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EFFECTS OF MOLECULAR OXYGEN ON LIGNIN DEGRADATION BY PHANEROCHAETE CHRYSOSPORIUM

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SUMMARY: Research has demonstrated that shallow stationary cultures of white-rot wood-destroying Basidiomycetes degrade lignin (^{14}C-lignin → $^{14}CO_2$) at much higher rates under O_2 than under air. The present study, conducted with Phanerochaete chrysosporium, showed that the effect on rate is a dual one: a) Immediately preceeding appearance of the lignin-degrading system the partial pressure of O_2 determines the amount of ligninolytic activity that develops in cultures; and b) after the system develops, the partial pressure of O_2 affects the rate of oxidation.

INTRODUCTION

Culture parameters and physiological events important for lignin oxidation to CO_2 by the white-rot wood-destroying fungus Phanerochaete chrysosporium (Basidiomycotina, Aphyllophorales, Corticiaceae) have been described (1-3). When the fungus is grown in stationary liquid cultures in a medium containing excess carbon source and limiting nutrient nitrogen, the medium nitrogen is depleted within 48 hours, DNA synthesis peaks at 24 to 30 hours, and protein synthesis peaks at 48 to 72 hours. These events reflect the cessation of primary growth. Ligninolytic activity appears during the transition to subsequent secondary metabolism, and is maintained as a part of the secondary phase (4). Lignin does not induce the system that degrades it, and its presence in cultures does not affect the activity of the ligninolytic system that is formed (2).

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Research has shown that cultures maintained under O_2 degrade lignin much faster than cultures in air (1,5,6), and that cultures grown in 0.05 atm of O_2 do not develop ligninolytic activity, despite good growth (1). The strong effect of oxygen is in accord with the oxidative nature of lignin degradation (7,8), but it is not clear whether the O_2 affects the amount (activity) of the ligninolytic system formed in cultures or the rate of oxidation by the system after its formation, or whether the effect is a general one on oxidative systems in the fungus. This question has been answered by the research described here.

MATERIALS AND METHODS

Cultures (10 ml) of P. chrysosporium ME-446 (ATCC 35540) were grown at 39° C in 125-ml Erlenmeyer flasks containing a dilute mineral salts-vitamin medium with glucose as growth substrate, L-asparagine and NH_4NO_3 as N-sources (1), and 0.01M poly(acrylic acid), pH 4.5, as buffer (9). Other details have been described (1,2). Residual glucose was determined by the method of Nelson (10), and mycelial nitrogen by the micro-Kjeldahl procedure. Synthetic [ring-U-^{14}C]-lignin (9.0×10^5 dpm/mg (2,11) was added to cultures as a suspension in sterile water (0.5 ml/culture, 5.4×10^4 dpm) as described previously (2). [U-^{14}C]-glucose (Cal Atomic, 10 mCi/mmol) was added as a sterile aqueous solution (0.5 ml, 2×10^5 dpm/culture). Mixtures of O_2 in N_2 were obtained by use of calibrated flow meters to give O_2 partial pressures of 0.1, 0.2, 0.4, and 0.8 atm (total pressure = 1 atm). Cultures were flushed every third day, or as indicated, with the appropriate gas mixture at a rate of approximately 200 ml/min for 15 min. The oxidizing activities of the cultures were determined by measuring $^{14}CO_2$ evolved (1,2) 2.5, 5.0, and 7.5 h after addition of labeled substates. Evolution was linear from 2.5 to 7.5 h, and rates during this period were taken as a measure of ligninolytic activity. Four replicate cultures were used for each treatment.

RESULTS

In the first experiment, the effect of O_2 partial pressure during the transition between primary and secondary metabolism on the activity of the ligninolytic system was examined. Cultures were carried through primary growth (to day 2.5) under air, then incubated under either 0.2 or 0.8 atm of O_2. After an additional 5, 7, and 9 days, cultures were assayed for oxidizing activities after addition of cycloheximide to stop further protein synthesis. Cycloheximide has heen shown to be a potent inhibitor of protein synthesis in

Table 1.--Rates of oxidation of synthetic lignin and glucose by cultures of Phanerochaete chrysosporium after prior incubation under 0.2 or 0.8 atm of O_2 [1/]

Pre-assay O_2 partial pressure (atm)	Rate of oxidation to $^{14}CO_2$ (%$^{14}CO_2$/h/culture) [2/, 3/]	
	[^{14}C]-Glucose	[^{14}C]-Lignin
0.2	0.045 ± 0.004	0.043 ± 0.03
0.8	0.039 ± 0.001	0.219 ± 0.002

1/ Experimental details in text.
2/ Percent of radioactivity added as [^{14}C]-glucose or [^{14}C]-lignin.
3/ Means ± std. dev. for 4 replicate cultures for each of 3 harvest times (12 cultures total for each rate given).

P. chrysosporium and to inhibit appearance of ligninolytic activity when added to 2-3-day-old cultures (2). The lignin-oxidizing system was increased 5-fold by incubating cultures under the higher O_2 pressure, whereas the glucose-oxidizing system was unaffected (Table 1). No significant differences were found between cultures assayed after 7.5, 9.5, and 11.5 days, and the reported values are the means of all 12 cultures per treatment. No significant differences between the cultures at 0.2 and 0.8 atm O_2 were found in residual glucose content, growth (total mycelial N), or culture fluid pH (Table 2).

A second series of experiments examined the effects of various concentrations of O_2 on the activity of the ligninolytic system after its appearance in cultures. Cultures were grown as above for 2.5 days under air, and then maintained under 0.8 atm of O_2 for an additional 4 days. Cycloheximide was added, and the oxidation of lignin and glucose determined under different O_2 atmospheres (Fig. 1). Oxidation of both substrates was maximal at 0.4 atm O_2, and not significantly lower at 0.8 atm O_2. The optimum for both is probably about 0.5 atm O_2 under the conditions used. Under 0.1 atm O_2, cultures oxidized glucose at 50% of the maximum rate, whereas lignin was oxidized at only 28%

Table 2.--Mycelial nitrogen (growth), residual glucose and medium pH in cultures of Phanerochaete chrysosporium maintained under two different O_2 atmospheres[1/]

Parameter	O_2 Partial pressure (atm)	Time after inoculation (days) 3	7.5	9.5	11.5
Mycelial nitrogen	0.2	2.6	3.2	3.1	3.0
(mg/culture)[2/]	0.8	2.6	2.8	3.0	3.1
Residual medium	0.2	27	22	17	25
Glucose (mM)[3/]	0.8	27	23	31	19
Culture pH	0.2	4.0	4.1	4.2	4.2
	0.8	4.0	4.2	4.2	4.1

1/ Cultures were replicates of the ones in Table 1.
2/ Mycelial mats were recovered by centrifugation, washed with water, re-centrifuged, dried and analyzed. Total nitrogen supplied = 3.0 mg/culture.
3/ Original concentration = 56 mM.

of the maximum rate. Oxidation of both substrates was enhanced greatly by increasing O_2 partial pressure, although the effect on lignin oxidation was much more dramatic, particularly between 0.1 and 0.2 atm O_2.

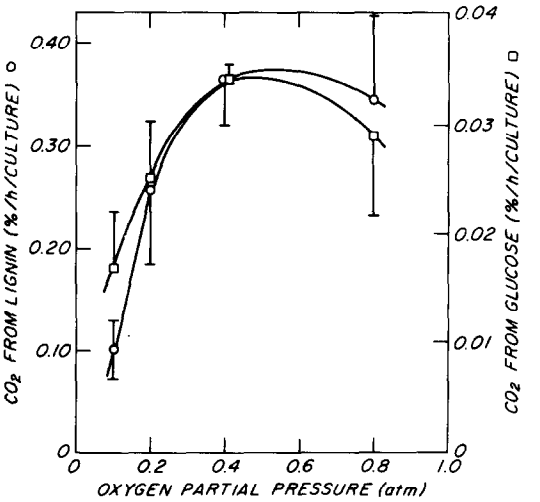

Fig. 1--Effect of O_2 partial pressure on the oxidation of [^{14}C]-glucose and [^{14}C]-lignin by ligninolytic cultures pre-grown in air (2.5 days) and then maintained under a partial pressure of 0.8 atm O_2 until assayed on day 6.

DISCUSSION

These results show that the O_2 concentration during the transition phase between primary and secondary metabolism strongly influences the activity of the ligninolytic system that develops. the Simplest interpretation of this finding is that O_2 induces the synthesis of one or more of the enzymes involved.

Unfortunately, the enzymes responsible for the oxidation of lignin have not yet been identified, although several of the oxidative reactions that take place in the polymer and in model compounds have been identified (8,9,12,13). A consideration of the heterogeneous structure of lignin and of the variety of modified lignins that are still readily degraded by white-rot fungi indicates that the degrading system is quite non-specific (12). It seems likely that key oxidative steps might not be carried out on enzyme surfaces. Instead, enzymatically generated oxidizing species might be the actual agents responsible for these reactions (12,14,15). Molecular O_2 but not lignin, therefore, might be expected to induce the responsible enzymes.

The results here indicate that high O_2 concentrations also enhance the rate of lignin degradation by the ligninolytic system after it has been formed. This cannot be attributed entirely to a general effect on oxidation. Presumably a substantial portion of the carbon of lignin pools with that of glucose in the final oxidative steps of metabolism. It is to be expected, therefore, that increasing the O_2 concentration will increase the rate of CO_2 formation from lignin if it does from glucose. This was observed here. However, the fact that the effect on the lignin was significantly greater than the effect on glucose indicates that reactions not shared by glucose oxidation were also affected. This, too, is not unexpected, because key reactions of lignin polymer metabolism are undoubtedly O_2-requiring (7). This second effect of O_2 is either directly on the action of the lignin-degrading system, or on the generation of required components.

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