

Bei Verwendung zweikomponentiger Fließmittel laufen gelegentlich mehrere Substanzen mit der Entmischungslinie und bleiben dann ungetrennt (vgl. Figur 2 und 3)²². Die Elutionskraft der mobilen Phase steigt offenbar an der β -Front zu stark an. Man muss in solchen Fällen ein Fließmittel wählen, dessen Komponenten sich nicht so stark in ihrer Polarität unterscheiden (versuchsweise auch die reine, unpolarere Komponente verwenden!), oder eine sehr leichtflüchtige polare Komponente (z.B. Methylchlorid, Äther, Methylformiat) einführen bzw. Trennkammervolumen vergrössern (Verwischung der β -Front durch Konzentrationsausgleich über die Dampfphase) oder aber zu vielkomponentigen Fließmitteln

übergehen. Wir beschränken uns im nachfolgenden II. Teil auf eine Untersuchung dieser dritten Möglichkeit.

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A Medium to Demonstrate the Ligninolytic Activity of some Fungi

Techniques for easily and rapidly demonstrating the ability of a microorganism to break down wood or its individual constituents are of economic and theoretical significance. Although there are several methods^{1,2} for rapidly determining the cellulolytic activities of microorganisms, using relatively unaltered cellulose as a substrate, few techniques exist for determining their ligninolytic abilities. This is probably because of the difficulty of readily obtaining unaltered lignin, due to its usually close association with cellulose in plant material.

SØRENSEN³ prepared lignin by chemical extraction from pine and spruce wood flour; he incorporated this in solid media and was able to determine the ligninolytic activities of bacteria by their ability to clear the resultant opaque media. The technique used to extract the lignin rather precludes the adoption of this clear-cut method for routine use.

Methods which have been used to determine the ligninolytic activities of fungi have usually relied either on the gross form of the rot produced, or on chemical analysis of the attacked wood, or on the production of polyphenol oxidases, which are often produced by ligninolytic Basidiomycetes.

In the method to be described, ball-milled beech (*Fagus sylvatica* L.) sawdust is used as the major carbon source in an agar medium; this is similar to the method of EGGINS and PUGH² to determine the cellulolytic activities of fungi by the use of ball-milled cotton cellulose. The resultant medium is fawn and opaque and sets in Petri dishes with the ball-milled wood particles evenly distributed. The ability of a fungal culture to break down lignin (as well as cellulose and hemicelluloses) is shown by its clearing of the medium, which can usually be observed within fourteen days from inoculation. As it is considered⁴ that ligninolytic fungi are usually cellulolytic as well, the presence of cellulose in the medium is not a marked disadvantage.

The medium has the following constituents: ammonium sulphate 0.5 g; L-asparagine 0.5 g; potassium dihydrogen phosphate 1.0 g; potassium chloride 0.5 g; crystalline magnesium sulphate 0.2 g; calcium chloride 0.1 g; ball-milled beechwood 10 g; water to 1 l. The beechwood is prepared by ball-milling for 96 h a 4% suspension in

water of sawdust (which has been passed through a sieve of aperture 0.15 mm). This suspension of ball-milled beechwood sawdust is added to the other constituents of the medium after they have been steamed and before autoclaving for 20 min at 10 lb pressure. The final pH of the medium is 5.0.

A number of known ligninolytic Basidiomycetes, e.g. *Peniophora gigantea* (Fr.) Massée, *Polystictus sanguineus* (L.) Mey, *P. versicolor* (Linn.) Fr. and *Lenzites trabea* (Pers.) Fr., have produced complete clearing of the medium showing that they are ligninolytic as well as cellulolytic; such fungi may also be shown to be cellulolytic when grown on the ball-milled cotton cellulose medium².

Although the effect of ball-milling on the association of lignin and cellulose has not yet been determined, it is considered that this technique may be of use in studying the deterioration by fungi of wood species in general; it may also be of use in toxicity testing of wood preservatives.

Zusammenfassung. Es wird hier eine neue Methode angeführt, um die holzauflösende Fähigkeit einiger Pilze zu bestimmen. Ein milchiges, homogenes Agarmedium wird hergestellt, indem man kugelmahlendes Buchensägemehl als hauptsächliche Kohlenstoffquelle benutzt. Wenn man in dieses Medium bestimmte holzerstörende Pilze einpflanzt, wird sowohl ihre holzspaltende als auch ihre zellulosezerstörende Wirkung dadurch gezeigt, dass das Medium unter dem Wurzelgeflecht sich aufklärt.

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¹ R. E. HUNGATE, Bact. Rev. 14, 1 (1950).

² H. O. W. EGGINS and G. J. F. PUGH, Nature 193, 94 (1962).

³ H. SØRENSEN, J. gen. Microbiol. 27, 21 (1962).

⁴ W. G. CAMPBELL, in Wood Chemistry (Ed. L. E. WISE and E. C. JAHN; Reinhold Publishing Corp., New York 1952), vol. 2, p. 1061.