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Kirk T K, Schultz E, Connors W J, Lorenz L F & Zeikus J G. Influence of culture parameters on lignin metabolism by *Phanerochaete chrysosporium*. *Arch. Microbiol.* 117:277-85, 1978.
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Several culture parameters were shown to influence mineralization of synthetic ^{14}C-lignin by the basidiomycete *Phanerochaete chrysosporium* in defined culture media. Important factors include molecular oxygen and trace element concentrations, pH and buffer, and especially culture agitation and nutrient nitrogen concentration. [The *SCI*® indicates that this paper has been cited in over 185 publications.]

Lignin Degradation by *Phanerochaete chrysosporium*

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This work has been widely cited because it was the first description of the rather curious factors that influence lignin mineralization by basidiomycete fungi. In the past 10 years, interest in lignin biodegradation has accelerated greatly, not only because of inherent scientific interest in an important and unusual process, but also because of its potentials for application. This paper provided guidelines for studying the process in defined cultures. An unequivocal assay for biodegradation based on synthetic ^{14}C-lignins made the study possible.[1]

My coauthors brought various skills to the study. Greg Zeikus and I had obtained National Science Foundation grants to clarify the microbiology of lignin biodegradation and had already obtained evidence that the process is obligately aerobic, that lignin does not serve as growth substrate for microbes known to degrade it (wood-decaying basidiomycetes), and that bacteria apparently do not degrade the polymer. Em Schultz was a postdoctoral associate with experience on the bacterial catabolism of aromatics. Bill Connors, an organic chemist at the Forest Products Laboratory (FPL) at the time, did most of the synthesis work on the ^{14}C-lignin precursors. Linda Lorenz is a support chemist at the FPL.

In this work, our assay for biodegradation was $^{14}C\text{-lignin} \rightarrow {}^{14}CO_2$. It was several years before the point in this conversion at which our "parameters" were exerting their influence could be determined. Recent results have shown that the culture parameters all affect at least the first step (degradation of the polymer) and probably subsequent steps as well.

The parameters reported in this paper as being important were in part not surprising: we showed that a complete chemically defined medium supports good growth and degradation, that the sources of nitrogen and carbon are not critical, that thiamine is the only vitamin required, that the optimum pH is about 4.5, that the choice of buffer is important, and that degradation is favored by high O_2 levels. But we also unexpectedly found two culture parameters to be critically important: (i) mineralization of the lignin only occurred when the cultures had depleted their nutrient nitrogen, and (ii) culture agitation, which is commonly used to increase O_2 availability, stopped degradation. The nutrient nitrogen discovery was serendipitous.

In the early 1970s, I had decided to prepare radioactively labeled lignins to develop the quantitative assays needed for meaningful study of biodegradation. Developing assays based on ^{14}C-lignins was ineluctable: lignin is so heterogeneous that assays based on other chemical and physical principles are inadequate. Preparing the synthetic ^{14}C-lignins was expensive in time and materials, and we were dismayed that the fungi ignored our precious lignins when they were added to growing cultures in liquid media. We eventually got the cultures to degrade the lignins, albeit slowly, by adding them to solid wood and allowing the fungi to decay the wood.

This was a great relief at the time because it showed that our lignins at least were being recognized by the degradative enzyme system of the fungi. But why were they not degraded in defined liquid media? We considered all kinds of possible reasons and were testing various hypotheses when the problem solved itself: in one experiment, for no reason that was immediately apparent, we saw the first efficient degradation of the lignin to $^{14}CO_2$. Fortunately, we were soon able to deduce from the laboratory notes that the basal culture medium, but not the carbon source, had been made up 10 times more dilute than intended.

Subsequent experiments showed that by limiting nutrient nitrogen alone we could trigger the ligninolytic system. Interestingly, subsequent work showed that the ligninolytic system is under N-regulation as a part of secondary metabolism[2] and that carbon limitation and, under some conditions, sulfur limitation[3] also trigger it.

An intellectually satisfying explanation for the connection between lignin degradation and secondary metabolism has not been advanced, although this seems to make sense intuitively. Wood is a very poor source of nitrogen, and the fungi can have only a very transient phase of primary metabolism. The culture agitation problem has been overcome with the addition of detergents to the culture medium, but the basis for this curious influence of agitation is still not clear.

Understanding the microbiology, physiology, chemistry, and biochemistry of lignin biodegradation has advanced far past the work described in this "culture parameters" paper. Several recent reviews describe those advances.[4-6]

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2. **Keyser P, Kirk T K & Zeikus J G.** Ligninolytic enzyme system of *Phanerochaete chrysosporium*: synthesized in the absence of lignin in response to nitrogen starvation. *J. Bacteriology* 135:790-7, 1978. (Cited 120 times.)
3. **Jeffries T W, Choi S & Kirk T K.** Nutritional regulation of lignin degradation in *Phanerochaete chrysosporium*. *Appl. Environ. Microbiol.* 42:290-6, 1981. (Cited 55 times.)
4. **Buswell J & Odier E.** Lignin biodegradation. *CRC Crit. Rev. Biotech.* 6:1-60, 1987.
5. **Kirk T K & Farrell R L.** Enzymatic "combustion": the microbial degradation of lignin. *Annu. Rev. Microbiol.* 41:465-505, 1987.
6. **Tien M.** Properties of ligninase from *Phanerochaete chrysosporium* and their possible applications. *CRC Crit. Rev. Microbiol.* 15:141-68, 1987.

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