

lignin has been digested, the lignin ratios in Table 1 are numerically equal to the weight of frass derived from a gram of ingested wood. Therefore, to obtain α -cellulose values of frass samples as a percentage of the wood ingested, the α -cellulose values obtained by analyses of frass samples have been multiplied by the lignin ratios in Table 1. The α -cellulose values of wood and the corrected α -cellulose values of frass, together with relevant calculations of digestibility of wood and α -cellulose, are given in Table 2.

From Table 2 it is apparent that differences in α -cellulose content of matched wood and frass samples account for all but a few per cent of the calculated total digestion. The wood digested, other than α -cellulose, must include some protein and probably starch and free sugars since an amylase is known to be present in the gut extracts¹.

Since only about one-third of the α -cellulose present in the wood is digested, it is interesting to speculate whether the amount of some substance or substances present in wood determines the extent to which α -cellulose can be utilized. Such material is unlikely to be part of the carbohydrate complex but could well be protein and/or accessory growth-factors, the significance of which in the nutrition of *A. punctatum* is as yet unknown.

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² Falck, *Cellulosechem.*, **11**, 89 (1930).

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⁴ Spiller and Denne, *N.Z. J. Sci. and Tech.*, **30**, B, 129 (1948).

⁵ Purves, in Ott: "Cellulose and Cellulose Derivatives", 141 (New York, 1943).

⁶ Wise, Murphy and D'Addieco, *Paper Trade J.*, **122**, 35 (1946).

⁷ Wise and Ratliff, *Anal. Chem.*, **19**, 459 (1947).

Zinc Requirements of *Aspergillus niger*

ZINC is indispensable for the growth of all the higher plants, fungi, Actinomycetes and bacteria which have been examined, yet little is known of the role of zinc in metabolism^{1,2}. Evidence has been presented recently, however, that phosphatase of *Penicillium chrysogenum* is a zinc-containing enzyme³.

The zinc requirements of fungi have been determined largely from experiments in which glucose or sucrose has been used as the source of carbon. In the experiments described below the zinc requirements of *Aspergillus niger* were determined when the latter was grown on media involving a number of different carbon sources. The fungus was grown in surface culture in 100-ml. 'Hysil' or 'Pyrex' conical flasks in triplicate and harvested after 4 days incubation at 28° C. The basal medium contained ammonium nitrate, 0.3 per cent; dipotassium hydrogen phosphate, 0.05 per cent; magnesium sulphate, 0.05 per cent; iron, 0.5 p.p.m.; copper, 0.1 p.p.m.; and manganese, 0.05 p.p.m.

The media containing glucose, glycerol and peptone as carbon sources were purified free from zinc by extracting with chloroform solutions of diphenylthiocarbazonate at pH 7.6⁴, and those containing citric and gluconic acid by extracting with chloroform solutions of 8-hydroxyquinoline at pH 5.2⁵. Zinc was added as ZnSO₄·7H₂O in graded amounts and growth response curves obtained.

Table 1 shows that for a given carbon source such as glucose there is a constant relationship between the amount of carbon used, the amount of growth made and the minimum amount of zinc required.

Table 1

Sole source of carbon (gm %)	Mycelial weight (gm.)	Minimum zinc requirement for optimum growth (p.p.m.)	Zinc requirement per gm. of mycelium (p.p.m.)
Glucose 0.25	0.018	0.015	0.83
Glucose 2.0	0.14	0.1	0.715
Glucose 5.0	0.38	0.3	0.79

Table 2 shows the relative zinc requirements for growth with different carbon sources.

Table 2

Sole source of carbon (gm. %)	Mycelial weight (gm.)	Minimum zinc requirement for optimum growth (p.p.m.)	Zinc requirement per gm. of mycelium (p.p.m.)
Glucose 2	0.14	0.1	0.715
Glycerol 5	0.069	0.05	0.725
Gluconic acid 5	0.15	0.015	0.10
Citric acid 5	0.074	<0.01	<0.13
Peptone 1	0.02	0.05	2.5

It will be seen that zinc is essential for growth on all the carbon sources; but considerably less is required for the metabolism of organic acids than for glucose or glycerol. It would appear, therefore, that zinc is chiefly required for some process involved in the metabolism of glucose or glycerol. It will also be seen that the zinc requirements for the utilization of glucose and glycerol, while being considerably greater than for the utilization of acids, are similar to each other. This may indicate that the utilization of glucose and glycerol involves the same metabolic pathway which would be expected if the Embden-Myerhof scheme exists in fungi, and that the zinc requirement occurs in that part of the metabolic path involved in the utilization of both substances; that is to say, after the formation of the triose phosphates in the Embden-Myerhof scheme.

The apparently high zinc requirement for the metabolism of peptone may be due largely to the formation of zinc complexes with the latter, thus diminishing the available concentration of zinc.

If the similar zinc requirements for the utilization of glucose and glycerol and for gluconic and citric acid do imply a similarity of metabolism, it may be that a study of minimum trace element requirements would be of value in determining whether two substances are metabolized by similar or different pathways.

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