MICROBIOLOGICAL CORROSION OF METALS—MARINE WOOD BORERS—RODENT ATTACKS ON STORED PRODUCTS—FOULING OF SHIPS BY BARNACLES—DETERIORATION OF STONE BY BACTERIA—ROTTON 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

BIODETERIORATION INFORMATION CENTRE
THE UNIVERSITY OF ASTON IN BIRMINGHAM
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We regret that, owing to an error in the layout of the title pages of articles in this issue it has not been possible to trim covers of reprints to the standard A4 size. It will be seen that the titles are set too low on the page to be visible through the apertures provided on the covers.

Authors may wish to trim off the bottom edges of these reprints to bring them to A4. This will remove half of the University of ASTON crest and the word "England" which is untidy but may be worth while to facilitate handling and filing of reprints.

We believe our printing troubles are now at an end and that errors and changes in style which have beset recent issues will not be repeated.

Professor T. A. Oxley
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INTERNATIONAL
BIODETERIORATION BULLETIN

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NOTES
FOR CONTRIBUTORS

The International Biodeterioration Bulletin is published four
times per year (Spring, Summer, Autumn, and Winter). Typescript contributions should be sent to the Editor, Professor
T. A. Oxley, at the above address.

The Bulletin acts as a vehicle for the publication of original
works, including reviews, on all aspects of biodeterioration,
i.e. deterioration of materials, artefacts or facilities, of economic
importance by living organisms, which is taken to include
micro-organisms, insects, rodents, birds, higher plants, etc.
Articles on biodegradation, that is conversion of materials to
more easily disposable, or higher value products, by the same
agencies, are also published.

Contributions are published only in English. Each article
must be accompanied by a summary in 50-150 words which
will be translated into French, German and Spanish. Native
speakers of these languages are invited to submit their summaries
in their own language; in certain circumstances complete
articles may be submitted in French, German or Spanish and
will be translated into English for publication.

Illustrations must be very clearly drawn in black on white
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independent referees for advice on their clarity, originality,
and general suitability for publication, but the final decision whether
or not to publish an article rests with the Editor and Editor-in-
Chief of the B.I.C. journals. If articles are rejected the substance
of the referee's report will usually be communicated to the
author and in suitable cases the Editor will be pleased to help
authors to improve their papers with a view to possible
publication.

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Reese and Levinson (1952), or Darby et al (1968)
and in the bibliography:
Reese, E. T. and Levinson, H. G. (1952). Comparative study of
the breakdown of cellulose by micro-organisms. Physiol.
Plant., 5, 354-366.

Darby, R. T., Simmons, E. G. and Wiley, B. J. (1968). A
survey of fungi in a military aircraft fuel supply system. Int.
Biodeterior., 4, (1) 39-41.

(In the latter reference note the correct abbreviation of the

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BIODETERIORATION SOCIETY NEWSLETTER

Forthcoming Symposium on "Recent Research on Household Pests" at Pest Infestation Control Laboratory, London Road, SLOUGH, Berkshire.

Papers are still sought urgently for presentation at this meeting. Please write to the programme secretary with offers and for further information. Pests in this context can be interpreted widely, to include insects (including parasites), arachnids, rodents, birds, molluscs, etc. Papers on any of these will be acceptable provided that they refer to the domestic situation.

Programme secretary: Dr. R. H. Tilbury, Tate & Lyle Group Research & Development, Philip Lyle Memorial Research Laboratory, P.O. Box 68, READING, Berkshire RG6 2BX.

Subscriptions 1977

Subscriptions to the Society are due on 1st April each year. The treasurer urges all those who do not pay by Banker's Order to send their subscriptions for 1977 to him immediately. The rates are: £3.50 U.K. and Ireland; £2.00 elsewhere. If subscriptions cannot be remitted in sterling, please increase the amount to the equivalent of £2.50 to cover bank charges.

Treasurer: Dr. K. J. Seal, Biodeterioration Information Centre, University of ASTON, 80 Coleshill Street, BIRMINGHAM B4 7PF.

Biodeterioration at the British Association for the Advancement of Science

Members of the Society and others took the opportunity of the visit of the British Association to the University of ASTON to stage a one day symposium on Biodeterioration. Under the chairmanship of Dr. M. B. Green the morning session was introduced by Dr. H. O. W. Eggsin who dealt with the range of deteriogens and with the costs of biodeterioration and its control. Mr. Genner of Cardiff gave a review of current work on the microbiological attack on hydrocarbon lubricants and fuels. Dr. Flannigan of Edinburgh dealt with biodeterioration of stored grain and Dr. Allsopp of Birmingham dealt with the deteriorative effects of higher plants.

In the second session, chaired and introduced by Professor T. A. Oxley, Dr. D. J. Dickinson of Imperial College, London, introduced the subject of deterioration of wood and Mr. J. M. Baker of the Building Research Establishment, reviewed the specific problems of deterioration of wood in buildings. Finally, Mr. B. A. Richardson of Winchester spoke on the colonisation of, and damage to, stone and brick building surfaces by algae, lichens, and other organisms.

The meeting was well attended by members of the non-specialist scientific public and discussions were lively.
Biodeterioration Society Newsletter (3) 1977

Papers Presented at the Summer Scientific Meeting
HERIOT-WATT UNIVERSITY, EDINBURGH
14 – 15 July 1977
Symposium on
BIODETERIORATION PROBLEMS IN SCOTLAND

<table>
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<tr>
<th>Title</th>
<th>Differences between the saprophytic abilities of separate isolates of Serpula lacrymans</th>
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<tr>
<td>Author</td>
<td>S. A. Hutchinson</td>
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<tr>
<td>Address</td>
<td>Botany Department, The University, GLASGOW G12 8QQ.</td>
</tr>
</tbody>
</table>

**ABSTRACT**

Separate isolates of *Serpula lacrymans* showed differences of up to 10X in their ability to cause loss of dry weight and of cross-grain breaking strength of pieces of *Pinus sylvestris* sapwood in controlled laboratory conditions.

The activities of monokaryotic strains are mostly higher than those of their parent dikaryons and inheritance is mostly controlled by an additive genetic system. The differences have been correlated with differences in the ability to produce extracellular carboxymethylcellulase, not with differences in growth rates of the hyphae in the conditions tested.

<table>
<thead>
<tr>
<th>Title</th>
<th>Problems associated with contact lenses</th>
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<tr>
<td>Author</td>
<td>R. J. McBride</td>
</tr>
<tr>
<td>Address</td>
<td>Department of Pharmacy, Heriot-Watt University, 79, Grassmarket, EDINBURGH, EHI 2HJ.</td>
</tr>
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</table>

**ABSTRACT**

Wetting solutions containing polyvinyl alcohol or polyvinyl pyrrolidone are used to give a hydrophilic surface on hard hydrophobic polymethyl methacrylate (PMMA) corneal lenses before insertion. When not being worn, PMMA lenses are kept in a disinfecting solution to inhibit any potential pathogens after treatment with a cleaning solution on removal from the eye.

Soft hydrophilic hydroxyethyl methacrylate (HEMA) lenses were thought to overcome the anoxic and low tolerance defects of the PMMA lenses. Sterilisation of HEMA lenses by storage in chemical solutions is unsatisfactory due to elution of anti-microbial agent into lachrymal fluid. Microbiological evaluation indicate soft lens solutions are slow to sterilise, while heating techniques makes lenses difficult to clean.

<table>
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<tr>
<th>Title</th>
<th>Agricultural wastes - pollution control and energy production.</th>
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<tr>
<td>Author</td>
<td>P.N. Hobson</td>
</tr>
<tr>
<td>Address</td>
<td>The Rowett Research Institute Microbiology Department, Greenburn Road, Buckburn, ABERDEEN, AB2 9SB.</td>
</tr>
</tbody>
</table>

**ABSTRACT**

Agricultural wastes, particularly slurries of excreta from intensive farming units, can cause pollution. Legislation exists to control such pollution, and although perhaps not rigorously enforced at the moment, the future may well bring greater measures of control. Present opinion looks not only to control of environmental pollution but also the recycling of, or production of useful materials from, wastes. In the latter context farm wastes may be re-used as animal feedstuffs, although this is unlikely to be a method of pollution control and, indeed, itself may cause pollution. Animal wastes have an increasing value as fertiliser and recycling of wastes in this way while controlling pollution from the waste is the object of investigations into farm waste management and treatment. Anaerobic digestion can reduce pollution while retaining the fertiliser value of wastes and also generate energy of use on the farm.

<table>
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<tr>
<th>Title</th>
<th>Physiological studies on the deteriogen Aureobasidium pullulans</th>
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<tbody>
<tr>
<td>Authors</td>
<td>Miss Jan Webster and J.E. Smith.</td>
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<tr>
<td>Address</td>
<td>Department of Applied Microbiology University of Strathclyde, GLASGOW G1 1XW.</td>
</tr>
</tbody>
</table>

**ABSTRACT**

*Aureobasidium pullulans* can act as a deteriogen on wood, painted surfaces and soft fruits amongst others. It has also been found as a secondary invader of human tissue. It is dimorphic, able to exist as a single celled yeast form, or a filamentous form depending on the environmental conditions. Several intermediate forms exist between the two extremes. Factors influencing morphology are being studied using continuous culture and it seems that the nitrogen source is important as ammonium sulphate gives mycelial growth and sodium nitrate, the yeast form. Other variables will be studied including carbon source, temperature and aeration. The production of the extracellular, polysaccharide pullulan, under varying conditions, may also be investigated.

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

W. Blyth
Department of Botany, Experimental Mycological Unit, University of Edinburgh, The King's Buildings, Mayfield Road, EDINBURGH, EH9 3JH.

ABSTRACT
Many fungi and actinomycetes causing biodeterioration of stored materials such as grain and hay may be pathogens and/or allergens of man and animals.

Aspergillus fumigatus may cause various forms of bronchopulmonary aspergillosis ranging from simple airway colonisation to necrotising infection or the formation of aspergillomata (fungus balls). The fungus may also provoke asthma by production of allergens.

Micropolyspora faeni is a well known cause of farmer's lung, (a form of extrinsic allergic alveolitis) giving micronodular shadowing in the lung which may be followed, as attacks proceed, by irreversible fibrosis and deterioration in function. Allergens are again responsible.

The degradation of the insoluble organic component of industrial effluent in estuarine sediments.

N.J. Poole and R.J. Parkes
Department of Microbiology, University of Aberdeen, Marischal College, ABERDEEN AB9 1AS

ABSTRACT
Shallow estuaries, stressed by the presence of excess cellulose, often become anoxic due to increased microbial activity. Evidence was presented to show that:
1. Anoxic sediments are important in determining the oxygen concentration of the overlying water.
2. A correlation exists between the activity of the sulphate-reducing bacteria and the sulphide concentration. Sulphide is important in maintaining anoxic conditions.
3. The toxicity of sulphide, which is a factor of temperatures, controls the activity of other microorganisms and hence the degradation rate of cellulose.
4. Temperature is an important factor in controlling the release of H2S from the sediment.

Public health aspects of recent trends in microbial infections of engineering lubricants.

R. S. Holdom
Department of Applied Microbiology, University of Strathclyde, GLASGOW, G1.

ABSTRACT
Since 1972, when most engineering lubricants and coolants were formulated with solvent refined oil (excepting so-called "synthetic oils") there has been an increase in the severity and incidence of microbial infections of engineering plant in which oil-water emulsions are circulated. The modern oils contain a wide range of additives (for engineering reasons) but these are all biodegradable and therefore support microbial growth in normal use.

At present double standards of health and hygiene seem to apply. On the one hand it is simply not permitted for pathogens to exist in foods, cosmetics, water, and other consumer products, while it seems to be permitted that engineering workers co-exist with emulsions bearing a largely unknown range of microbial species. Emulsions and body fluids compare as growth media; aerosolisation of works emulsion ensures that the microbes are disseminated by the most effective means known, and the particle size in the aerosol ensures that the deepest parts of the alveolar region of the lung are challenged with inoculum. Biocides are used too empirically and monitoring methods must be improved.

The effect of the degradation of cellulose on the sedimentary ecosystem of a Scottish sea loch.

T. H. Pearson and S.O. Stanley
Dunstaffnage Marine Research Laboratory, P.O. Box No. 3 OBAN, Argyll.

ABSTRACT
Long term studies on the response of a sea-loch ecosystem to effluent input from a pulp and paper mill have shown that changes in the distribution and composition of the benthic micro- and macrofauna can be closely correlated with changes in the physical/chemical nature of the sediment as a result of the microbial degradation of cellulose in the bottom sediments. Studies of the pathways involved in cellulose breakdown and their relationship to changes in the benthic ecosystem at various organisational levels are aimed at defining degradation rates in relation to differing environmental factors.

The provision of these abstracts by authors does not constitute formal publication. Those interested should write direct to authors, not the society.
Title: Quantification of microbial colonisation of wood.

Author: B. King

Address: The Rowett Research Institute, Microbiology Department, Greenburn Road, Bucksburn, ABERDEEN, AB2 9SB.

ABSTRACT

The nitrogen content of small wood blocks buried in soil has been shown to increase during microbial colonisation. It has also been shown that, in a typical fertile but not recently fertilised, soil, movement of nitrogen into wood is not a physical process. It is therefore presumed that the observed nitrogen increases are due to increase of microbial tissue deriving its nitrogenous nutrition directly from the soil.

The nitrogen content of sporophores and mycelium of representative wood colonising organisms ranges between 3% and 6% of tissue dry weight (Cowling & Merrill, 1966) with 4% a typical mean value. Subtracting the known initial nitrogen content of the wood from that observed in the decayed wood (using initial dry weight as a common basis) a difference remains which is presumed to represent microbial biomass. In this way it has been shown that, for example, Lime blocks which had been buried for 18 weeks in soil and having 65% weight loss consisted of up to 25% by dry weight of microbial material. It is suggested that conventional isolation techniques do not reflect this proportion of microbial virulence in wood and that other more appropriate techniques e.g. comminution types, might provide a more accurate assessment of numbers of micro-organisms present.

SESSION OF SHORT ORIGINAL CONTRIBUTED PAPERS 15th JULY 1977

Title: Occurrence of biological growth on concrete dams in north Scotland.

Author: A. F. Bravery

Address: Building Research Establishment, Princes Risborough Laboratory, Princes Risborough, AYLESBURY, Bucks HP17 9PX.

ABSTRACT

The paper described the main types of algal growth, more than seventeen species of lichen and six species of mosses encountered on the various aspects of 26 concrete dams located within the region of the North of Scotland Hydro-electric Board.

Data on the incidence and extent of growth were analysed in relation to the age of the structures, their height, altitude, or orientation with respect to the prevailing wind, rain and incident sunlight, and also the cement content and the types of sand and aggregate used in the concrete. On the main downstream faces the number of species was greatest towards the sheltered shoulders of the dam walls and fewest in the centre at the base. Areas protected from wetting by overhang were comparatively free from growths. The frequency and extent of wetting were apparently less important than the duration of the periods of wetness; the evidence for this is the fact that faces which received least direct sunlight were most heavily colonised. In the main, environmental exposure parameters were more significant in influencing the patterns and extent of growth than were properties of the concretes themselves.

Title: Isolation and enumeration techniques for marine film forming bacteria.

Authors: J. Carson and D. Allsopp

Address: Biodeterioration Information Centre, University of ASTON in Birmingham, 80, Coleshill Street, Birmingham B4 7PF.

ABSTRACT

The significance of the microbial film in the marine environment is outlined together with the limitations of previously suggested sampling, culturing and enumeration techniques. A new type of slide rack is described together with the development and use of a suitable washing technique for the removal of extraneous bacteria from the microbial film. The use of Acridine orange epifluorescence techniques for the enumeration of marine bacteria is discussed.

Title: Growth potential of microbes in commercial soluble oils.

Authors: R. S. Holdom and D. Ritchie

Address: Department of Applied Biology and Microbiology, University of Strathclyde, GLASGOW, G1 I XW.

ABSTRACT

(Abstract not received)

The provision of these abstracts by authors does not constitute formal publication. Those interested should write direct to authors, not the society.
**Title:** Differences in saprophytic abilities of *Coniophora puteana*  
**Authors:** A. B. McPhee and S. A. Hutchinson  
**Address:** Botany Department, The University, GLASGOW G12 8QQ.

**ABSTRACT**

34 isolates of *Coniophora puteana* showed wide variation in their ability to cause loss in dry weight and cross-grain breaking strength of pieces of *Pinus sylvestris* sapwood in controlled laboratory conditions. Some, but not all of these differences have been correlated with growth rate and it appears likely that they will also be correlated with extracellular cellulase production. Further work will elucidate this aspect.

Differences were found in the activity of isolates of the BSI test strain (11E) obtained from separate laboratories. The Society was asked to examine the possibility of establishing a standard national/international procedure for testing and recording the maintenance of specified properties of standard cultures in national collections.

---

**Title:** Thermophilous cellulolytic fungi of imported softwoods and of in-service timber joinery.  
**Authors:** L. H. G. Morton and H. O. W. Eggins  
**Addresses:** Dr. Morton, Division of Biology, Preston Polytechnic, PRESTON, Lancs.  
Dr. Eggins, Dept. of Biological Sciences, University of ASTON in Birmingham.

**ABSTRACT**

Sampling work undertaken on six imported softwoods is described and a list of fungi isolated over a wide range of temperatures is submitted. The cellulolytic activity and temperature tolerance range of each isolate is recorded. The isolation of cellulolytic fungi including known soft rot organisms from beneath the surface of imported timber may provide a case for considering that some timber may be infected when it goes into service.

The isolation of thermophilous fungi from painted and unpainted in-service timber joinery is described and the results show the presence of organisms which are capable of causing soft rot.

---

**Title:** Detecting the first mouse  
**Author:** T. A. Oxley  
**Address:** Biodeterioration Information Centre, University of ASTON in Birmingham, 80 Coleshill Street, BIRMINGHAM B4 7PF

**ABSTRACT**

The high standards of hygiene which now exist in some premises make it realistic to speak of totally mouse free areas. The effort required to maintain such a condition would be much eased if a reasonably certain method existed for detection of the first mouse to enter the area.

A simple device for this purpose was described. This takes advantage of the behaviour of an exploring mouse, which is somewhat different from that of a mouse foraging in familiar premises. This device gives a clear light signal as soon as the passage of a mouse is detected. A few such devices, strategically placed, will enable the entry of the first mouse to be detected very quickly and easily whereas the usual system based on close inspection of large numbers of bait boxes may not give any sign of infection until a breeding colony is already well established on the premises.

---

**Title:** The development of an amylolytic microflora on barley during malting.  
**Authors:** B. Flannigan  
**Address:** Department of Brewing and Biological Sciences, Heriot-Watt University, EDINBURGH EH1 1HX.

**ABSTRACT**

Changes in the number of viable units of bacteria, moulds and yeasts during an 8-day floor-malting process were examined by a spread-plate method. After 8 days, bacterial numbers were seven times those on dry barley, moulds had increased four-fold, and yeasts 20-fold. The most abundant moulds were *Aureobasidium pullulans*, *Cladosporium* spp. and *Verticillium lecanii*, and the common yeasts included *Candida edax* and *Rhodotorula glutinis* (both non-amylolytic), and *Cryptococcus flavus*, *C. uniguttatus* and *Sporobolomyces roseus* (with low amylolytic activity). All moulds except *V. lecanii* were amylolytic, but only *A. pullulans* produced amylases constitutively and appeared likely to contribute to starch degradation in the presence of glucose and maltose released by barley amylases.

The provision of these abstracts by authors does not constitute formal publication. Those interested should write direct to authors, not the society.
Title: Farmer's lung disease in Ayrshire dairy farms
Author: Violet E. Wardrop
Address: Department of Botany, Experimental Mycoses Unit, University of Edinburgh, The King's Buildings, Mayfield Road, EDINBURGH, EH9 3JH.

ABSTRACT

Two groups of dairy farms, 7 with a case of farmer's lung (FLD farms) and 5 without (non-FLD farms) were analysed microbiologically by sampling air of buildings, hay, grain and dusts from bruising machines. Concentrations of mesophiles and of thermophilic fungi were similar in both types of farm, but FLD farms gave higher counts of thermophilic actinomycetes, notably of M. faeni. No correlation was established between farmer's lung and seropositivity to fungal extracts, but 91% of all cases were positive to extracts from various strains of Micropolyspora faeni. Fifty-four per cent of sera from personnel on FLD and non-FLD farms were seropositive, but 69% of workers on FLD farms were also symptomatic. Occurrence of disease correlated with high environmental concentrations of M. faeni.

Title: Maltworker's lung in Scotland.
Author: W. Blyth
Address: Department of Botany, Experimental Mycoses Unit, University of Edinburgh, The King's Buildings, Mayfield Road, EDINBURGH, EH9 3JH.

ABSTRACT

During a survey of respiratory disease in Scottish maltworkers, 70% of 574 sputum smears were positive for higher plant cells, mycelia and/or spores of common environmental fungi. Penicillium spp. (90%), Rhizopus stolonifer (40%) and yeasts (33%) were dominant in 699 sputum cultures and in 327 samples of grain, malt, culms and dusts from 56 maltings. Twenty per cent of 711 men were precipitin-positive for Aspergillus fumigatus, 20% for A. clavatus, 10% for A. niger, 16% for Chadosporium spp., 6% for Penicillium cyclopium and 3% for R. stolonifer.

The incidence of extrinsic allergic alveolitis due to A. clavatus in the workforce was 5.2%. The allergenic fungus was found in 21% of maltings.

Title: Wood — a practical man's evaluation
Author: C. J. Chapman
Address: 50 Rothsay Avenue, Lenton Sands, NOTTINGHAM NG7 1PU.

ABSTRACT

Use of wood, in most spheres of manufacture, is the result of thousands of years of development before the advent of the scientist. Scientists' evaluation of wood has been independent of trade expertise and biased by Chemical industry finance. Wood has been further devalued by some Marine Surveyors accidentally or by design. Wet rot fungi are selective only of the portion of wood conditioned by a fault established over many months culminating in attraction by aromatic emanations. There is urgent need for co-operation of scientist, marine surveyor, and conventional boatbuilder to produce a treatise defying adverse criticism.

Title: Preservation of animal feedingstuffs by addition of alkali.
Author: C. S. Stewart
Address: Rowett Research Institute, Bucksburn, ABERDEEN, AB2 9SB.

ABSTRACT

Experiments at the Applied Nutrition and Microbiology departments of the Institute have been done to investigate changes in the digestibility of animal feeds after treatment with sodium hydroxide. In addition to increasing digestibility, NaOH treatment greatly reduced the incidence of microorganisms on the feeds. A number of microorganisms were able to survive in alkali treated feeds at a pH of between 10 and 12, notably the fungus Verticillium dahliae f. restrictum, sporeforming Bacillus species, a number of Gram positive cocci and coryneform rods of some small Gram negative rods.

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Dr. D. Allsopp, Biodeterioration Information Centre,
The University of Aston in Birmingham,
80 Coleshill Street, Birmingham, England B4 7PF.
A LONG TERM FIELD TRIAL OF GAMMA-HCH/DIELDRIN SMOKE AGAINST DEATH WATCH BEETLE (XESTOBIUM RUFOVILLOSUM) IN AN ANCIENT OAK ROOF

E.C. Harris

Summary. This long-term trial was started to determine whether annual smoke treatments, applied shortly before each beetle emergence, could eventually eliminate the beetle population by preventing annual re-infestation. The application rate for all treatments, apart from the first two, was 95 mg gamma HCH plus 197 mg dieldrin per cubic metre. The average surface deposits varied annually according to the weather conditions but on average were: 46 mg/m² gamma HCH and 214 mg/m² dieldrin on upward-facing surfaces, 4.8 mg/m² gamma HCH and 13.4 mg/m² dieldrin on vertical surfaces, and 3.8 mg/m² gamma HCH and 2.4 mg/m² dieldrin on downward-facing surfaces.

The annual emergence of beetles declined from over 1300 prior to 1963 to under 100 from 1973 onwards. The long period required to achieve this result is related to the long and variable period which the larva spends in the wood, unaffected by surface deposits, until its emergence as adult. The total cost of the thirteen annual treatments is under £300.

Introduction

Long-standing attack by the death-watch beetle (Xestobium rufovillosum Deg) in the 15th century roof of the chapel of King's College, Cambridge was first brought to the attention of the Building Research Establishment's Princes Risborough Laboratory (PRL) in 1956. The ancient oak roof, low pitched and lead covered, forms a chamber approximately 86 m² wide above the well-known stone-vaulted ceiling; the height varies from 1.8 m at the side walls to 3.3 m at the apex and the volume is approximately 3 100 m³. The chapel is regularly surveyed for structural integrity. The roof was stabilised in 1900 by the insertion of metal...
A long term field trial of gamma-HCH/dieldrin smoke against death watch beetle, E.C. Harris.

tie rods and was reloaded on the south side in 1957. There have been no recent renewals of the timber roofing members.

Initial tests

Assessment of the level of beetle activity and its distribution was started in 1959, using the annual emergence of adults as an indication of the need for remedial measures. The beetles were collected at the end of each emergence period - late March to early June - after they had fallen from the roof timbers on to the upper surface of the vaulted ceiling, and their numbers and distribution were recorded (Figure 1).

In 1963 an exploratory treatment with gamma-HCH/dieldrin smoke generators was carried out shortly before the onset of beetle emergence. The observations (Harris 1964) showed the following effects on the emerging beetles:

1. The normally well-separated peaks of dropping of the two sexes from the timbers were changed, both sexes dropping earlier than normal and nearly concurrently.

2. The sex ratio, normally around 1:1 was altered in favour of females, suggesting that some males had not been able to complete the cutting of their exit holes.

3. Weekly collections of beetles from sample areas showed that (a) 100 per cent of males and 93 per cent of females were in a condition of irreversible "knock-down" or dead at the time of collection, and (b) 74 per cent of females had failed to mate and no mated females had laid eggs.

4. The treated wood surfaces were sufficiently toxic to prevent boring by first-stage larvae in the event of any fertile eggs being laid.

Three further annual tests were carried out, the results being in close agreement with the initial findings (Harris 1967 and 1969) and justifying a continuation of the annual smoke treatments as a means of slowly reducing the larval population on the assumption that emerging adults are prevented from reinfesting the timbers. The subsequent programme was therefore restricted to (a) the annual treatment and analysis of insecticide retentions, usually at the end of March or first week in April, and (b) collecting and recording the annual beetle emergence at the end of June or early July.

Extended trials

Following the initial test at King's College in 1963, smoke trials were extended to many other buildings in co-operation with architects, surveyors and church authorities and have included tests with gamma-HCH alone. The work has aroused considerable discussion regarding the long-term efficacy of the method compared with conventional liquid formulations, its suitability of application to different types of buildings, and possible toxic hazards attending the liberation of smokes based on chlorinated hydrocarbons. Since this development work was based on the initial test at King's College, it is considered that the cumulative results obtained to date at this test site are worth reporting and assessing to determine whether the predictions made following the initial test are being borne out.

Application rate and surface retentions of insecticides

Thirteen annual treatments have now been given. Fumite canisters, size No. 10, each evolving 40.8 g dieldrin plus 19.6 g gamma-HCH were used for all the treatments. Ten such canisters were used for the first two treatments but numbers were increased to 15 from 1965 onwards. They were laid equidistantly along the upper surface of the vault. Before each treatment, open arches along the side walls and at the gable ends of the roof space were sealed with plastic-covered frames, most of which were left in position until the subsequent beetle emergence was completed. Each treatment was carried out in mid-morning and the roof space left undisturbed until the following day. Insecticide retentions were determined by placing filter papers on selected roof trusses to present an upward, downward and vertical face to the smoke particles. After each treatment they were folded into sealed tubes and analysed by gas-liquid chromatography. Temperatures at the start of each treatment varied from year to year between 8.3°C and 13.8°C.

Results

The mean retentions of the two insecticides on the roof timbers are shown in Table 1.

Figure 2 shows the annual emergence total from 1959 to 1962 (the pre-treatment years) and from 1963 to 1975 when the smoke programme was in operation. The ratio of males to females for each year is included.

Discussion

The surface retentions of the insecticides illustrate the characteristics of smoke particles, i.e. the heavy amounts settling out on to upward-facing surfaces and the much lighter amounts adhering to downward-facing and vertical surfaces. Gamma-HCH shows less disparity in this respect than dieldrin.

The annual fluctuations in the actual amounts of insecticide deposited are attributable to the varying weather conditions during the treatments. Although the temperatures were relatively consistent, winds were variable in direction and strength and, not unnaturally, in a chamber 21 m from the ground, caused some leakages of smoke.

In 1961 and 1962 the annual beetle emergence was above 1300 and had fallen to 80 by the time the thirteenth annual treatment was given in 1975. The lower proportion of males emerging compared with the
A long term field trial of gamma-HCH/dieldrin smoke against death watch beetle, E.C. Harris.

Roof bays in alphabetical order west - east
Figures in circles indicate number of beetles in north and south half of each bay
Fig. 1. King's College Chapel, Cambridge. - Outline plan of roof showing distribution of Death Watch Beetles emerging summer 1960

Fig. 2. Annual emergences of Death Watch Beetle adults from chapel roof
pre-treatment years is clearly shown. In 1971 and 1974, for instance, males comprised only 29 per cent of the total and in most other years were well under 40 per cent.

The rate of reduction in the annual emergence is a reflection of the long and variable length of the larval life. The speed of development of Xestobium is dependent on the presence and extent of fungal decay in the wood, which is particularly important to the establishment of first-stage larvae. In laboratory studies (Fisher 1940) the minimum life-cycle at normal temperatures in oak sapwood decayed by Fomes cryptarum was three years. In the same timber species free from decay, larvae were from ½–3% grown after 7 years but the life-cycle was not completed even at temperatures of 22–25°C.

In old oak roofs fungal decay is extremely variable and tends to be concentrated at certain points in the structure, notably at the ends of tie beams, feet of rafters and in wall plates. Under such conditions, larvae, although establishing themselves in decayed wood, could subsequently bore in wood varying from heavily decayed to undecayed and their rate of development will vary accordingly. Although it can be assumed that a small but unquantified proportion could emerge as adults in three years, the remainder would grow at varying rates, some very slowly, and for these the maximum life cycle could be well in excess of 10 years.

At the time of the first treatment in 1963 larvae at all stages of development would already be present in the timber. These larvae would not have been affected by the smoke deposits which are essentially surface treatments. Elimination of eggs and new larvae by the treatments in 1963 and subsequent years could not be reflected in the annual emergence for at least three years, i.e., until 1966 at the earliest. During this period the reduction in the annual totals must be ascribed solely to some adults being prevented from completing their emergence, probably mostly males.

The sudden drop in the 1967 total, after three years at a fairly consistent level, suggests that it was then, rather than in 1966, that the reduced larval population first showed itself in terms of a lower beetle emergence. Apart from a slight rise in 1968, the annual reduction in emergence proceeded smoothly, eventually resulting in totals of under 100 from 1973 onwards. A continuation of the programme will clearly be necessary before it can be decided whether any further annual beetle

### Table 1

#### APPLICATION RATES AND RESULTING γ-HCH/DIELDRIN RETENTIONS

<table>
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<tr>
<th>Year treated</th>
<th>Application Rate mg/m²</th>
<th>Ambient temp °C at start</th>
<th>Mean retentions of insecticides on differently orientated roof surfaces mg/m²</th>
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<th>Dieldrin</th>
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<tr>
<td></td>
<td></td>
<td></td>
<td>Upward</td>
<td>Vertical</td>
<td>Downward</td>
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<tr>
<td>1963</td>
<td>63 132</td>
<td>10.5</td>
<td>Sampling procedure not comparable with that of subsequent years.</td>
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<td>1964</td>
<td>63 132</td>
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<td>8.3</td>
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A long term field trial of gamma-HCH/dieldrin smoke against death watch beetle, E.C. Harris.

Emergences are derived from larvae already present in the wood in 1963 or whether the treatments have been less than completely successful in preventing reinestation each year.

A period of 13 years to achieve the present level of control would be quite unacceptable against most wood-boring pests but is reasonable within the context of the death-watch beetle problem in historic buildings. Even if 20 annual treatments are required for complete elimination this is insignificant when the problem is put into perspective. Field experience shows that in the majority of historic buildings there is little doubt that the death-watch beetle was introduced in pockets of decayed wood within the timbers at the time of building. Because the oak used would have been "green" and slow to dry naturally in the large sizes employed, the decay would doubtless have flourished, often at the centre of large beams, until eventual drying out halted its progress. It is this reservoir of old fungal decay which has enabled the death-watch beetle to survive away from its natural habitat in the form of inbred populations within old buildings, despite the periodic changes and restorations to which the buildings have been subjected over the centuries. It must be stressed (Anon 1976) that the presence of long-standing fungal decay and death-watch beetle damage can affect the structural stability of an ancient timber roof. A thorough survey must therefore be carried out before a decision is made to embark on a programme of smoke treatments and the necessary repairs or renewals should be made either before or during the course of the treatments.

Conclusions
Thirteen annual treatments with gamma HCH/dieldrin smokes, applied at a rate of 95 mg gamma-HCH plus 197 mg dieldrin per m³ except for the first two, have succeeded in reducing the death-watch beetle population in the chapel roof to a very low level. In view of the ease and cheapness of the method, entailing virtually no equipment or preparation, the programme will be continued in order to determine whether complete elimination of the death-watch beetle can be achieved and, if so, within how long a period. To date, the total cost of the thirteen treatments, including a small initial outlay for making the plastic-covered frames for sealing, is approximately £280.

Acknowledgements
Thanks are due to the Council and the maintenance staff of King's College for their co-operation regarding the use of the chapel roof as a test site and to the Laboratory of the Government Chemist for carrying out numerous GLC analyses of the insecticide retentions.

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Microbial Succession on a Wooden Surface Exposed to the Sea
Anthony M. Cundell and Ralph Mitchell

Summary. The microbial succession on wood discs placed in an
embayment at Fort Lauderdale, Florida for 12 weeks was
studied using a scanning electron microscope. Bacteria, pennate
diatoms, stalked diatoms and amoeboid and ciliated protozoa
were the dominant microorganisms observed in the succession.
Green macroalgae, wood-digesting invertebrates and barnacles
were well established after six weeks. The mechanism of
attachment of the bacteria and their role in the settlement of
the later organisms in the succession were investigated.
Cellulolytic bacteria were a major component of the
heterotrophic population.

Introduction
Since the growth of marine bacteria on solid surfaces
exposed to the sea was first recognized ( zoBell and
Allen, 1935), considerable work has been undertaken on
the microbial succession on immersed glass surfaces
(Corpe, 1970; Marshall et al., 1971; DiSalvo and Cobet,
1974) and other structural materials (O'Neill
and Wilcox, 1967; Dexter et al., 1975). Bacteria have been
implicated in primary film formation. Periphytic communities colonize these surfaces aided by
extracellular mucopoly-saccharides laid down by the
bacteria (Marshall, 1972; Fletcher and Floodgate,
1971; Tosteson and Corpe, 1975). With a wooden
surface, colonization by cellulolytic bacteria and fungi
can be expected within a heterotrophic succession
(Wood, 1967; Gordon et al., 1969). The role of
cellulolytic microorganisms in marine boring processes
remains problematic (Eltringham, 1971). This study was
conducted to determine the sequence of the microbial

succession on wood placed in a coastal environment in
order to gain better understanding of marine boring and fouling processes.

Materials and Methods
Exposure of wooden discs. Sugar pine sapwood discs
(dimensions 5 x 300 mm in diameter) were suspended
within the first 1 m of the water column in an
embayment adjacent to the Nova University
Oceanographic Laboratory, Fort Lauderdale, Florida
on December 10, 1975. Small portions of wood were
cut from the wood surface three, six and twelve weeks
after immersion. Wood samples were also placed in the
water during other visits to Fort Lauderdale. In early
January we investigated the microbial succession during
the first ten days of exposure to determine the sequence
of marine fouling. During the last week in February
wood discs were placed in the embayment to examine

1 Laboratory of Applied Microbiology Division of Engineering and Applied Physics Harvard University Cambridge,
Massachusetts 02138.

(Received April 1977)
Microbial succession on a wooden surface exposed to the sea

Anthony M. Cundell and Ralph Mitchell.

the initial attachment of bacteria to the surface after 16 and 36 hours in the sea water. To prevent the settlement of detritus and pennate diatoms, wood strips were included in a fine mesh nylon bag. Using this technique the nature and mode of attachment of individual bacteria could be seen when wood samples were examined with a scanning electron microscope.

Isolation of cellulolytic bacteria The presence of cellulolytic bacteria on the surface of the wood discs was confirmed by isolation in enrichment culture on a modified Kadota medium with filter paper as the sole carbon source (Kadota, 1956).

Scanning electron microscopy Sections of the wood cut from the discs were placed in 2.5% glutaraldehyde in 0.1 M phosphate buffer, pH 7.0, and conveyed to our laboratory. The wood sections were washed in phosphate buffer, post-fixed in 0.5% osmium tetroxide for one hour, washed with buffer, dehydrated by serial transfer through graded acetone solutions (25, 50, 75% and absolute acetone) and critical point dried. The dried material was mounted on stubs, coated with carbon, followed by palladium-gold alloy in a Hummer sputter coater and examined with an AMR electron microscope operating at 20 Kvolts (Cundell et al., 1976).

X-ray analysis: The presence of teredine borers and marine isopods attacking the wooden discs was demonstrated by x-ray analysis after six and twelve weeks exposure in the sea, using a Picker Industrial x-ray machine (two seconds exposure at 41 Kvolts and 50 milliamps).

### TABLE 1: Dominant periphytic organisms on the wood surface with increasing exposure time.

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<td>3</td>
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<tr>
<td>7</td>
<td>Bacteria and pennate diatoms</td>
</tr>
<tr>
<td>21</td>
<td>Cellulolytic bacteria, pennate diatoms, stalked diatoms, and amoeboid and ciliated protozoa</td>
</tr>
<tr>
<td>42</td>
<td>Cellulolytic bacteria, pennate diatoms, macroalgae, and molluscan wood borers</td>
</tr>
<tr>
<td>84</td>
<td>Cellulolytic bacteria, pennate diatoms, macroalgae, colonial protozoa, barnacles, molluscan wood borers and wood boring isopods.</td>
</tr>
</tbody>
</table>

Results

The microbial succession on wooden discs exposed at Fort Lauderdale for 12 weeks was determined using scanning electron microscopy. The dominant organisms colonizing the wood with increasing exposure time are listed in Table 1. Cellulolytic bacteria of the genera *Cytophaga* and *Pseudomonas* were routinely isolated from the discs after four days of immersion.

During the first day of exposure individual bacteria settled on the wood and laid down polymeric filamentous structures to anchor themselves to the surface. Bacteria tend to exploit the irregularities of the wood surface to colonize the wood. Populations of bacteria associated with detritus are clearly attached to particles by filaments or are embedded in a matrix of polymer (Paerl, 1973). Examples of bacteria attached to the wood surface after two days of immersion are shown in Figure 1.

After one week in the water, copious amounts of bacterial polymer covered the surface and pennate diatoms were common. Close inspection of wood surface suggested that the bacteria were perforating the wood. They were recessed into the surface and may have been exhibiting cellulolytic activity (Kohlmeyer, 1969). Thick layers of pennate diatoms, bacteria, and detritus developed on the surface after three weeks exposure (Figure 2). Stalked diatoms colonized the wooden discs and beneath the microbial film the wood was being eroded by cellulolytic bacteria. In some areas on the wood surface pennate diatoms showed signs of degradation. The absence of bacteria on the collapsing diatoms suggests that the process is one of autolysis.

The bacterial film was continually grazed by protozoa. Examples of amoeboid and ciliated protozoa were obtained from wood samples maintained in an aquarium for three weeks to illustrate the diversity of these grazing organisms (Figure 3).

Considerable honey-combing of the wood surface, probably by cellulolytic bacteria, occurred after six weeks in the embayment. Diatoms were lost from the wood surface and were being continuously replaced. The settlement of macroalgae, particularly *Ulva*, occurred at this time.

After twelve weeks a crust of partially digested wood was on the surface. When the wood sample to be prepared for scanning electron microscopy was cut from the wood disc, part of the surface was sheared, exposing the underlying wood tissue. Observation showed that cellulolytic bacteria had penetrated into the interior of the wood surface (Figure 4). At this time, marine invertebrates such as barnacle larvae had settled on the wood surface. A number of colonial protozoa of the genera *Vortilithium* and *Zoohamnium* (Figure 5) were observed in the vicinity of a barnacle. Limited signs of boring activity by the marine isopod *Liunoria triuncatata* were observed. X-ray analysis of the wooden discs revealed an extensive network of calcium carbonate lined burrows and individual marine borers of the genus *Teredo* (Figure 6).
Microbial succession on a wooden surface exposed to the sea

Anthony M. Cundell and Ralph Mitchell.

Figure 1 Initial settlement of bacteria on the wood surface immersed in an embayment at Fort Lauderdale, Florida.

a) Microcolony of short rod-shaped bacteria. Note the active division of individual bacteria, anchoring filaments and the neighboring stalked bacterium. 16 hours exposure. Marker indicates 1 um.

b) Rod-shaped bacteria exploiting irregularities in the wood surface for attachment. Note the anchoring filaments. 16 hours exposure. Marker indicates 1 um.

c) Bacteria enmeshed in copious amounts of polymer. 36 hours exposure. Marker indicates 1 um.

d) Bacteria associated with detrital material attached to the wood surface. Note the filaments binding bacteria to the detritus and the large spirillum. 36 hours exposure. Marker indicates 2 um.

DISCUSSION

The periphytic community on wood exposed to the sea developed according to the following succession: adventitious individual bacteria, bacterial microcolonies, pennate diatoms, ciliated protozoa, cellulolytic bacteria, stalked diatoms, sessile protozoa, macroalgae, and marine invertebrates such as barnacles, marine wood-boring isopods and mollusks. Similar patterns of colonization of surfaces exposed to the sea have been reported by earlier workers (Skerman, 1956; Wood, 1967; Floodgate, 1971). The absence of marine fungi colonizing the wood surface during the first twelve weeks should be emphasized. A number of reports in the literature maintain that the presence of cellulolytic fungi within the exposed wood is necessary for the nutrition of marine borers (Meyers and Reynolds, 1957; Becker, 1958). However, this study demonstrates that prior to fungal colonization of the wood surface, cellulolytic bacteria were actively degrading the wood and may play a more significant role in facilitating the penetration of marine borers into the wood than fungi.

The mode of attachment of the primary film-forming bacteria was apparent. The mechanisms included end and side-on attachment of an individual bacterium, the formation of stalks and the exploitation of irregularities in the surface to aid attachment. Production of bacterial polymers was used to anchor individual bacteria. Microcolonies of bacteria to the wood surface was noted during the first 16-48 hours.
Microbial succession on a wooden surface exposed to the sea. Anthony M. Cundell and Ralph Mitchell.

Figure 2 Attachment of bacteria and pennate diatoms to the wood surface.

a) Filaments associated with the bacterial colonization of the wood surface that aid attachment of detritus and diatoms. One week exposure. Marker indicates 5 µm.

b) Initial attack on the wood by cellulolytic bacteria. Note the bacteria are recessed into the wood surface. One week exposure. Marker indicates 1 µm.

c) Pennate diatoms attached to the wood surface. One week exposure. Marker indicates 5 µm.

d) Thick piles of diatoms and coccal bacteria. Three weeks exposure. Marker indicates 2.5 µm.

Although the bacteria may proliferate on the surface at the expense of absorbed dissolved and particulate organic matter, little or no growth of bacteria on the diatoms was observed. After the first week cellulolytic bacteria were seen embedded in the wood and the localized cellulase activity associated with each microorganism led to perforation of the wood fiber. The mechanisms of bacterial degradation of pine wood have been described (Koylmeyer, 1969). Deterioration of the wood cells begins at the lumina or along the rays with the bacteria attacking the cell wall. The tertiary wall (S₃) is penetrated, the secondary wall (S₂) which contains a preponderance of cellulose swells then disintegrates as the cellulolytic bacteria form cavities in the wall. Rupture of the remaining secondary wall (S₁), primary wall, and middle lamella gives the degraded wood a swiss-cheese like appearance when viewed with a scanning electron microscope. Similar perforation of cellulose membranes has been described by Sieburth (1975).

The settlement of pennate diatoms on the wood surface occurred after the first 48 hours exposure. Wood (1967) has noted that the diatom fouling surfaces are not commonly found in plankton tows and are also found on macroalgal surfaces. Ciliated protozoans appear early in the microbial succession but may be lost during the preparation of the wood sample for scanning electron microscopy. Stalked protozoans like Vorticillium and Zoothamnium probably graze the water column and not the wood surface.

The piles of bacteria and pennate diatoms on the wood surface after three weeks exposure to the sea resembled the microbial film that develops on well-aerated trickling filters (Mack et al., 1975). The microorganisms probably are exploiting the
concentration of dissolved organic matter at the wood surface while the diatoms contribute to the maintenance of the oxygen level within the film and many obtain vitamin B₁₂ from the bacteria (Haines, 1974). The film is detached and replaced continuously from the wood surface.

The interaction between the microflora on the wood surface and marine borers remains undefined. The arguments contained in the literature on the one hand supporting the nutritional role of marine fungi and on the other hand maintaining fungal colonization of the wood is unnecessary for borer attack have been reviewed by Eltringham (1971). Work is continuing in our laboratory to determine the influence of prior colonization of wood samples with pure cultures of cellulolytic microorganisms and treatment with cellulase on the rate of settlement and boring activity of marine borers such as the isopod Limnoria and Teredo.

Acknowledgements

This investigation was supported by the Office of Naval Research, Contract No. N00014–76–C–0042.

The authors thank Mr. Edward Seling of the Scanning Electron Microscopy Laboratory.

References


Microbial succession on a wooden surface exposed to the sea

Anthony M. Cundell and Ralph Mitchell

Figure 4 Degradation of the wood after 12 weeks of exposure in the sea.

a) Wood surface showing exposed subsurface. Marker indicates 25 um.

c) Subsurface showing cellulolytic bacteria perforating the interior of the wood. Marker indicates 2 um.


b) Degraded outer surface colonized by pennate diatoms. Marker indicates 5 um.

d) Stalked protozoans of the genera Vorillium and Zoohamnium growing in the shelter of a barnacle larva. Marker indicates 25 um.


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THE INFLUENCE OF WATER ACTIVITY AND OXYGEN TENSION UPON THE SURVIVAL OF ASPERGILLUS AND PENICILLIUM SPECIES ON TABLETS

A.R. Fassihi1 and M.S. Parker1

Summary. Tablets were prepared from sterile raw materials. Standard inocula of spores of Aspergillus niger and Penicillium species were added to tablets which were equilibrated with water vapour at 25°C in air and under vacuum. The effect of oxygen tension was examined by equilibrating tablets in various oxygen-nitrogen mixtures. The results indicate over 90% loss in viability when tablets were maintained at aw below 0.44. Tablets stored under vacuum showed a rapid fall in viable count at all aw levels comparable to that seen at low aw levels in air. Tablets stored at low oxygen tensions (less than 6%) and high aw (0.94) showed above 95% loss in viability in four weeks. The general moisture sorption isotherm has been determined as a reference for water content of tablets.

Introduction

In tablet preparation the active ingredients are usually mixed with diluent such as lactose, a binder, commonly a gum, a disintegrant, usually starch and a lubricant, magnesium stearate. These materials will, under the appropriate conditions of water activity, temperature and oxygen tension readily support the growth of microorganisms. There are many reports of organisms surviving on tablets and the conditions during the granulation process could be conducive to microbial proliferation. The raw materials may themselves be contaminated with bacterial and fungal spores and a variety of vegetative cells. Thus Pedersen and Ulrich (1968) examined 226 batches of 84 different pharmaceutical raw materials used in tablet making and reported that synthetic or semisynthetic materials had microbial counts of less than 100/g, whereas products of plant and animal origin often contained much larger numbers, e.g. 33 batches were contaminated in the range of 103 to 104 organisms/g. Fischer et al. (1968) found that contaminated tablets had counts ranging from 100-300 per tablet, whilst White and Bowman (1968) reported even higher levels of contamination in some antibiotic containing tablets.

The significance of such contamination as a health hazard was stressed by Kallings and his colleagues.

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(Received April 1977).
The influence of water activity and oxygen tension upon survival of *Aspergillus* and *Penicillium* on tablets. A.R. Fassihi and M.S. Parker.

(1966) in their report upon salmonellosis originating from tabletted raw materials. It is known that tablets of a very low microbial count can be produced using raw materials of good quality, and a carefully controlled manufacturing process. The problem which then remains is the maintenance of these tablets free from contamination or, if contaminated, in conditions to minimize microbial growth. To investigate this problem, tablets manufactured from sterile raw materials under carefully controlled conditions, including the use of sterile punches and die, were contaminated with spores of *Aspergillus niger* and *Penicillium* species, and viability determined under various conditions of water activity and oxygen tension.

Materials and Methods

The tablet ingredients (Lactose powder, Acacia, Potato starch and Magnesium stearate) all of B.P. quality, were sterilised in an ethylene oxide chamber (Getinge Automatic Autoclave model 22447) (12 hours exposure at 45° and 65% R.H.). A sieve of 16 mesh was used to prepare granulate for preparation of tablets on a single punch tabletting machine, (Manesty type F3) fitted with sterile 12.5 mm flat punches. The sieve, hook blade mixer (Kenwood Major A707), hopper, etc., were sterilized by dry heat. The tablet ingredients (Lactose powder, Acacia, Potato starch and Magnesium stearate) all of B.P. quality, were sterilised in an ethylene oxide chamber (Getinge Automatic Autoclave model 22447) (12 hours exposure at 45° and 65% R.H.). A sieve of 16 mesh was used to prepare granulate for preparation of tablets on a single punch tabletting machine, (Manesty type F3) fitted with sterile 12.5 mm flat punches. The sieve, hook blade mixer (Kenwood Major A707), hopper, etc., were sterilized by dry heat. The concentration of oxygen in the storage atmosphere was measured by gas liquid chromatography (PYE Panchromatograph). Final composition of tablets was Acacia 5%, Starch 5%, Magnesium Stearate 0.5% and Lactose to 100%.

**Preparation of spore suspensions**

Plate cultures were prepared of *A. niger* and *Penicillium* sp on malt extract agar at 25°. After 21 days incubation, spores were washed from cultures with sterile buffer, pH = 7, (Buhlmann, 1968) containing 0.01% v/v Tween 80 as a wetting agent. The spore suspension was filtered through a No. 3 sintered glass filter to remove clumps (Gerard et al 1960) and centrifuged (6000 R.P.M. for 20 minutes) washed with buffer, re-centrifuged and re-suspended in 10 ml sterile buffer. Suspensions were freshly prepared for these experiments but were stored for a short time in a refrigerator at 4°. They were standardised to contain *A. niger* 8 x 10^6 and 1.75 x 10^6 spores per ml, *Penicillium* 1.425 x 10^7 and 4 x 10^6 spores per ml.

**Experimental procedure**

Granules were prepared with aseptic precautions in a room supplied with filtered air using acacia solution (5% w/v) in sterile water as granulating liquid added to lactose powder, with mixing continued until a uniform dispersion was attained and the binders activated. The mass was discharged through a sterile 16 mesh screen and wet granules were dried in a hot air forced convection type oven at 50°C for 6 hours. Potato starch (5% w/w) and disintegrant and magnesium stearate (0.5% w/w) as lubricant were added and mixed with the granules in a sterile glass tumbler mixer. Tablets prepared aseptically under low applied pressure (54 MN m^-2) were inoculated with the standardised spore suspension (0.02 ml per tablet), placed in sterile petri dishes in desiccators or a special container (fig. 1) for storage. Three conditions of storage were examined:

1. Desiccators containing normal air.
2. Desiccators evacuated.
3. Containers (fig. 1) filled before being sealed with various mixtures of oxygen and nitrogen to give a range of oxygen concentrations from approximately 1% to 20%. The containers were designed to facilitate gas chromatography determinations and be suitable for transit to the equipment used.

Water activities in the desiccators were controlled by including sulphuric acid solutions of given strengths as described by Robinson and Stokes (1959). In series 1 and 2 the water activities thus produced were in the range of 0.96 to 0.12. In addition a_w = 0.0 was produced by inclusion of anhydrous P_2O_5 in one desiccator in each series. In series 3 all containers contained sulphuric acid solution of a strength to produce a_w = 0.94.

Survival of the spores on the tablets in all three series was determined by removing tablets periodically, dissolving in sterile buffer, pH = 7, and plating on to malt extract medium. Colonies were counted after the plates had been incubated at 25°C for five days. Three to five replicates were counted and the coefficient of variation ranged from 2.4 to 9.2.
The influence of water activity and oxygen tension upon survival of *Aspergillus* and *Penicillium* on tablets.

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The general moisture sorption isotherm for a tablet was determined by placing tablets prepared at low (54 MN m⁻²) and high (86 MN m⁻²) applied pressure in an oven at 100°C for 4 hours, and then further drying over *P₂O₅* under vacuum at room temperature to obtain a minimum residual water content. (Tablets were initially oven dried to remove water from capillaries. Although pore size distribution in tablets is not known it is likely that at aₕ below 0.5 most of the water is present in capillaries of < 100Å diameter). Tablets thus obtained were stored at different water activities as described above. Equilibrium was considered as reached when constant weight was obtained. Weighings were continued for 10 days in some instances and constant weight was always found to be established within four days. The amount of sorbed water vapour was measured by drying the tablets over *P₂O₅* at room temperature until constant weight was obtained (Tables 1 and 2).

Results

Results are shown in figures 2–7.

Although there are some differences in resistance between *A. niger* and *Penicillium* sp in that the former is the hardier organism, the response to imposed environmental conditions was similar. In air there was a decrease in viability of the spores on the tablets as aₕ levels were decreased such that storage below aₕ 0.44 was lethal to *Penicillium* within some 4 weeks, depending upon the level of counts achieved (figures 2, 3). This effect was enhanced for tablets stored under vacuum with counts falling more rapidly (figures 4, 5).

When tablets were stored at reduced oxygen levels the lethal effect was comparable to that achieved by conditions of low aₕ (figure 6).

The general moisture sorption isotherm for a tablet is shown in figure 7 which can be considered as a reference curve for the water content of tablets. The curves may be divided into two areas. The upper part of the curves (B) includes an aₕ close to 1.0 over a wide range of moisture contents which support microbial growth. The lower part of the curves (A), that is aₕ less than 0.5, corresponds to conditions in which tablets will support little or no microbial growth.

Discussion

The storage conditions for the tablets as they determine water availability and oxygen tension are critical to the survival of microbial contamination. Bacteria require relatively high levels of aₕ (above 0.90) for their growth and therefore, play a minor role in spoilage of tablets. At aₕ levels of between 0.80 and 0.85 spoilage occurs readily by a variety of fungi. Below this level microbial spoilage is unlikely. In practice spoilage due to moulds is usually found as surface growth, and in general, storage conditions which allow the marked growth of moulds upon tablets are associated with definite changes in their water vapour sorption.

In region (A) of the moisture sorption isotherm water is tightly bound or unavailable for reaction. In region (B) water is relatively free for reaction and hence microbial

Table 1

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<th>% moisture content (mg)</th>
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* Each figure is mean of FIVE tablets
The influence of water activity and oxygen tension upon survival of *Aspergillus* and *Penicillium* on tablets. A.R. Fassihi and M.S. Parker.

**Figure 2** Effect of $a_w$ at 25°C upon survival of *A. niger* on tablets.

**Figure 3** Effect of $a_w$ at 25°C upon survival of a *Penicillium* species on tablets.

**Table 2**
The percent moisture content of tablets prepared at (86 MNm$^{-2}$) at various $a_w$ levels

<table>
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* Each figure is mean of FIVE tablets
The influence of water activity and oxygen tension upon survival of *Aspergillus* and *Penicillium* on tablets.

A.R. Fassihi and M.S. Parker.

Figure 4: Effect of $a_w$ at 25°C upon survival of *A. niger* on tablets under vacuum.

Figure 5: Survival of *Penicillium* sp on tablets maintained at different $a_w$ levels under vacuum at 25°C.

Figure 6: Effect of oxygen tension upon survival of *A. niger* (O) and *Penicillium* species (O) on tablets stored for 4 weeks at 25°C and $a_w = 0.94$.

Figure 7: The general moisture sorption isotherm for a tablet (Each point is mean of 5 tablets)
- Tablets prepared at 54 (MNm$^{-2}$)
- Tablets prepared at 86 (MNm$^{-2}$)
The influence of water activity and oxygen tension upon survival of *Aspergillus* and *Penicillium* on tablets. A.R. Fassihi and M.S. Parker.

Proliferation. Applied pressure on tablets has an effect upon moisture adsorption, the higher the compaction pressure the lower vapour adsorption into the tablet probably due to the reduction of size and number of capillaries in the mass of tablets.

The observed effects of storage conditions upon microbial growth upon tablets suggests that isotherm changes could be used as a predictive index of the stability of tablets to spoilage.

As practical measures in the packaging of tablets the use of desiccants and containers impermeable to air will afford protection by maintaining low $a_w$ level even in multidose containers, provided resealing is efficient. In the case of single dose containers vacuum packs allowing storage at low oxygen tensions will give good protection.

Acknowledgement

The authors are grateful to Upjohn Limited, Crawley, U.K., for the ethylene oxide sterilization facilities.

References


THE CELLULOLYTIC ACTIVITY OF SOME INTESTINAL BACTERIA OF TERMITES

O. Krelinova¹, V. Kirku¹, and J. Skoda²

Summary. Bacteria associated with the hind-gut of eleven termite species were isolated and screened on the basis of their enzymatic capability to degrade and utilise native cellulose when grown on a selected medium in pure cultures. Although all the species of termite tested possess cellulose degrading hind-gut bacteria, there are differences in the activity of individual bacterial strains and in the dependence of their ability to decay cellulose on the conditions of their cultivation. A common morphological form of the strains isolated is the coccus. In almost all cases the isolated bacterial strains are Gram-negative.

Introduction

Cellulose is probably the most abundant organic compound in nature. For this reason it is highly important to open a new approach to the utilisation of large quantities of cellulose contained in domestic and agricultural wastes, cardboard, newspapers, etc. Saccharification of these materials with the intention of producing sugar at low cost in naturally a very desirable objective. The practical saccharification of waste cellulose requires a stable cell-free enzyme preparation with adequate levels of all essential components of a cellulose complex (Mandels, 1975), with good stability and activity at high temperatures, and with the ability to attack substrates that have not been milled or chemically pretreated. Some fungi and bacteria have been reported to produce high levels of cellulose complex and many laboratories have aimed at obtaining microbial strains producing cell-free cellulolytic systems of this character.

The purpose of our study was to isolate and screen the hind-gut bacterial microflora of different termite species on the basis of their enzymatic capability to digest and utilise native cellulose when grown on a selected medium in pure culture. In this the prime objective was to examine the possible industrial value of these organisms, not to elucidate their role in the living termite.

The hindgut paunch of some termite species is inhabited by a variety of different microorganisms of which the protozoa are the best known. The bacteria can live both intracellularly (within protozoa) and free in the gut fluid. The termite species compared in this study were selected on the basis of their taxonomy in relation to the expected free bacterial endosymbiotes. The significance of symbiotic organisms in termite digestion has already been investigated by many workers. Experimental data, however, are still scanty and controversial (Beckwith and Rose, 1929; Dickman, 1931; Mannesmann, 1969, 1972; Krelinova et al., 1977).

(General reviews on this subject: Wilke, 1975; Bevers, 1976).

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²Institute of Organic Chemistry and Biochemistry, Czechoslovak Academy of Sciences, 166 10 Prague, Czechoslovakia.

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The cellulolytic activity of some intestinal bacteria of termites. O. Krelinova, V. Jirku and J. Skoda.

Materials and Methods

The choice of the particular families of termites studied was made on the basis of information available that species in the families Kalotermidae and Rhinotermitidae harbour protozoa in the digestive tract. However, the presence of a bacterial microflora has not been confirmed. In addition, species of the family Termitidae harbour microflora only in the digestive tract (Honigberg, 1970).

The termite species finally chosen for isolation of the hind-gut bacteria, and their origin in collection, were:

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<td>Rhinotermitidae</td>
</tr>
<tr>
<td>Neotermes castaneus</td>
<td>Oct. 1973 Cuba</td>
<td>Kalotermidae</td>
</tr>
<tr>
<td>Neotermes jouteli</td>
<td>Sept. 1973 Cuba</td>
<td>Kalotermidae</td>
</tr>
<tr>
<td>Cryptotermes canifrons</td>
<td>Dec. 1972 Cuba</td>
<td>Kalotermidae</td>
</tr>
<tr>
<td>Nasutitermes costalis</td>
<td>Dec. 1964 Cuba</td>
<td>Termitidae</td>
</tr>
<tr>
<td>Proritillotermes junciferus var. sanmuensis</td>
<td>Aug. 1962 France</td>
<td>Rhinotermidae</td>
</tr>
<tr>
<td>Incestitermes schwarzi</td>
<td>Jan. 1973 Cuba</td>
<td>Kalotermidae</td>
</tr>
<tr>
<td>Neotermes sp.</td>
<td>Aug. 1963 Cuba</td>
<td>Kalotermidae</td>
</tr>
<tr>
<td>Neotermes sp.</td>
<td>Oct. 1963 China</td>
<td>Kalotermidae</td>
</tr>
<tr>
<td>Neotermes bosei</td>
<td>Dec. 1968 India</td>
<td>Kalotermidae</td>
</tr>
</tbody>
</table>

The termites listed above were obtained from the collection of the Institute of Entomology, Czechoslovak Academy of Sciences. In all tests individuals of the workers' caste were used.

The guts from the termites under study were removed by using sterilised entomological tweezers. Before this procedure the host's body surface was sterilised by UV light because this investigation was concerned with the digestive tract flora only and contamination with surface microflora could not be allowed. The UV irradiation (60 seconds) was done at a distance of 300 mm from the centre of the source (germicide tube, Philips TUV 15 watt at an intensity of 5 x 10^-7 J/sec). Immediately after the UV irradiation, the guts were removed and transferred into the cultivation media. The aim was to obtain the hindgut, which is always well developed. It was removed together with the midgut and foregut, the whole procedure being done under low magnification.

The following media were used for cultivation of the isolated bacteria. Quantities in grams per litre of distilled water.

| Medium A | KH₂PO₄ 0.136; (NH₄)₂SO₄ 2.0; MgSO₄ 0.2; |
| Medium B | KH₂PO₄ 1.0; NH₄Cl 1.0; MgCl₂·6H₂O 0.2; NaCl 5.0 |

1 ml of solution TES was added to 1 litre of medium B

T | FeCl₃·6H₂O 2.7; H₃BO₃ 0.1; ZnSO₄ 0.1; Co(NO₃)₂·6H₂O 0.05; CuSO₄ 0.005; MnCl₂ 0.005. The pH was adjusted to 7.0

The isolated culture was applied to agar plates containing all components of medium A or B in the concentrations shown and 2% Oxoid Agar No 3 (agar base only).

Cultivations in liquid media were carried out in a water-bath shaker. Anaerobic cultivations were carried out under sterile paraffin oil.

The examination of growth of isolated bacterial populations was done either by measuring optical density in a Spekol photo-colorimeter at 465 nm or by the determination of dry weight.

The total reducing power was determined by the Nelson-Somogyi method, (Nelson, 1971; Somogyi, 1952). The degradation of native cellulose was also followed by using a simple dye-release method. The blue dye (Remazol Brilliant Blue) which is bound to some of the free hydroxyl groups of cellulose polymers, is released by enzymatic solubilisation of this dye-cellulose conjugate. The level of released dye was determined photometrically at 595 nm. Cellulose-Azure (Calbiochem) was used as solid substrate in this method.

Cellulose substrates used in other experiments were: microcrystalline cellulose for chromatography (Whatman), pure paper wool, and soluble carboxymethyl cellulose (CMC).

Proteins were determined by the method of Lowry and coworkers (1951).

A heat-fixed smear of isolated bacterial population was stained by using Rapid Staining Kit for bacteriological staining according to the method of Gram.

Photography through the light microscope was done by phase contrast with microscope NU 2 Zeiss equipped with a camera.

Experiments and Results

1. Microflora of the digestive tract of Copotermes formosanus and Nasutitermes costalis.

The digestive tracts removed from these termites were transferred aseptically in 3 ml of the media A and B, each containing one of the following carbon sources:

<table>
<thead>
<tr>
<th>Carbon Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose (0.3% w/v)</td>
</tr>
<tr>
<td>Cellobiose (0.3% w/v)</td>
</tr>
<tr>
<td>Carboxymethylcellulose (0.3% w/v)</td>
</tr>
</tbody>
</table>

or one of the combinations:

<table>
<thead>
<tr>
<th>Component</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose (0.3%) + yeast extract (Difco) (0.03%)</td>
</tr>
</tbody>
</table>
The cellulolytic activity of some intestinal bacteria of termites. O. Krelinova, V. Jirku and J. Skoda.

Carboxymethylcellulose (0.3%) + yeast extract (Difco) (0.03%)
Celllobiose (0.1%) + Whatman filter paper No. 1 (20 mm strip)

Cultivation was carried out under aerobic and aerobic conditions.

In this experiment the time to attain the biomass corresponding to optical density 0.08 was determined during stationary cultivation at 30°C. The digestive tracts of both these termites contain bacterial strains which can grow and multiply in all conditions of cultivation under study. However, differences in the time of attaining any given value of optical density which were observed indicates that the growth of the microflora isolated is, in some combinations of C-sources, dependent on the manner of the cultivation and on the composition of the medium used. The most intensive growth and cell division on media A and B was found in the presence of glucose and yeast extract. It was found that the cells isolated were able to grow and multiply on the medium containing CMC and the combination of celllobiose-Whatman paper No. 1, respectively, which indicates that the bacteria isolated can synthesise cellulase complex or at least some of its components.

2. The ability of the microorganisms isolated from Nasutitermes costalis and Coptotermes formosanus to degrade native cellulose

100 ml of the medium B containing 0.15 g of Cellulose-Azur was inoculated with a cell population obtained by cultivation of the microflora isolated in the medium B which was supplemented with CMC up to a final concentration of 0.3%. During subsequent cultivation at 30°C under aerobic conditions the biomass concentration (measured as turbidity) and A₅₉₅ absorption were measured at 24 hrs. intervals (Fig. 1).

Because both determinations were carried out on the same sample, the individual samples were filtered by suction on Synpor 6 membrane filter and the A₅₉₅ absorption was measured on the filtrate obtained. Optical density (turbidity = biomass) was measured on the suspension of cells which were quantitatively washed away from the surface of the membrane filter and suspended in the original volume of the sample.

---

**Fig. 1.** Degradation of Cellulose-Azur in cultures of bacterial strain isolated from Nasutitermes costalis (1, 2) and Coptotermes formosanus (3, 4).

*△* absorbancy at 595 nm (Remazol brilliant blue);

*○ △* absorbancy at 465 nm (turbidity of cells).
The cellulyotic activity of some intestinal bacteria of termites. O. Krelinova, V. Jirku and J. Skoda.

In the cultures of the micro-flora isolated an increase in the values of A₅₉₅ absorption was found against a background of different growth of the biomass concentration (Fig. 1). These results imply that the microorganisms isolated have an ability to degrade native cellulose. The character of the time course of change in A₅₉₅ absorption values which was found, against the background of linear growth of the biomass concentration in the culture of the bacterial population isolated from the digestive tract of Nasutitermes costalis, indicates the possibility of a production of an extra-cellular cellulase complex. On the other hand, a small capacity to grow and multiply is evident during the cultivation of the microflora isolated from Coptotermes formosanus. In this culture, after a certain period of cultivation, cell lysis becomes evident. As seen from figure 1, the drop in the total number of cells (turbidity) precedes the increase in A₅₉₅ absorption values. This situation indicates that the degradation of cellulose-azure could be caused by cellulase complex which is released from the cells owing to their lysis.

In the light of this, the ability of the isolated micro-flora to grow and multiply on the surface of native cellulose was studied. In an agar layer containing the components of medium B, pure paper wool was fixed in such a way as to restrict the growth and cell division in a spread inoculum of isolated microorganisms to the area of direct contact of cells with native cellulose. The inoculum was prepared by cultivation of the isolated microorganisms in medium B containing CMC. The agar plates were incubated for 24 hours at 30°C.

The positive results of this experiment (Fig. 2) confirmed that the microorganisms isolated from the digestive tract of both termites under study can grow and multiply on the surface of native cellulose if the components of the medium B are available.

3. The ability of microorganisms from other species to degrade native cellulose

On the basis of the foregoing results the bacterial microflora from the digestive tracts of the other nine species of termites was isolated. In isolating the microflora from these species we have used for the multiplication of bacterial cells medium B supplemented by CMC up to a final concentration of 0.3% only. Cultivations were done under both aerobic and anaerobic conditions but for all subsequent experiments, and for single colony isolations, only cultures obtained by cultivation under aerobic conditions were used.

By multiplication of the bacterial population which is a part of the content of the digestive tract a mixed culture of bacterial strains is probably obtained. For this reason we isolated pure cultures. Isolation of single colonies was combined with selection based on the ability of the microflora to multiply on the surface of an agar layer containing the components of medium B and microcrystalline cellulose as a single C-source. In this way we have obtained a large number of pure cultures of the intestinal bacterial of all the eleven species under study. The strains obtained have been maintained on agar slants containing components of medium B and microcrystalline cellulose at 4°C and also at room temperature. In addition, the capacity of all isolates to multiply on the surface of paper wool fixed in agar (as described above) has been confirmed. From the results we can conclude that individual strains are different as far as the intensity of biomass growth and the morphology of colonies are concerned.

4. The ability of isolated strains to degrade native cellulose in liquid medium.

The ability to grow and multiply in a liquid medium has been studied in cultures of eleven strains which represent the isolated microflora from the eleven species of termites. These strains were cultivated in the liquid medium B containing microcrystalline cellulose (0.5% final concentration) as a single C-source. During cultivation the time course of change in the total reducing power and the change in the total extra-cellular protein level were studied in cell-free filtrates of samples taken for the determination of the total dry weight.

If we consider the graphical illustrations of the results obtained on cultures from three species of termites (Figs. 3–5) on the basis of the presumption that an increase in the total dry weight proves the capability of the strains studied to degrade and utilize cellulose, it may be seen that this ability was confirmed in each case. However, the growth of total dry weight ceases after approximately 4–7 days and in some cases a decrease in total dry weight was found during subsequent cultivation. The time course of change in the total protein level is very similar to that of dry weight.

![Fig. 2. The growth of a bacterial strain isolated from the digestive tube of Nasutitermes costalis on the surface of paper wool fixed in agar layer containing components of the medium B.](image-url)
The cellulolytic activity of some intestinal bacteria of termites. O. Kreinova, V. Jirku and J. Skoda.

Fig. 3. Degradation of cellulose in the culture of bacterial strains isolated from Neotermes castaneus.

- dry weight;
- extracellular protein;
- reducing sugars.

Fig. 4. Degradation of cellulose in the culture of bacterial strain isolated from Proctotermes simplex.

- dry weight;
- extracellular protein;
- reducing sugars.

Fig. 5. Degradation of cellulose in the culture of bacterial strain isolated from Neotermes sp. - China.

- dry weight;
- extracellular protein;
- reducing sugars.

On the other hand, the time course of the change in the total reducing power follows no consistent pattern. From this point of view, all the strains under study can be sorted into three groups. Figures 3 - 5 demonstrate the three types found. In the first group, i.e. the strains isolated from Reticulitermes lucifugus, Neotermes castaneus, Neotermes jouteli, Neotermes Sp. (Cuba), Nasutitermes costalis and Coptotermes formosanus, the time course of change in total reducing power is identical with that of dry weight and protein (Fig. 3). In the second case, i.e. strains isolated from Proctotermes simplex and Incisitermes schwarzii, a drop in the level of reducing power precedes its increase (Fig. 4). The course thereafter is seen to be similar to that of the first case. In the third case, i.e., strains isolated from Cryptotermes cavifrons, Neotermes Sp. (China), and Neotermes bosei, the change in total reducing power is not appreciable (Fig. 5).

5. The effect of the composition of the culture medium on the cellulolytic activity of the isolated strains.

The ability of the isolated strains to degrade native cellulose and to use the products of this degradation has been studied in relation to the composition of the medium B containing cellulose as sole C-source. In this experiment the concentration of individual components was reduced or some components were omitted. The growth requirements were assayed by the auxanographic plate technique. The agar plates were incubated for 30 hrs. at 30°C.
The cellulolytic activity of some intestinal bacteria of termites. O. Krelinova, V. Jirku and J. Skoda.

The results of this experiment showed that the ability of the strains under study to grow and multiply, which is solely due to their cellulolytic activity, is dependent on the presence of one or a few trace elements of the TES complex. On the other hand, a reduction of the concentration of P- or N-source, or the omission of Na+ and Mg++ ions does not result in any change of the ability of these strains to grow on native cellulose. It became evident that the strains isolated could be distinguished on the basis of their sensitivity to the changes in the composition of the medium used into strains relatively sensitive and strains relatively insensitive. The sensitive strains are those which were isolated from Incisitermes schwarzi, Neotermes Sp. (China) and Coptotermes formosanus.

6. Characteristics of the isolated strains

The Gram reaction of each of the isolated strains has been identified. With the exception of the strains isolated from Coptotermes formosanus which are Gram-positive, all the isolated strains are Gram-negative.

Microscopic inspection of cell population of the individual strains reveals that a common morphological form of a cell is coccus or a very short rod. These rod-shaped cells are suggestive of the morphological form referred to as coccobacilli. (See for example, Davis et al 1969, p.24).

Discussion

Although attempts have been made to demonstrate the mutualism of termite and its intestinal symbiotes: protozoa and bacteria (e.g. Mannesman, 1972), no exact information is available on the part played by cellulolytic activity. In view of the host-symbiote relationship it seems possible that protozoa themselves can produce complete or incomplete cellulase complex or an activator of an enzymatic bacterial symbiote. The presence of protozoa can also be important for space localisation of cellulolytic bacterial microflora and thus for more intensive local production of cellulose complex and vice versa. The fact that protozoa harbour bacterial cells supports such a notion. On the other hand, from some results it can be concluded that the role of symbiotes in the process of degradation of cellulose is marginal only and the termite itself is a producer of cellulase complex (Potts and Hewith, 1973) or at least some of its components (Retief and Hewith, 1973; Potts and Hewith, 1972; Hewith et al., 1974).

The isolations performed in the present work have confirmed the presence of a bacterial microflora in the digestive tract in all of the chosen species of termites. The ability of the isolated strains to grow and multiply on the surface of native cellulose confirms the ability of these strains to synthesise a cellulase complex containing the C_1- and C_2- components. In the light of these results it may be stated that the bacterial microflora of the digestive tract of all species of families under study includes strains which are able to degrade and utilise native cellulose when grown on a selected medium in pure culture.

However, a comparison of the ability of the isolated strains to grow and multiply in liquid and solid medium indicates that a fixed contact between cell and cellulose is advantageous to effective cellulolysis.

In the cultures growing in a liquid medium consisting of inorganic compounds and cellulose a decrease in the growth of total dry weight was found after some days of cultivation. The same was found in the time course of the change in the total extra-cellular protein level.

The unexpected drop in the level of total dry weight is generally explicable by cell lysis or by appreciable degradation of insoluble native cellulose. In this connection we could expect that the cell lysis will result in an increase in the total extracellular protein level. Similarly, a breakdown of native cellulose would result in an increase in the total reducing power of the cell-free medium used. On the other hand the observed decrease in extracellular protein level can be explained by quick proteolysis or by a linkage of proteins to an insoluble component which is separated when the cell-free filtrates are prepared. It is impossible to derive more precise conclusions from the results obtained. However, it may be stated that in seven out of eleven cultures of strains under study the drop in the total dry weight is accompanied by an appreciable increase in the total reducing power.

The study of the effects of changes in the composition of the medium on the ability to utilize cellulose has revealed that cellulolytic activity is dependent on the presence of a trace element or elements. In view of these results it is appropriate to make reference to the fact that in the bodies of some termites a high concentration of zinc has been found (Abushama and Kambal, 1976) and zinc enzymes have been described (Valee, 1976).

In spite of the fact that the light microscope reveals a more or less spherical form of cell and in almost all cases the isolated strains are Gram-negative, the isolated microflora is probably not identical taxonomically.

From the above results it can be concluded that in the digestive tract of all termites under study the cellulolytic activity is fully or partly conditioned by the presence of bacterial strains. In view of the fact that bacterial strains are technically favourable organisms for any industrial application, the strains which have been isolated will be studied in regard to the regulation of the synthesis and activity of the individual components of the cellulase complex, as well as from the aspect of physiological properties.

References

Abushama, F.T. and Kambal, M.A. (1976). The role of
The cellulolytic activity of some intestinal bacteria of termites. O. Krelinova, V. Jirku and J. Skoda.

...
Evaluation of fungicidal activity of volatile compounds in a Warburg apparatus

B.J. Rytych and B.J. Zyska

Summary. A modification of a manometric method for evaluation of fungicidal activity of volatile compounds is outlined and compared with the Findlay-Vernon method. The fungicidal activity of seven volatile compounds and 1:1 and 1:1:1 mixtures of some of them are briefly discussed.

Introduction

Several techniques for testing of volatile fungicides have been published (Findlay and Vernon 1951, Mateus 1957, Nemcova 1962, Cobb, Kstic and Zavarin, 1968, Strzelczyk 1968), however all these techniques require considerable expenditure of time and effort.

Moreover results achieved are of limited value since they provide information only for activity of single species of fungi and not for the whole microflora colonizing a given material. These techniques are based on subjective criteria which rely ultimately on visual evaluation of growth of the test fungus. For developing methods of preventing microbial deterioration of electro-insulating materials in coal mines there was a need for a quick and accurate method for evaluating the fungicidal activity of volatile compounds.

In many cases good results have been obtained using a manometric technique measuring the respiration of fungi and bacteria responsible for the deterioration of materials in order to evaluate the microbicidal or microbistatic activity of chemical compounds or for assaying microbiological susceptibility and microbicistatics of materials (Baker et al. 1941, Ordal and Borg 1942, Sevag and Ross 1944, Siu 1951, Darby 1958, Bornside et al. 1964, Burgess and Darby 1964, van der Toorn, Adorna and Hueck 1965, Harris 1968, Zyska, Rytych, Bozena, Zankowicz and Fudalej 1972, Smith 1975). A modification of a manometric method was developed for evaluation of fungicidal activity of volatile compounds in a Warburg apparatus and results were compared with those found using the Findlay-Vernon method (1951). The modified method is based on the assumption that for preventing microbial colonisation and deterioration of electro-insulating materials, the volatile compounds should comply with the same requirements as for soil fungicides. It was taken that if the volatile compound is toxic for the soil microorganisms in a layer of soil it will be even more effective as a fungicide for the electrical equipment used in coal mines.

Experimental

The soil was prepared according to the requirements laid down for the soil-burial test (PN-63/P-04731, DIN 53933) and screened on a DIN 1171 sieve with mesh 2.0 mm. Seventeen and a half grams of screened soil was added to each Erlenmayer 500 ml flask and wetted with 0.9 ml of Czapek medium without agar. The contents were accurately mixed with a glass rod. The pH of the soil was established at 4.1 by addition of the necessary quantity of 30% lactic acid, chemically pure, and determined by the method of Bralewski (1968).

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(Received April 1977).
In the centre of the flask bottom a 5 ml weighing bottle with 3 ml of water was placed to ensure 100% relative humidity of the air throughout the test. A piece of filter paper treated with a known quantity of the fungicide was hung in each Erlenmeyer flask. The flask were closed with rubber stoppers, sealed outside with polyethylene sheathing, and then kept for 6 days at 30°C. For evaluation of the effect of volatile fungicide on the respiration of soil microorganisms, at the end of this period 2.5 g of moist soil was weighed into each of 4 Warburg bottles and the respiration of soil microorganisms was measured in the Warburg apparatus four hours daily during two consecutive days. In each of the samples the amount of dry soil was determined. For CO₂ adsorption 0.5 ml of 30% KOH was added to the centre well with a filter paper wick. The measurements were conducted at 30°C, with a shaking speed of 54 oscillations per minute. On the second day of measurements vessels were aerated in the morning for 15 minutes. The oxygen uptake was measured in microlitres per hour and calculated for 1 g dry-weight of soil. Taking the respiration of the control sample as 100%, decrease in respiration of soil microorganisms to below 10% was taken as the criterion of effective fungicidal activity of the volatile compound.

The evaluation of the fungicidal effect of the volatile compounds on fungi colonizing the electro-insulating materials was conducted by the Findlay-Vernon method (1951). For these tests strains isolated from electro-insulating materials at the Prezydent coal mine were used, i.e. Aspergillus niger strain No. 110, A. ochraceus strain No. 36, A. ustus strain No. 33, Cephalosporium sp. strain No. 33a, Gliocladium roseum strain No. 93, Gliocladium chrysogenum strain No. 150, Penicillium stoloniferum strain No. 107, Scopulariopsis brevicaulis strain No. 106a, and Trichoderma viride strain No. 165. The test fungi were cultured on a carrot-potato agar, containing 2 per cent of glucose in 500 ml Erlenmeyer flasks and incubated at 25°C. The chemicals were dissolved in benzene or acetone and the solution was added dropwise to the filter paper in quantities of 5, 10, 15, 20, 50, 100 and 200 μg/cm² volume of the Erlenmeyer flask. After 6 days of exposure of test fungi to the volatile compounds transplants were taken from the culture to determine if the fungus had been killed. For each test and each concentration parallel control tests in flasks without the volatile compounds were made. The fungicidal concentration was set as the limit of concentration at which some growth was observed and that at which the fungus had been killed.

**Results and Discussion**

Table 1 presents the respiration activity of soil microorganisms after 6 days exposure to the volatile compound with soil pH 4.1. The most effective of the 7 volatile compounds tested was methoxyethylmercuric chloride, giving inhibition of respiration at a concentration of 20 μg/cm², while p-chlorophenol, p-chloro-m-cresol and 2,4-dichlorophenol were effective at a concentration of 50 μg/cm³. Fig. 1 presents a comparison of results of evaluation of the fungicidal activity of volatile compounds by the Findlay-Vernon method (1951) and by the respiration method. Bearing in mind that in the Findlay-Vernon method two individual fungi were tested and in the respiration method a single complex of soil microorganisms was tested, the agreement of results found would appear to be more than coincidental. The correlation of results between the two different methods also gives evidence of the reliability of the suggested new method, which enables a quick and accurate evaluation of the efficiency of fungicidal action of the volatile phase of the tested compound.

Using the new method tests were conducted to discover what mixtures of the evaluated compounds would give a synergistic effect. For this purpose 2,4-dichlorophenol, p-chloro-m-cresol, o-nitrophenol and p-chlorophenol were selected. A total of 9 mixtures were tested.

1:1 mixture of the following compounds were tested:
1. 2,4-dichlorophenol + p-chloro-m-cresol
2. 2,4-dichlorophenol + o-nitrophenol
3. 2,4-dichlorophenol + p-chlorophenol
4. p-chloro-m-cresol + o-nitrophenol
5. p-chloro-m-cresol + p-chlorophenol
6. o-nitrophenol + p-chlorophenol

1:1:1 mixtures of the following compounds were tested:
7. 2,4-dichlorophenol + p-chloro-m-cresol + p-chlorophenol
8. 2,4-dichlorophenol + p-chloro-m-cresol + o-nitrophenol
9. p-chloro-m-cresol + p-chlorophenol + o-nitrophenol.

Fig. 2 presents the comparative results of evaluation of the respiration activity of soil microorganisms exposed to the action of the volatile phase of mixtures of compounds and their individual components at concentration 20 μg/cm². The activity of each mixture or individual component is expressed as a percentage of the respiration of the control soil microorganisms, that is the average of measurements on two consecutive days in the Warburg apparatus. The highest values of fungicidal activity were found for mixtures 2, 3, 6, 7 and 9, for which respiration does not exceed 3.7%. Mixtures 4 and 8 gave less fungicidal effect, the respiration values being 24.4% and 13.1% respectively. In mixtures 1 and 5 no synergistic effect was found.

This method developed for testing volatile compounds for fungicidal or fungitoxic activity could also be useful in further studies on volatile fungicides as protectants of various materials. This new method, based on test conditions as for soil fungicides, gives rapid and accurate results and good agreement with the Findlay-Vernon method. It may be recommended both for evaluation of individual volatile compounds and for testing the synergic effect of their mixtures.
Evaluation of fungicidal activity of volatile compounds. B.J. Rytych and B.J. Zyska

<table>
<thead>
<tr>
<th>CHEMICAL COMPOUND</th>
<th>METHOD OF TESTING</th>
<th>FUNGICIDAL TOXIC LIMIT mg/l</th>
</tr>
</thead>
<tbody>
<tr>
<td>P-CHLOROPHENOL</td>
<td>FINDLAY-VERNON</td>
<td></td>
</tr>
<tr>
<td></td>
<td>RESPIRATION</td>
<td></td>
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<tr>
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<td>FINDLAY-VERNON</td>
<td></td>
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<tr>
<td></td>
<td>RESPIRATION</td>
<td></td>
</tr>
<tr>
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<tr>
<td></td>
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<td>RESPIRATION</td>
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Figure 1.

Comparative fungicidal toxic limits of volatile compounds evaluated by Findlay-Vernon and respiration method.

Test fungi in the Findlay-Vernon method:

1. Aspergillus niger, strain No. 110
2. Aspergillus ochraceus, strain No. 36
3. Aspergillus ustus, strain No. 33
4. Cephalosporium sp., strain No. 33a
5. Cladosporium sp., strain No. 156
6. Gliocladium roseum, strain No. 93
7. Penicillium chrysogenum, strain No. 150
8. Penicillium stoloniferum, strain No. 107
9. Scopulariopsis brevicaulis, strain No. 106a
10. Trichodema viride, strain No. 165
Table 1

Respiration activity of soil microorganisms after six days of exposure to the volatile phase of compounds in the soil adjusted to pH 4.1

<table>
<thead>
<tr>
<th>Concentration µg/cm³</th>
<th>Period of measurements day</th>
<th>p-chloro-phenol</th>
<th>p-chloro-m-cresol</th>
<th>cyclo-hexyl-amine</th>
<th>p-dichloro-phenol</th>
<th>2,4-dichloro-phenol</th>
<th>methoxy-ethyl-mercure-chloride</th>
<th>α-nitro-phenol</th>
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</thead>
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<td>100.0</td>
<td>100.0</td>
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<td>100.0</td>
<td>100.0</td>
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<tr>
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References


Smith, R.S. (1975). Respiration methods to follow wood decay and evaluate toximetric potential of wood.
Evaluation of fungicidal activity of volatile compounds. B.J. Rytych and B.J. Zyska

Table 2
Respiration activity of soil microorganisms after six days of exposure to the volatile phase of single compounds and their mixtures of concentration 20 μg/cm³

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<th>No. of the mixture</th>
<th>Compounds in the mixture</th>
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<th>Results for the mixtures</th>
<th>Expected result if the compounds act independently</th>
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BOOK REVIEWS

METHODS IN MICROBIOLOGY Vol. 9.
J. R. Norris (Ed).
Price £9.

The first volume of Methods in Microbiology was published in 1969 and the series was soon recognised as a literate and well produced compendium of modern microbiological methods. The editors had intended that the publication of one more volume in the general series would be appropriate.

Volume 9 is concerned with three distinctly different areas of study. The first three chapters discuss developments in techniques in taxonomy characterisation and identification. Firstly there is a concise account of the use of substrate specificity to detect aminopeptidase profiles and this is followed by a long, three part paper by Heden, Illeni and Kühn on their novel methods for the mechanised identification of microbes. This paper is not a set of instructions. It introduces and describes an approach to characterisation of microbes which eliminates traditional culture apparatus by using narrow strips of agar gel. These are inoculated mechanically and then can be incubated in special containers and the results recorded photoelectrically. This approach is a logical development of various multipoint inoculation techniques which have been described in recent years and may well become important where there is a need for taxonomic, ecological or applied purposes to characterise very large numbers of isolates. The final chapter in the taxonomic section is a comprehensive and clear introduction to actual and potential applications of gas liquid chromatographic techniques to microbial detection, characterisation and identification.

Two chapters in this volume are concerned with transmission electron microscopy. The first gives specific instructions for a series of basic techniques used in the examination of bacteria and goes on to discuss recent developments in cryomicroscopy and freeze etching. The second is concerned with the specialist techniques involved in the examination of very small particles. The final chapter provides a clear and detailed account of isolation, enumeration and other techniques which have been developed for the study of the genus Bdellovibrio.

Although this volume is not concerned specifically with topics of biodeterioration it contains much information which workers in this field may wish to have available and it continues the high standards of the previous volumes. The series as a whole is certainly a major contribution to the microbiological literature and the editors must be congratulated on its success.

G. Ayerst

PROCEEDINGS OF THE THIRD INTERNATIONAL BIODEGRADATION SYMPOSIUM SESSIONS II AND VIII BIODETERIORATION BY INSECTS, BIRDS, RODENTS AND ANIMALS
J. M. Sharples and A. M. Kaplan, Editors.
LONDON, Applied Science Publishers vi + 70 + index. £4.

The full Proceedings of the Symposium has already been reviewed in this journal (vol. 12 (4), 1976). It is a weighty, expensive volume and few individual workers will hope to own it. The publication of individual sections separately is certainly worthwhile. Sessions II and III are concerned with biodegradation by insects and by rodents and other vertebrates respectively.

The two previous symposia contained little reference to animal biodeteriogens and perhaps this is why so few papers were presented on the topic this time. There is no doubt that the number of papers is disproportionately small in relation to the economic importance of the subject and to the amount of research devoted to it.

The three papers in session II, on factors affecting termite attack, insect resistant packaging and proofing of wool against insect attack, are all short reviews and report virtually no new observations. They would certainly be useful to workers needing an introduction to the topics. Three of the session VIII papers are concerned with rodents. The first is a short report of a specific study on the susceptibility of packages to rat damage, the second reviews some information about recent developments in anticoagulant resistance and the third is a clear and useful survey of new perspectives in control of rodents and other mammals, it also contains some unusually vigorous invective against the (lack of) U.S. government support for research in this field. The single paper on biodeterioration by birds attempts to present an overall picture of the topic and succeeds in this in a most entertaining way within a short space.

As a whole this little book provides useful summaries of a range of topics but it would be dangerous to think of it as being a general indication of the situation with regard to the study of animal biodeteriogens. Of the seven papers, five are from United States authors and two from the continent of Europe. It is unfortunate that the large amount of work in progress in Britain is unrepresented. There is also no mention of insect or mite pests of stored food except with regard to packaging.

G. Ayerst
MICROBES, OUR UNSEEN FRIENDS.
Harold W. Rossmoore
pp 228 + vi Wayne State University Press, Detroit. 
$12.95.

There have been several popularisations of the roles of various of the microorganisms but the present work has advantages over all that this reviewer has seen. It has the advantage of gusto, in which it reflects the personality of the author, and it has the advantage of being eminently readable while being packed with fact. The human connection is emphasised at every point and no layman could read this book without becoming intensely aware of the relevance of micro-organic activity to most facets of his life.

The book has a short Prologue which the author describes as a glossary, since it introduces the technical terms which he uses, but it becomes a short course in microbiology which any young science student might be grateful for. Subsequent chapters deal, under somewhat fanciful titles, with wine, beer, bread, cheese and other dairy technologies, other foods in which microorganisms play a part, microbiological syntheses, the role of microorganisms in the cycles of nature, and with disease (and biological control).

The amount of amusing, interesting, and important fact contained in these lively chapters is astonishing. This reviewer certainly learned a lot and would not be ashamed to use the book as a handy reference work for quick access to information on each of the technologies with which the book deals. There is a reasonable amount of suggested reading in amplification of each of the chapters so that the inevitable deficiencies of such a short work covering such a wide range, are easily remedied.

The author could hardly contain himself within the chapters he planned so that he felt obliged to add an appendix of 28 pages containing considerable blocks of information, and chat, more or less relevant to the subject matter of each chapter. And in addition there are several pages of 'notes' which seem also to serve the function of allowing the author to go on after he should, perhaps, have stopped. But enthusiasm is infectious and attractive; this reviewer is glad he went on. This book is strongly recommended reading for the non-microbiologist science student, including the non-specialist biologist, and for the interested layman.

T. A. Oxley
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