers⁶⁵ and Zadrazil⁶⁶ have studied the conversion of straws into animal feed with lignin-degrading basidiomycetes. Daugulis and Bone⁶⁷ have studied conversion of barks into fungal protein, and Matteau and Bone⁶⁸ have described preliminary results on direct fermentation of wood to protein using a ligninolytic basidiomycete. (Other investigations have used non-lignin-degrading microorganisms, usually following steam or chemical treatment of lignocellulosics, to produce feeds (see Detroy *et al.*)⁶⁹

Technical problems in using lignin-degrading fungi to produce feeds appear to be the same as those in pulping of wood chips mentioned above. The importance of these problems is exacerbated by the relatively low value of the product feeds. On the other hand, production of feeds on a small scale for local uses could be expected to change the economic attractiveness somewhat. In common with the potential pulping applications, too, is a relative lack of study of feed production.

Production of edible mushrooms from wood and straw is a multimillion-dollar industry in Japan and other Asian countries¹³ and has considerable, as yet largely unexplored, potential in the West.⁷⁰ Techniques for cultivating mushrooms (*Lentinus edodes*, *Pleurotus* spp. etc.) on wood residues have recently been developed.⁷¹ Processes that yield valuable mushrooms as well as increasing the digestibility of the substrate to ruminants should be investigated further. Apparently, all of the commercial mushroom-forming fungi which grow on straw or wood are ligninolytic; as mentioned, non-lignin-degrading microorganisms cannot metabolize most lignocellulosics without pretreatment to disrupt the lignin.

Lignin products

By-product lignins from the chemical pulping of wood are currently used in polymeric form to make a variety of commercial products, including dispersants, oil-well-drilling muds, etc., and via chemical degradations to make vanillin, dimethyl sulphoxide, and a few other chemicals.⁷²⁻⁷⁵ Unfortunately, however, there are relatively few uses for by-product lignins, so that only a minor portion is converted to products. Most of the currently produced by-product lignins are burned for recovery of inorganic pulping reagents, returning only a small net yield of energy due to their high water content. Large quantities of 'hydrolysis' or 'solvolysis' by-product lignins are anticipated in the near future as lignocellulosics are increasingly converted to fuels and chemicals via fermentation of the saccharides hydrolysates.

Biological approaches to utilizing these available by-product lignins are only beginning to be researched. Two kinds of potential products can be envisioned: those that retain the polymeric structure of lignin and those of low molecular weight. Changes in the functionality of by-product lignin by microbial action could conceivably provide

materials with a fairly high value. For example, selective demethylation as by brown-rot fungi⁷⁶ could produce a polymer which might function well in phenol-formaldehyde type adhesives. Whether demethylation or other polymer biomodification is in fact, a promising approach awaits further study. It has been shown that low molecular weight aromatic products are formed during the fungal degradation of lignin.^{42,43} Practical use of such products, however, would seem to be especially difficult because of their diversity, even if they can be produced in good yields and at attractive rates. This subject has been discussed in more detail by Crawford and Crawford.²⁰

Waste treatment

In order to comply with future water pollution regulations, the pulp and paper industry will be required to reduce substantially the colour of its waste water. The bleach plant effluent is of special concern to the industry since the effluent is a major source of colour and is not easily contained within the mill due to its high content of corrosive chloride. Polymeric lignin degradation products, the main contributors to colour of bleach plant effluents, are resistant to the current bacteria-based biological effluent treatment processes. Alternative treatment processes known for effective colour removal, such as ultrafiltration, carbon adsorption, and massive lime precipitation are quite expensive.

It was demonstrated five years ago that *P. chrysosporium* rapidly decomposed a model bleach plant effluent prepared by chlorination and alkali treatment of kraft-cooked synthetic ¹⁴C-lignins.⁴⁶ Fukuzumi,¹⁰ using several white-rot fungi, and our group using *P. chrysosporium*,^{11,77} have since shown that the kraft bleach liquors can be decolonized readily. The data in Figure 7 demonstrate decolonization

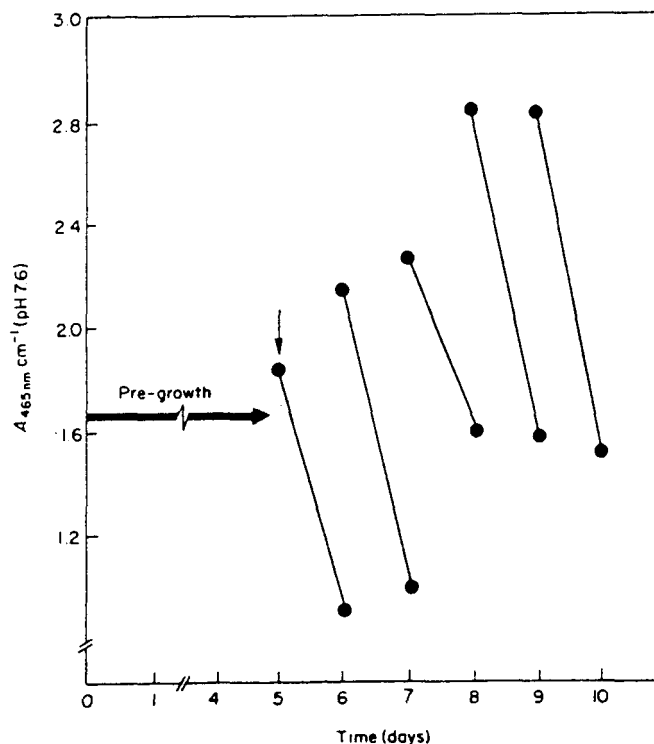


Figure 7 Decolorization of kraft bleach plant effluent with ligninolytic mycelium of *Phanerochaete chrysosporium*.¹¹ Initial growth (pregrowth stage) followed inoculation of the fungus into a cellulose medium. Coloured effluent was then added (vertical arrow). As colour was reduced, additional effluent was added (upper points on lines)

of a bleach plant effluent from the first alkali extraction stage. Latest results have shown that ~80% colour reduction can be achieved in one day, that a thin-film type of reactor gives the highest rates, and that the cellulose-containing primary sludge from the pulp mills and paper mills can serve as the required growth substrate.⁷⁷ Tire decolonizing mycelial mats have been used continuously for over (50 days with addition of only carbohydrate.¹¹¹ Decolorization results both from decomposition of the chromophore-bearing polymer and degradation of the chromophores in the polymer.⁷⁸ Current research is aimed at bench-scale evaluation of a process based on a rotating biological contactor.⁷⁹ Toxicity reduction as well as decolonization, and BOD and COD reduction, are being studied.

Lignin-derived wastes that will arise from proposed new processes for lignocellulose conversion are also expected to be resistant to currently used waste treatment systems. Processes of the type described above might thus have the potential for broad applicability. Improved waste treatment processes might well be the first intentional application of bio-ligninolytic systems.

Acknowledgements

Portions of the research reported here were supported by NSF grant PFR-7522789A03 to TKK.

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