
INTERNATIONAL
BIODETERIORATION
BULLETIN

A QUARTERLY JOURNAL OF BIODETERIORATION

BIODETERIORATION INFORMATION CENTRE
THE UNIVERSITY OF ASTON IN BIRMINGHAM
ENGLAND
CATOMANCE LIMITED
manufacturers of mystox* for the preservation of timber, textiles, paper, cordage, plastics and specialised applications

*mystox is the registered trade mark of Catomance Limited

94 BRIDGE ROAD EAST, WELWYN GARDEN CITY, HERTS., ENGLAND. Telephone: Welwyn Garden 24373/8
INTERNATIONAL
BIODETERIORATION
BULLETIN

CONTENTS

NEWS AND COMMENT 139-140

MYCOTOXINS 141-147
M. O. Moss

A METHOD FOR THE CONVENIENT PREPARATION OF ARTIFICIAL GAS MIXTURES 149-152
IN CLOSED CONTAINERS
J. H. Walsh

EXPERIENCE WITH BIOLOGICAL TESTS IN THE FIELD OF THE BIODETERIORATION 153-161
OF MATERIALS. 4. THE INFLUENCE OF CARBON AND NITROGEN SOURCES IN MILDEW
TEST MEDIA
H. J. Hueck and W. Hazeu

A PERFUSION TECHNIQUE TO STUDY THE COLONISATION OF A CELLULOSIC 163-168
SUBSTRATE BY FUNGI
K. A. Malik and H. O. W. Eggins

CUMULATIVE INDEX, 1965-1969 196-170
# Table of Contents

<table>
<thead>
<tr>
<th>Title</th>
<th>Pages</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>TABLE DES MATIERES</strong></td>
<td></td>
</tr>
<tr>
<td>Nouvelles et commentaires</td>
<td>139-140</td>
</tr>
<tr>
<td>MYCOTOXINES</td>
<td>141-147</td>
</tr>
<tr>
<td>M. O. Moss</td>
<td></td>
</tr>
<tr>
<td>UNE METHODE POUR OBTENIR UNE BONNE PREPARATION DE MELANGE DE GAZ</td>
<td>149-152</td>
</tr>
<tr>
<td>ARTIFICIELS DANS DES RECIPIENTS HERMETIQUES</td>
<td></td>
</tr>
<tr>
<td>J. H. Walsh</td>
<td></td>
</tr>
<tr>
<td>EXPERIENCE AVEC DES ESSAISS BIOLOGIQUES DANS LE DOMAINE DE LA</td>
<td>153-161</td>
</tr>
<tr>
<td>DETECTION BIOLOGIQUE DES MATERIALS. 4. L'INFLUENCE DES SOURCE DE</td>
<td></td>
</tr>
<tr>
<td>CARBONE ET D'AZOTE DANS LE MILIEU D'ESSAI DU MILDEW</td>
<td></td>
</tr>
<tr>
<td>H. J. Hoeck et W. Hazee</td>
<td></td>
</tr>
<tr>
<td>UNE TECHNIQUE DE PERFUSION POUR ETUDIER LA COLONISATION D'UN SUBSTRAT</td>
<td>163-168</td>
</tr>
<tr>
<td>CELLULOSIQUE PAR LES CHAMPIGNONS</td>
<td></td>
</tr>
<tr>
<td>K. A. Malik et H. O. W. Eggins</td>
<td></td>
</tr>
<tr>
<td><em>Indice, 1965-1969</em></td>
<td>169-170</td>
</tr>
</tbody>
</table>

**INHALT**

<table>
<thead>
<tr>
<th>Title</th>
<th>Pages</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nachrichten und Kommentar</td>
<td>139-140</td>
</tr>
<tr>
<td>MYCOTOXINE</td>
<td>141-147</td>
</tr>
<tr>
<td>M. O. Moss</td>
<td></td>
</tr>
<tr>
<td>EIN VERFAHREN ZUR GEEIGNETEN VORBEREITUNG VON KUNSTLICHEN GASMISCHEN</td>
<td>149-152</td>
</tr>
<tr>
<td>HUNGEN IN GESCHLOSSENETEN BEHALTERN</td>
<td></td>
</tr>
<tr>
<td>J. H. Walsh</td>
<td></td>
</tr>
<tr>
<td>ERFahrungen mit biologischen Prüfungen auf dem GEBEIT DER ZERSTÖRUNG</td>
<td>153-161</td>
</tr>
<tr>
<td>von Materialien durch Organismen. 4. der Einfluss von Kohlenstoff-</td>
<td></td>
</tr>
<tr>
<td>und Stickstoff-Quellen auf Schimmel-Prüfnährboden</td>
<td></td>
</tr>
<tr>
<td>H. J. Hueck und W. Hazee</td>
<td></td>
</tr>
<tr>
<td>EIN PERFUSIONSVERFAHREN ZUR UNTERSUCHUNG DER BESIEDLUNG VON</td>
<td>163-168</td>
</tr>
<tr>
<td>CELLULOSE-HALTIGEN SUBSTRATEN</td>
<td></td>
</tr>
<tr>
<td>K. A. Malik und H. O. W. Eggins</td>
<td></td>
</tr>
<tr>
<td><em>Register, 1965-1969</em></td>
<td>169-170</td>
</tr>
</tbody>
</table>

**CONTENIDO**

<table>
<thead>
<tr>
<th>Title</th>
<th>Pages</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nuevas y Comentarios</td>
<td>139-140</td>
</tr>
<tr>
<td>MYCOTOXINS</td>
<td>141-147</td>
</tr>
<tr>
<td>M. O. Moss</td>
<td></td>
</tr>
<tr>
<td>UN MÉTODO PARA PREPARAR DE MANERA CONVENIENTE LAS MEZCLAS ARTIFICI-</td>
<td>149-152</td>
</tr>
<tr>
<td>ALES DE LOS GASES EN LOS RECEPTACULOS CERRADOS</td>
<td></td>
</tr>
<tr>
<td>J. H. Walsh</td>
<td></td>
</tr>
<tr>
<td>EXPERIENCIA CON PRUEBAS BIOLOGICAS EN EL RAMO DE LA BIODETERIORACI-</td>
<td>153-161</td>
</tr>
<tr>
<td>ON DE LOS MATERIALES. 4. EL INFLUENCIA DE LAS FUENTES DE CARBON Y DE</td>
<td></td>
</tr>
<tr>
<td>NITROGENO EN LOS MEDIOS EMPLEADOS PARA INDAGAR EL MOHO</td>
<td></td>
</tr>
<tr>
<td>H. J. Hueck y W. Hazeu</td>
<td></td>
</tr>
<tr>
<td>UNA TÉCNICA DE PERFUSION PARA ESTUDIAR LA COLONIZACION DE UN</td>
<td>163-168</td>
</tr>
<tr>
<td>SUBSTRATO CELULOSICO POR LOS HONGOS</td>
<td></td>
</tr>
<tr>
<td>K. A. Malik y H. O. W. Eggins</td>
<td></td>
</tr>
<tr>
<td><em>Indice, 1965-1969</em></td>
<td>169-170</td>
</tr>
</tbody>
</table>
ACKNOWLEDGMENTS TO SUSTAINING ORGANISATIONS

Financial support for the International Biodeterioration Bulletin from the following organisations is gratefully acknowledged:

ALBRIGHT & WILSON (MFG) LTD., Oldbury Division, P.O. Box 3, Oldbury, Warley, Worcestershire.

AVON RUBBER COMPANY LIMITED, Melksham, Wiltshire; manufacturers of tyres and miscellaneous rubber products.

BORAX CONSOLIDATED LIMITED, Borax House, Carlisle Place, London, S.W.1. (Tel. Victoria 9070); miners, refiners and suppliers of borax, boric acid, boron ores, boron products and allied chemicals.

B.D.H. CHEMICALS LIMITED, Laboratory Chemicals Division, Poole, Dorset; manufacturers of laboratory chemicals, biochemicals, industrial fine chemicals and microbicides.

BRITISH INSULATED CALLENDERS CABLES LIMITED, 38 Wood Lane, London, W.12.

THE BRITISH PETROLEUM COMPANY LIMITED.

B.X.L. PLASTICS MATERIALS GROUP LIMITED, Tyeseye, Birmingham.

CATOMANCE LIMITED, Welwyn Garden City, Hertfordshire; manufacturers of specialty chemicals for the textile, paper, timber, leather industries, etc., including fungicides, bactericides and insecticides.

CENTRAL LABORATORY TNO, Delft, The Netherlands; research, analysis and testing facilities in materials science and technology, including biodeterioration and marine research. Sponsored by government agencies and by international industries.

CHIS FOREIGN FOODS WHOLESALERS LTD., Stories Mews, Stories Road, Camberwell, London S.E.5.; tropical foodstuffs importers.

CIBA LIMITED, 4000 Basle 7, Switzerland.

COALITE AND CHEMICAL PRODUCTS LIMITED, P.O. Box No. 21, Chesterfield, Derbyshire; manufacturers of chlorinated and alkylated phenols, phosphate plasticisers and bactericides. Extensive research work and development carried out in all these fields and customer service facilities are available for bactericides.

COMMERCIAL PLASTICS GROUP OF COMPANIES, Industrial Division, Berkeley Square House, Berkeley Square, London W.1.; specialists in the production of film and sheeting in PVC for all types of applications.

COURTAULDS LIMITED, Coventry.

CUPRINOL LIMITED, Adderwell, Frome, Somerset; manufacturers of preservatives for wood, textiles, cordage, paper, adhesives, paints, leather, plastics, and paint driers.

THE DUNLOP COMPANY LIMITED.

FARBENFABRIKEN BAYER A.G., Leverkusen, Germany; manufacturers of dyestuffs, industrial chemicals, synthetic fibres, pharmaceutical and agricultural chemicals and preservatives for wood, foodstuffs and technical products.

FISONS LIMITED, Cambridge Division, Saffron Walden, Essex; makers of agricultural chemicals Research on control of weeds, plant diseases, spoilage organisms, and agricultural and livestock pests.

FORESTAL INDUSTRIES (UK) LTD. Incorporating Dearborn Pittam and Feedwater Specialists. Both companies are suppliers of industrial water treatment service and chemicals, including a wide range of biocides for control of micro-organisms in cooling water.

GAGLIARDI RESEARCH CORPORATION, East Greenwich, Rhode Island, U.S.A.; sponsored industrial research in textile chemical dyeing and finishing products and processes.

GALLOWAY & BARTON-WRIGHT, Haldane Place, London, S.W.18.; consultants in industrial microbiology and microbiological deterioration.

GEIGY (U.K.) LIMITED, Simonsway, Manchester, 22.

J. R. GEIGY S.A., Basle, 21, Switzerland; manufacturers of dyestuffs, industrial chemicals, pharmaceuticals and agricultural chemicals.

GLAXO LABORATORIES LIMITED, Greenford Road, Greenford, Middlesex; manufacturers of pharmaceuticals, industrial enzymes and fine chemicals.

ARTHUR GUINNESS SON & COMPANY (DUBLIN) LIMITED.

HICKSON & WELCH (HOLDINGS) LTD., Ings Lane, Castleford.

IMPERIAL CHEMICAL INDUSTRIES LIMITED, Agricultural Division, Billingham, Co. Durham.

ARTHUR D. LITTLE LIMITED, with offices at Berkeley Square House, Berkeley Square, London, W.1.; and offices and laboratories at Inversk Gate, Musselburgh, Midlothian; independent sponsored research organisation.

MAY & BAKER LIMITED, Dagenham, Essex; chemical manufacturers.

RECKITT & SONS LIMITED, Hull.

RENTOKIL LABORATORIES LIMITED, East Grinstead, Sussex.

REVERTEX LIMITED, Harlow, Essex.

SCIENTIFIC CHEMICALS INC., 1637 South Kilbourne Ave., Chicago, Illinois, 60623, U.S.A.; leading manufacturers of industrial fungicides and bactericides who maintain substantial research and development facilities to assist customers in the development of final products geared to meet government and industry standards.

SHELL INTERNATIONAL CHEMICAL COMPANY LIMITED.

STAUFFER CHEMICAL COMPANY RICHMOND RESEARCH CENTER, 1200 South 47th Street, Richmond, California 94804, U.S.A.

VINYL PRODUCTS LIMITED, Butter Hill, Carshalton, Surrey; manufacturers of synthetic resins, specialising in emulsion polymers for adhesives, building, paper, surface coating and textile applications.

YARSLEY LABORATORIES LIMITED, Clayton Road, Chinnor, Oxfordshire; independent research and testing facilities.
NOTES FOR CONTRIBUTORS

The International Biodeterioration Bulletin is published four times per year (Spring, Summer, Autumn and Winter). Typescript contributions in triplicate should be sent to the Editor, Dr. H. O. W. Eggins, at the above address.

The Bulletin acts as a vehicle for the publication of works on all aspects of biodeterioration, i.e. the deterioration of materials of economic importance by micro-organisms, insects, rodents, etc.

Contributions may be in English, French, German or Spanish and should be submitted in triplicate on international A4 size paper (21.0 cm × 29.7 cm or 8.27 in. × 11.69 in.); typewritten on one side of the paper only. A summary of 25-100 words should accompany each contribution.

Illustrations should be clearly drawn in Indian ink or should be photographed. The reduction desired should be clearly indicated and illustrations when reduced are not to exceed 17 cm × 26 cm. Where figures are to be inserted in the text the approximate position for each one should be clearly marked in the typescript.

The bibliographic references are to be indicated in the text as, e.g.:

Reese and Levison (1952).

and in the bibliography:


Authors are requested to abbreviate journal titles according to the conventions of the World List of Scientific Periodicals.

Proofs will not be sent to authors before final publication.

25 reprints will be sent free of charge to each author. Additional reprints are obtainable on application to the Publications Editor at a charge of £5 ($12) per 100.
A meeting of the International Biodegradation Research Group took place in The Netherlands, on 13th-15th October, 1969. The Working Group on Plastics met in Delft and discussed the programme for the next year, after which the biology facilities of the Central Laboratory TNO were visited.

The Plenary Meeting reviewed and approved the work of the different Working Groups. A proposal for the establishment of a Working Group on Paints and Paint Films was discussed. It was concluded that this could be an important venture of the Research Group; a small programme committee consisting of Mrs. C. Skinner (The Paint Research Station, Teddington), Mr. J. J. Elphick (Admiralty Materials Laboratory, Poole, Dorset) and Mr. C. E. Hoey (Ministry of Technology, Directorate of Materials, London WC2) will consider which problems should be studied.

After the successful first International Biodegradation Symposium the Standing Committee for the Organization of Biodegradation Symposium is pleased to announce that the Second International Biodegradation Symposium will be held in The Netherlands, 13th-18th September, 1971, the Central Laboratory TNO acting as host.


A consultancy service dealing with the design and manufacture of plastic packaging particularly in relation to the prevention of biodegradation in developing countries is being provided by Intertech P.P. It is considered desirable for developing countries to manufacture their own packaging as far as possible and emphasis has, therefore, been placed on the design of production plant which can economically handle relatively small quantities of a wide variety of packaging products.

Further information can be obtained from: Intertech P.P., Greensleeves, Woodlands Road, Bickley, Kent, England. Telephone: 01-467 5245.

**THE BIODETERIORATION SOCIETY**

Widespread interest has recently been shown in forming a body to promote the study of biodegradation problems. As a result of several meetings between scientists interested in this field, it has been decided that this may best be accomplished by establishing an independent scientific society, to be known as the Biodegradation Society. Although this Society is being initiated in the U.K., it is stressed that it is not regarded as a solely British venture, and it is hoped that it will become truly international.

The Society's aims will be to promote the science and technology of biodegradation:

a) by the organisation of meetings, Symposia, etc. on aspects of biodegradation—at the moment it is anticipated that there will be about four meetings per year, held in the U.K.
b) by the reorganisation of workers in the field of biodegradation in the activities of other organisations throughout the world.

In scope the Society will embrace all aspects of biodegradation of natural products and manufactured materials. Because of adequate coverage elsewhere, however, it is at present envisaged that coverage of aspects of plant and animal pathology and the deterioration of manufactured foodstuffs will be minimal.

The annual subscription to the Society to individual members will be £1. All those interested are invited to complete the enclosed application form and send it together with their remittance to the Acting Honorary Treasurer:


The first scientific meeting will be held during the Autumn of 1970. Details of this, together with copies of the draft constitution will be sent to those who return their application forms in due course.

It is hoped that the Society will be able to arrange for the International Biodegradation Bulletin to act as its official organ, and that it will then contain the Society news, notes, etc. The Biodegradation Information Centre plans to increase its charges for its publications in 1971 so that they are more in line with current rates. Members of the Society will be able to obtain the publications and services of the B.I.C. at extremely advantageous rates.

Dr. H. J. Banker, Acting Honorary President, 17, Radnor Road, Twickenham, Middlesex, England.

J. J. Elphick, Esq., Acting Honorary Secretary, Admiralty Materials Laboratory, Holton Heath, Poole, Dorset, England.

A number of three-day courses on the control of insects and rot in buildings have been organised by the Forest Products Research Laboratory for 1970. These are intended for surveyors of buildings and supervisors and foremen who are concerned with the eradication and control of wood-destroying insects and fungi. Further information can be obtained from: The Director, Forest Products Research Laboratory, Princess Risborough, Aylesbury, Buckinghamshire, England. Telephone: Princess Risborough 3101.

The major German farm chemicals manufacturer, Badische Anilin- und Soda-Fabrik A.G., has recently announced the establishment of a marketing organisation in the United Kingdom. The new company is called B.A.S.F. United Kingdom and has its headquarters at Ipswich, Suffolk. (Abstracted from The Times.)

Information on the behaviour of chemicals in sewage treatment processes and on their toxicity to fresh-water life may be obtained from the Information Service on Toxicity and Biodegradability (INSTAB) which is operated by the Water Pollution Research Laboratory of the Ministry of Technology. The List of Substances on which information is available is now in its third edition and may be obtained on request. The service is normally free, but a charge is made for overseas enquiries, and also for enquiries from the U.K. if the weight of information required takes more than a day to assemble. Enquiries should be addressed to: The Director, Water Pollution Research Laboratory, Elder Way, Stevenage, Herts, England.

The problems of the corrosion of steel and other materials in the coal industry are of paramount importance, so much so that the costs of materials used in mines amount to 20 per cent of mining costs.

To acquaint a wider circle of mining engineers with this problem a course in the prevention of corrosion in mines was organized.
in September 1969 at Gliwice, Poland by the Silesian Centre for Technical Progress. In this course, which covered the different subjects on corrosion in mines 33 engineers from the coal industry took part. The syllabus covered 80 lectures, 6 of them being devoted to micro-biological deterioration of materials. Particular attention was drawn to the symptomatology of deteriorated materials and to the prevention of deterioration by bacteria and fungi. The lecturer, Dr. B. J. Zyska of the Central Mining Institute, Katowice gave examples of materials deteriorated by micro-organisms in mines, viz.: conveyor belting, rubber cables, ropes, mine timber and emulsions.

The profits of Hickson and Welch for the second half of 1969 were up by 19 per cent over the first half, bringing their total profit for the year to £1,601,000, an increase of 12 per cent over the previous year. This figure exceeds the previous profit peak of 1966. (Abstracted from The Times).

A new marine biological laboratory has recently been opened at Fawley on Southampton Water (U.K.) to study the interactions between a large electricity generating station and the marine environment. This is a joint project of Southampton University and the Central Electricity Research Laboratories at Leatherhead.

As well as dealing with aspects of the effect of power stations on the environment such as thermal pollution, investigations will be conducted into the fouling of inlet tunnels by marine organisms, particularly mussels. The mussels not only reduce the flow of water through the tunnel but may be swept into the power station and cause considerable damage to the condenser tubes. One control method which is under investigation is the introduction of low concentrations of chlorine into the water.

Birds caused another aeroplane crash at Kingsford Smith Airport, Sydney, Australia, at the beginning of December. The pilot was forced to take emergency action when the airliner, a Boeing 707 of Pan American World Airways, ran into a flock of birds as it was speeding for take-off on a flight to Honolulu and New York. Ingestion of the birds into two of the engines caused a loss of power and, in attempting to stop the take-off the pilot overshot the runway by 200 feet. Apart from the destruction of its landing gear, the aircraft was not badly damaged and only two of the 125 passengers were hurt. (Abstracted from The Times.)

On 1st October, 1969 two Netherlands research institutes, ITBON (Institute for Biological Field Research) at Arnhem and RIVON (State Institute for Nature Conservation Research) at Zeist, were amalgamated to form the Rijksinstituut voor Natuurbeheer (Research Institute for Nature Management). The new institute has the task of conducting research in the fields of nature conservation, landscape protection and the rational use of natural resources.

The amalgamation was brought about because the fields of research of both institutes were closely related and the growing importance of the care of the biosphere and the management of the environment made a concentration of forces desirable.

Prof. Dr. D. J. Kuuen has been appointed General Director of the new Institute while Prof. Dr. M. F. Mörzer Bruyns and Dr. C. W. Stortenbeker are respectively Directors of the Zeist and Arnhem establishments.

The former RIVON section will soon be moving from Zeist to new accommodation at Leersum. Both centres will remain in use with laboratory facilities concentrated at Arnhem and facilities for field research at Leersum.

The Fourth Vertebrate Pest Conference sponsored by the California Vertebrate Pest Technical Committee is being held on 3rd-5th March, 1970 at the El Rancho Motel, Sacramento, California. Further information as well as the proceedings of the second and third conferences (1964 and 1967) can be obtained from: Rex E. Marsh, Acting Secretary/Treasurer, Vetebrate Pest Conference, Department of Animal Physiology, University of California, Davis, California 95616, USA. The proceedings of the first conference (1962) are available from: National Pest Control Association, 250 West Jersey Street, Elizabeth, N.J. 07202, USA.
Summary Recent advances in the study of mycotoxins are reviewed.

Mycotoxines On passe en revue les résultats récemment obtenus dans l'étude des mycotoxines.

The metabolites produced by fungi have fascinated biochemists and have, for many years, offered a challenge to chemists interested in structural elucidation. The fact that some of these metabolites show various kinds of biological activity, as antibiotics and as physiologically active compounds, stimulated this interest. More recently the potential importance of some of these metabolites as toxins, when excreted by moulds into human or animal foods, has added further stimulus to an already active field.

The study of mycotoxins has gone through a phase of enormous field and laboratory activity into what one might describe as a review phase and there have been many reviews, as well as a book, published on this subject over the past few years. These review articles are listed as a separate part of the bibliography for the convenience of the reader. It would not be useful for the present author to redescribe material covered in these several reviews but perhaps it would serve some purpose to outline a few topics to which significant contributions have been made over the past few years.

Results of surveys of the fungal flora associated with a wide range of materials for human and animal consumption continue to appear in the literature. Weltt and Lucas (1968) have described the fungi isolated from damaged flue cured tobacco; the fungal flora of flour and refrigerated dough products is covered in these several reviews but perhaps it would serve some purpose to outline a few topics to which significant contributions have been made over the past few years.

A toxic strain of Trichoderma lignorum isolated from melon stems used as fodder was shown to produce abortions in pregnant guinea pigs by Stankush et al. (1966). Toxic strains of Aspergillus wentii (Rabie et al. 1965) and Penicillium glumae (Rice & Barrow, 1967) have been described. In a detailed study of the possible association of fungi with kidney disease in domestic animals Krogh and Hasselager (1968) conclude that a strain identified as Penicillium viridicatum could produce an active nephrotoxin. Such activity has also been associated with strains of Aspergillus fumigatus in studies by Rutquist and Persson (1966) and Thornton et al. (1968). The literature on the aflatoxins continues to increase and is usefully covered in a bibliography produced by the Tropical Products Institute (1968). Of particular interest has been the various suggestions that the aflatoxins are produced by species other than Aspergillus flavus and A. parasiticus. Scott et al. (1967) suggested the formation of aflatoxin by A. ostiarius, Walbeck et al. (1968) claim that the metabolites are produced by a species of Rhizopus and a strain of A. ochraceus. Kulik and


Mycotoxins.

Mycotoxines

M. O. Moss

Mycotoxins Es Wird eine Übersicht über die neueren Ergebnisse von Mycotoxin-Studien gegeben.

Mycotoxins Progresos recientes en el estudio de los mycotoxins se pasan en revista.

A toxic strain of Sclerotium rolfsii, the sterile mycelium of the basidiomycete Peltiglaria rolfsii, has been isolated by Terblanche and Rabie (1967). Strains of this fungus are known to produce oxalic acid but these authors claim that the toxicity they observe is not due to that particular metabolite. Of 53 isolates of the pyrenomycete Chaetomium globosum made by Christensen et al. (1966) nearly one half were lethal when grown on autoclaved corn and fed to rats. The toxicity of this material to rats seemed to be remarkably specific and it had, for example, no detectable effect when fed to pigs. This example underlines the fact that several toxic substances show a species specificity in their activity. Amongst the fungal metabolites sporidesmin, the toxin produced by Pithomyces chartarum is relatively atoxic to mice (Moss 1966). This metabolite is toxic to guinea pigs (Thornton and Percival 1959) and rabbits (Worker and Dodd 1960) but it shows a quantitative and qualitative difference in activity when fed to rats (Slater et al. 1964).

The number of sporidesmins which have now been characterised is at least six. As well as sporidesmins A, B and C (I, II and III) see Hodges and Shannon 1966), there are Sporidesmin D (IV) (Rahman and Taylor 1967) E, (V) (Brewer et al. 1968) and F (VI) Ali et al. 1968).

The number of species of fungi shown to produce toxic metabolites is now so large that it is perhaps worth recording that some caution is needed in assessing the significance of many of them in the field. However, even accepting this need for caution, there is no doubt that the work on mycotoxins over the past decade has further underlined the involvement of fungi in the illness and even deaths of animals.

1Department of Biological Sciences, University of Surrey, 14 Falcon Road, London S.W.11, England.
Holoday (1966) also suggest that aflatoxins are metabolites of several fungi. Hodges et al. (1964) claim in particular that aflatoxin is produced by Penicillium puberulum, a claim which is refuted by Wilson et al. (1967) who are currently studying a toxin produced by this organism which, although it has fluorescent properties similar to Aflatoxin G, is distinct in its other physical and chemical properties. A further study of strains outside the A. flavus group reported to produce aflatoxins led Wilson et al. (1968) to conclude that only the A. flavus group can presently be certified as sources of these toxins.

There is no fundamental reason why moulds of different species, or even from different genera, should not be found to produce aflatoxins. Amongst the mycotoxins, Xanthocillin-X (VII) has been found to be produced by Aspergillus flavus (Coveney et al. 1964).
Mycotoxins. M. O. Moss.

1966) and it has been known for some time as a metabolite of *Penicillium notatum* (Rothe 1954).

However work with the aflatoxins offers special difficulties in the ease with which the observation of fluorescence on thin layer chromatograms is susceptible to the presence of artifacts and the reviewer feels that more work is necessary to remove this subject from the realm of controversy.

The other aspect of aflatoxin studies which has aroused interest is that of the biosynthesis of these intriguing molecules. A very interesting hypothetical scheme for the biosynthesis of the aflatoxins, and of related mould metabolites, has been proposed by Biollaz, Büchi and Milne (1968) following a detailed study of the incorporation of radio-active label from both 1$^{14}$C and 2$^{14}$C acetate into the aflatoxin molecule. A further slightly toxic metabolite, Aspertoxin, has been isolated from strains of *A. flavus* and characterised as O-Methyl sterigmatocystin (VIII) (see Rodricks *et al.* (1968) & Waiss *et al.* (1968). This metabolite is a significant factor in discussions on the Biosynthesis of the Aflatoxins.

Wilson, Wilson and Hayes (1968) have reported the production of an interesting tremorgenic toxin from a strain of *Penicillium cyclopium* growing on food materials. Mass spectroscopic studies indicate a molecular formula C$_{37}$H$_{44}$NO$_4$Cl thus differentiating it completely from the toxic metabolite Cyclopiazonic acid (IX) isolated from the same species by Holzapfel (1968).

Arising from studies on the aetiology of mouldy corn toxicosis, the literature on which is described by Wilson and Wilson (1962), studies have been made by Dr. Wilson’s group at Vanderbilt University, by Dr. Wogan and Professor Büchi at M.I.T. and in the laboratories of the Tropical Products Institute on the toxic metabolites elaborated by strains of *Penicillium rubrum*. Two such metabolites have been isolated by Townsend Moss and Peck and called Rubratoxins A and B. The isolation and the purification of the latter has been described in detail by Hayes and Wilson (1968) and its production in deep fermentation has been described by Wogan & Mateles (1968). The structures (X) and (XI) have been suggested for Rubratoxins A and B respectively (Moss *et al.* 1968 & 1969).

The LD$_{50}$ values of these metabolites given to mice by the intraperitoneal route using propylene glycol as the solvent are 6 - 7 and 3 - 3.5 mg/KG respectively and experiments with derivatives prepared during the structural studies show that any alteration to the molecule of Rubratoxin B results in a decrease, or even a complete loss, of toxicity.

The number of toxigenic species of the genera Aspergillus and Penicillium has always been impressive but a large number of papers have more recently appeared implicating species of Fusarium in mycotoxicoses. It had been suggested by Brian *et al.* (1961) that metabolites produced by Fusarium species might be implicated in the general problem of mouldy
Mycotoxins. M. O. Moss.

Figure 5

Figure 6

This same derivative of diacetoxyscirpenol was also isolated from toxic strains of *Fusarium tricinctum* by Bamburg *et al.* (1968) along with diacetoxyscirpenol (XIV) itself.

The taxonomy of the genus *Fusarium* is difficult and a large number of species have been reduced to synonomy by various authors. Over 1,000 species were reduced to about 65 by Wollenweber (see Wollenweber & Reinking 1935). These in their turn were reduced to nine species by Snyder (see Snyder & Toussoun 1965), although other studies have resulted in positions perhaps intermediate between these two (see for example Booth 1966).

As an example the species *F. sporotrichioides* and *F. poae*, which occur very frequently in the Russian literature in connection with Alimentary toxic aleukia,
Mycotoxins. M. O. Moss.

are members of Wollenweber's Group, *Sporotrichiella*, this group being considered (by Snyder) as synonymous with the species concept *F. Tricinctum*.

Toxic strains of *Fusarium culmorum* associated with sickness in dairy cattle were described by Fisher et al. (1967) and the related species *F. graminearum* has been the subject of a number of papers. Curtin and Tuite (1966) described the refusal of maize contaminated with *Gibberella zeae* (*F. graminearum*) by pigs. The same material caused emesis and uterine hypertrophy in these animals. Vulvo-vaginitis in sows was associated with the same organism growing on feeds in the work of Bugeac and Berbinschi (1967). The uterotrophic activity of strains of *Gibberella zeae* (*F. graminearum*) had already been described by Stob et al. (1962) and the isolation of a toxic substance responsible for this activity was described by Christensen et al. (1965). The structure of this metabolite, called zearelenone (Urry et al. 1966), and its absolute configuration (Kuo et al. 1967) are shown in formula (XV).

The total synthesis of zearelenone has been achieved by Taub et al. (1968) and Eppley (1968) has worked out an analytical screening method which allows the determination of zearelenone as well as of aflatoxin and ochratoxin in mixtures of all three.

Studies on a disease of cattle called red clover disease, or slobbers, (see Aust and Broquist (1965) and Smalley et al. (1962) led to the isolation, and eventually to the characterisation, of a small molecular weight alkaloid called slaframine from strains of *Rhizoctonia leguminicola*. The structure XVI was recently proposed by Gardiner et al. (1968) in place of the original structure XVII proposed by Aust et al. (1966).

Some of the interesting pharmacological properties of slaframine are described in a paper by Crump et al. (1967).

*Claviceps purpurea* and its alkaloids have long been associated with ergotism, a disorder which is fortunately rarely encountered in man today, although reports of the poisoning of animals do still occur. (see for example Swarbrick & Swarbrick (1968) who described a suspected outbreak of ergotism in ducks). *Claviceps fusiformis*, a newly described species (Loveless 1967), has been shown to be responsible for the failure of the development of the mammary glands of pigs during late pregnancy. The agents responsible for this fairly specific physiological phenomenon appear to be the water soluble alkaloids produced, by the fungus.
Amongst the higher fungi, a species of Ramaria (≡ Clavariella) has been shown to be responsible for a disease of cattle known as "mal do eucalipto" in which salivation, loss of hair, blindness, abortion and paralysis of the rumen are among the symptoms (Bauer et al. 1967).

I consider this sketchy preamble to be an introduction to a list of the more recent reviews on mycotoxins, a list which, it is hoped, will be of some value to readers of this bulletin.

Review articles


Tyler, V. E. (1963) Poisonous mushrooms, Progress chem. Toxicology, 1, 339.


Wogan, G. N. & Mateles, R. I., (1968) Mycotoxins, Progress in Industrial Microbiology, 7, 149.

References


Mycotoxins. M. O. Moss.

Rothe, W., (1950) Pharmazie, 5, 190.
Swarbrick, O., Swarbrick, J. T., (1968) Vet. Record, 76.
Wogan, G. N., Mateles, R. I., (1968) Progress Industrial Microbiology, 7, 149.
A METHOD FOR THE CONVENIENT PREPARATION OF ARTIFICIAL GAS MIXTURES IN CLOSED CONTAINERS

J. H. Walsh

Summary Mixing gases by pressure at constant volume is shown to be a simple and accurate method of preparing artificial atmospheres for incubating microorganisms.

Une méthode pour obtenir une bonne préparation de mélange de gaz artificiels dans des récipients hermétiques. Mélanger des gaz en maintenant une pression à un volume constant se trouve être une méthode simple et sûre pour la préparation d'atmosphères artificielles pour l'incubation de micro-organismes.

Variations in oxygen and carbon dioxide concentrations are important ecological factors which may determine the occurrence and extent of microbial growth or degradation in particular situations. Investigations of the influence of these factors have been made most often by measuring growth, or degradative or other activities of the microorganism in static cultures (test or growth tubes, or petri dishes) incubated in a container filled with the desired atmosphere. The atmosphere is generally renewed daily to keep it relatively constant.

In my opinion the volume of work on this topic has been limited by difficulties in obtaining accurate gas mixtures, and the time involved in their daily renewal. In the main, two ways of getting the desired mixture have been used.

(i) Mixing of gases by volume at constant pressure over water. This is a slow, often messy method, and the volume which can be prepared is generally limited to 10-20 litres. There are several sources of error e.g. the difficulty of measuring large volumes of gas accurately; errors due to slight pressure differences; and particularly at low oxygen concentrations, errors due to equilibration with oxygen dissolved in the displacing water.

(ii) Purchase of ready mixed, accurately analysed gas mixtures from a number of companies which provide them. This avoids all the errors and difficulties above but the cost of these mixtures often has certain consequences. In any one experiment only 4 or 5 mixtures are used giving rather distant points for plotting a graph. More important, in a series of experiments the same mixtures tend to be used, even though early results may suggest that a different set of mixtures would be more suitable for later experiments in the series.

This note outlines a simple method of mixing gases by partial pressures at constant volume and gives some examples of analyses of mixtures made in this way. These show that the method is relatively accurate besides being very rapid and clean, and requiring no specialised apparatus. Since mixing is done directly in the container in which the cultures are to be incubated, the size of the container is the only limit on the volume of gas mixed. The method involves evacuation of this container and so it must be capable of evacuation without collapse. The author has used standard anaerobic jars for most work and redundant autoclaves for larger experiments. Vacuum desicators and vacuum ovens would be equally suitable.

A consequence of evacuation is that liquid media may 'boil'. This is normally unimportant if the tubes are not overfull, but it would be unsuitable for experiments where growth was required in the form of floating mats on liquid media.

Method

Fig. 1

Ein Verfahren zur geeigneten Vorbereitung von künstlichen Gasmischungen in geschlossenen Behältern. Es wird gezeigt, dass das Mischen von Gasen bei gleichbleibendem Volumen ein einfaches und genaues Verfahren ist, Künstliche Atmosphären für die Entwicklung von Mikroorganismen vorzubereiten.

Un método para preparar de manera conveniente las mezclas artificiales de los gases en los receptáculos cerrados. Se demuestra como método sencillo y exacto para preparar las atmósferas artificiales para incubar los microorganismos.

The chosen container with the cultures under test inside, is connected to a mercury manometer and the whole connected to a vacuum pump as shown. The connections between the container and the manometer should be as short as possible (see sources of error). The pump may be either mechanical or a filter pump as ultimate vacuum is not required. A mechanical pump is much more rapid, especially if a large container is used.

1. The container is evacuated to 700 m.m. Hg below atmospheric (i.e. the column in the manometer is 700 m.m. high). The figure of 700 m.m. has been chosen because it is easily achieved by any well maintained pump under conditions within the normal ranges of atmospheric pressure and water vapour pressure.

2. The tap between pump and container is closed. The desired gases are introduced separately in such a way that the proportion of the 700 m.m. vacuum lost as each enters equals the proportion of that gas required in the final mixture e.g. a 1:1:1 proportion of three gases would require falls of 700-466 m.m., 466-233 m.m. and 233-0 m.m. on the manometer.

3. Stages 1 and 2 must be repeated twice more to ensure that the contribution of the unevacuated 60 m.m. is negligible (see results). In the first two mixings manometer falls need only be approximately correct. For the third mixing the manometer must be adjusted accurately for a specific mixture, though it is generally sufficient to adjust it approximately, and by reading it accurately to obtain exact figures for the mixture prepared.

### TABLE 1

<table>
<thead>
<tr>
<th>O.F.N. added m.m. Hg pressure (= 700 - x)</th>
<th>Air added m.m. Hg pressure (= x - 0)</th>
<th>Air added corrected for water vap. press. m.m. Hg pressure</th>
<th>% Oxygen</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Theoretical</td>
</tr>
<tr>
<td>700</td>
<td>0</td>
<td>0</td>
<td>0:00</td>
</tr>
<tr>
<td>660</td>
<td>40</td>
<td>39:5</td>
<td>1:19</td>
</tr>
<tr>
<td>634</td>
<td>66</td>
<td>65</td>
<td>1:95</td>
</tr>
<tr>
<td>613</td>
<td>87</td>
<td>86</td>
<td>2:58</td>
</tr>
<tr>
<td>588</td>
<td>112</td>
<td>110:5</td>
<td>3:32</td>
</tr>
<tr>
<td>566</td>
<td>134</td>
<td>132</td>
<td>3:97</td>
</tr>
<tr>
<td>527</td>
<td>173</td>
<td>171</td>
<td>5:14</td>
</tr>
<tr>
<td>481</td>
<td>219</td>
<td>216</td>
<td>6:51</td>
</tr>
<tr>
<td>441</td>
<td>259</td>
<td>256</td>
<td>7:71</td>
</tr>
<tr>
<td>414</td>
<td>286</td>
<td>282</td>
<td>8:51</td>
</tr>
<tr>
<td>380</td>
<td>320</td>
<td>316</td>
<td>9:53</td>
</tr>
<tr>
<td>313</td>
<td>387</td>
<td>382</td>
<td>11:54</td>
</tr>
<tr>
<td>257</td>
<td>443</td>
<td>437</td>
<td>13:22</td>
</tr>
<tr>
<td>206</td>
<td>494</td>
<td>488</td>
<td>14:77</td>
</tr>
<tr>
<td>168</td>
<td>532</td>
<td>525</td>
<td>15:91</td>
</tr>
<tr>
<td>126</td>
<td>574</td>
<td>567</td>
<td>17:18</td>
</tr>
<tr>
<td>80</td>
<td>620</td>
<td>612</td>
<td>18:57</td>
</tr>
<tr>
<td>51</td>
<td>649</td>
<td>641</td>
<td>19:45</td>
</tr>
<tr>
<td>0</td>
<td>700</td>
<td>691</td>
<td>21:00*</td>
</tr>
</tbody>
</table>

*Machine calibrated against dry air = 21:00% oxygen.

**Note 1. Calculation of water vapour pressure correction.**

At the time of the experiment temp = 18·5°C. Relative humidity = 60%: barometric pressure 750 m.m. Hg.

At 18:5°C relative humidity 100% = c. 16 m.m. water vapour pressure.

So R. H. 60% = 9·6 m.m. water vapour pressure.

This represents \( \frac{9·6 \times 100}{750} = 1·28\% \) of total pressure.

So all air pressures must be reduced by 1·28% to correct for moisture content.

**Note 2. Calculation of theoretical value for % oxygen.**

The mixture was dried over silica gel before entering the Servomex so water vapour can be ignored after the above correction.

Theoretical % oxygen = \( \frac{\text{m.m. Hg Air (corrected)}}{\text{m.m. Hg O.F.N. + m.m. Hg Air (corrected)}} \times 21 \)

4. All taps on the container are closed, it is disconnected from manometer and pump, and placed at the required temperature.

The method is exactly the same whether a mixture is being prepared for the first time, or merely renewed.

It is most satisfactory if the last gas is added to a manometer reading of exactly 0 m.m. Hg. To achieve this oxygen is added in the form of air wherever possible, and is added last. Where all gases are added from cylinders under pressure special care is required to avoid adding the last to a positive pressure, which could not be measured on the manometer, yet would alter the mixture produced.

Results

To verify the theoretical soundness of this method, as well as to estimate its accuracy in practice, a series of test mixtures of oxygen free nitrogen (O.F.N.) and oxygen (in air) were prepared and oxygen concentrations determined on a D.C.L. Servomex oxygen analyser (Type 83). O.F.N. was added first, air last, as recommended above. Gas was displaced from the jar, for analysis, by liquid paraffin.

Table 1 gives the oxygen concentration obtained for each mixture, together with details of the pressures of O.F.N. and air added to prepare the mixture. A theoretical value has been calculated from these pressures in order to estimate the deviation of observed figures from the theoretical. The air used was not dried and so a corrected pressure is given for air with the contribution of water vapour removed. A note on these calculations follows the table.

The results in Table 1 show that the method can be used with accuracy, and that results do not diverge appreciably from theoretical values at any point in the range. The average difference is 0.05% oxygen and there is a fair scatter of both high and low readings.

Tests were also made to investigate the approach of observed values to the calculated values with successive evacuations and mixings. Table 2 gives the figures obtained for two widely separated oxygen percentages after one, two and three evacuations.

<table>
<thead>
<tr>
<th>O.F.N. added m.m Hg pressure</th>
<th>Air added m.m Hg pressure corrected for water vap. press.</th>
<th>% Oxygen</th>
<th>No. of evacuations</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Theoretical calculated as for Table 1</td>
<td>True Theoretical calculated as below</td>
<td>Observed</td>
</tr>
<tr>
<td>------------------------------</td>
<td>----------------------------------------------------------</td>
<td>----------</td>
<td>-------------------</td>
</tr>
<tr>
<td>523</td>
<td>175</td>
<td>5.27</td>
<td>6.32</td>
</tr>
<tr>
<td>&quot;</td>
<td>&quot;</td>
<td>&quot;</td>
<td>5.34</td>
</tr>
<tr>
<td>&quot;</td>
<td>&quot;</td>
<td>&quot;</td>
<td>5.27</td>
</tr>
<tr>
<td>162</td>
<td>531</td>
<td>16.09</td>
<td>16.42</td>
</tr>
<tr>
<td>&quot;</td>
<td>&quot;</td>
<td>&quot;</td>
<td>16.11</td>
</tr>
<tr>
<td>&quot;</td>
<td>&quot;</td>
<td>&quot;</td>
<td>16.09</td>
</tr>
</tbody>
</table>

Note 1. Calculation of true theoretical value for individual evacuations.

Barometric pressure = 750 m.m. Hg.

\[
\text{% oxygen (1 evacuation)} = \left( \frac{175 \times 21}{698} \right) \frac{700 + (50 \times 21)}{750} = 6.32.
\]

\[
\text{(2 evacuations)} = \left( \frac{175 \times 21}{698} \right) \frac{700 + (50 \times 6.32)}{750} \quad \text{etc.}
\]

From Table 2 it is clear that three evacuations are sufficient to replace all but a negligible fraction of the original contents of the container by the desired mixture.

Sources of Error.

(i) Water vapour pressure. Water vapour pressure in the air used to prepare the mixture can be corrected for as in the above results, or more simply the air can be dried in a tube of silica gel or other desiccant. It is important that this tube be very loosely packed so that the pressure in the container can reach exactly atmospheric pressure. Using dry air, oxygen concentration is very simply calculated as

\[
\text{pressure of air added} \times \frac{21 \times 100}{700} \quad \text{i.e. } \times 3
\]

Gas in cylinders is normally free of water vapour.

Where an aqueous medium is used for incubation, a water vapour pressure will also exist in the container. If such a container is evacuated to 700 mm Hg and the tap from the pump closed, the vacuum falls by 10 mm Hg or so as evaporation replaces water vapour removed. If the vacuum is readjusted to 700 mm Hg it will now be stable as saturation has been restored. The effect of water vapour pressure is therefore nil. If liquid water is present the 10 mm fall occurs rapidly, if water is in the form of agar, it is slow. Where the fall is so slow as to occupy a much longer time than refilling with the desired gases, then there is no need to wait for equilibration. The important thing is that the desired gases should be added to a relatively stable vacuum of exactly 700 mm Hg. Repeated evacuation has not appreciably accelerated the drying out of cultures in the authors experiments.

(ii) Rigidity of the Container. This method depends on mixing pressures at constant volume so the container must be effectively rigid. The anaerobic jars used do show a contraction of 0.3% under a pressure of 14 lbs./sq. inch, but this is not sufficient to appear as a consistent error in the results. Probably vessels designed for use under vacuum will be satisfactory in this respect, but it is a point to bear in mind if other containers are being modified for use. The result of appreciable contraction on evacuation would be that less gas would be added from 700-350 mm than from 350-0 mm.

(iii) Displacement. This error will occur if the space where the gases are mixed is not in the form of one main cavity. If two cavities are present with a narrow connexion then addition of the second gas to one will sweep through a disproportionate amount of the first gas to the other cavity. The two cavities will then contain different mixtures. This can be illustrated by a system where a 250 ml. Buchner flask was attached to the main 3000 ml. anaerobic jar, gases being introduced through a port in the anaerobic jar. O.F.N. was added first, then air to give a theoretical oxygen concentration in the system of 14.77%. Analysis of the gas in the jar gave a figure of 15.25% due to nitrogen being pushed into the Buchner when air was admitted. It is for this reason too that the volume of the manometer and connexions must be negligible compared to the volume of the container.

Conclusion

The above measurements show that with proper safeguards accurate gas mixtures can be prepared by partial pressures. All the above examples refer to mixtures of O.F.N. and atmosphere, and the practical range of such mixtures is 1-20% oxygen. If instead of the atmospheric component a 1% O\textsubscript{2}/99% N\textsubscript{2} prepared mixture is used (which can be purchased as mentioned above) the range can be extended down to 0.05% oxygen. It is with such a purpose in mind that this note has tried to establish that no major errors occur, which would make preparation of such low oxygen concentrations unrealistic. The author has found in preliminary experiments that with several fungi it is not until the oxygen concentration is reduced to below 0.5% that cellulolytic activity (tensile strength measurements) begins to fall appreciably.

Thus by the use of two gas cylinders only, an infinite range of oxygen concentrations from 21 - 0.05% could be prepared accurately, conveniently and quickly enough to make the daily replacement of the atmospheres of 10 jars only an hours work. The addition of other components to the mixture could be performed equally accurately and with only a little extra trouble.

The author wishes to acknowledge the help of Mr. E. G. Jefferys and Mr. J. C. Swait, of I.C.I. Pharmaceuticals Division, Alderley Park, Cheshire in providing facilities for, and advice on the oxygen determinations.
Experience with biological tests in the field of the biodeterioration of materials. 4.

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

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

H. J. Hueck¹ and W. Hazeu²

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. Carboxymethyl-cellulose 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 to be the most suitable nitrogen source when compared with pepton, ammonium chloride and sodium nitrate.


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.

²Central Laboratory TNO, Delft, Netherlands.

¹Central Laboratory TNO, Delft, Netherlands, present address: Laboratory for Microbiology, Technological University, Delft, Netherlands.
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 cm² 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

<table>
<thead>
<tr>
<th>Species</th>
<th>Strain</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aspergillus fumigatus</td>
<td>QM 6b</td>
</tr>
<tr>
<td>Aspergillus niger</td>
<td>CM.L. 17454</td>
</tr>
<tr>
<td>Aspergillus ustus</td>
<td>C.B.S. b</td>
</tr>
<tr>
<td>Chaetomium globosum</td>
<td>ATCC 6205</td>
</tr>
<tr>
<td>Memnoniella echinata</td>
<td>ATCC 9597</td>
</tr>
<tr>
<td>Myrothecium verrucaria</td>
<td>ATCC 450</td>
</tr>
<tr>
<td>Trichoderma viride</td>
<td>ATCC 8676</td>
</tr>
</tbody>
</table>

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/m²) 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 >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:

\[ B_t = \frac{S_o - S_t}{S_o} \times 10 \]  

where

\[ B_t = \text{breakdown index for time} \quad t \]
\[ S_o = \text{breaking strength before exposure} \]
\[ S_t = \text{breaking strength after exposure during period} \quad t \rightarrow t \]
\[ t = \text{time recorded in days} \]

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 cm² 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
Experience with biological tests in the field of the biodeterioration of materials. 4. Hueck, H. J. & Hazen, W.

Table 2 Composition of media used in the investigation

<table>
<thead>
<tr>
<th>Compound (g/l)</th>
<th>No. 1</th>
<th>No. 2</th>
<th>No. 3</th>
<th>No. 4</th>
<th>No. 5</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>VI</td>
<td>CCCT</td>
<td>Czapek</td>
<td>QM</td>
<td>CL</td>
</tr>
<tr>
<td>NH₄NO₃</td>
<td>2-0</td>
<td>3-0</td>
<td>-</td>
<td>6</td>
<td></td>
</tr>
<tr>
<td>NaNO₃</td>
<td>-</td>
<td>-</td>
<td>2-0</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>K₂HPO₄</td>
<td>-</td>
<td>2-5</td>
<td>-</td>
<td>2-2</td>
<td>1-18</td>
</tr>
<tr>
<td>K₃HPO₄</td>
<td>1-0</td>
<td>2-0</td>
<td>2-0</td>
<td>2-8</td>
<td>-</td>
</tr>
<tr>
<td>Na₂HPO₄</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>1-0</td>
</tr>
<tr>
<td>MgSO₄·7H₂O</td>
<td>1-0</td>
<td>-</td>
<td>0-5</td>
<td>0-2</td>
<td>0-5</td>
</tr>
<tr>
<td>KCl</td>
<td>-</td>
<td>-</td>
<td>0-5</td>
<td>-</td>
<td>0-5</td>
</tr>
<tr>
<td>Cellulose powder</td>
<td>specified in text</td>
<td>specified in text</td>
<td>-</td>
<td>specified in text</td>
<td>5-0</td>
</tr>
<tr>
<td>Saccharose</td>
<td>-</td>
<td>-</td>
<td>30</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>K. bitartrate</td>
<td>1-0</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Yeast extract</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>0-1</td>
<td>-</td>
</tr>
<tr>
<td>Trace elements</td>
<td>1)</td>
<td>-</td>
<td>2)</td>
<td>-</td>
<td>3)</td>
</tr>
<tr>
<td>Agar</td>
<td>20</td>
<td>20</td>
<td>15</td>
<td>15</td>
<td>-</td>
</tr>
</tbody>
</table>

1) added as 1 mg/l of Fe₂(SO₄)₃; ZnSO₄; CuSO₄; MnSO₄
2) added as 10 mg/l of FeSO₄
3) added as 1 mg/l of FeSO₄; ZnCl₂; MnCl₂

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".
Experience with biological tests in the field of the biodeterioration of materials. 4. Hueck, H. J. & Hazeu, W.

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

<table>
<thead>
<tr>
<th>Medium</th>
<th>Carbon source g/l</th>
<th>Growth</th>
<th>Breakdown index</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Cellulose suspension</td>
<td>Aspergillus niger</td>
<td>Myrothecium verrucaria</td>
</tr>
<tr>
<td>1=VI</td>
<td>+ 0 0</td>
<td>++++</td>
<td>6 5 6 7 5</td>
</tr>
<tr>
<td>1=VI</td>
<td>- 20 0</td>
<td>± ++</td>
<td>6 4 5 7 4</td>
</tr>
<tr>
<td>2=CCCT</td>
<td>+ 0 0</td>
<td>++++</td>
<td>6 5 8 7 6</td>
</tr>
<tr>
<td>2=CCCT</td>
<td>- 20 0</td>
<td>++++</td>
<td>6 4 8 7 4</td>
</tr>
<tr>
<td>2=CCCT</td>
<td>- 0 1</td>
<td>++ ±</td>
<td>6 6 6 7 6</td>
</tr>
<tr>
<td>2=Czapek</td>
<td>- 0 10</td>
<td>++++</td>
<td>7 5 8 8 5</td>
</tr>
<tr>
<td>3=Czapek</td>
<td>- 0 30</td>
<td>++++</td>
<td>0 0 8 5 0</td>
</tr>
</tbody>
</table>

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 inoculum 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 $t$ — the diameter $D$ remained constant, after that period, $D$ increased linearly with time: $D = (t - t) \tan \alpha$. 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 $r$.

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 sawdust 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
Experience with biological tests in the field of the biodeterioration of materials. 4. Hueck, H. J. & Hazen, W.

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

<table>
<thead>
<tr>
<th>Medium</th>
<th>Carbon source g/l</th>
<th>Growth</th>
<th>Breakdown index</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Filterpaper</td>
<td></td>
<td>Black cotton B₁</td>
</tr>
<tr>
<td></td>
<td>Cellulose-suspension</td>
<td>+ 0 0</td>
<td>± + + + +</td>
</tr>
<tr>
<td></td>
<td>Ma-CMC</td>
<td>++ ++ ++ + ++</td>
<td>0-5 7 9 5</td>
</tr>
<tr>
<td></td>
<td>Aspergillus niger</td>
<td>++ ++ ++ + ++</td>
<td>1 8 8 5</td>
</tr>
<tr>
<td></td>
<td>Chaetomium globosum</td>
<td>+ + + +</td>
<td>0-5 4 4 1</td>
</tr>
<tr>
<td></td>
<td>Myrothecium verrucaria</td>
<td>+ + + +</td>
<td>0 6 4 3</td>
</tr>
<tr>
<td></td>
<td>Trichoderma viride</td>
<td>+ + + +</td>
<td>0 6 4 3</td>
</tr>
</tbody>
</table>

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 containing different types of cellulose

<table>
<thead>
<tr>
<th>medium</th>
<th>carbon source</th>
<th>concentration g/l</th>
<th>growth</th>
<th>breakdown-index</th>
</tr>
</thead>
<tbody>
<tr>
<td>1=VI</td>
<td>Cellulose-suspension</td>
<td>5 1-4</td>
<td>0-1</td>
<td>7 0-5</td>
</tr>
<tr>
<td>1=VI</td>
<td>&quot;</td>
<td>20 1-5</td>
<td>0 8</td>
<td>0-5</td>
</tr>
<tr>
<td>1=VI</td>
<td>Cellulose-powder S&amp;S 123</td>
<td>20 1-4</td>
<td>0 8</td>
<td>1</td>
</tr>
<tr>
<td>1=VI</td>
<td>&quot;</td>
<td>20 1-5</td>
<td>0-2</td>
<td>8 0-5</td>
</tr>
<tr>
<td>1=VI</td>
<td>&quot;</td>
<td>100 1-4</td>
<td>0 3</td>
<td>0-5</td>
</tr>
<tr>
<td>1=VI</td>
<td>&quot;</td>
<td>100 1-5</td>
<td>0-8</td>
<td>2 0-5</td>
</tr>
</tbody>
</table>
Experience with biological tests in the field of the biodeterioration of materials. 4. Hueck, H. J. & Hazen, W.

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

<table>
<thead>
<tr>
<th>medium</th>
<th>source of carbon</th>
<th>conc. g/l</th>
<th>growth</th>
<th>cellulase-production (C.U.)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Aspergillus fumigatus</td>
<td>Clathrosporium globosum</td>
</tr>
<tr>
<td>no. 5, table 1</td>
<td>cellulose powder</td>
<td>1</td>
<td>++</td>
<td>+++</td>
</tr>
<tr>
<td>&quot; &quot;</td>
<td>S&amp;S 123</td>
<td>3</td>
<td>++</td>
<td>+++</td>
</tr>
<tr>
<td>&quot; &quot;</td>
<td>wheat bran</td>
<td>10</td>
<td>+++</td>
<td>+++</td>
</tr>
<tr>
<td>&quot; &quot;</td>
<td>&quot;</td>
<td>30</td>
<td>+++</td>
<td>+++</td>
</tr>
<tr>
<td>subtotal</td>
<td></td>
<td></td>
<td>9</td>
<td>12</td>
</tr>
<tr>
<td>&quot; &quot;</td>
<td>saw-dust</td>
<td>1</td>
<td>+</td>
<td>++</td>
</tr>
<tr>
<td>&quot; &quot;</td>
<td>&quot;</td>
<td>3</td>
<td>+</td>
<td>++</td>
</tr>
<tr>
<td>&quot; &quot;</td>
<td>&quot;</td>
<td>10</td>
<td>+</td>
<td>++</td>
</tr>
<tr>
<td>&quot; &quot;</td>
<td>&quot;</td>
<td>30</td>
<td>++</td>
<td>++</td>
</tr>
<tr>
<td>subtotal</td>
<td></td>
<td></td>
<td>5</td>
<td>8</td>
</tr>
<tr>
<td>&quot; &quot;</td>
<td>&quot;</td>
<td>1</td>
<td>±</td>
<td>++</td>
</tr>
<tr>
<td>&quot; &quot;</td>
<td>&quot;</td>
<td>3</td>
<td>+</td>
<td>++</td>
</tr>
<tr>
<td>&quot; &quot;</td>
<td>&quot;</td>
<td>10</td>
<td>±</td>
<td>++</td>
</tr>
<tr>
<td>&quot; &quot;</td>
<td>&quot;</td>
<td>30</td>
<td>±</td>
<td>++</td>
</tr>
<tr>
<td>subtotal</td>
<td></td>
<td></td>
<td>2·5</td>
<td>6</td>
</tr>
</tbody>
</table>

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 NH₄NO₃. 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
Experience with biological tests in the field of the biodeterioration of materials. 4. Hueck, H. J. & Hazeu, W.

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 NH₄Cl and NaNO₃ 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.

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

<table>
<thead>
<tr>
<th>Source of nitrogen g/l</th>
<th>No. 5 (Table 2)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pepton (Difco)</td>
<td>5·0 — — — — — —</td>
</tr>
<tr>
<td>Urea</td>
<td>— 0·6 3·0 6·0 — — — —</td>
</tr>
<tr>
<td>NH₄Cl</td>
<td>— — — 2·65 2·00 0·66 — —</td>
</tr>
<tr>
<td>NaNO₃</td>
<td>— — — — 1·06 3·20 4·25 —</td>
</tr>
<tr>
<td>NH₄NO₃</td>
<td>— — — — — 3·00 —</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>ph of medium</th>
<th>before growth</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aspergillus fumigatus</td>
<td>7·2 6·6 6·6 6·6 6·3 6·4 6·5 6·6 6·5</td>
</tr>
<tr>
<td>Chaetomium globosum</td>
<td>8·3 7·8 8·4 8·4 3·8 4·9 5·8 6·9 6·2</td>
</tr>
<tr>
<td>Memnoniella echinata</td>
<td>8·5 8·0 8·7 8·7 3·9 4·2 5·3 6·9 6·0</td>
</tr>
<tr>
<td>Myrothecium verrucaria</td>
<td>8·7 7·8 8·6 8·7 5·4 6·3 6·4 7·3 6·5</td>
</tr>
<tr>
<td>Penicillium funiculosum</td>
<td>6·7 8·0 8·1 7·9 4·3 4·7 5·5 6·5 5·7</td>
</tr>
</tbody>
</table>

| growth                 | — — — — — — — — |
| Aspergillus fumigatus  | ++ + ++ ++ ++ ++ + ++ +++ |
| Chaetomium globosum    | +++ +++++ ++++ ++ ++ +++++ +++++++ |
| Memnoniella echinata   | +++ + + + ++ ++ +++ +++++ ++ + |
| Myrothecium verrucaria | +++ + + + + + + + + + + + + |
| Penicillium funiculosum| ++ + + + + + + + + + + |

<table>
<thead>
<tr>
<th>cellulolytic activity in cellulose units (C.U.)</th>
<th>Aspergillus fumigatus</th>
<th>Chaetomium globosum</th>
<th>Memnoniella echinata</th>
<th>Myrothecium verrucaria</th>
<th>Penicillium funiculosum</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0·0 1·0 1·6 1·9 0·6 0·7 1·3 1·2 2·2</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>1·2 1·1 0·6 0·4 0·6 1·0 1·2 0·6 0·8</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>0·5 0·8 0·0 0·0 0·0 0·2 0·6 0·4 0·0</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>0·6 2·2 0·5 0·3 1·9 3·0 2·1 0·7 2·9</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>1·5 0·0 0·0 0·0 1·3 1·3 1·3 1·0 1·5</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

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.

In these experiments, yeast-extract and trace elements were omitted from the basic medium.
Experience with biological tests in the field of the biodeterioration of materials. 4. Hoeck, H. J. & Hazeu, W.

Table 9

<table>
<thead>
<tr>
<th>pepton</th>
<th>urea</th>
<th>NH₄Cl</th>
<th>NaNO₃</th>
<th>NH₄NO₃</th>
<th>score for</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>growth</td>
</tr>
<tr>
<td>5.0</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>13</td>
</tr>
<tr>
<td>—</td>
<td>0.6</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>9.5</td>
</tr>
<tr>
<td>—</td>
<td>3.0</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>7.5</td>
</tr>
<tr>
<td>—</td>
<td>6.0</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>6.5</td>
</tr>
<tr>
<td>—</td>
<td>—</td>
<td>2.65</td>
<td>—</td>
<td>—</td>
<td>8</td>
</tr>
<tr>
<td>—</td>
<td>—</td>
<td>2.00</td>
<td>1.06</td>
<td>—</td>
<td>9</td>
</tr>
<tr>
<td>—</td>
<td>—</td>
<td>0.66</td>
<td>3.20</td>
<td>—</td>
<td>12</td>
</tr>
<tr>
<td>—</td>
<td>—</td>
<td>—</td>
<td>4.25</td>
<td>—</td>
<td>12</td>
</tr>
<tr>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>3.0</td>
<td>12</td>
</tr>
</tbody>
</table>

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 NH₄NO₃. 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 NH₄Cl and NaNO₃.

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 NH₄NO₃ 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 NH₄NO₃ as the sole source of N is justified and needs no amendment.

It will be noted from Table 2, recipe I, 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.

Acknowledgment

The experiments described in this publication were mainly carried out by Miss M. Siebenhar, Miss J. Holsteijn and Miss S. J. v.d. Voort, whom we thank for their able assistance.

160
Experience with biological tests in the field of the biodeterioration of materials. 4. Hueck, H. J. & Hazeu, W.

6. References


![Diagram](image)

**FIGURE 1** Growth of mycelium of test fungi in Petri-dishes (explanation in text)
A perfusion technique to study the colonization of a cellulosic substrate by fungi.

Summary. A perfusion technique has been described in order to study the colonization of cellulosic substrate buried in soil. The colonization pattern of different fungi has also been elucidated.

Une technique de perfusion pour étudier la colonization d'un substrat cellulosique par les champignons. Une technique de perfusion a été décrite en vue d'étudier la colonization d'un substrat cellulosique enfoui dans le sol. Le processus de colonization de différents champignons a également été élucidé.

Introduction

Much work has been done on the physiological and nutritional aspects of fungi growing on cellulose (Siu & Sinden, 1951). Many of these studies have been carried using media with simple sugars as carbon sources, (Foster 1939, Steinberg 1939); the relationship of nitrogen and carbon assimilation has also been studied by many workers (Jensen 1931, Waksman 1938). Such studies have often been carried out with pure cultures of fungi and thus eliminate the influence of other factors present in the original habitat of the organisms. Some workers have attempted to study these environmental factors involved by introducing different forms of substrates into the soil, (Dubos 1928, Skinner & Mellam 1944, Tribe 1957, Keynan, Henis & Keller 1961, Went 1959, Griffith & Jones 1955). Soil enrichment studies have helped to isolate maximum number of cellulolytic organisms, but these studies do not show clearly the ecological factors at work in soil and their effect on the soil mycoflora.

To achieve such an ecological understanding a model culturing and isolating system has been developed, which is capable of continuous enrichment and constant maintenance of specific nutrients within a cellulose or a porous substrate.

Materials and Methods

The practical details of the technique revolve around the transport principle of paper chromatography and the property of glass sleeving to conduct nutrients by capillarity. Thus the substrate used is 3 mm chromatography paper having a heat rolled polythene backing (Eggins & Lloyd 1968). The polythene backing facilitates handling of the substrate even when highly degraded; the glass fibre sleeving consists of fine glass fibre, woven together and is normally used for insulation of high voltage electric cables. This glass fibre sleeving conducts the nutrients on to the paper where these are perfused and evaporated on the other end of the glass fibre sleeving. A perfusion set consists of five 9 cms diameter petri dishes. Each such petri dish contains 5 mms. wide polythene backed cellulose strip with the polythene side facing downwards. One end of the strip is joined to 4 mm wide glass fibre sleeving which is threaded through a 3 mm wide silicone tubing; this leads to a 100 ml conical flask containing nutrients. (See Figure 1). The other end of the strip is similarly joined to another small length of glass fibre sleeving threaded through silicone rubber tubing. The glass fibre sleeving is exposed for a distance of 5 cms.

The glass sleeving conducts nutrients from the conical flask to the cellulose strip where it is perfused and is evaporated at the other end. The strip in the petri dish is covered with a 2 cms wide glass fibre cloth (Figure 2) tape which screens the cellulose substrate from direct contact with soil, while allowing the fungi to grow through this inert fabric.

Three such sets are put in a petri dish carrier (Figure 3). These are then autoclaved after filling the flasks with an appropriate nutrient solution. After autoclaving, soil is added in the petri dishes and then incubated at the required temperature.

After incubation, the cellulose substrate is released after different intervals, the glass fibre sleeving being cut to release the cellulose strip, which is then cut aseptically into small pieces and inoculated on E. & P.'s cellulose agar (Eggins & Pugh 1962). The resulting fungi are then recorded.

The above described technique has been employed to study the colonization and deterioration of buried cellulose strip when perfused with E. & P. salt solution.

The soil was collected from a pasture field in Clent, near Stourbridge, Worcestershire, England. The average pH of the soil was 6-8 and had a 23% moisture content.

The process of cellulose deterioration was determined by estimating the weight loss of cellulose strip.
A perfusion technique to study the colonization of a cellulosic substrate by fungi.
Malik, K. A. and Eggins, H. O. W.

Each time 7 cms of the cellulose strip out of the perfusion device was cut and oven dried at 110°C for twelve hours. Appropriate controls were also kept. To allow enough replication, 40 perfusion devices were set up. Every time four perfusion devices were sacrificed; out of these, three devices were used to estimate weight loss and the fourth device was used to isolate fungi by cutting the strip aseptically and inoculating on to six E. & P. cellulose agar plates. The plates were observed after incubation for seven days. The resulting fungi were recorded. Each was given a positive record if it appeared on any of the replicate petri dishes.

Table 1  Percentage frequency of the fungi colonizing the cellulose strip perfused with E. & P. salt solution.

<table>
<thead>
<tr>
<th>FUNGI</th>
<th>Days of Incubation</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>12 hrs</td>
</tr>
<tr>
<td>Zygorhynchus moelleri</td>
<td>64</td>
</tr>
<tr>
<td>Mucor globosus</td>
<td>16</td>
</tr>
<tr>
<td>Fusarium solani</td>
<td>64</td>
</tr>
<tr>
<td>Trichoderma viride</td>
<td>16</td>
</tr>
<tr>
<td>Gelasinospora cerealis</td>
<td>64</td>
</tr>
<tr>
<td>Dactylella sp.</td>
<td>16</td>
</tr>
<tr>
<td>Arthrobotrys sp.</td>
<td>64</td>
</tr>
<tr>
<td>Phoma sp.</td>
<td></td>
</tr>
<tr>
<td>Gliocladium roseum</td>
<td></td>
</tr>
<tr>
<td>Paecilomyces varioti</td>
<td>32</td>
</tr>
<tr>
<td>Penicillium sp.</td>
<td>16</td>
</tr>
<tr>
<td>Pupalaspora sp.</td>
<td></td>
</tr>
<tr>
<td>Aspergillus fumigatus</td>
<td></td>
</tr>
</tbody>
</table>

Results

The results of colonization of cellulose strip by fungi over a period of 35 days are summarised in Table 1. The initial colonization started as early as twelve hours but macroscopically visible fungal growth did not appear until after three days.

Fusarium solani and Trichoderma viride were found to be the main fungi involved in the deterioration of cellulose buried in the soil. Fusarium solani was isolated after twelve hours of incubation and then remained dominant at all times. T. viride also had similar occurrence but its frequency was comparatively low.

The non-cellulolytic sugar fungi, namely Zygorhynchus moelleri and Mucor globosus, made their appearance between two to six days of incubation. Gelasinospora cerealis appeared after three to nine days of incubation and was seen growing on the strip. The other fungi isolated between six and eighteen days of incubation were Arthrobotrys sp., Dactylella sp. and Paecilomyces varioti. During this period nematodes were also found ramifying in the cellulose fibres. These might justify the presence

Table 2  Percentage frequency of the fungi colonizing the cellulose strip perfused with E. & P. salt solution.

<table>
<thead>
<tr>
<th>FUNGI</th>
<th>Days of Incubation</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>4:50</td>
</tr>
<tr>
<td></td>
<td>6:10</td>
</tr>
<tr>
<td></td>
<td>8:10</td>
</tr>
<tr>
<td></td>
<td>12:7</td>
</tr>
<tr>
<td></td>
<td>17:3</td>
</tr>
<tr>
<td></td>
<td>20:2</td>
</tr>
<tr>
<td></td>
<td>24:7</td>
</tr>
<tr>
<td></td>
<td>26:4</td>
</tr>
<tr>
<td></td>
<td>30:0</td>
</tr>
</tbody>
</table>

S.D. = Standard deviation
A perfusion technique to study the colonization of a cellulosic substrate by fungi. Malik, K. A. and Eggins, H. O. W.

of the nematode catching fungus Arthrobotrys sp. which is cellulolytic as well.

When the strip was highly degraded a variety of fungi were isolated e.g. Papulaspora sp., Gliocladium roseum, Penicillium sp. and Aspergillus fumigatus.

The weight loss results are summarised in Table 2. The % loss is plotted against the days of incubation (Figure 4). The weight loss increased steadily as the period of incubation prolonged. The weight loss percentage was maximum after 26 days of incubation i.e. 56·7%. This percentage decreased to 51·1% after 30 days, presumably due to the increase of fungal mycelium on the paper strip.

Discussion

The perfusion technique worked quite successfully throughout the period of incubation, maintaining a constant flow of nutrients through the cellulose strip until its complete deterioration. The screening of the cellulose substrate protected it from direct contact with soil particles, thus avoiding any direct isolation from the soil. This also helped in the estimation of weight loss since no soil was adhering to it.

The isolations showed some kind of colonization pattern with distinct early colonizers and late developers. Fusarium solani and T. viride were shown to be the early colonizers and the main causes of cellulose deterioration. The late developers comprised Gelasinospor pro cerealis, Arthrobotrys sp., Gliocladium roseum and Paecilomyces variotii, whereas a number of other fungal spp. were isolated when the strip was completely deteriorated.

The absence of some well known cellulolytic fungi namely Chaetomium spp. is indicative of the fact that the role of individual species in a pure culture can give misleading indications of their importance in nature; and this fact makes such ecological studies with mixed cultures all the more important.

This technique can also be employed to study various ecological factors like pH, nitrogen and carbon sources, etc., by changing the nutrients in the flask feeding the perfusion sets. The efficiency of biocides can also be tested by simply coating them on to the cellulose substrate and then incorporating it into the perfusion devices.

References


A perfusion technique to study the colonization of a cellulosic substrate by fungi.
Malik, K. A. and Eggins, H. O. W.

Figure 1
A perfusion technique to study the colonization of a cellulosic substrate by fungi.
Malik, K. A. and Eggins, H. O. W.
A perfusion technique to study the colonization of a cellulosic substrate by fungi.
Malik, K. A. and Eggins, H. O. W.

Figure 4
CUMULATIVE INDEX
VOLUMES 1-5, 1965-1969

AUTHOR INDEX

Adema, Dorothea M. M. .... 1(1): 15
3(1): 29
Ayerst, G. .... 1(2): 13
Baker, P. W. .... 2(2): 59
4(1): 59
Becker, G. .... 5(1): 25
Bernard, J. M. .... 5(1): 21
Bomar, M. .... 2(2): 151
4(1): 43
Bull, A. T. .... 3(1): 3
Butler, N. J. .... 1(1): 20
Calderon, O. H. .... 1(1): 59
1(2): 33
Chapin, J. C. .... 3(1): 13
Costa, C. .... 4(2): 93
Costello, J. A. .... 5(3): 101
Coursey, D. G. .... 4(2): 29
5(1): 27
Darby, R. T. .... 3(2): 65
4(1): 39
Desai, A. G. .... 3(1): 13
Domanski, S. .... 3(2): 79
Eggs, H. O. W. .... 1(2): 46
2(2): 135
4(1): 29
5(1): 63
5(4): 163
Elphick, J. J. .... 1(1): 8
1(2): 56
Evans, D. M. .... 4(2): 89
Forse, W. J. .... 5(2): 67
Gardiner, N. .... 2(2): 149
Garnier, G.... 1(2): 27
Ginocchio, J. C. .... 2(1): 22
Goldblatt, J. L. A. .... 1(1): 41
Nissen, T. V. .... 3(2): 77
Nowakowska, A. .... 4(2): 101
Olich, A. .... 3(2): 79
Osborne, D. G. .... 2(2): 149
1(2): 15
5(6): 153
Pucic, H. .... 4(2): 93
Pociti, J. .... 1(2): 74
Parbery, D. G. .... 4(2): 79
Payen, B. .... 1(2): 64
2(1): 36
Robertson, J. A. .... 1(1): 41
Rogers, M. R. .... 5(2): 95
Ruszel, J. D. .... 5(1): 27
Rytych, B. J. .... 5(1): 3
Savory, J. G. .... 4(2): 83
5(2): 77
Savulescu, A. .... 4(2): 75
Schmid, W. .... 4(1): 49
Sharp, R. F. .... 4(1): 63
Siebenhar, M. .... 2(1): 14
Simmons, E. G. .... 4(1): 39
Sorkin, M. .... 1(2): 61
Staffeld, E. E. .... 1(1): 59
4(1): 23
Stewart, C. S. .... 5(1): 15
Thivend, P. .... 3(2): 67
Townsend, R. .... 3(2): 47
Traxler, R. W. .... 1(1): 22
5(2): 43
5(1): 21
Tropicanski, J. .... 5(3): 119
Van der Toorn, J. .... 1(1): 13
1(1): 31
Wallbridge, Ann .... 1(2): 46
Walsh, J. H. .... 1(2): 30
5(1): 15
5(4): 149
Wassiebauer, R. .... 3(1): 1
Waterman, H. A. .... 1(1): 43
Williams, S. T. .... 2(2): 125
Zenger, C. .... 3(2): 43
Zinkenagel, R. .... 3(1): 21
4(1): 49
Zyska, B. J. .... 1(2): 56
22(2): 121
4(2): 109

SUBJECT INDEX

Actinomycetes: role in biodeterioration .... 2(2):125
Aflatoxin: extraction from groundnut meal .... 1(1):41
Aircraft: bird impacts .... 8(2):67
Aircraft fuel supply systems: fungi .... 4(1): 39
5(2): 35
Alkanes: utilisation by Pseudomonas aeruginosa .... 5(1): 21
Aluminium: corrosion .... 4(2): 79
Antibiotics: food preservation .... 5(2): 39
Antifungal substances: interaction with surface active agents .... 2(2):151
Argil C: anti-rot protection of cotton .... 2(1): 22
Artificial gas mixtures: preparation .... 5(4):149
Bactericides in rubber .... 5(1): 3
Bacteristats: testing .... 1(1): 15
Bermuda grass: frost-killed: toxicity .... 2(1): 5
Biodeterioration research at BAM, Berlin-Dahlem .... 5(3):125
Biodeterioration research in France .... 1(1): 27
Biodeterioration research in Israel .... 2(1): 1
Biodeterioration research in Poland .... 2(2):121
Biodeterioration research in Romania .... 4(2): 75
Biodeterioration research in the U.S.A. .... 3(2): 43
Biological tests: a survey .... 1(2): 38
Biological tests: general laboratory techniques .... 2(1): 7
Birds: aircraft .... 5(2): 67
Bitumen: degradation .... 1(1): 22
When you need a paint fungicide, you should look to Nuodex.

Why?
Because we have the most versatile line around.

**SUPER AD-IT** The recommended universal paint fungicide for both oil and aqueous systems.

**PMO-10** For mildew resistant oil, oleoresinous and alkyd paints.

**PMA-18** For use in standard aqueous systems as a fungicide and/or preservative.

**PMA-60** For use in emulsion paint systems to prevent bacterial spoilage during liquid paint storage and to protect the applied paint film against mildew attack. Packaged in 4 oz. and 8 oz. water-soluble packages.

**FUNGITROL 11** Fungicide-bactericide for non-aqueous paint systems. Effective for meat-packing plants, breweries, dairies, etc. Also imparts fungus resistance to baking enamels.

**NUODEX** products are available from the following affiliates and licensees:

- **ARGENTINA** • Plastica Bernabo S.A., Terrada 658/664, Buenos Aires; **AUSTRALIA** • Nuodex (Aust.) Pty. Ltd., 49-61 Stephen Road, Botany, N.S.W.; **BRAZIL** • Nuodex S.A. Industria, E. Comercio de Secantes, Rua Dom Gerardo 80-1º and., Rio de Janeiro; **ENGLAND** • Nuodex Limited, Birtley, County Durham; **FRANCE** • Nuodex France S. A. R. L., 14, Rue de Moscou, Paris 8e, France; **WEST GERMANY** • Gebr. Borchers A.G., Elizabethstr. 14, Dusseldorf; G. Siegle & Co. GmbH, Sieglestrasse 25, Stuttgart-Feuerbach (Stabilizers only); **HOLLAND** • N.V. Chemische Fabriek Servo, Delden; **ITALY** • Nuodex Italiana, S. P. A., Piazza Della Repubblica 11/A, Milan; **JAPAN** • Harima, Kasei Kogyo Ltd., Mizuashi, Noguchi-cho, Kakogawa, Hyogo; **MEXICO** • Nuodex Mexicana, S. A., Durango 209, Desp. 101, Mexico 7 D. F.; **NEW ZEALAND** • A. C. Hatrick (N.Z.) Ltd., 65 Main Road, Tawa, Wellington; **SOUTH AFRICA** • Poly-Resin Products, Ltd., Durban; **SPAIN** • Nuodex Espanola, S. A., Av. Jose Antonio 55, Madrid.

In any language NUODEX is your symbol for quality and service.

**TENNECO CHEMICALS, INC.**
**NUODEX DIVISION INTERNATIONAL DEPT.**
P.O. Box 2, Piscataway, N.J.

Published by the Biodeterioration Information Centre

£4 per annum

Printed by Suttons (Printers) Ltd., Birmingham