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Invited Speakers

Current and Future Perspectives in Research on Lignocellulose Biodegradation

D. A. WOOD AND J. M. LYNCH

Glasshouse Crops Research Institute, Worthing Road, Littlehampton, West Sussex, BN16 3PU, UK

ABSTRACT

The present article is an attempt to briefly summarize the findings of the meeting held at the Glasshouse Crops Research Institute on the 15th and 16th September, 1983. Over 120 people from a range of scientific disciplines attended and both formal presentations were given and poster sessions took place.

Currently the lignocellulose biodegradation field is at an exciting stage where breakthroughs in one or several areas of the fundamental work will have immediate effects on the range of technologies that are currently possible for the utilization of plant wastes.

FUNDAMENTALS OF LIGNOCELLULOSE BIODEGRADATION RESEARCH

We review both the basic aspects of lignocellulose biodegradation research and the applied or the technological aspects. It is salutary to begin by considering the historical perspective of this field and the continuing relevance of the statement below.

> The dead vegetation of the world might of course be converted into carbon dioxide and water by burning it but its accumulated energy can be turned into better account and is, in fact, being far more economically utilized in the natural processes of decay. [From A. C. Thaysen and H. J. Bunker (1927) *The Microbiology of Cellulose, Hemicelluloses, Pectin and Gums*, Oxford University Press, Oxford.]

Decay processes can be examined either for upgrading.lignocellulosic materials accumulating in the environment, e.g., straw, forestry wastes, paper industry wastes, etc., and as importantly for understanding such processes with a better view to providing strategies for overcoming decay. Clearly, decay is useful for upgrading lignocelluloses and conversely it requires control when biodegradation is economically damaging. Here, we briefly review the state of the art, considering lignin and cellulose separately and in combination, in terms of the type of approaches that are being undertaken to study biodegradation. Also considered are some of the outstanding problems that still remain unsolved, and where future work might lead to better understanding.

There are four types of approach to lignocellulose biodegradation that are currently being undertaken—chemical, biochemical, genetical, and lastly what is referred to here as structural and organismal. These last two are intended to describe what is known about the relationship of the organism to the substrate being degraded and what is known about the types of microorganisms involved in such degradations. For each type of approach, certain knowledge is available about the substrates, the hydrolytic agents responsible for the biodegradation of the plant biopolymers, and the products of such biodegradation.

For cellulose biodegradation the picture is well-advanced (see Table 1). Substrate chemistry for cellulose has been reasonably well described. The biochemistry and genetics of cellulose production is a problem for plant biochemists, geneticists, and breeders. There may well be some future in considering genetical and biochemical approaches to the nature and production of the substrate itself.

A wide range of organisms are capable of cellulose degradation in the broad sense of the term; that is, many organisms are known that have some degree of cellulolytic properties.

It is known now for many cellulolytic organisms that the hydrolytic agent is the cellulase–enzyme complex. There are still some unsolved problems and questions about certain microbial groups, such as the brown-rot fungi, which apparently do not produce the complete complex of the cellulase system, but nevertheless produce rapid strength loss in lignocellulosic materials; clearly there is a much to be gained by looking

Table 1
Current Knowledge of Cellulose Biodegradation[a]

	Chemical	Biochemical	Genetical	Structure and organisms
Substrate	+	±	±	+
Hydrolytic agents	+ Brown rots? Anaerobes?	+ Regulation/control	± Cloning	+ Where?
Products	± Brown rots?	±	±	Saccharification Thermophiles? Bacilli?

[a]+ indicates current knowledge substantial; − indicates lack of knowledge.

at that group. In addition, the anaerobic cellulase systems have also not been completely defined.

Much remains to be done in understanding the biochemistry of the control of product formation and the regulatory controls operating during degradation. With the genetic approach, mutant selection procedures have now shown that various classes of regulatory mutants can be isolated, and structural genes for these enzymes can be cloned in noncellulolytic organisms such as, for example, *E. coli*. Clearly, the marriage of mutant selection techniques and molecular genetics will be a growth point in this field in the future.

Despite cellulolysis having been studied for many years, little knowledge other than observational is yet available on the structural aspects of degradation in *intact* lignocellulosic materials in terms of *where* and *how* enzyme production is localized. Excellent work is available on the morphological *types* of degradation proceeding in lignocellulosic materials and on the interaction of isolated cellulase protein molecules with pure cellulose substrates. What is needed are techniques for looking at where the degradation is taking place and what is causing it.

The products of cellulolysis are now well-characterized, except in the case of brown-rot fungi. Genetic and biochemical control of product formation remains to be further examined. The range of organisms and types of product resulting from growth on lignocelluloses are still not fully explored. Microbiological research should continue to examine different environments for their range of cellulolytic organisms, particularly cellulolytic organisms growing to high temperature in other extreme environments.

The pattern of current knowledge for hemicellulolysis clearly resembles that of cellulolysis (*see* Table 2). One outstanding difference is that the chemical complexity of the hemicelluloses is obviously greater than that for cellulose. This greater complexity has an effect on the levels of knowledge of biodegradative processes of hemicelluloses.

Knowledge of biodegradation processes of lignin is still lagging behind that of the other two major biopolymers (*see* Table 3). Although the chemical nature of the substrate has been described in considerable detail

Table 2
Current Knowledge of Hemicellulose Biodegradation[a]

	Chemical	Biochemical	Genetical	Structure and organisms
Substrate	±	±	±	+
Hydrolytic agents	+	+	±	± Where?
Products	±	±	±	Thermophilic bacilli?

[a]+ indicates current knowledge substantial; − indicates lack of knowledge.

Table 3
Current Knowledge of Lignin Biodegradation[a]

	Chemical	Biochemical	Genetical	Structure and organisms
Substrate	+ some	?	?	Nature of
		Substrate specificity		"contact" range of types
Hydrolytic agents	±	±	±	± Where?
Products	±	±	?	Mixed cultures?

[a]+ indicates current knowledge substantial; − indicates lack of knowledge.

for some time for certain plant species, particularly, the conifers and the grasses, much remains to be done by way of analyzing lignins from other plant groups to determine whether significant differences occur within the three major lignin polymer types. Genetic and biochemical control of lignin production remains a task for plant geneticists and biochemists. Work here with plant mutants and plant cell culture lines may aid progress in this field.

For lignin biodegradation to occur, contact with substrate seems obligatory. This process needs to be analyzed to understand the biochemical basis for this requirement.

The exact role and number of enzymes involved in lignin biodegradation remains to be elucidated and it seems likely that answers to these questions will soon be forthcoming. Some knowledge is now accruing on the biochemical and genetic controls surrounding the production of the lignolytic system. As with the biodegradation of cellulose and hemicellulose, the fusion of work on mutant selection and molecular genetics should provide a rapid advance in the understanding of the processes involved. The structural organization of lignin biodegradation processes is unknown. The techniques of cell biology are required to give some understanding of where biodegradation is taking place and what agents are responsible.

Chemical analysis of lignin degradation products has and will continue to throw light on the nature of the depolymerizing processes involved. Little if anything is yet known about genetic control of product formation.

Microbiological research should now begin to examine ligninolysis with defined mixed-culture systems, since these may mimic the biodegradation processes that occur in natural environments.

APPLICATIONS OF LIGNOCELLULOSE BIODEGRADATION

The study of lignocellulolysis is relevant to industry and ecology. The two most abundant substrates are straw and wood; thus agriculture and forestry are the producer industries. Those concerned with wood decay and utilization have not collaborated in sufficient detail with those who are concerned with the decomposition of straw in environments such as the rumen, soil, fermentors, or mushroom beds. There is much common ground, and collaboration would be valuable.

Why study lignocellulolysis? The primary motivation has been the problems that the process creates. The study of wood decay has commercial importance. Plant pathologists have been concerned with lignocellulolysis in plant disease; but the formation of lignin is in fact a mechanism by which plants may produce resistance to disease. Straw decomposition can give rise to phytotoxic metabolites and this is one of the major reasons why the farmer burns his fields. Can a negative resource be converted into something with a positive value? Even the elimination of the negative value of straw would be advantageous in farming systems. The possible routes for upgrading lignocellulose wastes have been cataloged by Kirk in his recent review (Table 4). The production of fuel, particularly ethanol using cellulose as the primary substrate is a possibility. Pulp manufacture was not described in much detail during the course of the proceedings, but this is another route from cellulose. Chemical products were mentioned, but no identifiable chemical products are yet produced. These could come from either cellulose or lignin, and perhaps some quite exciting potentially high-value products might originate from the decomposition of lignin. Thus, there is considerable urgency in focusing attention on what chemicals might be desirable to produce from lignocellulosic wastes.

The most immediate application of lignocellulolysis industrially is for waste treatment. The impetus of research in this area should be to harness microorganisms to treat the various types of industrial waste and particularly, for example, food waste, which presents a considerable economic problem.

When utilization is considered, the type of fermentation system must also be considered. Microbiologists have become rather conditioned to submerged stirred cultures, including chemostats. There are potentially much simpler systems for use with solid substrates, such as

Table 4
Applications of Lignocellulose Biodegradation[a]

Application	Process	Currently used
Animal feed production	Use of ligninolytic fungi	–
Food production	Mushroom cultivation	+
Paper pulp production	Cellulase-less ligninolytic fungi	–
Chemicals	Lignocellulolytic microbes	–
Waste treatment	Lignocellulolytic microbes in fermenters	–

[a]*See* T. Kent Kirk (1983).

columns packed with lignocelluloses. On the farm, a silage tower would equate with this, and even simpler is the haystack. If such systems could be manipulated, economic processes might result.

In mushroom culture, composting is a solid substrate fermentation that is economic for a rather specific industry. Although this is an expanding industry, and it is producing a product that is the most important of the protected crops in Britain, it only utilizes straw on a relatively small scale. In some countries, other edible fungi are produced commercially on straw and wood wastes as substrates. Perhaps, on a cheaper scale, mushroom production technology might be employed to produce other types of products on a large scale. If there were a ban on straw burning in Britain, the incentive to do this would be greater.

One effective simple solid-substrate system being used in the Palouse region of the USA is the slot mulch technique. Bales of straw are incorporated in lines in the sloping hillsides to conserve the soil by virtue of the microbial polysaccharides produced.

A simple and mobile solid-substrate fermenter for use in the field is the rumen. It is difficult to find a more efficient or more economic solid-substrate fermentor. Would it be more efficient to build fermentors in the field or increase the stocking rate on the land?

It is perhaps disappointing that there are few economic costings of the commercialization of research findings. For example, although there is some controversy on the cost of alcohol production from lignocelluloses, it is an important exercise and also can be a stimulus to further experimentation. The value of fundamental studies *per se* is dubious without there being at least some economic prospect for a process. When a value is indicated, we should be able to exploit the recent potentials from biotechnology in optimizing lignocellulolysis.

REFERENCE

1. Kent Kirk, T. (1983), Degradation and Conversion of Lignocelluloses, in *The Filamentous Fungi*, Vol. IV. *Fungal Technology*, Smith, J. E., Berry, D. R. and Kristiansen, B., eds., Edward Arnold, London.