MICROBIOLOGICAL CORROSION OF METALS—MARINE WOOD BORERS—RODENT ATTACKS ON STORED PRODUCTS—FOULING OF SHIPS BY BARNACLES—DETERIORATION OF STONE BY BACTERIA—ROTting OF WOOD BY FUNGI—BACTERIAL BREAKDOWN OF ASPHALT—MILDEWING OF LEATHER—INSECT DAMAGE TO BOOKS—BIRD HAZARDS TO AIRCRAFT—FUNGI IN JET FUEL TANKS—TERMITES IN TIMBER—MICROBIOLOGICAL ATTACK ON RUBBERS, PLASTICS AND PAINTS,—FUNgal ETCHING OF GLASS.

INTERNATIONAL BIODETERIORATION BULLETIN

A QUARTERLY JOURNAL OF BIODETERIORATION
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INTERNATIONAL BIODETERIORATION BULLETIN

Biodeterioration Information Centre, Department of Biological Sciences, The University of Aston in Birmingham, B4 7PP.

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Professor T. A. Oxley

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


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CIBA-GEIGY (U.K.) LIMITED, Dyestuffs Division, Clayton, Manchester, England, M11 4AR.


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

FOSROC INTERNATIONAL LTD., 36 Queen Anne's Gate, London, England SW1H 9AR.


HALDANE CONSULTANTS LIMITED, 27 Dawkins Road, Poole, Dorset, BH15 4JB; consultants in industrial microbiology and microbiological deterioration.

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

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

LUCAS AEROSPACE LTD., Shaftmoor Lane, Birmingham, England B28 8SW.

MARKS & SPENCER LTD., Michael House, Baker Street, London, England W1A 1DN.

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NATIONAL COAL BOARD, Coal House, Lyon Road, Harrow, Middlesex, England.


VENTRON CORPORATION, Congress Street, Beverly, MA 01915, 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.

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, Professor T. A. Oxley, 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 Levinson (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. 30 reprints will be sent free of charge with each article. Additional reprints are obtainable: scale of charges available on application to the Editor.
BIODETERIORATION SOCIETY NEWSLETTER

Annual General Meeting 1975

This year's AGM was held during the 3rd International Biodegradation Symposium at the University of Rhode Island, USA.

Reports by the Honorary Secretary, Honorary Treasurer and Honorary Programme Secretary were presented and an addition to the Constitution formally incorporating the Standing Committee for International Biodegradation Symposia within the Society was made. The full minutes of the meeting, together with copies of the reports presented will be circulated to all members at a future date. Changes in the membership of the Council, resulting from the election in April, which were announced at the AGM are as follows:

President: Professor T A Oxley
Honorary Treasurer: Dr K J Seal
Council Member: Dr A F Bravery

Dr H J (Bill) Bunker

It is with much regret that we record the death of Dr H J (Bill) Bunker, who died in August.

Bill Bunker had a long and distinguished career in biology and in microbiology in particular. In biology he came to be regarded as epitomising the first rate applied biology consultant, and all those connected with this field of work were delighted when he was elected President of the Institute of Biology, a position which he filled with vigour and distinction. His achievements in various fields of microbiology were marked when he was made President of the Society for General Microbiology, having been a founding member of the Society.

He could be regarded quite truly as a Father of biodegradation, publishing in 1927 a book with Thaysen on the microbiology of fibres. For some years he worked in the brewing industry, earning a reputation in the handling and selection of yeasts, as well as in the microbiological problems of beers. Eventually he chose to follow the path of the consultant solving a wide range of problems including investigations on the microbiological problems of the oil industry, long before these became commonplace.

For those of us in biodegradation, we will always count ourselves particularly lucky in Bill Bunker's agreement to act as Chairman of the First International Biodegradation Symposium held at Southampton in 1968. From this Symposium arose the Biodeterioration Society and under his guidance as the first President, the Society flourished and did not fall into the unfortunate position of apparently competing with other societies with a fringe interest in biodegradation.

The Biodeterioration Information Centre at Aston University owes a particular debt to Bill Bunker—he showed considerable interest in the work of the Centre in its early days and became the first Chairman of its Consultative Council.

Those of us concerned with biodegradation will sadly miss Bill's presence at our meetings, as we did at the University of Rhode Island at the 3rd Symposium in August. He brought a lifetime's experience in industrial microbiology and biology generally to our meetings, but above all we will remember him for his jovial outlook on life and for his kindly gentlemanship.

A full, detailed obituary will be published in the IBB in a subsequent issue.

Mr A E Darby

It is with much regret that we announce the untimely death of Mr Albert Darby, who died on the 6th August 1975.

Mr Darby who was an employee of Ciba-Geigy (UK) Ltd, will be remembered as an active member of the Society and of the International Biodegradation Research Group. He will be missed not only as a scientist but also as a warm-hearted and friendly colleague.

Mr Darby leaves a widow, Jennifer, and a baby son, Anthony, and our sympathy goes out to them in their loss.

3rd International Biodegradation Symposium

The 3rd International Symposium was held at the University of Rhode Island, Kingston R.I., USA from 17th-23rd August. The title "Biodegradation" was chosen in preference to "Biodeterioration" because the former title more closely reflected the main emphasis of the papers invited and contributed.

Organisation and facilities were excellent and some idea of the amount of support received may be gained from the fact that there were 25 sessions with from three to eight papers each, and four plenary sessions. Attendance at the sessions was generally good even though there were at times three running simultaneously. There were 225 registered participants of whom 160 were from USA. The next largest contingent was 28 from UK. Twelve attended from other European countries and eleven from Canada, Japan, India and several other countries were also represented.

The Society owes a considerable debt to Dr. Richard Traxler, the local secretary, and his committee of nine others who did an immense amount of work to make the symposium a success. At the final plenary session Professor Gunther Becker announced that the 4th Symposium will be held at his Institute in Berlin in 1978.

Members of the Society attending the 3rd symposium were very grateful to the initiative of Dr. Harold Rossmore of Wayne University who, at short notice, organised a Society Dinner at the Larchwood Inn, a few miles from the University campus, on Friday 22nd August. This was a most enjoyable evening.
### Papers Presented at a Symposium on

**MECHANISMS OF BIOCIDAL AND BIOSTATIC ACTIVITY**

(Jointly with the Industrial Group of the Biochemical Society)

University College of Wales, Aberystwyth. 16th April 1975.

<table>
<thead>
<tr>
<th>Title:</th>
<th>Mechanisms of Bacterial and Bacteriostatic Activity</th>
</tr>
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<tbody>
<tr>
<td>Author:</td>
<td>Dr. W. B. Hugo</td>
</tr>
<tr>
<td>Address:</td>
<td>Department of Pharmacy, University of Nottingham, University Park, Nottingham NG7 2RD.</td>
</tr>
</tbody>
</table>

**ABSTRACT**

The mechanisms of bactericidal and bacteriostatic action are considered from the cellular targets, wall, membrane and cytoplasm. Special emphasis is placed on recent work on membrane-active compounds from the point of view of Mitchell's chemiosmotic theory. Attempts to distinguish between mechanisms of bactericidal and bacteriostatic action are made.

<table>
<thead>
<tr>
<th>Title:</th>
<th>Formulation and Biological Activity</th>
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<tbody>
<tr>
<td>Author:</td>
<td>H. S. Bean</td>
</tr>
<tr>
<td>Address:</td>
<td>Pharmacy Department, Chelsea College, University of London, London SW3 6LX.</td>
</tr>
</tbody>
</table>

**ABSTRACT**

Activities of antimicrobial agents are frequently determined in distilled water but they may be very different in formulated products. The presence of a drug may increase or decrease the activity of preservatives as may an increase in pH. Low concentrations of surface active agents (e.g. Tween 80) may increase activities whilst larger concentrations decrease. In an emulsion the concentration of preservative in the water may be either greater or less than the total concentration depending on the O/W partition coefficient of the preservative and the extent of binding with the emulgent. The extent of binding and partitioning is reflected in the preservative activity.

<table>
<thead>
<tr>
<th>Title:</th>
<th>Fungicides and Fungistats</th>
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<tbody>
<tr>
<td>Author:</td>
<td>Dr. R. N. Smith</td>
</tr>
<tr>
<td>Address:</td>
<td>The Hatfield Polytechnic, Hatfield, Herts.</td>
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</tbody>
</table>

*(Abstract not received)*

<table>
<thead>
<tr>
<th>Title:</th>
<th>Algicides and Algistats</th>
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<tbody>
<tr>
<td>Author:</td>
<td>Dr. K. H. Goulding</td>
</tr>
<tr>
<td>Address:</td>
<td>Department of Biological Sciences, The Hatfield Polytechnic, Hatfield, Herts.</td>
</tr>
</tbody>
</table>

**ABSTRACT**

Relatively few studies have been carried out on the mechanisms of algicide toxicity. Of those which have been examined the majority appear to act upon the photosynthetic process and in particular upon electron transport. Details of mode of action will be discussed where they are reasonably well established.

<table>
<thead>
<tr>
<th>Title:</th>
<th>Insecticides</th>
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</thead>
<tbody>
<tr>
<td>Author:</td>
<td>M. J. Van den Heuvel</td>
</tr>
<tr>
<td>Address:</td>
<td>Ministry of Agriculture, Fisheries and Food, Pest Infestation Control Lab., London Road, Slough, Berks.</td>
</tr>
</tbody>
</table>

**ABSTRACT**

All pesticides, including insecticides, act by interfering with, or blocking, one or more basic life "processes" within the target organism. Whilst the mechanism of action of many insecticides is poorly understood, it is possible to classify them in a general way by their mode of action.

<table>
<thead>
<tr>
<th>Title:</th>
<th>Rodenticides</th>
</tr>
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<tbody>
<tr>
<td>Author:</td>
<td>A. D. Martin</td>
</tr>
<tr>
<td>Address:</td>
<td>Pest Infestation Control Lab., MAFF Hook Rise South, Tolworth, Surbiton, Surrey.</td>
</tr>
</tbody>
</table>

**ABSTRACT**

Poisoning with toxic chemicals is the most widely used method of achieving rodent control. While a variety of poisons can be used as acute (single dose) rodenticides, the chronic (multiple dose) rodenticides now used are all anti-coagulants. The relative merits of these two rodenticidal types will be discussed together with those details of their biological action that are currently known.

The provision of these abstracts by authors does not constitute formal publication. Those interested should write direct to authors, not the society.
Title: Antifouling Agents
Author: G. C. Jackson
Address: Central Dockyard Laboratory, H.M. Dockyard, Portsmouth PO1 3LZ.

ABSTRACT
Various antifouling techniques have been tried, but to date, the most successful are toxic paints based on metallic salts e.g. CuSO₄. Heterocyclic systems designed to release formaldehyde and piperidine derivatives in weakly acidic media are being studied as potential new antifouling agents. All potential compounds are screened for activity using laboratory and raft trials.

Title: Some Important Features in the Mechanism of Action of Herbicides
Author: Stuart M. Ridley
Address: Biochemistry Section, Jealott's Hill Research Station, Bracknell, Berks.

ABSTRACT
(Shortened from author's original)
The principal sites involved in the mechanism of action of herbicides may be loosely divided into three groups:
1. Interference with plant growth through direct effects on plant hormones, cell division, nucleic acid metabolism and protein synthesis. It is especially difficult with this group to sort out the primary actions from those of secondary effects.
2. Respiration, concerning interference with mitochondrial electron transport. The main group, the substituted phenolic herbicides, act by uncoupling oxidative phosphorylation; then weeds cannot produce the energy required for growth.
3. Photosynthesis, concerning interference with chloroplast development and function. The main action is by blocking the electron transport system close to the reducing side of photosystem II.

Papers Presented at the Annual Summer Meeting 1975
University of Reading

Symposium on Biodeterioration Problems in Agriculture 11th July 1975

Title: Fungal Growth in Stored Grain and Cereal Crops
Author: Dr. J. H. Clarke
Address: Biology Department, Pest Infestation Control Laboratory, London Road, Slough, Berks. SL3 7HJ.

ABSTRACT
Some of the fungi that grow in stored grain and cereal crops and the damage resulting from such growth are discussed. A few aspects of the physiology of these fungi and of methods which attempt to control them will then be given.

Title: Moulding of Hay and its Implications
Author: J. Lacey
Address: Plant Pathology Department, Rothamsted Experimental Station, Harpenden, Herts. AL5 2JO.

ABSTRACT
Grass standing in the field carries a varied inoculum of microorganisms. Which develop subsequently depends on conditions during drying and storage, especially the rate of drying, the water content at baling, and the degree of spontaneous heating. Besides depleting the nutrient content of the hay, several organisms can affect the health of man and animals, through allergy, infection or toxicosis.

The provision of these abstracts by authors does not constitute formal publication. Those interested should write direct to authors, not the society.
Title: Problems in Making and Feeding Silage  
Author: R. F. Wilson  
Address: Grassland Research Institute, Hurley, Berks, SL6 8ND.

**ABSTRACT**

When the water-soluble carbohydrate content of ensiled crops is low, lactobacilli are unable to produce sufficient lactic acid for preservation. Undesirable clostridial fermentations can then take place, proteins are severely degraded and feeding value of the silage reduced. Yeasts and moulds can cause serious deterioration in silage quality after the silo has been opened for feeding.

Title: Mycotoxins in Animal Feeds  
Author: D. S. P. Patterson  
Address: Central Veterinary Laboratory, New Haw, Weybridge, Surrey KT15 3NB.

**ABSTRACT**

Traces of aflatoxin, ochratoxin A, sterigmatocystin and zeamelenone were detected in a few feed-stuff samples examined at Weybridge but, at worst, only minor effects on animal production were observed. Certain recurring syndromes appeared to be mycotoxic diseases although the relevant toxins were not identified.

Public health aspects of mycotoxicosis and feed-stuff screening in ADAS were discussed briefly.

Title: Infestation of Grain and Cereal Crops by Insects  
Author: John M. Holborn  
Address: Entomology Department, Wellcome Research Laboratories, Berkhamsted Hill, Berkhamsted, Herts.

**ABSTRACT**

Pests of stored cereals and damage caused are described primarily applied to the U.K.

High standards of storage and hygiene are essential preventative measures, but not sufficient alone to prevent infestation.

The commercially available insecticides and fumigants and new developments are described. Grain protection involves using a variety of control measures from harvesting to delivery at the mill.

Title: Rodent Control on Farms  
Author: J. O. Bull  
Address: Plant Protection Division, I.C.I. Fernhurst, Hazlemere, Surrey.

**ABSTRACT**

Control of rodents on farms is still in the “do it ourselves” category since farmers and gamekeepers are accustomed to using various pesticides. Despite effective rodenticides, the numbers of rodents on farms are frequently too high; this is often the consequence of poor hygiene and building standards, inadequate understanding of rodent behaviour and, sometimes, misuse of rodenticides.

Title: Aquatic Weed Problems on Farms  
Author: P. R. F. Barrett  
Address: ARC Weed Research Organisation, Begbroke Hill, Yarnton, Oxford OX5 1PF.

**ABSTRACT**

Excessive weed growth in farm ditches reduces the drainage capacity of these ditches and results in more rapid silting. This can cause crop loss both by direct flooding and by delaying farming operations particularly drilling. The use of herbicides and weed cutting machinery is reducing the need for hand labour but regular maintenance is still necessary.

The provision of these abstracts by authors does not constitute formal publication. Those interested should write direct to authors, not the society.
Title: Culture Media for the Isolation of Fungi Causing Deterioration in Tobacco

Author: T. G. Mitchell

Address: British-American Tobacco Co. Ltd., Group R. & D. Centre, Regents Park Road, Southampton S09 1PE.

ABSTRACT

Fungal deterioration of tobacco is caused mainly by species of Aspergillus. The use of two media—Christensen's malt salt agar containing 10% sodium chloride and Littman ox-gall agar with streptomycin—has proved of particular value for the enumeration and identification of Aspergillus strains from cured tobacco.

Title: Testing the Crack and Crevice Injection System against German Cockroaches

Author: D. P. Blow

Address: Biology Laboratory, Protim Ltd., Fieldhouse Lane, Marlow, Bucks.

ABSTRACT

The paper outlines a field trial of the crack and crevice injection system at a site where a conventional propoxur spray failed to control the German cockroach (Blattella germanica) population. A laboratory trial to study the effective life of the insecticide applied by this system is then discussed.

Title: Detecting Wood Decay by Indicator Dyes

Author: Miss J. K. Carey

Address: Building Research Establishment, Princes Risborough Laboratory, Princes Risborough, Aylesbury, Bucks.

ABSTRACT

Use of pH indicators to determine the extent of fungal attack by detecting acids produced during decay was described. The pH range of the indicator selected must be compatible with the natural pH of the wood; bromocresol green (sodium salt) and bromophenol blue were successful on Scots pine and spruce and alizarin red S on Corsican pine and Abies sp.

The provision of these abstracts by authors does not constitute formal publication. Those interested should write direct to authors, not the society.
Title: The Production of Bacterial Protein from Chemically Treated Plastics
Authors: J. C. Jones and B. S. Brown
Address: Dept. Medical Biochemistry, Medical School, The University, Manchester.

ABSTRACT
Pyrolysing refuse plastics may yield a suitable substrate for the production of single cell protein. Polyethylene pyrolysis yields a range of hydrocarbons containing up to forty carbon atoms. Candida pseudotropicalis has been grown, in batch fermentation, on an emulsion of the pyrolysate.

The utilization of hydrocarbon and the production of cells have been monitored.

Title: The Microbial Colonisation of Selected Naturally Durable Tropical Timbers in the Sea
Author: S. E. J. Furtado
Address: Department of Biological Sciences, Portsmouth Polytechnic, King Henry I Street, Portsmouth PO1 2DY.

ABSTRACT
Ten selected naturally durable hardwoods were exposed at five sites to evaluate their resistance to the various marine borers common to each station. Marine fungi found colonising the different timbers were recorded monthly over a period of a year. The greatest number of fungal species recorded was at Vancouver and Sekondi with progressively fewer at La Rochelle, Follonica and Genoa.

The provision of these abstracts by authors does not constitute formal publication. Those interested should write direct to authors, not the society.

FORTHCOMING CONFERENCES, MEETINGS AND COURSES

<table>
<thead>
<tr>
<th>Dates</th>
<th>Title</th>
<th>Location</th>
<th>Contact</th>
</tr>
</thead>
<tbody>
<tr>
<td>24-26 November 1975</td>
<td>The Control of Insects and Rot in Buildings (3 day course, Fee £21.60)</td>
<td>Princes Risborough, England</td>
<td>Mrs. Susan Hobbs, Building Research Establishment, Princes Risborough Laboratory, Princes Risborough, Aylesbury, Bucks. HP17 9PX</td>
</tr>
<tr>
<td>14-17 October 1975</td>
<td>International Rubber Conference 1975</td>
<td>Tokyo, Japan</td>
<td>Secretariat, IRC '75—Tokyo, Society of Rubber Industry, 5-25 Motoakasaka, 1-chome, Minato-ku, Tokyo 107, Japan</td>
</tr>
<tr>
<td>20-25 October 1975</td>
<td>International Rubber Conference 1975</td>
<td>Kuala Lumpur, Malaysia</td>
<td>Chairman, Executive Committee IRC '75—Kuala Lumpur, Rubber Research Institute of Malaysia, P.O. Box 150, Kuala Lumpur, 01-02, Peninsular Malaysia.</td>
</tr>
<tr>
<td>14-19 June 1976</td>
<td>4th International Congress on Marine Corrosion and Fouling</td>
<td>Juan-les-Pins Antibes, France</td>
<td>Secretariat, Centre de Recherches et d'Etudes Oceanographiques 73777, rue de Sèvres 92100 Boulogne-sur-Seine</td>
</tr>
<tr>
<td>Late August 1977</td>
<td>Second International Mycological Congress</td>
<td>Tampa, Florida, USA</td>
<td>Secretary: Melvin S. Fuller, Department of Botany, University of Georgia, Athens, Georgia 30602</td>
</tr>
</tbody>
</table>
FUNGAL BIOLOGICAL FLORA
IN THE
INTERNATIONAL BIODETERIORATION BULLETIN

In Volume 7 No. 1 (1971) a short article by G. J. F. Pugh on p. 35 entitled “Fungi of Importance in Biodeterioration” drew attention to the scattered nature of the biological information on fungi which are of concern to those who study Biodeterioration. It was suggested that “fungal biological floras” should be prepared from time to time by workers familiar with particular genera, species, or groups, and submitted for publication in the International Biodeterioration Bulletin. A series of headings for all contributions was suggested and advice on length and scope was given.

Up to the present time, three such contributions have been published, viz:

1. *Gliomastix murorum* and *G. murorum* var. *Felina* by

2. *Epicoccum nigrum* (Link) by

3. *Curvularia* (Boedijn) by

Further contributions of a suitably high standard are invited. It is hoped eventually to reprint them in loose leaf or book form. No contributions have been commissioned but I would welcome early notice from any worker who is considering preparation of a paper for this series in order that duplication of effort may be avoided. Any firm undertaking to prepare a contribution to Fungal Biological Flora will be published together with the intending author’s name and address. I will be glad to send a photocopy of Dr. Pugh’s original article to any serious enquirer.

T. A. Oxley,
Editor,
International Biodeterioration Bulletin.
July 1975
MODEL TESTS ON THE MECHANISMS OF MICROBIAL DETERIORATION OF FILLED VULCANIZATES.

3. INFLUENCE OF SOIL MICRO-ORGANISMS ON THE SURFACE OF NATURAL RUBBER VULCANIZATES

J. Reszka, B. J. Zyska, P. S. Fudalej, and K. R. Reszka

Summary. Natural rubber vulcanizates containing structural changes in the rubber surface are found. The superficial structure were examined by scanning electron microscopy. A close relationship exists between the degree of microbial deterioration of the rubber surface and the exposure time. The higher the carbon black filler loading in the rubber the less structural changes in the rubber surface are found.

Tests spéciaux sur les mécanismes de détérioration microbienne de caoutchouc vulcanisé chargé - 3. Action des micro-organismes du sol sur la surface du caoutchouc naturel vulcanisé. On a enterré expérimentalement du caoutchouc naturel vulcanisé auquel on a ajouté du carbone noir en proportion de 0, 20 et 40 pour cent de caoutchouc, dans un sol stérile et sol normal pendant 30, 120, 180 et 360 jours. Après divers intervalles de temps on a examiné les changements dans la structure superficielle au moyen de "scanning electron microscopy" (méthode de microscope électronique). Il existe un rapport étroit entre le degré de détérioration microbienne de la surface du caoutchouc et la durée d'exposition. Plus la charge en carbone noir est grande dans le caoutchouc, le moins on observe de changements dans la structure de la surface du caoutchouc.

Introduction

In our previous work on the mechanisms of microbial deterioration of filled vulcanizates the changes in a model paraffin oil-carbon black system due to Pseudomonas sp. have been investigated (Zyska et al., 1971, 1973). This paraffin oil-carbon black suspension model made possible a study of the formation of spatial carbon black structures, similar to those formed during the interaction of the elastomer and carbon black filler. In microbiological tests this system proved to be very useful as the decrease in the structure-forming ability of carbon black after microbial deterioration of the paraffin oil could be demonstrated and the relation between the carbon black filler loadings in the suspension and the degrading influence of Pseudomonas on the carbon black structure of the model system could be elucidated. As the next step it was decided to trace the changes of the surface of filled natural rubber vulcanizates due to microbial deterioration. Every deteriorating effect of the microorganisms on the elastomer has its beginning at the surface, as this is the natural place of contact of the microorganisms with the material. Penetration of the microorganisms into the material follows in the further stage of deterioration, as is evidenced by more or less observable changes of properties. Phenomena occurring on the surface of vulcanizates seem to have a considerable influence on the further process of microbial deterioration.

Our hypothesis for the time-dependent mechanism of the microbial deterioration of elastomers postulates the cause of decreased strength properties as due to the following:

—destruction of primary network formed by the vulcanized elastomer chains, either directly by micro-organisms or indirectly by the microbial metabolites;

—impairment or decrease in number of filler-elastomer bonds due to the microbial metabolites;

—appearance of micro-defects and micro-cracks, decreasing the strength properties;

—acceleration of the ageing process due to the microbial metabolites.

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Explanation of the photographs

All the photographs are scanning electron microscope pictures of the surface of natural rubber specimens taken after varying periods of exposure to sterile soil or normal soil with microorganisms.

The scale in micrometres is given in the bottom left hand corner of each picture.

In the bottom right hand corner: 
- S = sterile soil
- A = normal soil with micro-organisms

The figure to the left of the sloping line indicates the filling in parts per hundred of rubber of carbon black.

The figure to the right of the sloping line indicates the number of days exposure.

Figures 1-4 Unfilled rubber exposed to sterile soil for 30, 120, 180 and 360 days × 1000
Figures 5-8 Unfilled rubber exposed to soil with micro-organisms for 30, 120, 180 and 360 days × 1000
Figure 9 As figure 7 × 3000
Figure 10 As figure 7 × 300
Figure 11 As figure 8 × 300
Figure 12 As figure 8 × 100
Figures 13-16 Filled rubber containing 20 parts per hundred of rubber of carbon black exposed to sterile soil for 30, 120, 180 and 360 days × 1000
Figures 17-20 Filled rubber containing 20 p.p.h.r. carbon black exposed to soil with micro-organisms for 30, 120, 180 and 360 days × 1000
Figures 21-24 Filled rubber containing 40 p.p.h.r. carbon black exposed to sterile soil for 30, 120, 180 and 360 days × 1000
Figures 25-28 Filled rubber containing 40 p.p.h.r. carbon black exposed to soil with micro-organisms for 30, 120, 180 and 360 days × 1000

Note on magnifications:

The magnifications given relate to the original photographs which measured approximately 110 × 90 mm. After reduction for printing the equivalent magnifications are:

Fig. 9 × 1920, Figs. 10 and 11 × 192. Fig. 12 × 64. All other figures × 640.

This hypothesis of the mechanism of the microbial deterioration of elastomers was discussed in detail during the Second Colloquium on microbial deterioration of materials held in October 1972 in Katowice, Poland (Fudalej, et al., 1975).

The micro-defects and micro-cracks, as one group of phenomena of microbial deterioration of elastomers, lead to increase of specific surface of the material without any change in the geometry of the samples. This surface development of the rubber also gives rise to changes in the capacity for sorption of gases and water vapour. In this case the increase of oxygen and water vapour sorption may be of importance as factors promoting the further growth of micro-organisms in the elastomer. It is noteworthy that water vapour sorption has a decisive influence on the dielectric properties of the elastomer used, for instance, in electric rubber cables. The appearance of micro-defects and micro-cracks in pipe joint rubber rings was described recently by Kerner-Gang (1973). It was reported that after a period of two years of exposure of samples to micro-organisms in the sludge of a sewage-treatment plant, deterioration of the rubber ring surface could be observed, shown as cavities and cracks in the material.

A more complete explanation of the microbial deterioration of elastomers would appear to require more detailed analysis of changes occurring on the surface of the material. The series of samples prepared for this purpose should be as nearly as possible uniform in composition, as variation in more than one component of the vulcanizate makes proper interpretation of the deterioration process very difficult. For this reason in our tests it was decided to expose to micro-organisms natural rubber vulcanizates with constant composition of the base mixture, varying only the carbon black filler loadings. The purpose of the tests was to investigate the influence of the quantity of filler in the vulcanizate on the superficial microbial destruction of the material.

Experimental

The natural rubber vulcanize was made up from the following constituents, given in dry parts by weight: white crepe 100; thiram 0.5; zinc oxide 1.0; sulphur 2.5; channel carbon black SAO. The quantities of carbon black in the series of formulations were respectively: 0 parts per hundred of rubber, 20 p.p.h.r. and 40 p.p.h.r. Channel carbon black SAO of specific surface 90.88 g/m² was used in these tests as in the previous investigations (Zyska et al., 1971, 1973). The mixes were moulded in a press into 1 mm sheets and cured for 2 min. at 151°C at a pressure of 50 at. For the tests in sterile soil and in normal soil burial, test samples 100 × 40 mm were cut and divided into two equal parts. The soil burial test was performed according to the standards given earlier (Zyska et al., 1971). The pH of the soil was 7.0 to 7.1. The sterile soil was prepared in glass jars of 1000 cm³, by sterilizing in the presence of 10 cm³ of 1% HgCl₂ solution for three consecutive days. The exposure periods of the samples in the sterile soil and soil burial tests were respectively 30, 120, 180 and 360 days. After the various time intervals the changes in the superficial structure were examined by scanning electron microscopy. The samples were evaporated at vacuum pressure 2 × 10⁻⁵ Torr and coated with carbon and gold in a vacuum evaporator JEE-4B. A Jeol JSM-S1 scanning electron microscope was employed.

Discussion of Results

In detailed scanning electron microscopy studies on the microbial deterioration of vulcanizates the elimination of additional ageing processes of the materials is of importance. Hence as a basis for analysis we employed a comparison of control specimens exposed for a given time to sterile soil with specimens exposed in the soil burial test.

Fig. 1, 2, 3 and 4 are scanning electron micrographs of the surface of unfilled natural rubber, after 30-360 days exposure to sterile soil. The surface of these natural rubber specimens remains relatively featureless. The corrugations and shallow pits to be observed are simply technological residues.

After 30 days of the soil burial test the soil micro-organisms caused remarkable changes in the sample A-0/30. The surface of the vulcanizate became distinctly loosened and lumpy, to be observed on scanning electron micrograph fig. 5. The extension of the exposure time to soil micro-organisms in the soil burial test up to 120 days is marked by formation of fissures up to about 20 μm long and 0.3 μm wide (fig. 6). Simultaneously numerous cavities 4.7 μm in diameter are formed on the rubber surface. After 180 days (figs. 7, 9 & 10) the length and width of the fissures increase approximately to 50 μm and 5 μm respectively. After 360 days of exposure to the activity of soil micro-organisms the surface of unfilled natural rubber vulcanizate shows the formation of cavities even up to a diameter of 380 μm (figs. 8, 11 & 12). The deterioration of the rubber surface is so advanced that on the scanning electron micrograph at magnification 100× cavities of approximately regular form are clearly visible (fig. 12). The inner surface of the cavities and also the remaining fragments of the surface, are corrugated as can be seen on scanning electron micrographs figs. 8 and 11, which are magnifications of fig. 12. Concluding the observations on the surface of specimens of unfilled rubber it was noted that the destructive activity of soil micro-organisms is time dependent. In parallel tests on control specimens of unfilled rubber exposed in sterile soil, these phenomena do not occur, and this difference may be assumed to be the effect of microbial degradation.
Separate tests on the penetration rate of soil microorganisms into the natural rubber vulcanizates are currently being performed. Specimens with carbon black filler loadings respectively 20 and 40 p.p.h.r., exposed in the sterile soil show no visible surface changes as may be seen on the scanning electron micrographs (figs. 13, 14, 15, 16 and 21, 22, 23, 24). The visible corrugations are technological residues.

The behaviour of filled natural rubber in the soil burial test is quite different due to the influence of soil microorganisms. In samples of filled rubber, containing 20 p.p.h.r. the surface exhibits no change after 30 days of exposure to soil microorganism (fig. 17). After 120 days of exposure there are only minor visible superficial changes (fig. 18), but the surface seems to be softened or scarified. Marked surface change of specimens occur after 180 and 360 days of exposure to soil micro-organisms (figs. 19, 20). Cavities of remarkable size may be observed in the sample A-20/360 about 150 μm long and 70 μm wide, and also a larger number of smaller pits may be seen. The scanning electron micrograph fig. 20 gives evidence of the deteriorated surface structure.

Only after 120 days of exposure in the soil burial test do the samples of filled rubber, containing 40 p.p.h.r. show certain visible changes in the surface structure, which can be described as a softening or scarifying of the surface (fig. 26). After 180 days of soil micro-organism activity (fig. 27) a few cracks and small pits have developed. After 360 days of the test samples of 40 p.p.h.r. carbon black filled natural rubber have an appearance typical of deteriorated rubber material, that is characteristic fissures and pits occur on the surface (fig. 28).

From observations of samples of natural rubber exposed to soil micro-organisms it may be concluded that a close relationship exists between the degree of microbial deterioration of the rubber surface and the exposure time. In a comparable time interval the higher the carbon black filler loading in the rubber, the less structural changes in the rubber surface are found. This effect may be most probably attributed to the increase of the total network density of the carbon black filled vulcanizate. It may be postulated that a high filler loading of natural rubber vulcanizates could effectively hinder penetration of microorganisms. Quantitative estimation of the degree of microbial deterioration of natural rubber vulcanizates would require further investigations into the changes of the network density of unfilled and carbon black filled vulcanizates.

References


Methods for Measurement of Biodegradability of Chemical Compounds

Drs. J. Blok

**METHODS FOR MEASUREMENT OF BIODEGRADABILITY OF CHEMICAL COMPOUNDS**

Summary. This survey aims at promoting the standardization of methods for determining biodegradability. The most important problems met in biodegradation research concern adaptation, toxicity, non-biotic eliminations and analytical restrictions. Consequent rules and recommendations can be divided into two groups, viz one group aiming at adaptation and another group at obtaining reliable measurements. From a classification of frequently used methods it appears that there are only two clearly different groups of methods which show a great many small differences. Adjustment of well known methods to the proposed rules and recommendations calls for an adaption procedure followed by a procedure for measurements.

Verfahren zur Messung der biologischen Ablaufbarkeit von chemischen Verbindungen. Dieser Überblick will zur Standardisierung von Verfahren zur Bestimmung der biologischen Ablaufbarkeit beitragen. Die wichtigsten Probleme, die bei der Erforschung der biologischen Ablaufbarkeit auftreten, sind Adaption, Toxizität, Ausschaltung nichtbiotischer Einflüsse und Beschränkungen im Hinblick auf die Analytik. Daraus sich ergebende Regeln und Empfehlungen können in zwei Gruppen eingeteilt werden, d.h. diejenigen, die sich mit der Anpassung befassen und diejenigen, die sich mit der Erreichung verlässlicher Messungen beschäftigen. Bei einer Klassifizierung der häufig verwendeten Methoßen stellt man fest, daß es nur zwei deutlich verschiedene Gruppen gibt, die sich durch viele kleine Unterschiede auszeichnen. Eine Angleichung der bekannten Verfahren an die vorgeschlagenen Regeln und Empfehlungen erfordert ein Anpassungsverfahren, aus dem dann ein Meßverfahren entwickelt wird.

Méthodes de mesure de la biodégradabilité des composés chimiques. Le but de cette étude est de promouvoir la standardisation des méthodes de détermination de la biodégradabilité. Les plus grands problèmes que rencontre la recherche en biodegradation se rapportent à l'adaptation, la toxicité, les éliminations non biotiques et les restrictions analytiques. On peut diviser ceux deux groupes les règles et recommandations en résultat, à savoir un groupe visant à l'adaptation et un autre à obtenir des mesures exactes. Suivant une classification des méthodes les plus souvent utilisées on ne trouve que deux groupes de méthodes clairement distincts présentant un grand nombre de petites différences. L'ajustement de méthodes bien connues aux règles et recommandations proposées demande un procédé d'adaptation suivi d'un procédé de mesures.

Introduction

Increasing interest in the environmental consequences of modern industrial life has led to the question of biodegradability of chemical products becoming a point of major importance.

In studying biodegradation it appears that it involves many problems, both as far as methods and interpretation of test results for conditions in practice are concerned. A rather large number of biodegradation test methods exist that are all different at one or more points. The results obtained with these methods often vary rather considerably. This may largely be attributed to the fact that the requirements for obtaining biodegradation often conflict with the requirements for obtaining a reliable measurement.

Several attempts have already been made to standardise test methods and for anionic detergents some standardisation has been achieved. These methods, however, are not generally applicable.

The problems which require consideration are only those presented by substances that are more or less poorly degradable. A great many chemicals have such a high degradability that with all tests methods practically the same positive results are obtained. In this paper first of all the fundamental aspects of the most important problems are discussed. Subsequently, a few consequent rules and requirements for a method of solving these specific problems are considered. Next is examined how far these requirements are satisfied by the methods mentioned in the literature. Finally, a standardisation procedure is proposed.

Any test method must have regard to the purpose for which biodegradation is required. It may be intended primarily to protect the aquatic environment or to protect sewage treatment works, or both, against damage to their biological fauna and flora. If the fauna or flora of a sewage treatment plant are damaged there will inevitably be pollution of the aquatic environment. Also, as is explained elsewhere in this paper, the hazard will differ depending on whether the discharge is more or less continuous (as, for example, detergents in domestic effluent) or occasional, as will occur in the event of accident. The test method must take account of these different circumstances.

Definitions

The following is a generally accepted classification of various forms of degradation:

Primary biodegradation (Swisher)

In this case degradation is detected by an analysis
specific for the original molecule. Hence, upon primary degradation of the molecule, degradation is in most cases not yet complete. Detergents are primarily degraded, for example, when their foaming activity has stopped, which stage may be reached as soon as the hydrophobic and the hydrophilic parts are separated.

Environmentally acceptable biodegradation (W.P.C.F. 1967)
This is also an incomplete degradation, i.e. to such a degree that the degradation products cause no further damage or inconvenience to the environment. The criterion for these last aspects, of course, does not necessarily apply in general. So this definition must be considered to be of relative merit only.

Ultimate biodegradation (Swisher) or mineralization
This is the complete conversion of the molecule to CO₂, H₂O and inorganic salts.

There also exists a more quantitative method of differentiating biodegradation in which the degradation is related to that of domestic sewage with consequently a "biodegradability index" ranging from one to zero.

The biodegradability of domestic sewage is not nearly the best there is, hence even indexes above unity may occur. The index, of course, varies with the method. The greater the "biodegradation potential" of the method, the higher will be the index of the substance. Reference to both a satisfactorily and a badly degradable substance might reduce the influence of the method on the results.

Relevance of tests
In most cases a biodegradation test is done for practical purposes and the method should therefore be so designed that natural or practical conditions are imitated as well as possible. If not, interpretation of laboratory test results will be very difficult. Environments in which biodegradation occurs can roughly be divided into four main groups:

1. Water: mostly aerobic; temperature 10°-20°C; pH 7 to 7.5; mainly saprophytic bacteria responsible for degradation.

2. Soil: varying between aerobic and anaerobic; pH varying with humic acid and minerals. Apart from bacteria, insects and other organisms play an important role here.

3. Anaerobic Environments: A specialised environment in which the degradation of organic materials follows a totally different biochemical pathway and is much slower than in an aerobic environment. Anaerobic conditions occur not only in the deeper parts of soil and at the bottom of lakes and ditches but also in sludge digesting tanks where the greater part of the digestion of solids takes place in many sewage purification plants. Irrespective of whether the chemical products under test are themselves biodegraded in anaerobic conditions it is important that they shall not damage the anaerobic flora of sludge digesting tanks. This requirement should be borne in mind when the results of tests of biodegradability are interpreted.

4. Plants-sunlight: The combined influence of light and plant growth, especially algae and lichens, is sometimes very great. As a result of the production of acids etc. plants often create micro-environments with an extremely high biodegradation potential. Saprophytic bacteria complete the degradation.

Test methods have been developed with conditions that resemble those of the natural environment. Most research has been done with water as the test medium for water-soluble substances. Deterioration of materials has mainly been studied in soil.

Test methods are not often found to use the other two types of environments. However, very frequent use is made of models of biological sewage purification plants and of river-water.

Only occasionally a study is made of degradation in seawater or sludge from the bottom of the sea, although it is supposed to represent a rather aggressive and general environment. This is probably to be attributed to the interest in biodegradation being limited to direct consequences for human beings. The most urgent reason for studying biodegradation has always been the purification of ground water and surface water to make it suitable for drinking.

Problems
In view of the extensive literature on the subject the problems are here dealt with only in a general review and a short discussion. The problems have been divided into four different groups, vis.:

1. Adaptation
2. Toxicity
3. Non-biotic elimination
4. Analytical limitations

1. Adaptation
There are two important questions related to adaptation.

1. When is the chance of adaptation greatest?
2. What does the presence or absence of adaptation in a laboratory test mean in practice?

These two questions may be combined because the natural environment gives the best opportunities for
adaptation and is at the same time the environment of interest to us. So the laboratory test should be designed in such a way that its resemblance to the natural environment is as great as possible. On the other hand it is conceivable that after a long selection procedure the degradation power of microorganisms cultured in the laboratory is better than is to be expected in river or in a purification plant. In most cases, however, it is not important that any degradation can take place in a laboratory, but that the receiving environment will develop the rate of degradation necessary to keep the concentration of the chemical compound to a harmless level. Therefore, the theoretical possibility that degradation can occur as a result of adaption and selection is not necessarily relevant to the real situation.

There may be circumstances that make it very difficult to interpret laboratory tests. When, for example, the spill of a substance in a purification plant is not continuous but only occasional, the possibility of biodegradation only after a period of adaptation is of little importance. Also in the case of a slow growth rate for a special species the chance of this species maintaining itself and not being diluted because of the fast growth of other species will very much depend on the type of purification plant. When in the laboratory test the substance to be tested is the only source of carbon, such effects do not occur, which makes the results of the test less directly valuable.

There is also confusion about the word adaptation. Every organism that is transferred to a new substrate needs a period of "acclimation" during this period passive organisms are activated and new enzyme systems induced. This period is normally short, viz; a few hours or a day. However, it may be very much lengthened by the presence of certain other organic substances. It is especially well known of sugars that they can inhibit the induction of new enzyme systems.

This kind of adaptation is very much dependent on the trophic level of the environment. A rather oligotrophic (i.e. food deficient) environment with an enormous diversity of substrates and very low degree of dynamics may offer an ecological niche for many species. Such an environment is especially suitable for obtaining adaptation. Activated sludge from oxidation ditches, sludge from the lower part of a trickling filter, river sludges, old compost or garden soil and sea bottom sludge are examples of such biologically rich environments. The addition of organic or inorganic compounds to such an environment immediately disturbs the sublecosystem to the benefit of only a few species. Such disturbance should be restricted to a minimum, because one cannot say that a special species will always multiply, when the specific substance to be degraded is present and the inoculum is sufficiently rich; there are too many other unknown factors that determine the growth of a microorganism. Also the disturbance of the original ecosystem generally leads to the death of the specialists and the multiplication of the generalists so that the chances of adaptation become smaller.

High-loaded purification plants, high-rate trickling filters, or industrial purification plants, contain a very limited spectrum of species because of the high food concentration and high degree of dynamics.

The same can be said for intensively fertilized soil, fresh manure, acid vegetable mould, alternately salt and fresh water or alternately wet and dry soil.

Generally, the water or soil in a laboratory test for degradation can be regarded as a dynamic and strongly disturbed environment.

In spite of the addition of all kinds of salts, trace elements, vitamins etc. it is almost impossible to create the number of ecological niches present in the oligotrophic natural environment.

As far as possible, the following conditions should be adhered to:

1. A very low food/biomass ratio e.g. 0.05g organic matter per g biomass per day.
2. A large amount of biomass e.g. $10^4$ to $10^6$ organisms/ml or 0.5 to 5 g dry matter/l.
3. A great diversity of species that each grow at a slow rate.
4. Presence of many different organic substrates in very low concentration.
5. Presence of many growth factors and all the trace elements.
6. A very balanced situation without discontinuities or other dynamics.
8. Concentration of the test substance 1-10 ppm but in any case at a subtoxic level.
9. Continuous contact with other environments to promote immigration of new species.
10. Consequences of the above-mentioned conditions: No dilution of the biomass, that can only select the fast growers, and provision of a very large adsorptive contact surface.
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2. Toxicity

The general term toxicity is difficult to handle, because in most cases it implies a very specific interaction between a chemical and a specific living organism. Both the way a substance enters an organism and its subsequent effect vary so widely from organism to organism that the general term toxicity can hardly be considered suitable. Therefore it cannot be expressed in a simple figure, as is possible with a boiling or melting point. A toxicity figure is always related to a special test with special organisms.

Toxicity can be the cause of many troubles in biodegradation research. One of the most important rules therefore is to work at subtoxic concentrations. However, sub-toxicity is difficult to determine beforehand because one does not know in advance what organism will play a role in a mixed system after selection and adaptation. The simplest procedure is to measure the degradation of a control substance in the presence of the test compound. When, under the same conditions, the degradation of the control substance is not inhibited one might conclude that there is no toxicity. Yet, this conclusion is not fully justified because the test has not been done on the organism that was to degrade the test substance but on the organism that degraded the control substance. In fact one is obliged to presume that these various species have the same sensitivity although it is generally well known that this is not true.

Far less valid is a control experiment under totally different conditions.

Minimum inhibitive concentrations determined with pure cultures and respiration inhibition measurements are not fully comparable with inhibitive effects in a degradation test. In spite of these problems, a toxicity control experiment is always necessary and it is advisable to adhere to concentrations that are well below the critical level. In fact a control experiment with lower concentrations of the test substance will offer the best guide, although it is sometimes difficult to carry out because of the analytical limitations. Because of the enormous adsorptive surface area in natural environments such as river sludge and soil these display a great detoxifying activity. Around the adsorbing particle arises a concentration gradient. This makes it possible for adaptation to occur at a low concentration followed by a desorption process, in that in the end the substance is totally degraded. This is an important reason why tests with only a small amount of biomass and no adsorbing surfaces often give a more negative result than other tests, although the concentration of the substance per unit volume is the same.

3. Non-biotic elimination

Generally, non-biotic degradation processes are of less importance. Yet there are several substances that disintegrate under the influence of light or hydrolysis in water. In other processes such as evaporation, precipitation and adsorption, the material is, in principal, not degraded but only replaced. Although of a very important nature, these processes may make it very difficult for the biodegradation to be measured separately. Therefore it would be of advantage to have a test method in which these non-biotic factors are excluded.

The conditions, however, for such a test do not always correlate with the optimum conditions for adaptation. In each case the incubations should take place in the dark, use should be made of sterile controls, the medium must be homogenized before the analysis, and aeration should be done in closed systems. These conditions are not difficult to achieve, but more difficulties arise with the exclusion of adsorption and precipitation, especially in the case of poorly water-soluble compounds.

The addition of organic solvents will greatly disturb the biosystem. Alcohol, acetone, etc. are toxic at high concentrations but have a high degradability at low concentrations, resulting in a rapid growth of a few species on these compounds. After this the apolar test compound will disappear from the solution in some way which in most cases means that it will escape from the analysis. Therefore only non-toxic and fully non-degradable emulgators are helpful.

4. Analytical Limitations

If there is need for a general test for biodegradation suitable for all kinds of substances and also for waste water samples with an unknown chemical composition or for products with unknown chemical structure, then a specific analytical method is of no use. As a rule, a specific analysis can only serve to measure the primary degradation. In several countries the disposal of organics is taxed according to the amount of oxygen demand needed for ultimate biodegradation or environmentally acceptable degradation. So the only advantage of specific analysis is its greater sensitivity, which makes measurements at very low concentrations possible.

Aspecific analytical methods such as COD (chemical oxygen demand) TOD (total oxygen demand) TOC (total organic carbon) etc. have a clear lower limit of concentration in their range of application, viz. 10 to 50 mg/l. These methods are limited also because of the constant presence of a basic concentration due to small amounts of other organics. As was explained under adaptation and toxicity, one should preferably test the degradation, and in any case during the adaptation, at concentrations below 10 mg/l.

Apart from the direct chemical analysis of the substance one could measure some indirect feature
typical of biological activity, such as CO₂ production, O₂ consumption and increase of biomass.

By far the most sensitive method is the oxygen demand test. The lower limit is at about 5 mg/l oxidable matter, because in that case a certain basic oxygen demand in a blank experiment is measured and because always only part of the lost organic substance is oxidized. It is rather risky only to use indirect measurements. A small stimulation of the basic oxygen demand by a substance that is not immediately decomposed may interfere with the results. Also other oxidative reactions such as those of the chemo-autotrophic bacteria (S~ → SO₄; S~ → S; Fe → Fe³⁺; NH₄ → NO₃) may interfere. Another fundamental problem is the question of the subtraction of a blank value. In some cases this has been shown to cause large errors, because a biosystem with food is basically different from one without food.

The above-mentioned analytical limitations, and problems with non-biotic processes, have their implications for a test method, which has resulted in the following recommendations.

1. The test substance should be the only source of carbon.
2. The concentration of the test substance should not be lower than 10-50 mg/l.
3. The test substance should be soluble in water or it must be emulsified with a non-toxic, non-degradable emulgator.
4. The presence of large surfaces with adsorptive properties should be avoided.
5. Before the analysis the inoculated medium should be entirely homogenized.
6. Aeration should be done with a limited amount of air, preferably in closed systems.
7. The test should be carried out in the dark.
8. A toxicity blank is required.
9. A toxicity control is necessary.
10. The degradation should be related to degradation of a control substance.
11. A blank experiment is necessary.
12. Activity of autotrophic bacteria should be avoided.
13. The amount of biomass used as the inoculum should be as small as possible.
14. It is preferred not only to do indirect measurements but also a direct chemical analysis and a measurement of the oxygen demand.

Methods

Considering that excellent surveys of biodegradation measurement methods are available, we will not try to improve on them. With the aid of a survey given by Swisher an effort has been made to re-arrange the methods in accordance with the above recommendations.

There are many varying methods, which differ in respect of volume, manner of aeration, addition of salts, presence or absence of control experiments and the kind of analysis. For example, there are a few dozen different respirometric methods. It will be clear that these differences are not of importance for the microorganisms. Therefore the methods can be classified only on the basis of microbiological criteria, the most important of which are listed below.

1. Concentration of the test substance below or above 10 ppm.
2. Test substance as the only source of carbon or not.
3. Biomass in high concentration (0.5 to 5 gr/l) or not.
4. A long adaptation period or not.

The most important point for a test method is that it should be carried out in an environment that offers enough scope for microbial adaptation, so that the degradation potential will be similar to that in purification plants, fresh surface water and soil. Accordingly, the four above-mentioned alternative conditions are relatively unimportant and may be neglected. Yet, almost every method calls for one or more concessions merely for analytical or technical reasons. Therefore, apart from the difference between biodegradation potentials, there is no sharp separation between primary and ultimate degradation. Roughly, however, the tests can be divided into two major groups, namely 1. inoculated die-away tests and 2. activated sludge test.

Group 1 The inoculated die-away tests

In this case a water, naturally or artificially enriched with salts, is inoculated with a small amount of biomass (5 to 50 mg/l). The test substance is added in combination, if desired, with another substrate, in a rather low concentration (≤10 ppm). The time of incubation varies from 5 to 40 days. Adaptation may be promoted in subsequent runs. Sometimes the inoculum is obtained from soil or sewage sludge, but it is also possible to use naturally infected waters without further addition of biomass.

This group comprises several more or less standardized methods, such as the provisional OECD open flask test (OECD 1968). The closed bottle test of
Method for Measurement of Biodegradability of Chemical Compounds
J. Blok.

Fischer (1963) the French IRChA (Institut de Recherches Chimiques Appliquées) test (Brebion 1966), The procedure adopted by the American SDA (Soap & Detergent Association) (1965) and by the Japanese (Japan Industrial Standard JIS K 3363, Toriyama 1969), The British test (Standard Technical Committee on Synthetic Detergents 1966) and by the Swiss (Eidgenössische Anstalt für Wasser und Abwasserforschung and Gewässerschutz).

Most methods are to be used for studying the biodegradation of detergents, the primary degradation being followed with specific analysis. The main disadvantage is that it is impossible for the ultimate biodegradation to be followed with a specific chemical analysis. To remove this drawback Fischer et al (1974) proposed that the concentration of the test substance be increased to up to 20 ppm.

Group 2 Test methods with activated sludge

The most important feature in these methods is the large amount of biomass, mostly activated sludge. Generally, this calls for the use of additional organic substrates and unless the concentration can be above 100 ppm a specific analysis is the only way of following the primary degradation. The systems are open and they are fed continuously, semicontinuously or discontinuously. Adaptation periods are generally kept short, i.e. up to 3 weeks. These systems are commonly believed to be easier to interpret and to be more relevant to practical circumstances, but this is to be doubted. Also this procedure is standardized. Several modifications have been introduced by: the Water Pollution Research Laboratory in Great Britain (Truesdale 1959), the South African National Institute for Water Research (Urban 1965), the German Emschergenossenschaft (Hussmann 1963). The latter developed a method modified, and later accepted, as the official test method of the German Government (1962) (Fischer 1965).

The Rumanian modification is restricted to three days’ adaptation (Vaicum 1967). A semicontinuous form was accepted by the Soap and Detergent association in 1965 (SDA 1965). A fundamentally identical, continuous method for small-scale use has been described by Davis (1962) and extensively used by Swisher (1964-1967).

Recommendations

Re-arrangement of the most frequently used methods has resulted in only two clearly different major groups. But quite a few fundamental and practical modifications have been introduced. When checked with the directives mentioned for obtaining adaptation, the procedures summarised in group 2 appear to come nearest to the ideal situation. Yet, some caution is required. The circumstances in the official German test, for example, are rather different from those prevailing in a natural environment. This because of the high sludge load (1.0 to 0.6 gr. substrate/ gr. biomass/day) and the simple composition of the substrate.

Consequently the number of bacterial species in the sludge will be restricted. On the other hand, the test conditions seem clearly to correspond to the conditions in some purification plants.

All the methods have the disadvantage that they are based on the primary degradation measurement of anionic detergents. But apart from that it seems that the activated sludge procedure, just like lysimeters (soil percolators) and trickling filters, offers a good possibility for adaptation.

The procedure followed in the methods of group 1 seems to be quite suitable for doing correct measurements both for ultimate biodegradation and for a specific analysis. For the purpose of adaptation, however, even the procedure with subsequent runs is not very attractive. For these reasons there is much to be said for dividing the methods into two parts:

An adaptation procedure, followed by a measuring procedure.

In the adaptation procedure the afore-mentioned directives may be adhered to, without there being any need for a measurement. With this cultured, adapted, biomass a die-away test medium is inoculated. For this second method the directives for correct measurement should be observed. It is, of course, of great importance that in the transition from the one procedure to the other the carefully adapted microorganisms should not be inhibited or killed. Therefore the compositions of the media should not differ too much.

In principle, the adaptation procedure could be made general and be internationally standardized. The measuring procedure, however, will depend on the solubility and the toxicity of the compound. In several cases a specific sensitive analysis will be needed to measure the compound at low concentrations. In the case of concentrations above 20-50 ppm, however, it would be advisable to use a universal measuring method.

An important secondary argument in favour of a universal biodegradation test is its simplicity and ease with which it may be described and followed. A rather cumbersome method which is labour intensive and calls for a great deal of experience is, for example, the official German test with activated sludge.

Its biggest problem is the large amounts of water to be treated. An attractive solution to this problem seems to be the use of a micromethod to be carried out with the David equipment (1962). Moreover, by increasing the mean retention time from 4 to 24 hours

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the amount of water is decreased by a factor of 60. A decrease of the sludge load and the introduction of a more complex substrate would contribute to the value of the method as adaptation procedure and would facilitate the treatment of the sludge.

After two or three weeks the adapted sludge can serve as the inoculum for a die-away test. The medium of this "die-away" test must have the same composition as in the adaptation procedure, except that the test compound is the only source of carbon.

A rather good starting point is formed by the methods described by Fischer (1963 and 1974). The concentration can be increased in two ways, viz. by raising the oxygen content of the system (pure oxygen or an air bubble) or by subsequent runs. An aspecific chemical analysis such as COD or TOC together with the measurement of the oxygen consumption by the bacteria will provide sufficient information to study the ultimate biodegradation.

These procedures can be worked out in details and standardized without much difficulty. The advantage is that they differ only little from other frequently used procedures.

References


Organisation for Economic Co-operation and Development Annex to letter W/DAS/SCI/ 68.971 appendix II.


Vaicum, L. and Iliescu, A. (1967). Biological degradation of the detergents and the methodology of their determination. Rev.Chim 18 (1); 6-12 (Rom.).


ON THE INTERACTION BETWEEN BACTERIA AND WOOD PRESERVATIVES

O. Schmidt, F. Wolf and W. Liese

Summary. Bactericidal concentrations of the wood preservative components boron, chromium, copper, fluoride and pentachlorophenol, as well as of a CF-salt (chromium-fluorine) were examined in water free from nutrients using Bacillus subtilis, Cellulomonas sp., Erwinia carotovora and Serratia marcescens. Boron and fluoride were less active, but chromium, copper, pentachlorophenol and the CF-salt showed medium to strong toxicity. The limiting concentrations were generally lower in water than those obtained earlier in nutrient liquid. Bacillus subtilis was the most resistant species. Erwinia carotovora was adapted to fluoride by successive subcultures. The composition of the culture medium influenced the NaF tolerance of Erwinia carotovora considerably.

Introduction

Bacteria are able to colonise wood and to degrade wood components (e.g. Seifert, 1967; Greaves and Foster, 1970; Karnop, 1972 a & b; Berndt and Liese, 1973; Rossell, Abbott and Levy, 1973; Schmidt and Dietrichs, 1975). The effects of preservatives against bacteria have been investigated (Greaves, 1973; Schmidt and Liese, 1974) as well as some reaction mechanisms of these microorganisms (Schmidt and Liese, 1975).

The following report deals with three topics: the viability of four bacterial species exposed to six wood preservatives dissolved in water without additional nutritive substances; the adaptation of Erwinia carotovora to fluoride; the influence of the carbon source on the toxicity of fluoride. This continues and expands earlier investigations on the effect of twelve wood preservatives against six bacteria in nutrient liquid (Schmidt and Liese, 1974), on the influence of the culture medium on the bacterial sensitivity, on the behaviour of mixed bacterial populations as well as on the germination of bacterial spores exposed to preservatives (Schmidt and Liese, 1975).

Materials and Methods

The investigations were done with Bacillus subtilis W23, Cellulomonas sp. DSM² 20108, Erwinia carotovora CCM¹ 1008 and Serratia marcescens DSM 47. As preservatives boron (as sodium borate, Na₂B₄O₇·10H₂O), chromium (as sodium dichromate, Na₂Cr₂O₇·2H₂O), copper (as copper sulphate, CuSO₄·5H₂O) fluoride (as sodium fluoride, NaF), pentachlorophenol (PCP-Na) and a commercial CF-salt (chromium-fluorine) were tested. The preservatives were sterilized by membrane filtration (0.2 μm) and added aseptically to distilled water in an exponential range of concentrations. Incubations were carried out with 50,000 (± 10%) cells per 5 ml suspensions in tubes with 30° inclination on a rotary shaker with 120 rpm and with constant temperature of 30.0°C. Culture volumes of 0.1 ml were transferred from the test tubes at different intervals of preservative influence to pour plates of peptone meat yeast extract agar, if necessary diluted with 0.86% NaCl yielding the viable cells. After seven days incubation colonies were counted and related to the time of exposure. A suspension of 6% NaCl served as control for the bactericidal effect of highly concentrated salt solutions.

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2Deutsche Sammlung von Mikroorganismen, Munchen,
3Czechoslovak Collection of Microorganisms, Brno.

For 'training' experiments Erwinia carotovora was cultivated in liquid inorganic medium with 0.5% galacturonic acid and increasing NaF concentrations. The growth was measured photometrically in the culture tubes at 578 nm wavelength (Schmidt and Liese, 1974). After growth had reached the stationary phase, successive subcultures were made by transferring 0.1 ml suspension to a new culture tube with NaF medium. The extent of any reversion behaviour was investigated after eightfold repeated subculture in a medium without preservative.

The mechanisms by which Erwinia carotovora becomes accustomed to NaF were analysed by the 'replica technique' of Lederberg and Lederberg (1952) and also by the method of 'diluted inocula' (Rippel-Baldes and Claus, 1955). In the latter method the rates of growth of successively diluted inocula in a normal medium are compared with the rates of growth from an undiluted inoculum on a medium containing increasing concentrations of the inhibitor being examined. The influence of the composition of the culture medium on the tolerance behaviour was tested with Erwinia carotovora and 0.05% NaF using the following media:

a. 0.5% NH₄Cl, 0.25% K₃PO₄, 0.1% NaCl, 0.001% CaCl₂ 2 H₂O, 0.001% MgSO₄ 7 H₂O, with 0.5% galacturonic acid;

b. salt solution as above with 0.5% glycerol;

c. complex nutrient broth of 0.5% peptone, 0.3% meat extract, 0.05% yeast extract, pH always 7.2.

TABLE 1. Bactericidal concentrations (per cent).

<table>
<thead>
<tr>
<th>Organism</th>
<th>Bacillus subtilis</th>
<th>Cellulomonas sp.</th>
<th>Erwinia carotovora</th>
<th>Serratia marcescens</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>LD 50</td>
<td>LD 100</td>
<td>LD 50</td>
<td>LD 100</td>
</tr>
<tr>
<td>Na₂B₂O₄· 10 H₂O</td>
<td>1</td>
<td>&gt; 5</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Na₂Cr₂O₇· 2 H₂O</td>
<td>0.001</td>
<td>&gt; 1</td>
<td>0.1</td>
<td>0.01</td>
</tr>
<tr>
<td>CuSO₄· 5 H₂O</td>
<td>0.0001</td>
<td>&gt; 0.1</td>
<td>0.0001</td>
<td>0.01</td>
</tr>
<tr>
<td>NaF</td>
<td>0.1</td>
<td>&gt; 4</td>
<td>1</td>
<td>0.1</td>
</tr>
<tr>
<td>PCP-Na</td>
<td>0.01</td>
<td>1</td>
<td>0.0001</td>
<td>0.01</td>
</tr>
<tr>
<td>CF-salt</td>
<td>0.01</td>
<td>&gt; 5</td>
<td>0.1</td>
<td>0.1</td>
</tr>
</tbody>
</table>

As a criterion for a bactericidal effect, the lethal dose 50 (LD 50) was used which is defined here as the decrease of living cells of ≥ 50% within 24 h incubation. Table 1 collates the results of the LD 50 together with the 100% bactericidal concentrations, i.e. LD 100.

Apparently the bacteria and also the tested inhibitors exhibit a varied reaction in their effect. For example the LD 100 of PCP-Na varies between Erwinia carotovora and Bacillus subtilis by the factor 1: 100,000. On the other hand, the efficacy of PCP and borax against Erwinia carotovora differs in the ratio 1: 500,000. Altogether boron and fluoride are less active against these bacteria, but chromium, copper, PCP and the CF-salt show medium to strong toxicity. Bacillus subtilis is the most resistant strain among the four bacteria; it tolerates sublethal inhibitor concentrations by sporulation.

Tests are essential for determining the growth inhibition of bacteria. In table 2 the bactericidal concentrations in inhibitor solutions without nutrients (Table 1) are compared with those obtained by Schmidt and Liese (1974) in nutrient liquids with these toxicants.

### TABLE 2. Bactericidal concentrations (LD 100) in water and synthetic nutrient liquid.

<table>
<thead>
<tr>
<th>Organism</th>
<th>Bacillus subtilis</th>
<th>Cellulomonas sp.</th>
<th>Erwinia carotovora</th>
<th>Serratia marcescens</th>
</tr>
</thead>
<tbody>
<tr>
<td>Salt (%)</td>
<td>water</td>
<td>nutrient liquid</td>
<td>water</td>
<td>nutrient liquid</td>
</tr>
<tr>
<td>Na₂B₄O₇ · 10 H₂O</td>
<td>&gt; 5</td>
<td>&gt; 50</td>
<td>1</td>
<td>&gt; 1</td>
</tr>
<tr>
<td>Na₂Cr₂O₇ · 2 H₂O</td>
<td>&gt; 1</td>
<td>0.1</td>
<td>0.01</td>
<td>0.05</td>
</tr>
<tr>
<td>CuSO₄ · 5 H₂O</td>
<td>&gt; 0.1</td>
<td>0.1</td>
<td>0.01</td>
<td>0.1</td>
</tr>
<tr>
<td>NaF</td>
<td>&gt; 4</td>
<td>&gt; 50</td>
<td>4</td>
<td>0.5</td>
</tr>
<tr>
<td>PCP - Na</td>
<td>1</td>
<td>1</td>
<td>0.001</td>
<td>0.01</td>
</tr>
<tr>
<td>CF-salt</td>
<td>&gt; 5</td>
<td>20</td>
<td>0.1</td>
<td>0.1</td>
</tr>
</tbody>
</table>

In general the bactericidal concentrations are higher in nutrient liquid than in water. *Erwinia carotovora* exhibits differences without and with nutritive substance up to 1:10,000. This might be explained by the ability of *Erwinia carotovora* to tolerate sublethal concentrations by 'training'.

### Adaptation to inhibitors

With increasing amounts of inhibitors in the culture medium, *Erwinia carotovora* extends its lag phase, although growth rate and maximum biomass eventually attain the same level as in the control (Schmidt and Liese, 1974; Liese and Schmidt, 1975).

Figure 2 shows the accustoming of *Erwinia carotovora* to 0.05% NaF, a normally sublethal concentration.

![Graph showing adaptation of *Erwinia carotovora* to 0.05% NaF](image)

**Figure 2.** Adaptation of *Erwinia carotovora* to 0.05% NaF by successive subcultures.  
- ○ — growth curve without NaF,  
- ■ — growth curve with 0.05% NaF,  
- □ — first subculture with NaF,  
- ■ — second subculture with NaF,  
- × — sixth subculture with NaF and growth after eight reversion tests.

With the sixth successive subculture *Erwinia* tolerates 0.05% NaF growing nearly without any elongated lag phase. By this method it was possible to increase the NaF tolerance up to 0.5%. In the first culture at this concentration the lag phase was 144 h, while after seven transfers it was reduced to 24 h; the wild strain of *Erwinia* however, did not show any growth within 20 days incubation with 0.5% NaF.

These adapted strains were subcultured for reversion tests in the same medium free from NaF eight times, and growth behaviour in the presence of NaF was tested again giving a result very close to growth after 6 sub-cultures in the presence of 0.05% NaF (Fig. 2). In this test its tolerance against NaF was maintained; however, it was lost after numerous subcultures in the complex nutrient broth.

In considering possible mechanisms of this tolerance, the 'replica technique' of Lederberg was applied, but did not yield any NaF tolerant mutant.

In figure 3 the results of the 'diluted inocula method' are shown.

![Graph showing calculation of NaF tolerance rate in *Erwinia carotovora*](image)

**Figure 3.** Calculation of NaF tolerance rate in *Erwinia carotovora* by 'diluted inocula'.  
- ■ — growth with 0.025% NaF,  
- ○ — growth with 0.05% NaF,  
- ■ — growth with 0.1% NaF.

Rates of development of NaF tolerance determined from these growth retardations are:

- 0.025% NaF = 4.8 x 10^{-2}
- 0.05% NaF = 8.0 x 10^{-4}
- 0.1% NaF = 3.2 x 10^{-5}

According to Schlegel (1974) spontaneous mutation rates for resistances are between 10^{-7} and 10^{-10}. Therefore an induction of one or more of the internal and external mechanisms of tolerance appears likely; Antonovics, Bradshaw and Turner (1971) describe 17 possible mechanisms.

**Influence of culture medium**

The influence of the culture medium on the tolerance is demonstrated in figure 4.

![Figure 4. Growth curves of *Erwinia carotovora* in different media with and without 0.025% NaF.](image)

Figure 4. Growth curves of *Erwinia carotovora* in different media with and without 0.025% NaF. Broken lines—growth curves without NaF, unbroken lines—growth curves with NaF. O — inorganic medium with glycerol, □ — medium as above with galacturonic acid, and • — complex nutrient broth.

The wild strain of *Erwinia carotovora* was incubated at identical conditions in two synthetic media with different carbon sources (galacturonic acid and glycerol) and a complex nutrient broth. Because *Erwinia carotovora* is a pectinolytic bacterium degrades pectin via galacturonic acid (reviewed by Berndt, 1972), the growth behaviour with that acid as sole carbon source was used as a standard of NaF efficacy.

Glycerol, an unusual carbon source for *Erwinia*, increased the toxicity of 0.025% NaF four times, in contrast the complex nutrient broth lowered it 12.5 times (fig. 4).

Investigations with *Erwinia carotovora* and a CCB (Copper/chromium/boron) wood preservative have revealed that by increasing concentrations of galacturonic acid from 0.1% to 0.5% the CCB tolerance was raised ten times (Schmidt and Liese, 1975). Therefore the inhibitor tolerance of microorganisms may depend on the energy metabolism in the cells as it seems to be similar with wood destroying fungi (Liese and Schmidt, 1976).

**References**


TOXICITY TESTS OF SOME CHEMICALS AGAINST CERTAIN WOOD-STAINING FUNGI

A. J. Cserjesi1 and J. W. Rolf1

Summary. Chemicals now in use to control pests were tested in the laboratory against moulds and sap stain fungi for their effectiveness to control the growth of these fungi on unseasoned lumber.

Tests de l' action toxique de produits chimiques sur certaines moisissures qui tachent le bois. On a testé en laboratoire l'action des produits chimiques utilisés a l'heure actuelle contre les organismes nuisibles sur des moisissures et champignons qui tachent le sève, en vue d'établir leur efficacité à contrôler la croissance de ces derniers dans du bois non conditionné.

Although wood is susceptible to fungal attack when the moisture content is more than 20%, large volumes of unseasoned lumber in the Pacific Northwest region of North America continue to be stored and shipped to overseas destinations in this condition for economic reasons and as a result of trade preference. To protect this material, particularly from sap-stain and mould fungi, chemical treatments are normally applied and have become an important step in lumber production (Roff et al., 1974). To minimize the cost and to avoid any fire hazard, sap-stain and mould preventive treatments in sawmills are carried out in water solutions. At present, the sodium salts of chlorinated phenols (sometimes with additives) are used exclusively for this purpose, as they are highly toxic to fungi and relatively resistant to leaching (Cserjesi and Roff, 1964). Chlorinated phenols have been used as fungicides and wood preservatives for nearly 50 years. While they are toxic, there are few records of actual damage to mammals, but they are particularly harmful to fish (LD50 = 0.2 mg/l) (Bevenue and Beckman, 1967). As many sawmills are located near fish-bearing waters, the use of chlorinated phenols for treating lumber requires constant care to prevent escape of the solutions into drainage systems.

Because of concern for the toxicity of chlorinated phenols, and because of the recent shortage of 1,2,3,4-tetrachloro-benzene in Canada, there is a constant search for other fungicides which would be readily available, equally (or more) effective against sap-stain fungi and moulds, but less hazardous in use (Butcher, 1973). This report summarizes results of tests of this nature carried out over two years.

1Department of the Environment, Canadian Forestry Service, Western Forest Products Laboratory, Vancouver, British Columbia V6T 1X2.

2These species have some slight resistance to attack by staining fungi, but experimental results were only accepted when untreated controls showed good growth.

3This publication is available free of charge from the authors.
TABLE 1. List of the Chemicals Tested (Test numbers refer to numbers in text under Test Chemicals and Results)

<table>
<thead>
<tr>
<th>Name of chemicals</th>
<th>Code (trade name)</th>
<th>Formula</th>
<th>Test No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>mono (methyl ethyl ketone) thio-carbonhydrazone</td>
<td>aMMEKT</td>
<td>$\text{H}_3\text{C} = \text{C}=\text{NNHC} \text{SNH} \text{NH}_2$</td>
<td>1</td>
</tr>
<tr>
<td>2,3-dihydro-5-carboxoanalido-6-methyl-1,4-oxathiin</td>
<td>bcarboxin (Vitavax)</td>
<td><img src="formula1.png" alt="Formula" /></td>
<td>2</td>
</tr>
<tr>
<td>2,3-dihydro-5-carboxoanalido-6-methyl-1,4-oxathiin-4,4-dioxide</td>
<td>boxycarboxin (Plantvax)</td>
<td><img src="formula2.png" alt="Formula" /></td>
<td>2</td>
</tr>
<tr>
<td>2,4-dimethyl-5-carboxoanalido thiazole</td>
<td>bG-696</td>
<td><img src="formula3.png" alt="Formula" /></td>
<td>2</td>
</tr>
<tr>
<td>2-(4-thiazoyl) benzimidazole</td>
<td>cThiabendazole</td>
<td><img src="formula4.png" alt="Formula" /></td>
<td>2</td>
</tr>
</tbody>
</table>
TABLE 1. (continued)

<table>
<thead>
<tr>
<th>Name of chemicals</th>
<th>Code (trade name)</th>
<th>Formula</th>
<th>Test No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>methyl-1-(butyl carbomoyl)-2-benzimidazole carbamate</td>
<td>Benomyl</td>
<td><img src="image" alt="Formula" /></td>
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</tr>
<tr>
<td>sodium dimethydithiocarbamate</td>
<td>Thiostop-N</td>
<td>Na-SCSN(CH₃)₂</td>
<td>2</td>
</tr>
<tr>
<td>bis (tri-n-butyltin) oxide + Hyamine 1622</td>
<td>TBTO</td>
<td>(C₄H₉)₃SnOSn(C₄H₉)₃</td>
<td>3</td>
</tr>
<tr>
<td>bis(tri-n-propyltin) oxide</td>
<td>TPLO</td>
<td>(C₃H₇)₃SnOSn(C₃H₇)₃</td>
<td>3</td>
</tr>
<tr>
<td>4-(2-nitrobutyl) morpholine(I)</td>
<td>Bioban P-1487</td>
<td>C₂H₅CHNO₂CH₂-N</td>
<td>4</td>
</tr>
<tr>
<td>4,4′-(2-ethyl-2-nitro-propylene dimorpholine (II) (prepared by dissolving II in I so that the mixture remains liquid at 0°C.)</td>
<td></td>
<td><img src="image" alt="Formula" /></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Name of chemicals</th>
<th>Code (trade name)</th>
<th>Formula</th>
<th>Test No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>1,1'-ethylene-2,2'-dipyridinium dibromide</td>
<td>aDiquat (Reglone)</td>
<td><img src="image" alt="Chemical Structure" /></td>
<td>5</td>
</tr>
<tr>
<td>1,1'-dimethyl-4,4'-di pyridinium di (methyl sulphate)</td>
<td>Paraquat (Gramoxone)</td>
<td><img src="image" alt="Chemical Structure" /></td>
<td>5</td>
</tr>
<tr>
<td>Zinc oxide-ammonium carbonate (5:6) in ammonium hydroxide solution</td>
<td>bZnO-CO³</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Zinc oxide-ammonium thiocyanate (approx. 1:2) in ammonium hydroxide solution</td>
<td>bZnO-SCN</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

bSupplied by Uniroyal Chemical, Division of Uniroyal Ltd., Elmira, Ontario.
cSupplied by Merck & Co. Inc., Rahway, N.J., U.S.A.
dCommercial sample of Benlate (wettable powder containing 50% benomyl).
eSupplied by M. & T. Products of Canada Ltd., Hamilton, Ontario, as an emulsion, of approximately 5% concentration.
fMarketed by Commercial Solvents Corp., New York. Obtained from Van Waters & Roberts Ltd., Vancouver, B.C.
gSupplied by Chipman Chemical Ltd., Hamilton, Ontario.
hSupplied by Eastern Forest Products Laboratory, Can. For. Serv., Ottawa, Ontario.
Toxicity tests of some chemicals against certain wood-staining fungi. A. J. Cserjesi and J. W. Roff.

TABLE 2. Visual growth of fungi on wood specimens after two weeks of incubation at 22°C

<table>
<thead>
<tr>
<th>Fungicide</th>
<th>Concentration of active ingredient (% w/w)</th>
<th>Cylindrocarpus fragrans</th>
<th>Trichoderma hamatum</th>
<th>Trichoderma virgatum</th>
<th>Penicillium sp.</th>
<th>Phialophora sp.</th>
<th>Aureobasidium pullulans</th>
<th>Cryptococcus sp.</th>
</tr>
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<tr>
<td>MMEKT</td>
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<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.7</td>
<td>4</td>
<td>3</td>
<td>4</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>1.4</td>
<td>4</td>
<td>2</td>
<td>4</td>
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<td>3</td>
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<td></td>
<td></td>
</tr>
<tr>
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<td>4</td>
<td>4</td>
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<td></td>
</tr>
<tr>
<td>Carboxin</td>
<td>0.017</td>
<td>2</td>
<td>4</td>
<td>4</td>
<td></td>
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1 Specimens inoculated with mixed spore suspension of T. virgatum, Penicillium sp. and Phialophora sp. and the ratings refer to the growth of all of these fungi.
2 Specimens inoculated with mixed spore suspension of all the test fungi and the ratings refer to the growth of all of these fungi.
3 Only the growth of C. fragrans (tolerant to chlorinated phenols) was observed on the test material.
Toxicity tests of some chemicals against certain wood-staining fungi. A. J. Cserjesi and J. W. Roff.

Test Chemicals and Results (Table 1 and 2)

1. MMEKT

This compound has been suggested for use on textiles against cellulose-destroying fungi (Anon, 1970: Wiles and Suprunchuk, 1970). Shields and Shih (Canada Eastern Forest Products Laboratory, private communication) found it to be somewhat inhibitory to moulds and decay fungi when tested on malt agar.

At concentrations up to 0.24%, its maximum solubility in water, this chemical was completely ineffective. When dissolved in ethanol in concentrations up to 2.8%, MMEKT did not prevent growth of any mould except *Philalophora* sp.

2. Chemicals of carboxoanllido and benzimidazole derivatives

These chemicals, belonging to the group called "systemic fungicides", are reported together because of their structural relationships. The toxic action of oxathiin and thiazole compounds (Mathre, 1971) and of thiabendazole and benomyl (Maxwell and Brody, 1971) was found to be similar. Edgington et al. (1971) suggested that the toxicity of thiabendazole was due to the benzimidazole portion of the molecules. The mammalian toxicity of this group of fungicides is low, LD₅₀ values varying from 1 g/kg to 15 g/kg.

Water solutions (up to maximum solubility) of carboxin, oxy-carboxin, G-696 and combinations of these chemicals showed little or no effectiveness against our test fungi. Thiabendazole inhibited all fungi except brown mould (*Cephaloascus fragrans*) when applied at a concentration of 0.1%. Benomyl was the most promising fungicide in this group, but it has the disadvantage of being a wettable powder and the solution therefore requires constant agitation during treatment. As a suspension at 0.6% in water, it inhibited practically all fungal growth. Somewhat improved toxicity to fungi was obtained when it was dissolved in DMSO (dimethylsulfoxide).

3. Organo-tin compounds

DaCosta and Osborne (1972) found that among the organo-tin compounds, the tri-butyl and tri-propyl derivatives have the highest toxicity to fungi, comparing favorably to the toxicity of pentachlorophenol. The organo-tin compounds react with wood components (Heertjes and de Jong, 1971), therefore their penetration into wood is negligible, which can be an advantage in treating lumber with a sap-stain and mould preventive. These compounds break down readily in soil and sunlight in the presence of oxygen.

These compounds are insoluble in water and were supplied as stable emulsions. In our test, TBTO preparations performed better than TPTO. However, the TBTO preparation contained an additional quaternary ammonium compound (Hamine 1622) with an unknown effect. Hamine alone was found in a later experiment to be ineffective at 2% concentration, but synergistic effect is still possible.

4. Bioban P-1487

This formulation is used to prevent bacterial growth in cutting fluids. Its LD₅₀ is in the neighbourhood of 0.4 g/kg.

Bioban was effective against the experimental fungi at concentrations above 1% in the treating solution.

5. Diquat-parquat

These compounds are used as herbicides where they are reported to break down rapidly in plants and in soil. They are highly toxic to mammals (LD₅₀ = 30 to 70 mg/kg), but information as to safe handling, volatility, and absorption by skin is unreliable, varying from safe (Melnikov, 1971) to unsafe (Condens. Chem. Dictionary, 1971).

Although they were ineffective in preventing the growth of other moulds in our test, they were very toxic to *C. fragrans*, the fungus which is most tolerant to chlorinated phenols.

6. Ammoniacal zinc oxides

Two compounds of zinc oxide, one with ammonium carbonate and one with ammonium thiocyanate, were supplied for test. These were reported by Shields et al. (1974) to control chemical brown stain in pine and had inhibited sap stain and mould on eastern white pine (*Pinus strobus* L.). In our tests, fumes from the ammonium hydroxide used to solubilize the zinc salts continued to escape from the wood after treating, so that the standard petri-plate method could not be used.

The zinc thiocyanate formulation appeared more effective than zinc carbonate, but both of these ammoniacal treatments produced an olive-green coloration which developed immediately upon lumber dipping and became olive-drab during storage. In addition, the chemical was deposited in grey-white spots upon more than half the lumber pieces. These colors, combined with frequent failure of the treatments to prevent fungal discolorations, gave the lumber a most unattractive appearance.

Odors of ammonia, emanating from lumber during dip treating, were monitored using a Drager detector (approved for use by British Columbia Workman's Compensation Board). Levels of 40 ppm at one foot above the dip tank, reducing to 5 ppm at a distance of 6 feet from the tank, were detected in still air during humid weather (65-70°F). This is below the danger level for safety, although the odor was penetrating and still persisted in stacked lumber 24 hours after piling.
Toxicity tests of some chemicals against certain wood-staining fungi. A. J. Cserjesi and J. W. Rolf.

Sodium tetrachlorophenate, which was used as a control treatment in this experiment, was only partially effective because of the development of C. fragrans, a fungus which is known to be tolerant of chlorinated phenols. Some degree of control of brown mould was obtained in the zinc carbonate-ammonium treatment, although this was difficult to assess precisely because of the occurrence of other fungi.

Conclusions

The chemicals tested tended to be selective in toxicity to different sap stain and mould fungi on wood and none exhibited the overall effectiveness of chlorinated phenols. In the Pacific Northwest, fungal genera such as Ceratocystis, Trichoderma, Penicillium and Cephaloascus occur widely on green lumber and treatments which cannot control these fungi would not be acceptable for use by the lumber industry. By combining some of the more promising new fungicides, it may be possible to obtain protection against a wide spectrum of wood-inhabiting fungi.

Chlorinated phenols, when used at commercial concentrations, are least effective against C. fragrans (brown mould) which is thus a potential problem for the lumber industry. Hitherto the fungus has been controlled only through addition of mercurials. Of the chemicals tested, only Paraquat appeared to inhibit brown mould at low concentrations. The substitution of Paraquat for the more toxic mercurials, in anti-stain treatments for lumber, would be more environmentally acceptable as the former is degraded rapidly in contact with the soil.

References


Factors influencing the growth of fungi in high-moisture corn treated with propionic acid.

FACTORS INFLUENCING THE GROWTH OF FUNGI IN HIGH-MOISTURE CORN TREATED WITH PROPIONIC ACID

R. E. Smith1 and K. R. Stevenson2

Summary. When corn (maize) treated with propionic acid (PA) was stored in unsealed laboratory-scale silos together with untreated corn, and exposed to wide daily fluctuations in ambient temperature, fungal growth which developed in the untreated corn spread to lower layers of treated grain. Analyses indicated that condensed water movement had lowered PA levels to a concentration which would not inhibit fungal growth. Results with sealed silos indicated that lack of air prevented the spread of moulds in PA-treated corn.

Introduction

Many reports have appeared which relate high moisture levels of stored grain to spoilage by fungi. For example, Kochler (1938), and Christensen and Gordon (1948), showed that corn (maize) with a water content of 10% or less was highly resistant to fungal growth, but at levels of about 18% or greater fungi grew profusely at room temperature. Machacek et al. (1961) reported similar results for stored wheat, oats and barley seed, and showed that high moisture levels in seed grain reduced or counteracted the effect of pre-storage treatment with chemical fungicides. However, high-moisture corn, ensiled in sealed stores to prevent fungal growth, is nutritionally superior for cattle to artificially-dried corn (McCaffree and Merrill 1968).

Certain volatile acids can be used to preserve corn with a high moisture content without reducing its value as an animal feed (Jones et al. 1970). Propionic acid (PA) at a concentration of 1.0% by weight will protect corn with 72% dry matter according to Jones (1970). Although this method of preservation is now widely used, pockets of mould occasionally develop in treated corn. These are thought to result from improper use of the PA applicator and uneven distribution of the acid (Stevenson, 1972). Burrell et al. (1973) believe that moisture condensation in wet maize stimulates the development of fungi, and that PA levels may decrease as a result of microbial activity. Since some uncertainty seems to exist, the study reported here was undertaken to obtain more information concerning the causes of fungal growth in PA-treated high moisture corn.

Materials and Methods

Ten pound representative samples of corn, treated by the addition of PA at the rate of 2 gal/ton, were obtained from the Crop Science Department, University of Guelph. These, and samples of untreated corn, were stored in sealed polythene bags at 5°C until required. Aliquots of the corn samples were packed into two 3.75" X 18" perspex columns by placing a 6" layer of untreated corn (Layer B) between two 6" layers of PA-treated corn (Layers A and C). Probes were inserted so that temperature could be monitored using a YSI scanning tele-thermometer (Yellow Springs Instrument Co., Yellow Springs, Ohio, U.S.A.) Column number 1 was lightly covered with aluminium foil, and a 1/2" hole was drilled through the wall 3" above the bottom for ventilation. Column number 2 was sealed with aluminium foil and tape.

Both columns were placed in a window with a southern exposure, and were allowed to remain throughout the winter from December until the following April. Total storage time was 120 days. At the end of this period, the columns were opened and the various layers of corn were transferred to individual plastic bags. Samples of the contents of each were assayed for percentage dry matter and PA. Aliquots consisting of PA-treated and untreated corn used as...
Factors influencing the growth of fungi in high-moisture corn treated with propionic acid.
R. E. Smith and K. R. Stevenson.

controls and stored at 5°C during the experiment, were analysed at the same time. PA content was determined by distillation as described in the A.O.A.C. handbook (1960), followed by titration with 0.01 N NaOH to pH 8.0. Moisture content was estimated using toluene distillation (A.O.A.C. methods, 1965).

Using a Waring blender and sterile distilled water, serial dilutions were made of samples of corn from the upper levels of Layer C (unsealed column) showing fungal growth, and 0.1 ml aliquots of these were used to surface-inoculate plates of Corn Meal Agar (Difco) for the isolation of fungi. Plates were incubated at 30°C. Isolates from these were tested for ability to grow at 30°C on Corn Meal Agar, supplemented with 1.0% glucose and 0.1% yeast extract, in the presence of 0.3, 0.5, 0.7 and 1.0% PA. The PA-supplemented media ranged in pH from 3.6 to 3.8. The PA was added to the molten agar before pouring into Petri plates. Replicate plates of solidified medium were point-inoculated, and growth response was evaluated by measuring colony diameters after 7 and 14 days.

Results and Discussion

During the storage period in the perspex silos, corn at the sides of the columns facing the window became chilled as outdoor temperatures fell, and rose again with increasing temperatures. This effect was most marked when nights were extremely cold and the days were sunny, resulting in a spread of corn temperature of as much as 30°C in 24 hours.

It was soon noted that water vapour condensed in the cold areas of the columns during the night, and partially vapourized again during the day. The amount of residual condensate increased as storage time progressed. Fungal growth was first evident in the untreated corn after about 2 weeks of storage, and spread quickly throughout Layer B in the unsealed column. Most rapid growth occurred in the corn adjacent to the window. Results were similar with the sealed column, except that growth occurred at a slower rate. Within a month, the kernels of corn in Layer B of the unsealed column appeared to be bound together with mycelium, and the fungi had begun to invade Layer C. By the end of the test period, the upper 25% of Layer C was heavily infested with fungi, with as much as 75% of the portion of this layer adjacent to the window infected (see Fig. 1). The segment of Layer C showing mould growth was removed and labelled C1, while the remainder consisting of uninfected kernels was labelled C2. These were analysed separately. No growth was evident in layers of treated grain in the sealed column. Results of the analyses of all layers are reported in Table 1.

It can be seen that the PA content of Layer A in both columns was considerably reduced after 120 days storage, and some acid appeared in Layer B. It is interesting to note that PA levels in Layer C were also lower (with greatest difference appearing in the unsealed column), resulting in what appeared to be a net loss of the acid. It was concluded that some had ended up in the condensate (which unfortunately was not analysed), possibly accounting for lower values in Layer C of the unsealed column as a result of the larger volume of condensate. These findings suggest

Figure 1. Laboratory column containing propionic acid-treated corn (layers A and C) and untreated corn, (layer B), after storage in an unsealed state for 120 days under conditions of widely fluctuating ambient temperatures.
Factors influencing the growth of fungi in high-moisture corn treated with propionic acid.
R. E. Smith and K. R. Stevenson.

that PA may become eluted out of treated corn by the movement of condensed water vapour, some of which is liberated by respiring fungi, lowering levels of acid below concentrations required to prevent fungal growth. In addition, some degradation of PA may result from microbial activity as suggested by Burrell et al. (1973). This effect would lead to rapid spread of fungi when oxygen and temperature were not limiting. Pockets of untreated corn resulting from improper application of PA would tend to favour this process by providing a concentrated inoculum of mould spores which could be transported in the moving condensate.

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<td>Stored at 5°C</td>
<td></td>
<td>77.0</td>
<td>1.08</td>
<td>0.83</td>
</tr>
</tbody>
</table>

**UNSEALED COLUMN:**

| 1A | PA-treated corn from top layer (uninfected) | 77.0 | 0.70 | 0.54 |
| 1B | Untreated corn from middle layer (infected) | 27.5 | 0.16 | 0.04 |
| 1C | PA-treated corn from bottom layer (infected portion) | 49.0 | 0.08 | 0.04 |
| 1C | PA-treated corn from bottom layer (uninfected portion) | 65.5 | 0.37 | 0.24 |

**SEALED COLUMN**

| 2A | PA-treated corn from top layer (uninfected) | 76.6 | 0.56 | 0.43 |
| 2B | Untreated corn from middle layer (infected) | 70.0 | 0.24 | 0.17 |
| 2C | PA-treated corn from bottom layer (uninfected) | 75.0 | 0.88 | 0.66 |

These postulates are supported by the information reported in Table 2. The fungi listed were the only species recovered from Layer C1 of the unsealed column, and most of these grew well on supplemented Corn Meal Agar containing 0.3% PA. Some grew slowly at 0.5% PA. Presumably, growth did not occur in Layer C2 which had a PA concentration of about 0.24% wet weight (see Table 1), either because environmental conditions were less favourable than on the agar plates (i.e., O2 may have been limiting), or because the column experiment was not continued long enough to allow spread of mycelium from Layer C1 to C2.

<table>
<thead>
<tr>
<th>TABLE 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Effect of various concentrations of propionic acid on the growth of fungi isolated from corn treated with propionic acid using Corn Meal Agar supplemented with glucose and yeast extract as the test medium.</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Fungal Species</th>
<th>Propionic acid Concentration % by weight</th>
<th>Net increase in mean colony diameter in 7 days</th>
<th>14 days</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aspergillus versicolor</td>
<td>0.3</td>
<td>0 mm</td>
<td>0 mm</td>
</tr>
<tr>
<td>0.5</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>0.7</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>1.0</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Aspergillus fumigatus</td>
<td>0.3</td>
<td>17</td>
<td>52</td>
</tr>
<tr>
<td>0.5</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>0.7</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>1.0</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Monascus purpureus</td>
<td>0.3</td>
<td>19</td>
<td>62</td>
</tr>
<tr>
<td>0.5</td>
<td>1</td>
<td>23</td>
<td></td>
</tr>
<tr>
<td>0.7</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>1.0</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Monascus ruber</td>
<td>0.3</td>
<td>20</td>
<td>62</td>
</tr>
<tr>
<td>0.5</td>
<td>1</td>
<td>12</td>
<td></td>
</tr>
<tr>
<td>0.7</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>1.0</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Scopulariopsis brevicaulis</td>
<td>0.3</td>
<td>20</td>
<td>62</td>
</tr>
<tr>
<td>0.5</td>
<td>1</td>
<td>12</td>
<td></td>
</tr>
<tr>
<td>0.7</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>1.0</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
</tbody>
</table>

Comparisons of results in sealed and unsealed columns were based on visual assessment of the relative amounts of mycelial development which occurred in 120 days. The differences were readily detectable. Fungal counts were purposely avoided, since the authors believe that such criteria are of little value in assessing mycelial mass of mixed populations of fungi. Marked differences in the numbers of spores produced by different species may provide misleading results when one attempts to assess the effects of environmental conditions.

It would seem, therefore, that the following factors are involved in the etiology of fungal infection of PAtreated high moisture corn stored for moderately long periods:

1. The presence of pockets of partially-treated or untreated corn in which growth of indigenous fungi can occur;
2. Fluctuating ambient temperatures, resulting in condensation of water vapour;
3. Sufficient oxygen to allow fungal growth.
Factors influencing the growth of fungi in high-moisture corn treated with propionic acid.
R. E. Smith and K. R. Stevenson.

References


MOISTURE CONTROL IN LABORATORY TESTS WITH WOOD-ROTTING FUNGI

J. K. Carey1 and C. Grant2

Summary. The use of soil and vermiculite as moisture-holding substrates for laboratory tests with wood-rotting fungi has been investigated. These substrates had widely differing water-holding capacities, but provided that they were moistened to the same extent, equilibrium moisture contents were achieved. Scots pine sapwood blocks reached almost identical equilibrium moisture contents. At substrate moisture levels below water holding capacity the equilibrium moisture content of both Scots pine sapwood and beech moved towards fibre saturation point. Above substrate water holding capacity the blocks became fully saturated. In contrast, the moisture content of standard and tempered hardboards increased gradually until saturation was reached, without any rapid change as substrate moisture content increased above the water holding capacity.

1 Introduction

Soil has been employed in laboratory tests with wood-rotting Basidiomycetes to serve solely as a water holding substrate (Leutritz, 1939 and 1946; ASTM D 1413-61; Nordic Standard 1.4.1.1.70) or additionally to provide infection with a mixed microbial population (Theden, 1961, Nordic Standard 1.4.1.2.70 and others as reviewed by Savory and Bravery 1970). Leutritz emphasised the need for soil moisture control for good test results and crudely adjusted soil moisture content by adding water until the soil cohered when squeezed. Later a standard method for determining water holding capacity (whc) of any soil was incorporated in the American method for testing wood preservatives (ASTM D 1413-61) which suggested the use of soil at 130 per cent, which, determined by this method, for Basidiomycete tests. Later Becker and Kaune (1966) studied the effect of moisture content on mixed microbial decay in soils with various water holding capacities. In this context water holding capacity may be defined as being the amount of water retained by a soil sample after


El Control de la humedad en pruebas en el laboratorio con los hongos destructores de la madera. Se ha indagado el empleo de la tierra y del vermiculito como substratos retentivos de la humedad en las pruebas en el laboratorio sobre los hongos destructores de la madera. Tales substratos se diferenciaban mucho en cuanto a su capacidad de retener el agua pero, con tal que se los humedeciese al mismo grado calculado como porcentaje de su capacidad de retener el agua, los bloques enterados de alburno de pino escocés alcanzaron un equilibrio casi idéntico de capacidad para retener el agua. A los niveles del substrato de humedad inferiores a la capacidad de retener el agua, el equilibrio del contenido de humedad del alburno de pino escocés además del haya se acercaron al punto de saturación de las fibras. A un nivel más elevado de la fibra, pero del substrato para retener el agua, los bloques se pusieron por completo saturados. Contrastando con esto, el contenido de humedad del tablazón de madera dura normal y templada se aumentaba gradualmente hasta llegar al punto de saturación, sin cambio rápido alguno mientras el contenido de humedad del substrato excedía la capacidad de retener el agua.

being subjected to a prescribed series of conditioning procedures and is expressed as:

\[
\frac{\text{soil oven dry weight}}{\text{soil wet weight after conditioning}} \times 100
\]

Work on soil substrate/wood test block moisture equilibria was undertaken to elucidate an instance of failure to decay due to waterlogging of blocks in a soil burial test system. Parallel data on a vermiculite burial system have been recorded following the observation by Kaune (1970) that this material was another suitable water-holding substrate. The opportunity was taken to compare the ASTM method for determining whc with a convenient, more rapid, method in use at this laboratory (PRL method).

2 Test Methods

Determination of substrate whc

The American method, as described in ASTM D 1413-61, consists in allowing a layer of soil 25mm deep to wet from below by capillarity until obviously

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Moisture control in laboratory tests with wood-rotting fungi. J. K. Carey and C. Grant.

wet on top and then allowing water to stand level with the surface for a further 12 hours (or overnight) before extracting on a Buchner funnel by suction for 15 minutes. This method was used to provide results for comparison with those established using the PRL method which is as follows:- A sample of moist material (200 g soil or approximately 15 g vermiculite) was placed in a 125 mm diameter Buchner funnel over a coarse (Whatman No. 4) filter paper. The sample was flooded with water, spread out evenly and a vacuum drawn for 10 minutes. The wet material was then transferred to a container of known weight, and the moisture content determined. The average moisture content at the whc was then established using the data from three replicate samples.

Equilibrium moisture content studies

Using soil. Quarter sawn test blocks of Scots pine sapwood or beech, measuring 30 x 15 x 5 mm (longitudinal x radial x tangential) were oven dried at 103°C for 18 hours. They were then sterilised by exposure to 1, 2-epoxypropane vapour for 24 hours followed by ventilation in a stream of sterile air for 48 hours. Two blocks were placed on edge 20 mm below the soil surface, and 20 mm apart in a 450 cc square glass jar containing 200 g of unsterilised air dry soil of known whc and moisture content. The soil moisture content was then adjusted to give a range of levels with 3 replicate jars at each level. The jars were loosely closed with an unvented cap and incubated at 30°C for 14 days. The blocks were then removed, freed of adhering soil, weighed, oven dried and reweighed. A sample of the soil from each jar was also dried to establish the final moisture content.

Using vermiculite. (Dupré vermiculite, grade DF). Hardboard blocks measuring 35 x 50 mm were cut from Bowater standard hardboard (Athy Mill) and Swedish Masonite tempered hardboard of nominal thickness 3.2 mm. Quarter sawn Scots pine sapwood blocks, measuring 35 x 50 x 3.2 mm were also used. 30 or 50 g samples of vermiculite were dispensed into 575 cc square glass jars and the moisture contents adjusted to a range of levels. Blocks were planted one per jar in a vertical position, 10 mm below the vermiculite surface; three replicate jars were used for each type of block at each moisture content level. The jars closed with a ventilated cap, were autoclaved for 30 minutes at 121°C then incubated at 22°C for 3 days. The blocks were then removed, freed of adhering vermiculite, weighed, oven dried and reweighed to allow calculation of the moisture content.

3 Results

Comparisons of the moisture content of soil at whc as determined by the ASTM and PRL methods are given in Table 1. The moisture contents of tempered hardboard blocks after different periods of incubation at 22°C following autoclaving whilst buried in moist vermiculite are recorded in Table 2.

The moisture contents of fully saturated test pieces are given in Table 3. The equilibrium moisture contents of Scots pine and beech in soil and Scots pine in vermiculite are presented graphically in Fig. 1 and those of standard and tempered hardboard in vermiculite are presented in Fig. 2. The moisture content of the substrate has been expressed as a percentage of the moisture content at the water holding capacity.

| TABLE 1 | Moisture content of soil at water holding capacity. |
| --- | --- | --- |
| Method | Moisture content % at whc | Mean value |
| ASTM | 27.3 | 31.0 | 29.0 | 29.1 |
| PRL | 24.6 | 24.0 | 24.2 | 24.3 |

| TABLE 2 | Moisture content of tempered hardboard after 3, 7 and 14 days incubation at 22°C following autoclaving |
| --- | --- | --- | --- | --- |
| % of whc | Moisture content % after incubation period |
| % absolute | 3 days | 7 days | 14 days |
| 36.3 | 110.5 | 31.1 | 34.9 | 37.4 |
| 70.8 | 215.8 | 53.2 | 53.8 | 51.0 |
| 105.3 | 321.1 | 71.7 | 71.5 | 71.0 |
| 139.9 | 426.3 | 73.8 | 73.1 | 72.7 |

| TABLE 3 | Moisture content of test pieces at saturation |
| --- | --- | --- |
| Test Material | Treatment | Moisture Content % |
| Scots pine sapwood | BS 838 impregnation | 173.6 |
| Beech | .. | 116.7 |
| Scots pine sapwood | BS 838 impregnation followed by autoclaving | 217.0 |
| Standard hardboard | .. | 75.4 |
| Tempered hardboard | .. | 66.3 |

4 Discussion of Methods Used

The ASTM method of determining whc involves moistening the soil by capillarity followed by soaking over a 12 hour period or overnight, therefore complete wetting should occur. With the PRL method complete wetting may not always occur, resulting in the lower equilibrium obtained by this method (Table 1). However, the PRL method provides consistent data more quickly and has therefore been used throughout the rest of the work.
Moisture control in laboratory tests with wood-rotting fungi. J. K. Carey and C. Grant.

Figure 1. Moisture content of wood buried in soil or vermiculite.
Moisture control in laboratory tests with wood-rotting fungi. J. K. Carey and C. Grant.

Figure 2. Moisture content of hardboard buried in vermiculite

Tests in soil were carried out according to the methods currently used to test the toxicity of preservatives against soft rot fungi by burial of treated wood blocks in unsterile soil. Equilibrium moisture contents could not be determined in autoclave sterilised soil because this changes the soil who. Use of non-sterilised soil limited the equilibration period to two weeks since it was known that longer periods would have resulted in significant attack of the test blocks. Autoclave sterilisation of the vermiculite was part of the test method being investigated; it was also thought that it expedited equilibration.

The period of incubation required after autoclaving to achieve equilibrium was investigated using tempered hardboard (Table 2). At 30 per cent who the moisture content was highest after 14 days incubation but at levels of 70 per cent who and above the moisture content had reached equilibrium after 3 days. Although moisture contents achieved at the lower who levels are slightly below the equilibrium moisture content, the differences are slight and hence the 3 day incubation period was used in subsequent tests with vermiculite.

The moisture contents of wood and hardboard test pieces at saturation (Table 3) were determined by a vacuum impregnation (BS 838:1961 method) followed where this process had been part of the test method, by autoclaving at 15 psi (121°C) for 30 minutes. The autoclaved set of Scots pine samples showed a higher saturation moisture content than the set which were not autoclaved. However, the two sets were drawn from different timber sources and the results obtained are similar to the highest equilibrium moisture content achieved when blocks were buried in the substrate (Fig 1).

5 Discussion of Results

Although the actual moisture content by weight of soil and vermiculite differ widely at the water holding capacity (soil approximately 27 per cent moisture content, vermiculite 30 per cent moisture content) the relation between the percentage who of the substrate and the equilibrium content of Scots pine sapwood followed the same pattern in both systems. Below the who the equilibrium moisture content remained almost constant just above the fibre saturation point, with some tailing off at very low levels. Above the who, presumably because free moisture is available from the substrate, the equilibrium moisture content rose rapidly, levelling off towards the saturation level. With beech in soil, the equilibrium moisture content rose less rapidly but otherwise followed the same form of curve as Scots pine sapwood.

The equilibrium moisture content of the two types of hardboard in vermiculite increased gradually and no rapid changes occurred as substrate moisture content exceeded who. The equilibrium moisture content of the tempered hardboard was consistently lower than that of the standard hardboard and
moisture contents at saturation agreed closely with the values achieved by impregnation.

6 Conclusions

Over a wide range of moisture levels both soil and vermiculite control the moisture content of buried test blocks at levels suitable for decay to take place.

For both beech and Scots pine sapwood, just above the whc the moisture content of the blocks rises rapidly and they become saturated. Test assemblies adjusted to a moisture content close to but not exceeding the who should maintain a moisture content in buried wood test blocks suitable for decay, even with some drying during the incubation period.

The equilibrium moisture content of both samples of hardboard was suitable to allow decay to occur over a wide range of the substrate moisture contents tested.

References


BOOK REVIEWS

INSECTICIDES OF THE FUTURE
Edited by Martin Jacobson

The use of the word 'insecticide' in the title is somewhat misleading: moreover, it is apparent that the authors do not have access to a crystal ball. After a brief introduction by the editor, the book comprises five papers (by eminent scientists and first published in Environmental Letters, 18, 1975) each of which deals with a pest control technique which the author considers worth pursuing in preference to, or in conjunction with, conventional chemical pesticides. Some of the techniques have been in use for many years and in no sense does the book break new ground. This volume has been prepared directly from typescript and displays a number of type faces and sizes.

The five subject areas covered are

a) Biological control of insects by predators and parasites (R. van den Bosch)
b) Entomopathogens as insecticides (C. M. Ignoffo)
c) Manipulating sex phenomena for insect suppression (W. Roelofs)
d) Control of insects by sexual sterilisation (A. B. Bořkovec)
e) Development of morphogenetic agents in insect control (J. J. Menn and F. M. Pallos)

The paper of most direct relevance to those concerned with insects involved in biodeterioration is that on the use of morphogenetic agents in pest control. The role of juvenile hormone and its analogues in this technique is treated at some length but reference is made to compound PH 60-40 which appears to affect target insects at the moult, due to an inhibition of chitin synthesis. Trials against agricultural insects have not been successful due to the fact that these juvenoids are not persistent in the field but evidence is presented which indicates that the technique may be much more applicable to stored products insects and those of medical and veterinary importance.

Any value that the other papers may have lies in the extent to which they will stimulate research in their particular line and to this end they vary somewhat in their contribution.

The first paper, which contains a multitude of flowery phrases, deals with biological control in the classic sense, involving the manipulation of parasitoids and predators as density dependant mortality agents. It contains no new material other than passing references to the possible role of certain chemicals (Kairomones) which are released by prey and increase the activity of natural enemies.

An interesting account of many of the technical problems associated with the development of a microbial insecticide forms the bulk of the paper on entomopathogens. Problems include difficulty in obtaining patent protection and the registration of products under US law. While there is evidence that many of the materials available are safe to vertebrates, nothing is mentioned about safety to beneficial insects.

The other two techniques described are those which interfere with the breeding biology of pests. Sex attractants have been used in pest control in a number of ways and these are extensively reviewed by Roelofs, together with their limitations. Although there are no references to biodeteriorating insects it should be pointed out that the methods described are equally applicable to this field and that a number of moths which are stored-products pests are known to have sex attractants.

Pest control by sexual sterilisation depends on the ratio between sterile and fertile individuals in a population and not their actual numbers. As with other authors Bořkovec feels that the best potential for the technique is as a part of a pest management situation. The release of large numbers of laboratory-sterilised (by chemicals or radiation) insects is best used to control low levels of a pest population while field applications of chemo-sterilants work best at high population number. Not all insects lend themselves to this technique which can be expensive to implement: a major limitation is the time taken for the system to become established at population level.

In many ways a quotation from the above paper sums up the theme of the techniques described in the book "that apparent simplicity of the principle is in sharp contrast with the complexity of its utilisation".

Richard C. Reay

BENCHMARK PAPERS IN BIOLOGICAL CONCEPTS: ANHYDROBIOSIS
Ed. John H. Crowe and James Clegg

This is the first of a proposed new series of books which are said by the publishers to "consist of the most critical papers in biology selected from the literature of past and present". The editors take a more modest view of their achievement and repeatedly indicate that papers are chosen to illustrate particular aspects of research or thought rather than for their exclusive importance.

This volume is concerned with the continuation of latent life, for which the term cryptobiosis is used, in dried organisms, and is therefore relevant to the
survival of some biodeteriogens. However the editors have attempted a very catholic selection of papers and biodeterioration is not mentioned specifically.

Apart from the bibliography and the indices the book consists of six sections. These are: points of view about cryptobiotes; the metabolic state of dried organisms; the longevity of dried cryptobiotes; the manner of death of cryptobiotes; adaptations associated with anhydrobiosis; metabolic changes associated with revival from anhydrobiosis. Each section contains photographic reproductions of a number of original papers and is introduced by the editors. The direct copying of the original papers means that the pattern and size of print changes with each of them. This provides a welcome variety in visual impact in a long book but the size of print in a few papers has been reduced to such an extent that I had recourse to a reading glass. Apart from this the reproduction is very clear and the volume is handsomely produced.

When a new type of publication is presented it is reasonable to ask “was it worth doing?” as well as “is it well done?”. It seems to me that this particular endeavour is misconceived. The sample of papers included in the book emphasise the time period directly before its publication. It became out of date soon after publication yet its time span and perspective is too short for a work of historical significance. Although there are some papers here that one is glad to have seen and might have missed in a casual scanning of the literature, the book as a whole seems an expensive substitute for a traditional critical review.

G. Ayerst
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