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EXPERIENCE WITH BIOLOGICAL TESTS IN THE FIELD OF THE BIODETERIORATION OF MATERIALS

4. THE INFLUENCE OF CARBON AND NITROGEN SOURCES IN MILDEW TEST MEDIA

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Summary. Different sources of C and N in mildew test media are compared. It is found that filterpaper strips may be advantageously replaced by cellulose powder. Carboxymethylcellulose is less favourable as a substitute and it is confirmed that the addition of saccharose adversely influences cellulolytic breakdown. More complex carbon sources do not seem to offer any advantages over cellulose. Ammonium nitrate appears the most suitable nitrogen source when compared with pepton, ammonium chloride and sodium nitrate.

Expérience avec des essais biologiques dans le domaine de la détérioration biologique des matériaux. 4. L'influence des sources de Carbone et d'Azote dans le milieu d'essai du Mildew. On compare les différentes sources de C et de N dans le milieu d'essai du mildew. On a trouvé que les bandes de papier filtre peuvent être avantageusement remplacées par de la poudre de cellulose. La cellulose-carboxymethyl est moins favorable en tant que produit de remplacement et il est confirmé que l'addition de saccharose influence la dégradation cellylolytique. Des sources plus complexes de carbone ne semblent pas offrir des avantages supérieurs à ceux de la cellulose. Le nitrate d'ammonium semble être la source d'azote la plus appropriée lorsqu'on le compare au peptone, au chlorure d'ammonium et au nitrate de sodium.

Erfahrungen mit biologischen Prüfungen auf dem Gebiet der Zerstörung von Materialien durch Organismen. 4. Der Einfluß von Kohlenstoff- und Stickstoff-Quellen auf Schimmel-Prüfnährböden. Verschiedene C- und N-Quellen in Schimmel-Prüfnährböden werden verglichen. Es hat sich gezeigt, daß Filtrierpapierstreifen gut durch Cellulose-Pulver ersetzt werden können. Carboxymethyl-Cellulose ist weniger günstig als Ersatz, und es wurde bestätigt, daß die Zugabe von Saccharose den cellulolytischen Abbau ungünstig beeinflußt. Komplexere Kohlenstoff-Quellen scheinen keine Vorteile gegenüber Cellulose zu haben. Ammoniumnitrat scheint im Vergleich mit Pepton, Ammoniumchlorid und Natriumnitrat die geeignetste Stickstoff-Quelle zu sein.

Experiencia con pruebas biológicas en el ramo de la biodeterioración de los materiales. 4. El influjo de las fuentes de carbon y de nitrógeno en los medios empleados para indagar el moho. Se comparan las fuentes diversas de C y de N en los medios empleados para indagar el moho. Se ha hallado que se pueden reemplazar con gran ventaja las listas de papel de filtrar por pólvora celulósica. La celulosa carboxy methyl se averigua como menos adecuada como reemplazo, y se confirma que el anadir sa carosa influye adversamente en la destrucción celulolitica. Las fuentes más complejas del carbón no parecen tener ventajas sobre la celulosa. El nitrato de amonio parece ser la fuente más apropiada del nitrógeno cuando se compara con el peptón, el clorido de amonio y el nitrato de sodio.

1. Introduction

A previous publication in this series (Hueck *et al.* 1966) gives a general discussion of mildew tests of textiles, as used in our laboratory, together with data on the influence of sterilization and variations in the inoculum on the results of such tests. In the present paper we present some findings on the influence of the composition of the medium, especially as to the sources of C and N, on mildew tests of textiles.

The relevant data were gathered in different periods, sometimes as a by-product of other investigations; the technique used, therefore, is not as homogeneous in the whole range of investigations as would be possible in a specific research project of short duration. We feel, however, that the time-consuming nature of the experiments makes it, nevertheless, worthwhile to report our experience, in which other investigators may be interested.

2. Experiments with Different Sources of Carbon

2.1 Introduction

The investigations reported in this chapter mainly deal with our specification Vitno Bio A3. This is a pure culture test with a defined inoculum in which the test specimen is laid out on a previously prepared mycelial mat growing on a synthetic medium containing cellulose as a homologous source of carbon (classification Hueck-Van der Plas, 1965, no. 3.4.2.-1.2.1.).

In the original specification, the cellulose is provided as a strip of filterpaper inserted into the Petri-dishes after cooling of the agar medium. In dealing with large numbers of dishes, it is technically easier to use a medium which, before being poured into the dishes, already contains the required amount of a carbon source. For this reason we started investigations on the incorporation into the agar of cellulose powders, such as used in chromatography, carboxymethylcellulose and similar sources of carbon. Certain

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powders that contain natural cellulose (e.g. saw dust) may also contain some substances more easily digested. Though the unfavourable influence of mono- and disaccharides on the cellulolytic performance of fungi had already been shown (cf. Siu, 1951, pp 201-203), we incorporated such substances for the sake of comparison. The ultimate aim of our investigation was merely practical in that we wished to obtain well growing test fungi, with good capacities for breaking down cellulosic textiles, on a medium that offers as few complications as possible in preparation, always bearing in mind that alterations in specifications must not be made lightheartedly.

2.2 **Methods**

The fungi we used are listed in Table 1. They were grown in 12 cm Petri-dishes containing 20 cm^3 of an agar medium. Growth was generally recorded according to an arbitrary scale:

— = no growth;
± = traces of growth;
\+ = slight growth;
++ = moderate growth;
+++ = abundant growth.

Table 1 Test fungi used in the investigation

Species	Strain
Aspergillus fumigatus	QM 6b
Aspergillus niger	CM.I. 17454
Aspergillus ustus	C.B.S. b
Chaetomium globosum	ATCC 6205
Memnoniella echinata	ATCC 9597
Myrothecium verrucaria	QM 450
Trichoderma viride	ATCC 8676

In one part of this study growth was recorded as the diameter of the surface covered with mycelium. Details of this method will be given in the appropriate place in the text. Activity, however, was usually determined by measuring the breakdown of cellulose, wherefore two methods were used. The first uses strips of our cotton standard fabric (240 g/m^2) and measures residual strength after a specified time of exposure to the action of the fungi. Residual strength is usually given as a percentage of initial strength. As the variation coefficient in this type of experiment is high, i.e. ranging from 3-4% in unexposed strips to $\geqslant 10\%$ in exposed strips, such a percentage gives too high an impression of accuracy.

For this investigation it was preferred to record the breakdown of strips rounded off to the next 10% and to represent each decade by the first figure.

In a formula this reads:

$$B_t = \frac{S_o - S_t}{S_o} \times 10 \qquad (1)$$

where

B_t = breakdown index for time = t
S_o = breaking strength before exposure
S_t = breaking strength after exposure during period tô →t
t = time recorded in days.

The other method uses a determination of cellulolytic activity according to Sumner and Somers (1966). In principle this consists in measuring the quantity of glucose released from carboxymethyl cellulose under the influence of enzymic action. The result is expressed as cellulase-units (C.U.), one C.U. being 0·4 mg "glucose", released under specified conditions.

The compositions of media used are shown in Table 2.

2.3 **Results**

In a first experiment, a comparison was made between strips of filterpaper on mineral agar and agar containing either suspended cellulose or saccharose. Five species of fungi were used. The cellulolytic capacities were measured with the strip method described in § 2·2. Growth was measured according to the arbitrary growth scale given in the same paragraph.

As cellulose suspension we used the liquid from a pilot plant for paper making, available in the Fibre Research Institute TNO, which liquid contained a suspension of fragmented cotton-linters prepared in a "hollander". We tried this, because it was expected that the cellulose in this suspension would have suffered little denaturation. The filterpaper used for the strips was Whatman no. 2. Each 20 cm^3 of medium received a strip weighing 3·9 g. The basic salt-solutions we used were drawn from specification Vitno Bio A3 (Hueck, 1958) (medium 1) indicated as VI, and from U.S. Federal specification CCC-T-191 b method 5750 (medium 2) indicated as CCCT. The well-known medium, Czapek solution, containing Saccharose as a carbon source was added for comparison (medium 3). The cotton test-strips were either untreated or impregnated with 0·75% Na- pentachlorophenol, the latter being henceforth indicated as PCP-cotton.

The results are shown in Table 3.

It will be noted from this table that the replacement of filterpaper strips by suspended cellulose has little influence. The differences between species, both as to growth and breakdown, are much larger than the effect within species of this change in carbon source. The influence of saccharose is, however, indeed remarkable, and it is of quite another nature: Saccharose furthering the growth of the aspergilli, whereas *Chaetomium* and *Trichoderma* grow more abundantly on cellulose. Only on Czapek-Dox agar, which had a high content of saccharose the cellulolytic activity was clearly inhibited in some fungi. Whereas the general

Table 2 Composition of media used in the investigation

Compound (g/l)	No. 1 VI	No. 2 CCCT	No. 3 Czapek	No. 4 QM	No. 5 CL
NH_4NO_3	2·0	3·0	—	6	Composition specified in text
$NaNO_3$	—	—	2·0	—	
KH_2PO_4	—	2·5	—	2·2	1·18
K_2HPO_4	1·0	2·0	2·0	2·8	—
Na_2HPO_4	—	—	—	—	1·0
$MgSO_4.7H_2O$	1·0	—	0·5	0·2	0·5
KCl	—	—	0·5	—	0·5
Cellulose powder	specified in text	specified in text	—	specified in text	5·0
Saccharose	—	—	30	—	—
K. bitartrate	1·0	—	—	—	—
Yeast extract	—	—	—	0·1	—
Trace elements	1)	—	2)	—	3)
Agar	20	20	15	15	—

1) added as 1 mg/l of $Fe_2(SO_4)_3$; $ZnSO_4$; $CuSO_4$; $MnSO_4$
2) added as 10 mg/l of $FeSO_4$
3) added as 1 mg/l of $FeSO_4$; $ZnCl_2$; $MnCl_2$

picture of the breakdown of PCP-treated strips is nearly the same for all media with most fungi, *Trichoderma viride* behaves differently. It shows a significant breakdown on agars that contain cellulose, but not on those containing saccharose; on these it grows rather poorly in our experiments. Along with this different behaviour, it is the only fungus which gives a significant breakdown of the strips treated with PCP, but only if pregrown on cellulose. In experiments not recorded here, we found this same deviating behaviour on strips impregnated with copper naphthenate.

In conclusion we may say that replacement of filterpaper strips by a cellulose suspension gives only rise to minor differences; they are far less important that the influence of other factors such as the choice of test-fungi.

These findings were corroborated in a second experiment, when as sources of carbon we compared filterpaper, cellulose suspension and Na-carboxymethylcellulose (CMC).

Furthermore, we changed to a medium with another composition of nutrient salts and yeast extract added. This medium was recommended to us (Quarter Master culture collection) as a remedy for the poor growth of *Myrothecium verrucaria* on medium 1. The composition of this QM medium is given in Table 2, as no. 4. The Na-CMC was available in two qualities with respectively low and high viscosity [1]). The type of experiment was the same as the one recorded above, except that *Aspergillus ustus* was dropped as a test fungus.

The results are shown in Table 4.

The data in this table shows that, again, a change from filterpaper to cellulose suspension has no significant effect on growth or breakdown properties. Both growth and breakdown are adversely affected by using the "soluble" Na-CMC as a source of carbon. The expected improvement in growth, as compared with VI, was indeed found with the QM medium, but it did not affect breakdown properties. Also in this experiment *Trichoderma viride* behaved differently as compared with the other fungi, in that it was rather insensitive to PCP. In conclusion we may say that a change from filterpaper to suspended cellulose is justified. Accordingly, we continued this investigation

[1]Hercules Powder Co. CMC-70 "high" and ditto "low".

Table 3 Comparison of growth of some test fungi on media containing different carbon sources, and breakdown of cotton strips exposed on these media

Medium	Carbon source g/l			Growth					Breakdown index									
									Blank cotton B_7					PCP-cotton B_{14}				
	Filterpaper-strip	Cellulose suspension	Saccharose	*Aspergillus niger*	*Aspergillus ustus*	*Chaetomium globosum*	*Myrotheciun verrucaria*	*Trichoderma viride*	*Aspergillus niger*	*Aspergillus ustus*	*Chaetomium globosum*	*Myrotheciun verrucaria*	*Trichoderma viride*	*Aspergillus niger*	*Aspergillus ustus*	*Chaetomium globosum*	*Myrotheciun verrucaria*	*Trichoderma viride*
1=VI	+	0	0	+		+	++	+++	6	5	6	7	5	1	1	1	1	6
1=VI	−	20	0	±		+	+	+++	6	4	5	7	4	1	0·5	1	1	5
2=CCCT	+	0	0	+		+	+++	+++	6	5	8	7	6	0·5	1	2	1	6
2=CCCT	−	20	0	++		++	+++	+	6	4	8	7	4	1	2	1	1	5
2=CCCT	−	0	1	+		+	±	±	6	6	6	7	6	1	2	0	2	1
2=CCCT	−	0	10	+++		+++	+	+	7	5	8	8	5	2	2	1	1	2
3=Czapek	−	0	30	+++		+++	+	±	0	0	8	5	0	1	0·5	1	0·5	1

with cellulose powders that were more easily prepared than the one just mentioned.

As such we tried cellulose powder as used in chromatography. We compared Schleicher & Schull nos. 123 and 124. The content of α-cellulose was found to be respectively 76% and 62%. These powders were mixed with the agar, care being taken to prevent precipitation during setting of the agar.

The set-up of the experiment was about the same as described above, but *Chaetomium globosum* was the only test fungus used. Furthermore, growth was checked in more detail. For the purpose we measured the diameter of the mycelial mat on consecutive days in 12 Petri-dishes cm with a standardized inoculum. The incoculum consisted of blocks of agar, covered with mycelium, punched from pregrown cultures of the test fungus, which blocks were put in the centre of the test medium.

During some time — the lag period τ — the diameter D remained constant, after that period, D increased linearly with time: $D = (t-\tau) \tan \alpha$. The fit of this graph with experiment is sufficiently accurate (correlation coefficient $r > 99\%$); it is shown in Fig. 1. Any experiment can be characterized by the two parameters $\tan \alpha$ and τ.

It will be seen from this table that hardly any difference in growth can be observed on the different types of cellulose used, be it that cellulose powder S&S no. 124 tends to introduce a short lag-period. After 8 days, however, its growth cannot be distinguished from that of the other media.

As to breakdown it appears that the only clear-out influence is that of the concentration of cellulose, a high concentration leading to a low breakdown of the added strips. From these experiments we concluded that we could adopt the incorporation of cellulose powder S&S no. 123 in a standard specification, which is our present practice.

In a final experiment about the influence of the source of carbon, we compared the influence of different concentrations of cellulose powder with that of more complex powders as saw-dust and wheat-bran. The experiment was carried out in shake cultures at 30°C and results recorded as growth and the production of cellulase (expressed as cellulase-units (C.U.)) after 14 days as described earlier.

The results are given in Table 7.

The summation of growth in Table 7 is given only as a rough approximation of overall performance. It will be noted from this table that the addition of

Table 4 Comparison of growth of some test fungi on media containing different carbon sources, and breakdown of cotton strips exposed on these media

Medium	Carbon source g/l			Growth				Breakdown index							
								Black cotton B_7				PCP-cotton B_{14}			
	Filterpaper	Cellulose-suspension	Ma-CMC	*Aspergillus niger*	*Chaetomium globosum*	*Myrothecium verrucaria*	*Trichoderma viride*	*Aspergillus niger*	*Chaetomium globosum*	*Myrothecium verrucaria*	*Trichoderma viride*	*Aspergillus niger*	*Chaetomium globosum*	*Myrothecium verrucaria*	*Trichoderma viride*
1=VI	+	0	0	±	+++	±	+++	0	(8)	9	6	0	0	0·5	6
4=QM	+	0	0	++	+++	+++	+++	0·5	7	9	5	0	(7)	1	6
4=QM	—	20	0	+++	+++	+++	+++	1	8	8	5	1	1	4	5
4=QM	—	0	20-low visc.	+	++	±	±	0·5	4	4	1	0·5	0·5	0	2
4=QM	—	0	20-high visc.	+	++	±	±	0	6	4	3	0	2	0·5	1

Legend: Data between brackets refer to strips visibly infected with other fungi than the pregrown test fungus. (Strips in this method are not sterilized.)

Table 6 Comparison of growth and breakdown properties of *Chaetomium globosum* on media contai ning different types of cellulose

medium	carbon source	concen-tration g/l	growth		breakdown-index	
			tan α	lag-period days	blank cotton B_7	PCP-cotton
1=VI	Cellulose-suspension	5	1·4	0·1	7	0·5
1=VI	,,	20	1·5	0	8	0·5
1=VI	Cellulose-powder S&S 123	20	1·4	0	8	1
1=VI	,, S&S 124	20	1·5	0·2	8	0·5
1=VI	,, S&S 123	100	1·4	0	3	0·5
1=VI	,, S&S 124	100	1·5	0·8	2	0·5

Table 7 Comparison of growth and cellulase production of test fungi grown on liquid media containing different concentrations of three sources of carbon

medium	source of carbon	conc. g/l	growth						cellulase-production (C.U.)					
			Aspergillus fumigatus	*Chaetomium globosum*	*Myrothecium verrucaria*	*Memnoniella echinata*	*Penicillium funiculosum*	all fungi together	*Aspergillus fumigatus*	*Chaetomium globosum*	*Myrothecium verrucaria*	*Memnoniella echinata*	*Penicillium funiculosum*	all fungi together
no. 5, table 1	cellulose powder S&S 123	1	++	+++	+++	++	±	10·5	1·7	0·4	2·0	0	0·7	4·8
,,	,,	3	++	+++	+++	+	+	10	2·1	0·6	2·8	0	1·4	6·9
,,	,,	10	++	+++	+++	−	++	10	2·3	1·2	3·3	0	1·8	8·6
,,	,,	30	+++	+++	+++	—	+	10	1·5	0·7	2·7	0	1·5	6·4
		subtotal	9	12	12	3	4·5	40·5	7·6	2·9	10·8	0	5·4	26·7
,,	wheat bran	1	+	++	+	++	+	7	0·4	0	0	0	0·6	1·0
,,	,,	3	+	++	+	++	++	8	0·7	0·1	0·3	0	0·6	1·7
,,	,,	10	+	++	++	+	+	7	0·9	0	0·7	0·3	0·4	2·3
,,	,,	30	++	++	++	+	+	8	1·4	0·5	0·9	0·3	0·7	3·8
		subtotal	5	8	6	6	5	30	3·4	0·6	1·9	0·6	2·3	8·8
,,	saw-dust	1	±	+	++	++	+	6·5	0·7	0	0·6	0	0	1·3
,,	,,	3	+	+	+	+	±	4·5	1·3	0·1	1·2	0	0	2·6
,,	,,	10	±	++	+	+	±	5	2·0	0·3	1·2	0	0·8	4·3
,,	,,	30	±	++	±	—	±	3·5	2·1	0·1	1·6	0	1·4	5·2
		subtotal	2·5	6	4·5	4	2·5	19·5	6·1	0·5	4·6	0	2·2	13·4

In subtotals growth is sum of number of crosses, counting ± as ½ cross.

these more complex sources of carbon has no advantage over cellulose, either from the point of view of growth or that of cellulase production.

Comparing the fungi, the low performance of *Memnoniella echinata* is notable. No correlation between growth characteristics and cellulase production is apparent. This is in line with the findings of Bravery (1968) that not only sugar but also other common ingredients of nutrient media such as asparagine and yeast-extract may inhibit cellulolytic activity. The use of such complex C-sources, therefore, appears not to be justified.

3. Experiments with Different Sources of Nitrogen

3.1 Introduction

If different specifications are compared, it can be seen, that basic media in these specifications, apart from the source of carbon, may show great differences as to the source of nitrogen used. Beforehand we may assume that it will make a difference whether nitrogen is present in organic or in inorganic form. Moreover, we may expect differences between ammonia salts and nitrates. In our usual specification, nitrogen is present as NH_4NO_3. It appeared to be worthwhile to check the usefulness of this approach. For the methods employed, § 2·2. may be consulted. In this part of the investigation growth and cellulase production (C.U. units) in shake cultures of 14 days duration were chosen as parameters.

The fungi used are described in Table 1. The basic medium used throughout this investigation was no. 5 of Table 2. The salts used were analytically pure. The pepton was obtained from Difco. Sterilization was done by autoclaving, except for urea, which was Seitz-filtered.

In a preliminary experiment it was found that urea decomposed by autoclaving, as indicated by an

increase in pH of the medium after sterilization (pH 6·6 → 8·2) and irregular and bad growth on such a medium.

3.2 **Results**

In a number of experiments were investigated growth and cellulase production on media containing pepton, different concentrations of urea and combinations of NH_4Cl and $NaNO_3$ as additions to basic medium 5. Moreover, the pH of the medium before and after growth was recorded. A summary of results is given in Tables 8 and 9.

By summation we may get a generalized "score" for both growth and cellulolytic activity (Table 9).

It can be seen from Tables 8 and 9 that pepton gives rise to luxurious growth and moderate cellulolytic activity. The pH, however, increases generally; except for *P. funiculosum*, which, in this respect, is an exception. Urea is no improvement as an organic source of nitrogen. Growth is only acceptable in the lowest concentration, and so is the cellulolytic activity. pH increases in all cases, indicating perhaps the release of ammonia and its subsequent use by the fungi.

Table 8 Growth, cellulolytic activity and change of pH of test fungi on media containing different sources of nitrogen.

		Basic medium	No. 5 (Table 2)								
Source of nitrogen g/l		Pepton (Difco)	5·0	—	—	—	—	—	—	—	—
		Urea	—	0·6	3·0	6·0	—	—	—	—	—
		NH_4Cl	—	—	—	—	2·65	2·00	0·66	—	—
		$NaNO_3$	—	—	—	—	—	1·06	3·20	4·25	—
		NH_4NO_3	—	—	—	—	—	—	—	—	3·00
ph of medium	before growth		7·2	6·6	6·6	6·6	6·3	6·4	6·5	6·6	6·5
	after growth	*Aspergillus fumigatus*	8·3	7·8	8·4	8·4	3·8	4·9	5·8	6·9	6·2
		Chaetomium globosum	8·5	8·0	8·7	8·7	3·9	4·2	5·3	6·9	6·0
		Memnoniella echinata	8·5	8·0	8·6	8·5	5·3	5·4	5·7	6·9	6·3
		Myrothecium verrucaria	8·7	7·8	8·6	8·7	5·4	6·3	6·4	7·3	6·5
		Penicillium funiculosum	6·7	8·0	8·1	7·9	4·3	4·7	5·5	6·5	5·7
growth		*Aspergillus fumigatus*	++	+	++	++	++	+++	+++	++	+++
		Chaetomium globosum	+++	+++	+++	++	++	++	+++	+++	+++
		Memnoniella echinata	+++	++	+	+	++	++	+++	+++	+
		Myrothecium verrucaria	+++	+++	+	+	+	+	++	++	+++
		Penicillium funiculosum	++	±	±	±	+	+	—	+	++
cellulolytic activity in cellulose units (C.U.)		*Aspergillus fumigatus*	0·0	1·0	1·6	1·9	0·6	0·7	1·3	1·2	2·2
		Chaetomium globosum	1·2	1·1	0·6	0·4	0·6	1·0	1·2	0·6	0·8
		Memnoniella echinata	0·5	0·8	0·0	0·0	0·0	0·2	0·6	0·4	0·0
		Myrothecium verrucaria	0·6	2·2	0·5	0·3	1·9	3·0	2·1	0·7	2·9
		Penicillium funiculosum	1·5	0·0	0·0	0·0	1·3	1·3	1·3	1·0	1·5

In these experiments, yeast-extract and trace elements were omitted from the basic medium.

Table 9 Scores for growth and cellulolytic activity of five test fungi on media containing different sources of nitrogen

pepton	urea	NH_4Cl	$NaNO_3$	NH_4NO_3	score for	
					growth	cellulolytic activity
5·0	—	—	—	—	13	3·8
—	0·6	—	—	—	9·5	5·2
—	3·0	—	—	—	7·5	2·7
—	6·0	—	—	—	6·5	2·6
—	—	2·65	—	—	8	4·4
—	—	2·00	1·06	—	9	6·2
—	—	0·66	3·20	—	12	6·5
—	—	—	4·25	—	12	3·9
—	—	—	—	3·0	12	7·4

With inorganic salts it is clear that the presence of nitrate is favourable for growth leading to a comparable growth as with pepton. However, only if both nitrate and ammoniacal nitrogen are present, cellulolytic activity becomes optimal. Apparently the best combination of both factors is present in NH_4NO_3. Additional advantages of this compound appear to be that changes in pH of the medium are slight; it is better defined than pepton, it can be sterilized better than urea and it requires only one salt as compared with mixtures of NH_4Cl and $NaNO_3$.

It must be remarked that change in pH may be affected by growth factors other than nitrogen compounds such as phosphates, minerals, source of carbon, etc. Buffering capacity is of course provided by the phosphates in the media, but generally this is insufficient for experiments exceeding one or two weeks. In conclusion we may state, however, that NH_4NO_3 appears to be the compound of choice for a source of nitrogen in test media of the type investigated here.

4. Discussion

It has been the aim of our investigations to arrive at a synthetic medium for mildew tests that is as simple as possible. The investigation of the influence of the source of carbon shows that such a simplification can be reached by using cellulose powder as specified, which circumvents the cumbersome technique of separately adding strips of filterpaper. The only drawback of the use of cellulose powder is a trivial one; the powder precipitates if the agar is left too long in the liquid state. The obvious remedy for this is to stir well before using and to take care of a rapid solidification after pouring plates. With the amount used (20 g/l) no undue settling need be feared.

Increasing the amount of cellulose powder is apparently of no use, as both growth and cellulolytic activity were influenced adversely under some conditions of test (Table 6). In practice the precipitation is found inconvenient if cellulose agars are sterilized in Petri-dishes or Roux or Kolle flasks and no time is available for individual handling of media. If plates are poured in sterile Petri-dishes from molten medium in tubes or flasks, there is no trouble. It must be remarked that insertion of sterile filterpaper strips in Petri-dishes requires individual handling of plates, with a greater chance of contamination, and more complex preparation, because of separate wrapping and sterilization of filterpaper strips. The investigation of the influence of the source of nitrogen shows that our choice of NH_4NO_3 as the sole source of N is justified and needs no amendment.

It will be noted from Table 2, recipe 1, that our present medium contains two further items which may appear to be of rather doubtful value, viz. the addition of K. bitartrate and trace elements. These complications which are absent in the CCCT recipe (Table 2) and in the Mildew Test Medium as advocated e.g. by the Difco manual (B 428) 9th edition (1953) will be the subject of a future publication in this series, together with a review of relevant literature.

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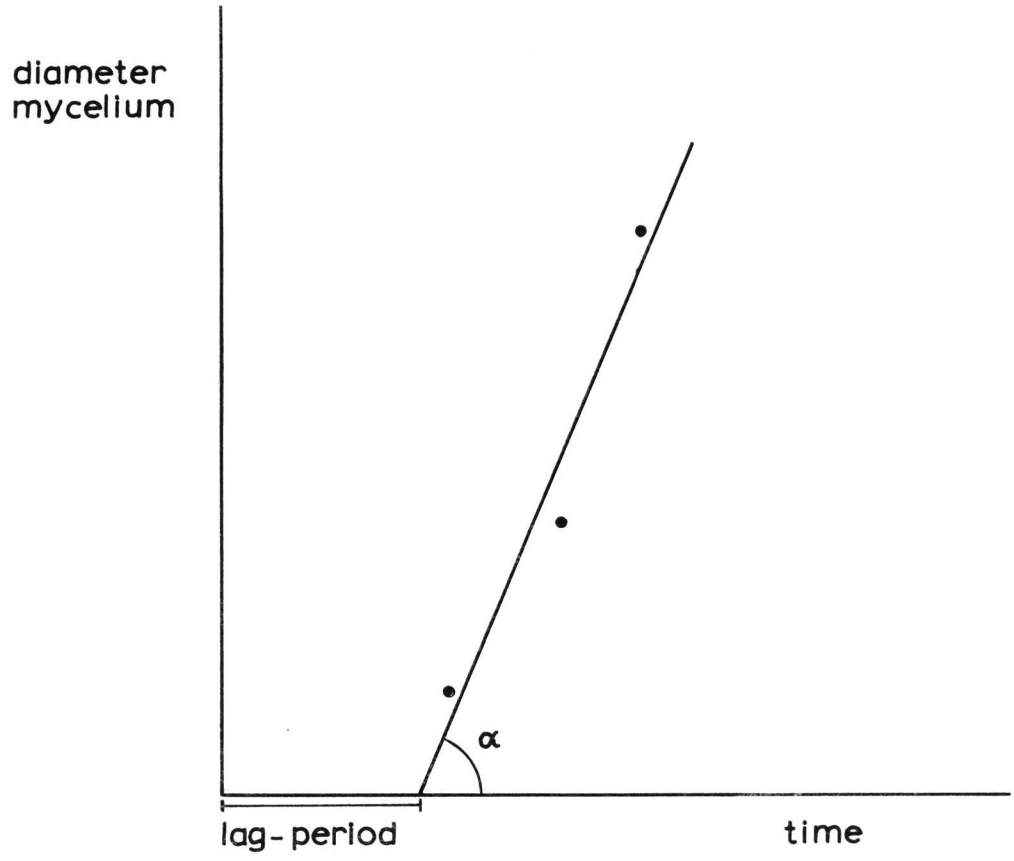

FIGURE 1 Growth of mycelium of test fungi in Petri-dishes (explanation in text)