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BIODETERIORATION CENTRE
UNIVERSITY OF ASTON
ST. PETER'S COLLEGE,
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The Editors are able to call upon the assistance of an Editorial Board whose members are in Britain, various countries of Europe, and the U.S.A.

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 include microorganisms, insects, rodents, birds, higher plants, etc. Articles on biodegradation, that is, conversion of materials to less objectionable, more easily disposable, or higher value products by living organisms, 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, normally larger than the size finally desired. The suggested final size should be clearly indicated but the Editor reserves the right to vary this in the interests of economy and clarity.

As far as possible diagrams will be reduced to single column width (80 mm) or to half page (170 mm). In any event, neither these nor half tone photographs can exceed full page (260 by 170 mm). Authors should bear in mind that it is generally more convenient for readers if legends which accompany diagrams or photographs appear with them on the same page and should proportion their illustrations accordingly. Lettering on diagrams will normally be inserted by the printer; authors are therefore asked to insert lettering or symbols in pencil on the originals or in ink on a copy.

All articles are submitted by the Editor to one or more 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 editors. 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.

Bibliographic references are indicated in the text by author names (no initials) and year only, viz: Reese and Levinson (1952); or: Darby et al., (1968) and in the bibliography in strict alphabetical order of first author's names, thus:

Reese E.T. and Levinson H.G. (1952)
Comparative study of the breakdown of cellulose by microorganisms.
Physiologia Plantarum 5: 354-366

or:
Darby R.T., Simmons E.G. and Wiley B.J. (1968)
A survey of fungi in a military aircraft fuel supply system.

References to books, conference proceedings, etc. should quote first the author(s) or editor(s), then the year of publication and title followed by the name of the publisher and the city in which it is published.

As far as possible titles of journals should be given in full except for such abbreviations as 'Journ.', 'Proc.', 'Trans.' etc.

20 reprints will be sent free of charge to the first named author unless otherwise instructed. Any number (normally not more than 50) of additional reprints may be purchased if ordered sufficiently in advance. An order form and price will be sent giving about one month's notice.

ACKNOWLEDGEMENTS TO SUSTAINING ORGANISATIONS

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

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BIODETERIORATION SOCIETY NEWSLETTER

The Bunker Memorial Prize

The Society has decided to establish an annual "Bunker Memorial Prize" in memory of its first president, Dr. H. J. Bunker. This will be open for competition by any person within four years of his or her first degree, who does not yet hold any higher degree. Each candidate must be nominated as a candidate for the Prize by a college or university teacher who is a member of the Society. The prize, of £50 together with a certificate, will be awarded for the best 10-12 minute presentation by a candidate of his or her own original work. The work may be either pre or post graduate and may, indeed, be part of the candidate's work for PhD or other higher degree. An important criterion for the Judges' award of the prize will be effectiveness and clarity in public presentation, in recognition of Dr. Bunker's own skill in this direction.

It is intended that presentations by candidates for the Bunker Prize will constitute a special session at the Society's annual summer meeting. A panel of judges will make their decision at the time and the result will be announced, and the prize presented, before the end of the summer meeting. It is hoped to hold the first Bunker Prize session, and to make the first award, at the 1982 summer meeting in Portsmouth. Nominations should be sent to Mrs. Joan Maw, secretary of the Society.

Meetings Secretaries in Canada

Dr. Dickson Liu, National Water Research Institute, Burlington, Ontario, has agreed to act as a meetings secretary for Eastern Canada.

Dr. Roger Smith, Forintek Canada Corporation, Western Laboratory, 6620 Marine Drive, Vancouver, B.C. V6T 1X2 is willing to act similarly for Western Canada.

Forthcoming U.K. Meetings Programme

The following programme of meetings has been arranged:

April, 1982, Thursday and Friday 15th. and 16th.
"Modern Methods for Detecting Microbial Spoilage"
Long Ashton Research Station, Bristol.

July, 1982, Thursday and Friday, 8th and 9th.
Annual Summer Meeting.
" Decay of Wood in the Marine Environment"

December, 1982, Monday and Tuesday, 13th. and 14th.

March, 1983, Tuesday 29th.
"Fungal Physiology" University of Nottingham

July, 1983, Thursday and Friday, 7th. and 8th.
"Post-harvest Deterioration of Crops" Venue not fixed.

December, 1983, Tuesday, 13th.
Venue: Probably London area.
"Packaging to Protect Against Biodeterioration"

April, 1984, Tuesday and Wednesday, 17th. and 18th.
"Developments in the Study of Microbial Growth" Hatfield Polytechnic

For particulars of any of these proposed meetings, contact Ms. Jo Ford, Hon. Programmes Secretary, Department of Biological Sciences, The Hatfield Polytechnic, P.O. Box 109, Hatfield, Herts. AL10 9AB (Phone: 07072 68100 Extn. 506).

International Biodeterioration Symposia

The Fifth Symposium, held in Aberdeen in September 1981, was generally considered to have been a considerable success. The Society is very grateful to Dr. J. M. Shewan and his local committee for their very hard and conscientious work in organising the event. The Proceedings, edited by T. A. Oxley, and Sheila Barry, will be published by John Wiley and Sons Ltd. Edited copy is with the publishers and sub-editing is now well advanced. It will be published under the main title of Biodeterioration 5, during summer 1982.

The date and venue for the fifth Symposium are not yet fixed. The Society feels that the regular triennial cycle should be maintained if possible and is therefore aiming at September 1984. It is considered that a venue in North America will be most convenient for the greatest number of specialists in the various aspects of biodeterioration and the Society is exploring this possibility.

Subscriptions 1982

Members are asked to remit their subscriptions to the treasurer, Dr. L.H.G. Morton, Division of Biology, Preston Polytechnic, Corporation Street, Preston, Lancs. England PRI 2TQ as soon as possible.

The worldwide subscription is £3.00 and members are asked to remit in sterling if possible. If unable to do this, please add the equivalent of 20.75 to cover bank charges.

Members in Britain pay £4.50 but, if not paid by 1st May in each year, the charge becomes £5.50.
Abstracts of papers presented at a meeting of the Society held at the Polytechnic of the South Bank, LONDON

December 11th, 1981

Deteriogenic Fungi and Algae in Culture and Testing

Attendance at this meeting was very much reduced by rail and other transport disruption caused by a severe blizzard. About thirty members were present but only three papers were formally presented. The abstracts of these papers follow:

Title: Fouling of Terrestrial Substrates by Algae
Author: Colin Grant
Address: Building Research Establishment, Princes Risborough Laboratory, Princes Risborough, Aylesbury, Bucks HP17 9PX

ABSTRACT:

The Paper described the main genera of algae causing fouling of terrestrial substrates, the types of growth and the nature of any deterioration caused. Many of the commonly encountered algae enter into lichen associations. Different problems result from lichenisation, including development of more resistant, tenacious growths and physical penetration into the masonry substrate.

Practices for the cleaning and maintenance of surfaces fouled by algal growth include the use of toxic washes containing biocides. Preliminary evaluation of biocidal treatments requires reliable laboratory techniques. A number of methods have been reviewed and a new technique using solid samples of masonry and a moist bed of vermiculite has been developed. A summary of findings from these studies was presented.

Title: Isolation, Identification and Cultivation of Spoilage Fungi
Author: Agnes H. Onions
Address: Commonwealth Mycological Institute, Ferry Lane, Kew, Surrey

ABSTRACT:

Spoilage fungi attack many of man's organic materials and foodstuffs, so it is necessary to isolate and identify them. Recognition of a mouldy product and subsequent isolation and growth of the causative fungi were described. Slides were used to illustrate the appearance of some commonly occurring spoilage fungi and the characters on which their identification is based.

Title: Rapid methods for Identifying Industrially Important Yeasts
Author: D. E. Odell
Address: School of Natural Science, Hatfield Polytechnic, P.O. Box 109, Hatfield, Herts. AL10 9AB.

ABSTRACT:

A brief discussion about the nature of yeasts, the various situations in which they are found, and physicochemical factors determining their occurrence and growth, was followed by comments on features used in their identification including microscopic appearance, morphology of sexual reproduction, and physiological activities. An outline of yeast classification was given.

Various approaches to the identification of yeasts were discussed: the merits and drawbacks of (a) 'Ladder,' (b) the 'guide' by Barnett et al., (c) Davenport's rapid method, and (d) Yeast test kits, were assessed with their application to industrial requirements in mind. Methods (c) and (d) were considered to be the most suitable for the industrial situation. Nevertheless, the works discussed under (a) and (b) are major sources of practical information and should be consulted.
APPLICATION FOR MEMBERSHIP

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Cheques should be made payable to the Biodeterioration Society.
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Secretary, The Biodeterioration Society
C. P. Snow Building,
The Hatfield Polytechnic,
P.O. Box 109
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AL10 9AB

* Please delete whichever does not apply.

£4.50 for members in Great Britain and Ireland (includes meetings fee)
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Reduced Rate Subscriptions to Journals Published by the Biodeterioration Centre

As a member of the Biodeterioration Society you will be entitled to a reduced rate personal subscription to the International Biodeterioration Bulletin and/or the bibliographic journal Biodeterioration Research Titles (B.R.T.) Currently this reduction amounts to about 44%. The rates are given inside the back cover of the International Biodeterioration Bulletin.

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ROLE OF THE MYCELIUM IN THE CORROSIVE ACTIVITY OF CLADOSPORIUM RESINAЕ IN A DIESE/WATER SYSTEM

D. J. Hansen¹, D. J. Tighe-Ford² and G.C. George³

Summary

Previous studies of the corrosion associated with the presence of C. resinae in fuel systems have not clearly distinguished between the effects produced when the mycelium was in contact with an alloy and those where the alloy was exposed solely to fungal metabolites. Two experimental sytems were employed to examine differences between these conditions and to ascertain whether the mycelium plays a significant role. In "drop-tests" the alloys were exposed to fungal growth, whereas in "total immersion" tests they were exposed solely to the metabolites. Of the two alloys examined, an aluminium — 4% copper (HE15) and a stainless steel (EN58AM), only HE15 was attacked. The nature and severity of corrosion was markedly different in the two experimental conditions. In the "total immersion" test there was only a light, general attack, whereas in the "drop-test" much more severe corrosion occurred which included major pits up to 0.6 mm deep. There were distinct zones of attack related to the contact between alloys and fungus, and pitting was restricted to the outer edge of the mycelium. The relationship between the different forms of corrosion and the nature of mycelial growth is discussed and possible mechanisms are proposed.

Rôle du mycelium dans l'ac
c
tion corrosive de Cladosporium resinae dans un système diesel/éau

Des études préalables de corrosion associée à la présence de C. resinae dans des systèmes au fuel n'ont pas clairement fait la distinction entre les effets produits par le mycelium en contact avec un alliage et ceux où l'alliage était seulement exposé à des métabolites de champignons. On a employé deux systèmes expérimentaux pour voir les différences entre ces conditions et pour s'assurer que le mycelium a un rôle significatif. Dans les "essais à la goutte", les alliages furent exposés à la croissance due champignon alors que, dans le "essai en immersion totale", ils furent seulement aux métabolites. Parmi les deux alliages examinés, un d'aluminium — 4% de Cuivre (HE15) et un acier inoxydable (EN58AM), seul le HE15 fut attaqué. La nature et la sévérité de la corrosion fut bien différente dans les deux conditions expérimentales. Dans l'essai en "immersion total", il y eut qu'une attaque légère générale alors que, dans l'essai "à la goutte", il y eut une corrosion plus sévère incluant des perforations importantes jusqu'à 0,6 mm de profondeur. Il y eut des zones distinctes d'attaque liées au contact alliage — champignon et la perforation fut limitée au bord externe du mycelium. On discute de la relation entre les différentes formes de corrosion et la nature de la croissance du mycelium et on propose des mécanismes possibles.

Introduction

Cladosporium resinae has been widely reported as the dominant fungal species in the microbiological contamination of aviation fuels and associated corrosion (Hazzard, 1961; Parbery, 1971; Sheridan, Nelson and Tan, 1971; Park, 1975). An early investigation by Hendey, (1964) reported that C. resinae perforated aluminium foil and postulated that secreted organic acids were responsible for the corrosion. Later workers were in agreement and it has been found that four tricarboxylic acids are produced by the fungus (Rivers, 1971; McKenzie, Akbar and Miller, 1976); dodecanoic, acetic, glycollic and glyoxylic acids were identified by Siporin and Cooney (1975). Studies of the corrosive effect of C. resinae have centred on aluminium and its alloys: pitting, surface exfoliation, blistering of the oxide layer, and intergranular corrosion have commonly been reported, as well as general weight loss (e.g. Fedrick, et al. 1965; Parbery, 1968; Rivers, 1973; Al-Haidary, 1977). Other studies have shown that the four

¹ Lieutenant, Canadian Armed Forces. ² to whom correspondence should be addressed. ³ Commander, Royal Navy.

(Received, August 1981)
tricarboxylic acids will cause micro-pitting and other corrosion (McKenzie, Akbar and Miller, 1976) and that metabolites lower the pitting potential (Ep) of aluminium and aluminium-copper alloy (e.g. de Mele, Salvarezza and Videla, 1979; Meybaum and Schiapparelli, 1980). It was reported by Schiapparelli and Meybaum (1980) that dodecanoic acid, alone of the organic acids examined, lowered Ep and concluded that this was dependent no upon pH but upon anion concentration.

Despite numerous such studies of the corrosive activity of C. resinae there has been no specific investigation of the role of the mycelium and the nature of growth. It was decided, therefore, to examine the effects of the fungus under two experimental systems; one where the effects of the fungus under two experimental systems; one where the mycelium developed directly upon the metal surface, and the other where the metal was exposed solely to the water phase under a mycelium growing at the water/fuel interface.

Two very different alloys were investigated, an aluminium-copper (well established as susceptible to C. resinae corrosive activity) and a stainless steel selected as being reasonably typical of a free-machining 18/8 type alloy such as might be used in service. As previous authors employed aviation kerosene as the carbon source it was decided, in the context of microbiological contamination of ship fuel systems, to use Dieso in this study.

Materials and methods

Organism

Cladosporium resinae f. avellaneum (ATCC 22771) was examined for its corrosive activity when growing in Dieso (Naval diesel fuel, Def. Stan. 91-7/2). The fungus was routinely cultured on malt extract agar. Quarter-strength modified Bushnell-Haas (4/4 BH) mineral salts solution:

\[
\begin{align*}
\text{NH}_4\text{NO}_3 & = 1.20 \times 10^{-3} \text{M} \\
\text{MgSO}_4\cdot 7\text{H}_2\text{O} & = 8.1 \times 10^{-4} \text{M} \\
\text{CaCl}_2\cdot 6\text{H}_2\text{O} & = 1.36 \times 10^{-3} \text{M} \\
\text{KH}_2\text{PO}_4 & = 7.35 \times 10^{-4} \text{M} \\
\text{Na}_2\text{HPO}_4 & = 7.94 \times 10^{-4} \text{M} \\
\text{FeCl}_3\cdot 6\text{H}_2\text{O} & = 6.10 \times 10^{-4} \text{M} \\
\text{Post autoclave pH} & = 6.95 \\
\end{align*}
\]

was employed as the water phase in the experimental systems. Spore suspensions were prepared from 14 days old cultures on malt-extract agar by rolling cotton wool swabs, wetted with 0.1% 'Nonidet' (non-ionic detergent) in distilled water over the cultures. Successive 'rolls' were swirled in 10 ml 'Nonidet' solution until a concentration of ~ 25 x 10^6 spores/ml, estimated by colour, was obtained. A spore count was made on a haemocytometer cell and an appropriate volume of suspension was added to 1/4 BH to give a concentration of 5 x 10^5 spores/ml.

Metal specimens

The composition, as percentages, of the aluminium-copper alloy (Spec. HE15 2B) was:

- Cu: 3.5-4.8
- Mg: 0.85 max.
- Si: 0.90 max.
- Fe: 1.0 max.
- Mn: 1.2 max.
- Al: remainder

The steel investigated was an austenitic (oil quenched from 1050°C) alloy of specification EN58AM/BSS303S21; its composition was:

- C: 0.12
- Cr: 17.0 - 19.0
- Ni: 8.0 - 11.0
- Mn: 1.0 - 2.0
- Si: 0.2 - 1.0
- S: 0.15 - 0.30
- P: 0.045 max
- Fe: remainder

Discs 1.5 mm thick were cut from 20 mm dia. bar stock and polished to 600 grit, followed by ultrasonic cleaning in acetone. They were then rinsed in ethanol and dried under ultraviolet light.

Experimental systems

Two systems were employed: a) a 'drop-test' simulating condensation or splashed water on a fuel tank surface, and b) a 'total immersion test', simulating a water bottom, with the alloy surface in contact with neither the fungal mycelium nor the oil. In the drop test, 0.1 ml spore suspension containing 5000 spores was placed on each disc forming a drop approximately 10 mm in diameter. The discs were transferred to glass petri dishes and covered with Dieso sterilised by 0.3 μm filtration. Further discs with non-inoculated 1/4 BH acted as controls. Two inoculated and one control disc of each alloy were removed weekly for approximately three months and fungal growth examined. This was followed by ultrasonic cleaning in acetone to remove adhering mycelia, fuel and corrosion products. In the total immersion test, discs were placed in 400 ml beakers containing 125 ml 1/4 BH and 250 ml Dieso. The discs were suspended vertically in the water phase by nylon thread attached to the edge by a spot of cyanacrylate adhesive. The water bottom of inoculated beakers contained 50,000 spores/ml. Discs were removed fortnightly and then treated as in the drop test. On each occasion measurements were made of the dissolved oxygen content and pH of 10 ml samples from the water bottom of each beaker; these were extracted, examined and transferred back, under sterile, nitrogen-flooded conditions. Measurements were carried out using an EIL 7020 pH/0 meter with a
colonies of growth on EN58A M had developed into covered with a thin colourless 'membrane' of inner somewhat wider and thicker than the outer (Fig. 2). The overall from of mycelial growth is represented in Fig. 3. The BH drops under the Dieso were drying standard combination electrode and an 8012-1 dissolved oxygen probe.

The experimental systems and all cultures were maintained at 30°C, which is generally considered to be an optimum temperature for C. resinae growth (E. G. Sheridan, Tan and Nelson, 1972).

Results

Growth in drop test

Growth was evident as a fine white mycelium at the fuel/BH interface on most stainless steel (EN58AM) and some aluminium-copper (HE15) discs by the fourth day and on all by the end of the week. On HE15 the mycelium formed a ring around the drop, whereas growth on EN58AM was in the form of isolated colonies. The mycelium subsequently developed both over the BH drop and as a thin, adherent film over the alloy surface into the fuel phase, extending up to 1.5 mm on EN58AM and 0.8 - 1.0 mm on HE15 after two weeks. At places on the circumference of the drop where growth was most dense, the hyphal film had apparently entrained BH to form a secondary fuel/water interface (Fig. 1). By three weeks the mycelial film over the alloy surface extended 1 - 2 mm from the drop, generally with greater growth on EN58AM. During the remainder of the experimental period there was relatively little increase in the width of this film, which attained a final width of 1.5 - 2.0 mm. Virtually all of the growth appeared to be in the fuel phase rather than in the BH; each drop was covered with a thin colourless 'membrane' of unknown nature. By about the fifth week the original colonies of growth on EN58AM had developed into a relatively smooth ring of mycelium similar to that on HE15. On both alloys the surface mycelial mat was in the form of two concentric zones around the drop, the inner somewhat wider and thicker than the outer (Fig. 2). The overall from of mycelial growth is represented in Fig. 3. The BH drops under the Dieso were drying out during the experimental period, presumably as a result of uptake into and evaporation from, the fuel. This was particularly evident around weeks 7 - 9 and some drops were completely desiccated by the end of 11 weeks.

Growth in total immersion test

Growth was evident at the fuel/BH interface in all inoculated beakers by the end of the first week. The interface in some was completely covered after three weeks, although there was relatively little growth in others; by the end of week 9, however, there was no discernible difference between mycelial development on the various discs. As in the drop test the growth associated with HE15 alloy differed from that with EN58AM, although the fungus was not in direct contact with the alloys; whereas the mycelium over HE15 was relatively smooth, that over the EN58AM developed as coarse, isolated colonies which later emerged to form an uneven, lumpy, mat.

The dissolved oxygen content of the BH dropped from approximately 1.5 ppm to 0.3 - 0.7 ppm over the first 4 - 6 weeks. Thereafter, when the fuel/water interfaces were completely covered with fungal mats, values remained relatively constant. The rate of fall was generally greater in association with HE15 than with EN58AM (Fig. 4). The pH of the BH fell to a mean value of 4.3 and 4.9 with HE15 and EN58AM respectively, after 14 weeks. Again, the rate of fall over the first four weeks was greater with HE15 (Fig. 5).

Corrosion of HE15 in drop test

Several forms of corrosion developed on the discs in distinct zones which could be related to fungal growth. The nature and sequence of corrosion can be summarised as follows: the first evidence of attack was discoloration of the disc under the drop, followed after several weeks by a general attack and micro-pitting of the surface inside, and to a greater extent,
Role of the Mycelium in the Corrosive Activity of *Cladosporium Resinae*. D.J. Hansen, D.J. Tighe-Ford and G.C. George.


outside, the drop. Major pilling was evident after approximately four weeks, but only at the outer edge of the hyphal film. Attack of the surface under individual hyphae was evident at about the same time, starting near the outer edge of the mycelium and spreading inwards with time. Major pilling later became more common and severe, but was still confined to the region of the mycelial edge. The five corrosion zones represented in Fig. 3 were, A - the area under the drop, B - a clear band, C - a roughened zone, D - a 'hyphal film zone', and E - a zone of major pitting.

A - area under the drop. By the end of the second week the specimen surface under the drop was discoloured, with the fuel/water interface evident as a distinct line (Fig. 6). A general, light, attack was evident after five weeks; spalling of what appeared to be adherent corrosion product in some areas revealed micro-pitting of the surface (Fig. 7). In general, the centre of the drop was less severely attacked than the periphery.

B - clear band. After two weeks a narrow band (100 - 150 μm) of apparently uncorroded metal was visible outside the drop (Fig. 6). This had a sharply defined inner boundary (the fuel/water interface) and gradually faded into a further zone of corrosion. Its width decreased slightly over the experimental period.

C - Roughened zone. This region, first observed during week two, when it was 100 - 150 μm wide, developed outwards to a width of 200 - 400 μm by the third week.

4. Total immersion test: dissolved oxygen content of water phase; numerals denote percentage of fuel/water interface covered by *C. resinae* mat.
5. Total immersion test: pH of water phase; • - HE15, ○ - EN58.

with an ultimate width of approximately 1 mm. After three weeks the zone was characterised by a general attack of the surface; in some areas spalling of corrosion products revealed the underlying matrix which had been attacked, producing micro-pits predominantly 0.1 - 0.3 μm diameter (Fig. 8). The zone was evident only where particularly well-developed mycelial growth was present on the water drop and was identified as being underneath the area of drop expansion, inside the ‘secondary interface’. The corrosion was not widespread and remained as shown in Fig. 8 throughout the experiment; at no time was major pitting evident.

D - Hyphal film zone. This zone was associated with the area of metal surface underneath the main hyphal film which grew out from the fuel/water interface. Several forms of attack were evident: a general surface corrosion, corrosion under hyphal threads, and most important, the formation of large, deep pits (discussed in the following section). Corrosion was evident after four weeks when there were isolated shallow areas of cracking and spalling of deposited corrosion products (Fig. 9a), together with micro-pitting (0.1 - 0.2 μm) of the underlying matrix (Fig. 9b) similar to that in the ‘roughened zone’. These areas were randomly distributed except that there were few around the location of major pits.

After four to five weeks it was evident that there was corrosion directly under hyphae in the outer region of the surface mycelial film. Extending inwards from near the outer edge of the mycelium, towards the drop, was a network of fine lines of corrosion, 1 - 2 μm wide and often branched (Fig. 10a). The relationship of these ‘hyphal valleys’ to individual hyphae is illustrated in Fig. 10b. This form of corrosion was not evident under hyphae at the very outside edge of the film nor close to the drop. On a highly polished disc (0.25μm) initiation of valleys was evident after two weeks’ growth and in localised areas there was extensive development of valleys up to 5 μm wide after four weeks (Fig. 11).

Subsequently corrosion spread rapidly in this zone and by the end of the experimental period extended back to the ‘roughened zone’. Attack under individual hyphae spread towards the drop and general perforation of the surface (Fig. 12) became more widespread.

E - Zone of major pitting. After four weeks one or two major pits were observed on each disc at, or just inside, the outer edge of the hyphal film. Each was covered by a small bubble, which had first been evident one week earlier, the diameter of which ranged from a few hundred micrometres up to 1 mm (Fig. 13). Pits were 100 - 200 μm across with a typical depth of 200 μm. Fig. 14 illustrates a typical pit after five weeks. Subsequently the pits deepened, with some increase in diameter. Examination of etched vertical sections showed that the grain boundaries were preferentially attacked.
8. Drop test: attack of oxide layer and attack of HE15 matrix in 'roughened zone' after three weeks of *C. resinae* growth.

9. Drop test: a - attack of HE15 surface in 'hyphal film zone' after four weeks of *C. resinae* growth; area X is magnified in b.

10. Drop test: a - 'hyphal valleys' in HE15 after five weeks of *C. resinae* growth; b - hyphal remnant over valley.
From six weeks onwards most pits occurred in groups in which one major pit was surrounded by numerous smaller ones, generally within a distance of 200 μm. Vertical sections showed that these pits were often connected beneath the surface and were up to 0.6 mm deep. By the end of the experimental period the incidence of major pitting was widespread, with 2 - 6 groups of pits on each disc. These groups were invariably overlain with bubble-like formations of up to 1mm diameter. The bubbles over early pits developed a clear amber, resinous appearance, later becoming opaque white. This may have been a consequence of degraded fuel and/or corrosion products. These larger bubbles often had smaller ones around their base and their 'skin' was thin and brittle; when this was removed, gas (possibly hydrogen) bubbled from the pits. Where developing hyphal valley met a pit, an extension of the pit often developed along the line of the valley (Fig. 15).

Corrosion of HE15 in total immersion test

Corrosion was much less severe than in the drop test and even after 14 weeks attack was still microscopic. The first visual change was after six weeks when a slight bluish discoloration developed evenly across the discs. This darkened with time, all specimens eventually becoming a dull grey colour. Electron microscopy showed a uniform corrosion in the form of micro-pitting (0.1 - 0.3 μm) of the underlying matrix, (Fig. 16), similar to, but more severe than, that in the drop test under the BH and in the 'roughened zone'. There was no evidence of the major pitting that occurred in the drop test.

Corrosion of HE15 controls

Late in the drop test there was a slight blistering, cracking and spalling of the surface in a narrow band, about 400 μm wide, outside the fuel/water interface; there was no evidence of corrosion under the drop itself. No corrosion was observed in the total immersion test controls.

Corrosion of EN58AM in drop and total immersion tests

There was no evidence of corrosion in either experimental system, except for a faint brown line at the fuel/water interface of inoculated discs in the drop-test and a slight grey discoloration towards the end of the total immersion test.

Discussion

The drop test allowed what is believed to be the first study of the relationship between the C. resinae mycelium and the occurrence and nature of corrosion. Severe corrosion of HE15 depended upon the presence of mycelium upon the alloy surface. Where the alloy was exposed solely to the fungal metabolites in the total immersion test there was only slight, uniform, corrosion, whereas in the drop-test there were distinct zones of attack and major pitting associated with the

11. Drop test: 'hyphal valleys' in localised area of highly-polished HE15 after four weeks of C. resinae growth.

12. Drop test: corrosion of HE15 in 'hyphal film zone' after seven weeks of *C. resinae* growth.

13. Drop test: bubble (arrowed) overlying a major pit in HE15 after four weeks of *C. resinae* growth.

14. Typical major pit in HE15 after five weeks of *C. resinae* growth.

15. Drop test: extension of a major pit in HE15 along a 'hyphal valley' (arrowed) after seven weeks of *C. resinae* growth.

16. Total immersion test: corrosion of HE15 after 14 weeks of *C. resinae* growth at fuel/water interface; arrows indicate segregates; remains of what appear to be thickened oxide film lie on surface.
mycelial presence. These observations raise a key question about the role of the mycelium in corrosion: why did major pitting occur only at the outer edge of the surface mycelial film? In considering why this region should be unique, three possible factors emerged: a) the nature of mycelial development over the alloy surface and associated acid production, b) drying out of the BH drop with time, and c) dissolved oxygen levels. These are reviewed in turn.

a) Early growth over the disc was relatively rapid, extending 0.8 - 1.0 mm from the drop within a fortnight, and in places had virtually reached the limits of its extension (1.5 - 2.0 mm) by the end of the third week. It was here that the first evidence of pit initiation occurred after a month, with major pits apparent within the next week. General corrosion, also, was apparent in this outer region and from week five spread back towards the water drop, becoming more severe and widespread. These observations would appear, in part, to be related to acid/metabolite production by the fungus. There is no direct evidence as to where secretion occurs along hyphae; it seems reasonable to suppose, however, that this is largely or wholly concentrated in the region towards the hyphal tip where compartmental protoplasm contributes to apical extension and where metabolism may be expected to be highest. The distribution of corrosion on HE15 discs in the drop test is consistent with this. It is suggested that accumulation of secreted acids occurred on the disc surface where mycelial growth had slowed down or stopped, resulting in a high, localized concentration which caused major pitting. Diffusion of acids from the outer region of the mycelium towards the water drop could explain the spreading of corrosion in the 'hyphal film zone'. Similarly, the relative lack of attack in the 'roughened zone' is consistent with the relatively rapid rate of growth across this region which resulted, presumably, in no accumulation of acids. Furthermore, the BH entrained behind the 'secondary interface' (Fig. 1) would result in dilution.

b) The second factor which may be involved in pitting at the mycelial edge is the drying out of the BH drop. The most likely restriction upon growth of the fungus into the fuel (a maximum of only 2 mm) is the availability of water from the drop. This could be supplied to the apical compartment either via internal transport or by absorption from outside the hyphae. The latter supply could arise in two ways: First, by the presence of an aqueous thin surface layer around the drop, under the fuel; this is indicated by the 0.4 mm ring of slight corrosion on control discs. Second, the hyphae may carry, individually or together, a surrounding film of BH from the drop as they grow into the fuel. Which of these is the limiting factor is uncertain. In relation to internal transport, Trinci (1971) reported that fungal septa become plugged a specific number of compartments from the hyphal tip, depending on species, thus limiting the distance over which the hypha contributes to growth. The situation in C. resinae in particular has not been reported. Once significant drying out of the BH drop began to occur, water associated with the hyphal film would be drawn away from the outer regions. Thus acids secreted into this area would suffer less dilution than elsewhere. This, again, would be consistent with the observed pattern of corrosion.

c) Consideration, also, of dissolved oxygen in different areas of the drop-test may help to explain the location and rapid development of major pits. Differential oxygen concentrations are well established as causing pitting corrosion. Attack in this test occurred under the non-polar Diesso, indicating that the necessary electrolyte was provided by secreted organic acids and by the mineral salts medium entrained within the mycelium. It is reasonable to suppose that the areas under the hyphal film, and, particularly, the water drop were oxygen depleted. The total immersion test (Fig. 4) illustrates the rapidity with which C. resinae takes up oxygen. At the mycelial edge, however, there probably existed a steep oxygen gradient where the outer area was well supplied with oxygen from the fuel and the inner was depleted by the metabolic activity. Such a gradient, in conjunction with acids, would provide suitable conditions for pitting. Within the drop itself, however, it appears that acids from the mycelium were diluted by a water volume which was without a marked oxygen gradient and low in dissolved oxygen. This was also the case with the total immersion test which resulted in similar, non-severe, corrosion. The importance of dissolved oxygen levels is indicated by the report that corrosion of aluminium alloys is less with de-aerated citric acid than with aerated (Rivers, 1973). It also seems likely that the 'hyphal valleys' in the drop test were formed by corrosion which was the consequence of a localised concentration of secreted acids and by oxygen depletion under the hyphae resulting in an oxygen differential cell.
of consequence in service. The formation of pits up to 0.6 mm deep in the drop test indicates that droplets of condensed or splashed water in fuel systems could cause similar, serious problems. The results also suggest that the most serious attack in a water-bottom region may occur at the fuel/water interface, in association with the mycelial mat.

Conclusions

1. While HE15 aluminium-copper alloy corroded readily in the presence of C. resinae there was no discernible effect upon EN58AM stainless steel.

2. The corrosion caused by the fungus depends upon whether or not the mycelium is in contact with the metal surface. Whereas there was major pitting under the surface mycelium in the drop test, there was much less severe, and uniform, attack under the total immersion conditions.

3. The fungal mycelium plays a significant role in corrosion, particularly in pit formation. The occurrence of major pits only at the outer edge of the surface mycelial film in the drop test may be attributable to (a) localised concentration of secreted acids/metabolites, (b) drying out of the drop, and (c) the presence of differential oxygen concentrations as a result of fungal metabolism.

References


Summary

This paper describes a number of tests for the laboratory evaluation of algicidal biocides for use on construction materials. The streaked plate, algal lawn and liquid culture methods all suffer disadvantages relating to interpretation and extrapolation of results although the liquid culture method may be useful in preliminary screening of candidate biocides. A novel technique employing building materials such as natural stone and mortar, dampened by contact with moist vermiculite offers a more realistic assessment of biocide performance. The technique provides conditions which permit interaction between substrates, organism and biocide, and enables residual toxicity to be evaluated by re-challenge with fresh algal inoculum. It is possible that the method could be used to determine the concentration of biocides which might be effective in service.

Evaluation de laboratoire de biocides algicides à utiliser pour des matériaux de construction. 1/ Essai de quelques méthodes d’essai courantes.

On décrit un certain nombre d’essais de laboratoire pour évaluer les biocides algicides pour matériaux de construction. Les méthodes de la plaque rayée, de la couche d’algue et de la culture liquide ont toutes des désavantages liés à l’interprétation et l’extrapolation des résultats bien que la méthode en culture liquide puisse être utile dans des criblages préliminaires de candidats biocides. Une technique nouvelle employant des matériaux de construction tels que de la pierre naturelle et du mortier humidifié par contact avec de la vermiculite humide conduit à une évaluation plus réaliste de la performance biocide. La technique fournit des conditions permettant une interaction entre substrat, organisme et biocide et permet d’évaluer la toxicité résiduelle par un contre-essai avec un inoculum frais d’algue. Il est possible que la méthode puisse être utilisée pour déterminer la concentration de biocides pouvant être efficace en service.

1 Introduction

The significance of algae as primary colonisers of stone and cementaccous building substrates has been acknowledged only in recent years. In a review of microbiological deterioration of porous building materials Hueck-van der Plas (1968) found scant reference to algae as deteriogens, although their role as precursors of higher organisms and and their symbiotic association with fungi in lichens formation was well known. However, subsequent research has shown the deleterious effects of algae on works of art (Palen and Curri, 1972) archaeological remains (Favali, et al, 1978), stone monuments (Fusey and Hyvert, 1966) and buildings and building materials (Dukes, 1972; Richardson, 1973 (a); Paleni and Curri, 1973; Lloyd, 1976). Strong evidence for direct algal deterioration of stone has been provided by Degelius (1962), while Trotet et al (1973) showed damage to the surface of a concrete runway in Brittany by species of blue-green algae (Cyanophyceae).

These findings have prompted investigation of treatments for the control of biological growths (Anon, 1977; Richardson, 1973 (b); Genin, 1973; Keen, 1976) and a list of chemicals and products used for their eradication and prevention has been compiled (Bravery, 1977, 1981).

An increasingly wide range of biocidal treatments for fouled building surfaces is becoming available. Because of this and the practical difficulties involved in conducting field trials a reliable laboratory method is needed to evaluate biocide efficacy. Hueck and Adema (1967) pointed out that whereas in vitro tests for algicidal compounds had been studied, methods for testing algiacidal-treated materials in the laboratory still had to be developed. They devised a test for the effectiveness of antifouling paints against algae and also developed a novel method for assessing
the algicidal properties of several compounds against *Chlorella pyrenoidosa* on treated samples of asbestos-cement. No further development or application of Hueck and Adema's work on building substrates appears to have occurred, although there have been many studies on the interaction of algae with biocides, pesticides and other chemicals (see reviews by Butler, 1977 and Wright, 1978).

Tests involving the culture of algae in liquid media have been widely used in the past (see, for example Palmer and Maloney, 1955). Developments in methodology are described in Stein (1973) and Skulberg (1978) and recently the use of an *in vitro* liquid culture laboratory screening test has been proposed by Morton (1979) for assessing the algicidal properties of biocidal washes intended for the elimination and prevention of algal growths on stonework and other surfaces. In this technique the persistence or otherwise of green pigmentation is used as the criterion for assessing the viability of the test algae and thus the toxic level of the biocide.

The present paper describes part of a programme of work to evaluate several different methods for testing the algicidal activity of biocides on stone and cementacious materials. In the course of the work a new vermiculite-bed technique was developed for the direct assessment of activity against algae on a range of such substrates. Some preliminary results using this development are presented here.

### 2 Materials

#### 2.1 Algae

The following algae employed in the various methods investigated were chosen either because they could be readily handled and cultured under laboratory conditions, or because they were known to grow on buildings or building substrates such as stone, mortar and cement.

- *Ankistrodesmus braunii* (*Chlorophyceae: Chlorococcales*) Strain No: CCAP 202/8c. Although strictly a freshwater alga and not one which is found on the above building substrates, *A. Braunii* was used because of its ease of handling in laboratory culture and widespread use in other fields or algal research (George, 1977).

- *Selenastrum capricornutum* (*Chlorophyceae: Chlorococcales*) Strain No: CCAP 278/4. The alga is a widely used organism (Skulberg, 1978) and is routinely employed in the US Environmental Protection Agency Algal Assay Procedure (Anon, 1971).

- *Stichococcus* sp. (*Chlorophyceae: Ulotrichaceae*) isolated from a brick wall at the Princes Risborough Laboratory. *Stichococcus* sp are known colonisers of stone (Hueck-van der Plas, 1968; Faval et al, 1978) and asbestos-cement (Lloyd, 1976).

- *Trebozia* sp. (*Chlorophyceae: Chlorococcales*) isolated from an asbestos-cement panel exposed on the roof of the Press Association Building, London. Culture supplied by Mr A O Lloyd who has noted *Trebozia* as a primary coloniser of asbestos-cement (Lloyd, 1976).

#### 2.2 Chemicals

Two chemicals were used: Benzalkonium chloride (BAC), a quaternary ammonium compound (alkyl-dimethyl-benzyl ammonium chloride), trade name Preventol R (Bayer Ltd) and a purified grade of sodium pentachlorophenoxtde (NaPCP). BAC was

![Figure 1](image-url)  
Figure 1 Streaked plate technique showing *A. braunii* after 9 days incubation.  
Top left: Control  
Top right: 1.0 µg ai ml⁻¹ BAC  
Bottom left: 5.0 µg  
Centre: 10.0 µg  
Bottom right: 100 µg
available as a water-miscible liquid concentrate containing 50% active ingredient.

3 Methods

3.1 Streaked plate technique. Developed by Drisko and Crilly (1973) for evaluating biocides for the control of algal growths on paint films, the method provides a subjective assessment of the ability of algae to survive and develop on a simple inorganic nutrient agar medium in which biocide has been incorporated. Pure calcium carbonate was included in the medium because this compound is used as an extender pigment in paints and is also a component of stone and cementitious building materials. Additionally, calcium carbonate ensures that the medium is sufficiently alkaline to prevent the growth of any contaminating fungi and produces a white background which facilitates observation of algae.

An appropriate amount of BAC biocide (para 2.2) was dissolved in sterile deionised water and added to freshly autoclaved batches of medium which were vigorously swirled to ensure thorough mixing, and poured into 9 cm diameter plastic petri dishes. Concentrations of BAC achieved were 100, 10, 5 and 1 μg active ingredient (ai) ml⁻¹ medium; control plates were prepared by the addition of sterile deionised water alone.

After storing for several days to allow the agar surface to dry, plates were inoculated with a streak of algal inoculum taken from active cultures of *A. braunii* and *Stichococcus* sp on agar slopes. Duplicate plates for each alga at each biocide concentration were placed in a random array in humidity cabinets and incubated for four weeks at 25°C under uniform lighting conditions of 2.5 k lux at the plate surface provided by 'colour matching' fluorescent lights.

At intervals plates were observed and the appearance of the algae relative to the control recorded using a numerical scoring system. See figure 1.

3.2 Algal lawn technique. Algal 'lawns' on nutrient agar medium in petri dishes have been used in the assay of herbicides (Wright, 1975) and to evaluate the toxicity of fuel oil components (Winters et al, 1977). The compound under study is usually applied to the lawn in a disc of absorbent material. After a suitable period of incubation the zone of growth inhibition around the disc is measured.

The technique used was essentially that of Wright (1975) and employed modified Knop's nutrient solution solidified with agar. However, two inoculation methods were investigated: spraying algal cell suspension directly on to poured plates (Wright's method) and seeding cooled medium prior to pouring. For the latter, 19 ml batches of autoclave-sterilised medium cooled to 42°C (Winters et al, 1977) were seeded with 1 ml algal cell suspension, rapidly mixed and poured into 9 cm diameter plastic petri dishes. Test algae were *A. braunii* and *capricornutum*.

Inoculated plates were incubated for 7 days in the light to allow a vigorous lawn to develop. BAC biocide (para 2.2) was applied to the lawns, absorbed into 6 mm diameter Whatman Antibiotic Assay Discs which were placed singly in the centre of each lawn. Biocide concentrations tested were 10,000, 500 and 1000 μg ai per disc; the control discs were treated with deionised water. The range of biocide concentrations was selected to encompass recommended application rates for BAC on fouled surfaces (Bayer, 1978).

Plates were randomly arranged in humidity cabinets and incubated at 25°C, 2.5 k lux for a total of 25 days. The inhibition zones around the treated discs were measured at intervals. See figure 2.

3.3 Liquid culture toxicity test. This involved growing algae in nutrient solution containing the substance under scrutiny and, after a period of incubation, determining the effect on the test organism. The test was performed using 150 x 18 mm Pyrex test tubes containing 8 ml modified Knop's solution (Wright, 1975). Tubes were closed with aluminium caps and autoclaved at 1.03 bar (121°C) for 20 minutes. Stock solutions of BAC biocide and NaPCP (para 2.2) were prepared in sterile deionised water and separately added to culture tubes in 1 ml aliquots to give the following final working concentrations:

- BAC: 50, 10, 5 and 1 μg ml⁻¹ medium; deionised water control.
- NaPCP: 20, 10, 5 and 1 μg ml⁻¹ medium; deionised water control.

The test alga *A. braunii* and *S. capricornutum* were separately added in 1 ml aliquots from actively growing cell suspensions to give initial cell densities of between 1 x 10⁴ and 1 x 10⁵ cells ml⁻¹ culture solution. The mean absorbance (optical density) of each alga/biocide treatment group at the start of incubation was recorded. Five replicates of each alga/biocide concentration were prepared and incubated for 7 days at 25°C in continuous illumination of 4.5 k lux at the culture tube face. A higher level of illumination was possible because the tubes were not screened inside a humidity cabinet as were agar plates in sections 3.1 and 3.2. The effect of biocides on algal growth was determined by measuring the absorbance of culture solutions (Sorokin, 1973) in a spectrophotometer at a wavelength of 441 nm.

3.4 Vermiculite-bed technique for construction materials

This technique was developed to provide exposure conditions similar to those under which biocides are used on construction materials in service. The method enables factors such as biocide/organism/substrate interactions, leaching, and repeated challenge of the biocide to be taken into account.
Laboratory Evaluation of Algicidal Biocides for use on Constructed Materials 1. C. Grant and A.F. Bravery.

Culture vessels consisted of transparent perspex boxes 220 x 110 x 80 mm with close-fitting non-ventilated lids. Each box was charged with 100 g air-dry vermiculite (Dupré vermiculite, grade DSF) which was lightly firmed down to give a depth of approximately 25 mm. Deionised water was added to raise the moisture content to 400 per cent based on the air-dry weight of vermiculite. Moisture levels up to ~ 450 per cent are possible with this grade of vermiculite, though above this, excess free water causes the vermiculite to become mobile and unable to support test blocks. Since the test system is not assembled or maintained under aseptic conditions, it is possible to add water during incubation to prevent drying out. See figure 3.

Figure 2  Algal lawn technique. Spray inoculated plates of *A. braunii* after 5 days incubation with BAC applied to discs in various concentrations.

3.4.1 Natural stone

Blocks (80 x 30 x 10 mm) of Locharbriggs sandstone and Monks Park limestone were bedded into the moist vermiculite (Plate 1). After equilibration for 3 days, blocks were spray inoculated with Knop’s solution containing a mixed suspension of *A. braunii*, *S. capricornutum*, *Stichococcus* sp and *Trebouxia* sp, and incubated at 25°C, 2.5 k lux illumination. After 4 weeks the blocks were extensively colonised and the amount of growth present on each was recorded using a numerical rating system. BAC biocide (para 2.2) at 10000, 5000 and 2500 μg a.i. ml⁻¹ (1, 0.5 and 0.25 per cent v/v a.i.) in deionised water was then lightly applied with a soft brush to the upper surface of replicate
blocks to achieve a coverage rate equivalent to approximately 200 ml m⁻²; deionised water alone was used as the control.

Treated blocks were incubated as before; algal growth was recorded after one week and then subsequently at four-weekly intervals following treatment. At intervals during incubation fresh inoculum was sprayed on to the blocks to provide a further challenge to the biocide and permit assessment of residual activity.

3.4.2 Mortar

Blocks of mortar 95 x 30 x 10 mm were cast in silicone rubber moulds using white Portland cement (to facilitate observation of algae), lime and soft sand (1:1:5 by volume). As the pH of freshly-prepared mortar is very high and strongly inhibitory to algae, accelerated carbonation using carbon dioxide gas was used to lower the pH prior to biocide evaluation.

For carbonation, blocks were placed inside a desiccator containing in its base a reservoir of saturated sodium bromide solution to provide some control of the atmosphere in the region of 58% relative humidity which is within the humidity range favouring rapid carbonation (Venuat and Alexandre, 1969). The lid of the desiccator was fitted with a rubber bung through which passed two tubes — one vented to the outside, the other coupled by flexible plastic tubing to a supply of carbon dioxide gas. Each block was spaced from its neighbours and the wall of the desiccator, and layers were built up using semi-rigid plastic mesh as staging.

Carbon dioxide was slowly admitted to the system and allowed to disperse air through the vent tube until it filled the desiccator. This was checked by allowing vented gas to bubble through a small reservoir of lime-water (Ca(OH)₂). The vent tube remained open during the course of carbonation and any condensation which formed on the wall and lid of the desiccator during the early stages was periodically removed and fresh CO₂ admitted. Subsequently the sytem was topped up with the gas daily and also after blocks removed for pH measurements were replaced.

The process of carbonation was monitored using narrow range indicator papers on the lightly moistened surface of sample blocks, while in-depth carbonation was checked using phenolphthalein indicator solution (1 g of phenolphthalein dissolved in 50 ml ethanol and diluted to 100 ml with water) on a freshly broken surface (Venuat and Alexandre, 1969; Anon, 1978). A purple-red colouration indicated where the mortar was still highly alkaline due to the presence of calcium hydroxide while the colourless area had a pH of less than ~pH 8.5. After between 10-14 days exposure to CO₂ a full desiccator charge of blocks (40) showed surface pHs of ~pH 8.

Following carbonation, mortar test blocks were soaked in deionised water which was changed daily. After 7 days the blocks were exposed in the vermiculite-bed culture vessels. Following colonisation by algae the blocks were treated with BAC at 10000, 5000, 2500 and 1250 µg a.i. ml⁻¹ (1, 0.5, 0.25 and 0.125 per cent v/v ai) as appropriate by the same methods as were used for stone blocks, then incubated and observed as described previously (3.4.1).

4 Results

4.1 Streaked plate technique

Algal growth is summarised in Table 1 and Figure 1 illustrates the appearance of A. Braunii after 9 days exposure to the biocide.

For both algae the effect of the biocide was clearly evident after only one day although the response did stabilise until 6 days had elapsed. Thereafter no change occurred in the condition of biocide-exposed cultures compared with controls.

Stichococcus was much more sensitive than Ankistrodesmus. The results indicate that at 10 µg ai ml⁻¹, BAC is toxic to Stichococcus, while at 5 µg ml⁻¹ it acts initially as an algalist becoming progressively algiloxic with time as evidenced by the reduction in growth and loss of colour which occurs. Ankistrodesmus was only marginally affected at 10 µg ml⁻¹, and at 100 µg ml⁻¹, although no growth occurred, still showed isolated patches of green after 27 days.

4.2 Algal lawn technique

The seeding method of lawn preparation failed to provide vigorous growth suitable for test purposes. S. capricornutum did not grow and A. braunii made only sparse and variable growth during 4 weeks incubation. It is likely that cell damage resulted from contact with agar at 42°C although other workers have successfully
Table 1

Growth ratings for two algae using the streaked plate technique

<table>
<thead>
<tr>
<th>Alga</th>
<th>Conc. BAC μg ai ml⁻¹</th>
<th>No of days incubation</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1 2 3 6 9 14 27</td>
<td></td>
</tr>
<tr>
<td>Ankistrodesmus braunii</td>
<td>10.0</td>
<td>1 3 3 3 3 3 3</td>
</tr>
<tr>
<td></td>
<td>5.0</td>
<td>1 3 3 3 3 3 3</td>
</tr>
<tr>
<td></td>
<td>1.0</td>
<td>1 3 3 3 3 3 3</td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>0 0 0 0 0 0 0</td>
</tr>
<tr>
<td>Stichococcus sp</td>
<td>10.1</td>
<td>1 3 3 3 3 3 3</td>
</tr>
<tr>
<td></td>
<td>5.0</td>
<td>1 3 3 3 3 3 3</td>
</tr>
<tr>
<td></td>
<td>1.0</td>
<td>1 3 3 3 3 3 3</td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>0 0 0 0 0 0 0</td>
</tr>
</tbody>
</table>

BAC = benzalkonium chloride.

Notes: Key: 0 = No growth and loss of original green colour.
1 = No growth, but persistence of original green colour.
2 = Slight growth, much less than control.
3 = Growth equal to that of control.

Ratings recorded as 0-1, 2-3, etc indicate that growth of the alga was intermediate between the two states.

* Ratings recorded as with oblique stroke between indicate different behaviour of the two replicates.

Table 2

Mean Diameter of inhibition zone (mm) using Algal Lawn Technique

<table>
<thead>
<tr>
<th>Conc. BAC μg ai disc⁻¹</th>
<th>Duration of incubation (days)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0 0 0</td>
</tr>
<tr>
<td></td>
<td>8 8 8</td>
</tr>
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<td></td>
<td>13 16 17</td>
</tr>
<tr>
<td></td>
<td>16 20 21</td>
</tr>
</tbody>
</table>

4.3 Liquid culture toxicity test

Results are summarised in Table 3. Detailed statistical analysis of the data from each of the treatments was carried out by shortest significant range testing (Parker, 1973) and showed that BAC significantly reduced the growth of S. capricornutum at 1 μg ai ml⁻¹, while growth of A. braunii was reduced at 5 μg ai ml⁻¹. Both algae were adversely affected by NaPCP at 1 μg ml⁻¹. Eloranta (1974) has reported (for another Ankistrodesmus species), 50 per cent destruction of biomass after 24 hours exposure to 1 μg ml⁻¹ NaPCP. At the highest concentrations tested (BAC 50 μg ai ml⁻¹; NaPCP 20 μg ml⁻¹) neither alga grew beyond inoculum levels, in fact reductions in absorbance occurred indicating loss of green pigmentation or destruction of cells; absorbance readings for these cultures are probably largely attributable to reduction in light intensity due to scattering of the beam in the spectrophotometer. Toxic concentrations for both algae therefore lie between 10-50 μg ai ml⁻¹ BAC and 10-20 μg ml⁻¹ NaPCP.

4.4 Vermiculite-bed technique

4.4.1 Natural stone

Figure 5 illustrates the effectiveness of various concentrations of BAC in eliminating and preventing recolonisation of two types of natural stone by algae. Even at 10000 μg a.i. ml⁻¹ the biocide failed to eradicate algae completely from Locharbriggs sandstone (Figure 5 (a)) and subsequent recolonisation occurred, especially following re-inoculation. However, even after 12 weeks incubation following application of biocide, blocks treated with 10000, 5000 and 2500 μg a.i. ml⁻¹ BAC showed less overgrowth than the untreated control. On Monks Park limestone (Figure 5 (b)), 10000 μg a.i. ml⁻¹ BAC eradicated algae and prevented regrowth during the
course of the experiment. Concentrations of 2500 and 5000 µg a.i. ml\(^{-1}\) permitted some recolonisation which was delayed at the higher level and represented only a trace of growth.

4.4.2 Mortar

Figure 6 illustrates the performance of BAC on Portland cement/lime/sand mortar test pieces. Four days after application of biocide all treatments had reduced the amount of overgrowth relative to the controls. Growths were not completely killed on all of the blocks even at 10000 µg a.i. ml\(^{-1}\) BAC and a trace persisted on two of the four replicates. Although no reinoculation was carried out, recolonisation occurred after 4 days with 5000 µg a.i. ml\(^{-1}\) treated blocks and some regrowth was evident after 16 days with 2500 and 10000 µg a.i. ml\(^{-1}\) treated blocks. Recolonisation to the level of the controls did not occur with any of the treated blocks even after 22 days incubation.

Table 3

Summary of absorbance measurements after 7 days incubation using liquid culture toxicity test

<table>
<thead>
<tr>
<th>Test alga</th>
<th>BAC Concentration µg a.i ml(^{-1})</th>
<th>Mean absorbance (5 replicates)</th>
<th>NaPCP Concentration µg a.i ml(^{-1})</th>
<th>Mean absorbance (5 replicates)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0 (control)</td>
<td>0.862</td>
<td>0 (control)</td>
<td>0.878</td>
</tr>
<tr>
<td></td>
<td>1.0</td>
<td>0.836</td>
<td>1.0</td>
<td>0.192</td>
</tr>
<tr>
<td></td>
<td>5.0</td>
<td>0.378</td>
<td>5.0</td>
<td>0.188</td>
</tr>
<tr>
<td>Ankistrodesmus braunii</td>
<td>10</td>
<td>0.248</td>
<td>10</td>
<td>0.198</td>
</tr>
<tr>
<td></td>
<td>50</td>
<td>0.162</td>
<td>20</td>
<td>0.050</td>
</tr>
<tr>
<td></td>
<td>at start of incubation</td>
<td>0.168</td>
<td>at start of incubation</td>
<td>0.112</td>
</tr>
</tbody>
</table>

|              | 0 (control)                          | 0.304                         | 0 (control)                          | 0.320                         |
|              | 1.0                                  | 0.238                         | 1.0                                  | 0.172                         |
| Selenastrus capricornutum | 5.0                                  | 0.174                         | 5.0                                  | 0.174                         |
|              | 10                                   | 0.138                         | 10                                   | 0.216                         |
|              | 50                                   | 0.100                         | 20                                   | 0.064                         |
|              | at start of incubation               | 0.121                         | at start of incubation               | 0.073                         |

Absorbances were read at 441 nm in 10 mm cells.

Vertical bars connect figures which do not differ significantly at the 95 per cent confidence level.
Laboratory Evaluation of Algicidal Biocides for use on Constructional Materials 1. C. Grant and A.F. Bravery.

5 Discussion

Although the streaked plate technique is likely to be of value as a screening test to evaluate and compare the activity of biocides against algae, the method is considered to be of limited use in determining working concentrations in practice and suffers uncertainties associated with incorporation and diffusion of biocides in agar. A similar criticism may be levelled at the algal lawn technique where again the differing diffusion characteristics of biocides, particularly those with different chemical structures, renders the technique unsuitable, especially as an indicator of comparative performance. Furthermore, a knowledge of the degree of growth inhibition of algae in agar culture does not necessarily indicate treatment concentrations which would be effective in practice. However, the technique could be useful as a quantitative assay of biocide concentration in, say, residues following biocide treatment.

The liquid culture toxicity test presents certain advantages over the agar-based techniques. Problems associated with incorporation and diffusion of water soluble biocides are avoided. Also, since even on solid substrates, water films are necessary for growth, the aqueous medium in which the test is conducted is more closely akin to the sphere of interaction of algae with biocides in natural environments. In addition, the method does enable the direct comparison of biocidal activity of different chemicals. However, emulsified biocide formulations may be difficult to disperse satisfactorily, and the absence of substrate interaction must be considered a major disadvantage. The extrapolation of laboratory results to working concentrations again presents difficulties.

The results from both tests using the vermiculite-bed technique with stone and Portland cement/lime/sand mortar confirms the potential of this technique as a method of biocide testing. Preliminary results indicate the differential effectiveness of BAC on different types of natural stone and mortar, although further work is desirable to substantiate these findings and to correlate effective concentrations determined in laboratory studies with those from field and service trials.

6 Conclusions

Tests conducted on agar media or in aqueous solution provide rapid results but are largely of value in screening or in tests to determine relative algicidal properties and potencies of compounds. The vermiculite-bed technique, employing building substrates, allows the more realistic assessment of biocide activity under conditions which permit interaction between substrate, organism and biocide, and enables residual toxicity to be evaluated by re-challenge with fresh inoculum. It is also possible that the method will allow determination of the concentration of biocides which might be effective under service conditions.

References


0 = No growth  
1 = Trace of growth  
2 = 1 - 10% surface colonised  
3 = 10 - 30%  
4 = 30 - 70%  
5 = > 70%  

- Control (untreated)  
× 0.125% v/v a.i. BAC  
□ 0.25%  
○ 0.50%  
△ 1.0%  
(R) Reinoculation of all blocks  

a) Locharbriggs sandstone  
b) Monks Park limestone  

Figure 5  Vermiculite-bed technique. Performance of biocide applied to natural stone test pieces.  

Figure 6  Vermiculite-bed technique. Performance of biocide applied to mortar test pieces (4 replicates per treatment).
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BIODETERIORATION CENTRE
SHORT COURSE PROGRAMME 1982

We are pleased to announce the following proposed Short Course Programme

VC 822 - 12 May 1982
Pest Control for Customers
The course will attempt to show the need for pest control and how the customer should interact with the contractor for best results. Both the pest control industry and customers will be represented in the panel of speakers and there will be time for detailed discussion.
Cost: including lunch and refreshments - £35.00

VC 823 - 25 May 1982
Biodeterioration Problems in the Antique Trade
The day's lectures and demonstrations will be given by Centre Staff and scientists and conservationists from London and Birmingham Museums. Fungal, insect and rodent damage will be explained and the problems associated with paper, textiles, wood and leather will be considered. No previous biological knowledge will be assumed.
Cost: including lunch and refreshments - £35.00

VC 824 - 8 June 1982
The Biodeterioration of Plastics and Rubber
This course is designed to cover recent developments in the biological breakdown of plastics and rubbers in a variety of user situations. The present interest in the fungal breakdown of polyurethanes will be reflected in the course; it will be of interest to both manufacturers and users of plastic materials and also those researching in this area.
Cost: including lunch and refreshments - £35.00

VC 825 - 5-14 July 1982
Biodeterioration
A comprehensive residential course of lectures, demonstrations, seminars, visits and practical work giving a basic background in many aspects of materials biology. The course, which is based on a previous UNESCO-UNEP sponsored course will be of benefit to postgraduate biologists and industrialists in biological industries, such as biocide manufacturers and consulting scientists.
Cost: including full board residential accommodation - £250.00

NOTE: Some sponsored places may be available to students from Developing Countries.

VC 826 - 27 & 28 September 1982
Moisture in Materials
Problems caused by moisture in materials and subsequent biological effects will be covered, together with techniques for monitoring and measurement. A second day on identification of spoilage fungi is planned as an optional extra.
Cost: including lunch and refreshments - £35.00
Identification course including overnight accommodation - £50.00 extra

VC 827 - 12 October 1982
Chemical Preservation of Materials
Lectures on various aspects of chemical preservatives for both solid and liquid systems will be given and there will be a commercial exhibition by manufacturers. This course will be held either at the Biodeterioration Centre or in a hotel in the Midlands.
Cost: including lunch and refreshments to be announced but probably in the region of - £50.00

VC 828 - 26 October 1982
Toxins in Food and Feedstuffs
The course will concern itself with toxic agents of biological origin in both human and animal foodstuffs. Special emphasis will be given to Mycotoxins.
Cost: including lunch and refreshments - £35.00

VC 829 - 9 November 1982
Weed Control
Various aspects of weed control (physical, chemical and biological) will be considered both in agricultural and amenity situations. Of interest to agriculturalists, local authorities and building conservators.
Cost: including lunch and refreshments - £35.00

All courses, unless otherwise stated, are held at the Biodeterioration Centre. Further details of courses and booking forms may be obtained from:
Dr D Allsopp, Course Organiser, Biodeterioration Centre, University of Aston in Birmingham, St. Peter's College, College Road, Saltley, Birmingham B8 3TE.
Telephone: 021-328-5950 Telex: 336997 (Mark for attention Biodeterioration Centre)
The Centre reserves the right to amend this programme and to cancel any courses should there be insufficient support to run them economically.

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INTRODUCTION

A wide range of chemicals and biocidal products has been proposed to eradicate and inhibit biological growths on fouled building surfaces (Anon, 1977; Bravery, 1977, 1981). Assessment of their potential value in practice would be aided by a rapid, reliable and reproducible laboratory test. A method employing actual building materials as test substrates has recently been developed (Grant and Bravery, 1981) to evaluate and compare such compounds. This second paper describes the use of the moist vermiculite-bed technique to assess the effectiveness of a quaternary ammonium biocide against algae colonising a range of natural stone and mortar test blocks. It is not yet possible to compare results using this test with results from field trials.

2 MATERIALS

2.1 Natural stone

Test blocks (80 x 30 x 10 mm) cut from a range of natural stones (Table 1) were rinsed and then immersed in deionised water for three days to become fully saturated.

2.2 Mortar blocks

Mortar test blocks (95 x 30 x 10 mm) were cast from a mixture of white Portland cement, lime and soft sand (1:1:5 by volume) (hereafter referred to as CLS mortar). Subsequent accelerated carbonation and conditioning were as described by Grant and Bravery (1981).

2.3 Culture vessels

Transparent perspex boxes 220 x 110 x 80 mm with close-fitting lids were charged with 100 g vermiculite, moistened to 400 per cent moisture content with deionised water.

2.4 Algae

Test algae were cultured separately in modified Knop's mineral nutrient solution (Wright 1975). Prior to use in the tests, equal volumes of cell suspensions of each alga were mixed to produce a dense inoculum.

The following algae were used:

2.4.1 Ankistrodesmus braunii Strain No: CCAP 202/8C

SUMMARY

The efficacy of a range of concentrations of a quaternary ammonium biocide in eliminating and controlling algal growth on natural stone and mortar test blocks has been evaluated by means of a moist vermiculite bed technique. At the highest concentration (1 per cent) the biocide was most effective on marble, certain limestones, and mortar, and least effective on sandstone samples.

EVALUATION DE LABORATORIO DE ALGECIDAS USADAS EN MATERIAL DE CONSTRUCCION. 2. USO DE UNA TECNICA DE LECHO DE VERMICULITA PARA EVALuar UN BIOCIDA DE AMONIO CUATERNARIO

Por medio de una tecnica de lecho de vermiculita humedo, se ha evaluado la eficacia de varias concentraciones de un biocida basado en amonio cuaternario para eliminar y controlar el crecimiento de algas sobre piedra y trozos de hormigon. A la mas alta concentración (1%) el biocida es mas efectivo sobre marmol, determinadas calizas y el hormigon, y la menos efectivas sobre muestras de piedra arenisca.

LABORATORIUMUNTERSUCHUNG VON Algiziden zum Schutz von Baustoff. 2. VERWENDUNG EINES VERMICULIT-VERFAHREN zur BEWERTUNG EINER QUATERNären Ammonium-VERBINDUNG

Die Wirksamkeit einer Reihe von Konzentrationen einer quaternären Ammonium-Verbindung zur Bekämpfung und Kontrolle von Algenbewuchs auf Naturstein-und Mörtelklötzen wurde in einem Verfahren mit feuchtem Vermiculit geprüft. Bei der höchsten Konzentration (1%) war das Biocid am wirksamsten auf Marmor, einigen Kalksteinarten und Mörtel und am wenigsten Wirksam auf Sandsteinproben.
Table 1
Natural stones used in evaluation of BAC

<table>
<thead>
<tr>
<th>Limestones</th>
<th>Sandstones</th>
<th>Marble</th>
</tr>
</thead>
<tbody>
<tr>
<td>(1) Clipsham stone (a Lincolnshire limestone)</td>
<td>(8) Spinkwell stone (a Millstone Grit)</td>
<td>(10) Carrara (a white marble)</td>
</tr>
<tr>
<td>(2) Savonnières (a French limestone)</td>
<td>(9) Locharbriggs stone (a New Red Sandstone)</td>
<td></td>
</tr>
<tr>
<td>(3) St Maximin Construction (a French limestone)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(4) Monks Park stone (a Bath stone of the Great Oolite series)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(5) Portland stone (Whit Bed)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(6) Tadcaster stone (a Magnesian limestone)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(7) Tercé (a French limestone)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 2
Overall performance of BAC in eradicating Algae and inhibiting recolonisation of the different types of natural stone

<table>
<thead>
<tr>
<th>Least effective</th>
<th>Most effective</th>
</tr>
</thead>
<tbody>
<tr>
<td>Locharbriggs sandstone/Spinkwell sandstone</td>
<td>Carrara marble</td>
</tr>
<tr>
<td>Savonnières limestone</td>
<td></td>
</tr>
<tr>
<td>Tercé limestone</td>
<td></td>
</tr>
<tr>
<td>Portland limestone/Tadcaster limestone</td>
<td></td>
</tr>
<tr>
<td>Clipsham limestone</td>
<td></td>
</tr>
<tr>
<td>Monks Park limestone</td>
<td></td>
</tr>
<tr>
<td>St Maximin Construction limestone</td>
<td></td>
</tr>
</tbody>
</table>

Fig 2. Effect of BAC on growth of algae on mortar blocks - Assessed by eye (for key see page 128).
2.4.3 Selenastrum capricornutum Strain No: CCAP 278/4

2.4.3 Stichococcus sp isolated from a brick wall at the Princess Risborough Laboratory was used in the inoculum for natural stone only.

2.4.4 Stichococcus bacillaris Strain No: CCAP 379/la was used in the inoculum for mortar test blocks only.

2.4.5 Trebouxia sp isolated by Mr A O Lloyd from an asbestos-cement panel.

2.5 Biocide

Benzalkonium chloride (BAC), a quaternary ammonium compound (alkyl-dimethyl-benzyl ammonium chloride), trade name Preventol R (Bayer Ltd), BAC was available as a water-miscible liquid concentrate containing 50 per cent active ingredient.

3 Methods

3.1 Exposure

3.1.1 Natural stone Test blocks were randomly assigned to culture vessels in groups of five. Each block was spaced clear of its neighbour and the walls of the box and pressed firmly into the moist vermiculite to approximately half the thickness of the block with one 80 x 30 mm face exposed horizontally. The test systems were equilibrated for 3 days at 25°C.

3.1.2 Mortar blocks Each culture vessel received four CLS mortar blocks, exposed as in 3.1.1 above.

3.2 Inoculation and incubation

A mixed algal suspension was sprayed onto the test blocks in situ in the culture vessels which were then closed and incubated at 25°C. Illumination at the block surface was 2.5 k lux provided by “colour matching” fluorescent lights. After four weeks the natural stone samples were overgrown by algae while six weeks incubation was necessary to achieve colonisation of CLS mortar blocks. At these times the amount of growth present on each block was recorded using a numerical rating which corresponded to the approximate percentage of the block surface colonised by algae. Although blocks of the different stone types were not always colonised to precisely the same extent, overgrowth was similar in most cases. All of the CLS mortar blocks were colonised over more than 70 per cent of the exposed surface at the time of biocide treatment.

3.3 Application of biocide

BAC biocide, diluted with deionised water to give a range of concentrations between 0.125 and 1.0 v/v active ingredient (ai) was applied to test blocks using a soft artist’s brush. The biocide was carefully brushed over the exposed upper face of each block almost to “run-off” but without dislodgement of algae to achieve a coverage rate of approximately 200 ml m⁻². Control blocks were treated with deionised water alone.

The blocks were incubated for a further 12 weeks (natural stone) and 16 weeks (CLS mortar) under the same conditions employed before treatment was carried out.

3.4 Recording and re-inoculation

During the course of incubation the amount of algal overgrowth on the test blocks was recorded using the numerical rating system referred to in section 3.2 following simple visual observation with the naked eye. Additionally, blocks were examined microscopically at X100 magnification using a UV incident-light microscope. This enabled small numbers of algal cells to be detected and also indicated viability by the presence of red autofluorescence from the chlorophyll pigments of live algal cells.

At intervals blocks were re-inoculated with mixed algal suspension to provide a re-challenging of the biocide.

4 Results

4.1 General

Microscopic examination of test blocks under incident UV light permitted observation of individual algal cells and small colonies which were not visible to the unaided eye. This was of considerable value on some stones where the colour of the stone itself or the nature of the algal overgrowth made it difficult to assess the effect of the biocide. The reddish-brown background of moist Locharbriggs sandstone presented particular problems and microscopic examination was necessary to reveal the true extent of colonisation. Also fluorescence microscopy made it possible to determine the viability of algae which became discoloured following treatment with BAC. Colonies on St Maximin Construction limestone were particularly discoloured and only microscope examination revealed that the algae had in fact been killed by BAC treatment.

Use of the microscope indicated that simple visual observations generally underestimated the full extent of algal colonisation. Nevertheless using the subjective rating system it was possible to identify and record the effects of different concentrations of BAC and differences due to the types of natural stone.

In the mixed inoculum Ankistrodesmus and Stichococcus were more successful colonisers than Selenastrum and Trebouxia. Individual cells of Ankistrodesmus and chains of cells of Stichococcus were clearly visible under UV illumination.
Fig. 1. Effect of BAC on growth of algae on natural stones - Assessed by eye
4.2 Natural stone

The growth ratings for algal colonisation are presented in Figures 1.1 to 1.10. The appearance after 12 weeks incubation of representative blocks from control samples and those treated with 1 per cent BAC is shown in Plate 1.

Visual observations one week after biocide application indicated that all the treatments initially reduced the level of algal colonisation of test blocks with the exception of 0.25 per cent BAC on St Maximin Construction limestone (Figure 1.3) and Spinkwell sandstone (Figure 1.8) and 0.25 per cent and 0.5 per cent BAC on Locharbriggs sandstone (Figure 1.9).

However, microscopic examination of the sandstones revealed numerous fluorescing colonies and single cells of the two main colonising algae at all concentrations of BAC. Even at 1 per cent BAC did not materially reduce colonisation on these stones.

Recolonisation of treated blocks during incubation was generally greater on those at lower concentrations of BAC. With Clipsham stone there was early failure of the 0.5 per cent BAC treatment (Figure 1.1). Both sandstones rapidly became recolonised as was apparent from simple visual observations on Spinkwell samples and microscopic examination of Locharbriggs blocks. Inhibition of algal growth following treatment with 0.5 and 1 per cent BAC (Figure 1.3) was sustained on St Maximin Construction limestone while on Carrara marble, algae were completely eradicated at 0.25 per cent BAC four weeks after treatment.

At the end of incubation all treatments maintained algal colonisation below the level of controls on all stones excepting Savonneries limestone treated with 0.5 per cent BAC (Figure 1.2) and the two sandstones at all levels of BAC treatment (Figures 1.8 and 1.9). On Clipsham stone, Monks Park stone, St Maximin Construction limestone and Carrara marble (Figures 1.1, 1.4, 1.3 and 1.10) algae were completely eliminated and recolonisation prevented at 1 per cent BAC. On St Maximin Construction limestone the lower concentration of 0.5 per cent BAC also proved effective.

An overall assessment of the efficacy of BAC on the different types of stone is given in Table 2. At the concentrations tested the biocide was largely ineffective on the sandstones while better performance was obtained on several of the limestones. BAC was most effective at eliminating and inhibiting the growth of algae on Carrara marble and St Maximin Construction limestone.

4.3 CLS mortar blocks

The results for the CLS mortar blocks are shown in Figure 2. Plates 2 and 3 show control blocks and those treated with 1 per cent BAC 16 days after application of biocide.

Reductions in algal overgrowth were directly proportional to the concentrations of BAC used. Figure 2 clearly shows that four days after treatment algae had been almost completely eliminated by 1 per cent BAC; in fact no algae remained on two of the four replicate blocks and only a trace was visible on the other two.

Gradual recolonisation occurred subsequently on blocks treated with 0.25, 0.5 and 1 per cent BAC. The amount of overgrowth on 0.125 per cent BAC treated blocks remained static for several weeks, probably because this concentration caused a smaller initial reduction in colonisation. After 13 weeks incubation no further recolonisation took place and the experiment was ended 16 weeks after biocide application.

Despite reinoculation with fresh mixed algal inoculum on three occasions during incubation, all the treatments reduced recolonisation well below the level in the controls. At the end of the experiment controls were covered to more than 70 per cent of the surface while blocks treated with 1 per cent BAC were colonised to between 10-30 per cent.

5 Discussion

The work has demonstrated that algal colonisation of stone and CLS mortar test blocks is reduced initially following treatment with BAC, and that the degree of subsequent recolonisation depends upon the concentration of biocide used and the nature of the substrate. Response to the biocide was fairly rapid and maximum reduction in overgrowth occurred within a week following application. Maximum recolonisation occurred by about the twelfth week of incubation. In general BAC was equally effective on both limestone and CLS mortar test blocks.

The different performance of BAC on the various types of natural stone is of interest as it indicates that higher treating solution concentrations may be necessary to achieve control of algae on sandstones than on some limestones and marble. However, results obtained in these laboratory investigations will need comparing with field trials to establish the extent of correlation. Limited in-service trial experience to date indicates that 1 per cent quaternary ammonium treatment alone can remain effective for up to four years (unpublished PRL data).

The differing effectiveness of BAC on limestones and sandstones may result from the different chemical composition of these stones. The disinfectant efficiency of BAC is known to increase with pH and the content of alkaline substances in the substrate to which it is applied (Bayer 1978). However, the pH of water in which blocks of each of the stone types had been soaked for 4 weeks was tested and found to lie in
Laboratory Evaluation of Algicidal Biocides for use on Constructional Materials 2. C. Grant and A.F. Bravery.

Fig 3  Natural stone test blocks, 12 weeks after treatment with biocide. Upper row - untreated controls, lower row - BAC at 1% ai.

<table>
<thead>
<tr>
<th>Locharbriggs</th>
<th>Monks Park</th>
<th>Savonnières</th>
<th>Tadcaster</th>
<th>Terce</th>
</tr>
</thead>
<tbody>
<tr>
<td>Portland</td>
<td>Clipsham</td>
<td>Carrara</td>
<td>St Maximin Construction</td>
<td>Spinkwell</td>
</tr>
</tbody>
</table>

Fig 4  Mortar test blocks 16 days after treatment - untreated controls

Fig 5  Mortar test blocks 16 days after treatment with BAC at 1% ai
the narrow range pH 7.4-8.2. The lowest pH was recorded for Clipsham limestone and the highest was Tadcaster magnesian limestone; Locharbriggs and Spinkwell sandstones gave readings of pH 7.8 and 8.1 respectively. On this evidence pH appears unlikely to account for differences in biocide efficacy.

Schaffer (1932) indicates that sandstones comprise ~90 per cent silica (SiO₂) with alumina (Al₂O₃) and iron oxide (Fe₂O₃) forming the bulk of the remainder, whereas limestones consist predominantly of calcium carbonate with variable amounts of magnesium carbonate (CaCO₃ and MgCO₃) and usually a small amount of organic matter. Quaternary ammonium compounds are very strongly adsorbed onto certain inorganic surfaces such as glass and clay (Mackrell and Walker 1978) both of which contain silica as principal constituents, and it is possible that adsorption onto the sandstones greatly reduced the biological activity of BAC.

The work has also shown that BAC was equally effective against algae on CLS mortar and limestone test blocks. It might be expected that since the CLS mortar blocks contained a high proportion of sand, itself predominantly silica, the algicidal activity of BAC would be broadly equivalent to that obtained on the sandstones. However, in mortar the majority of sand grains are in fact covered with cement paste. In the fully carbonated blocks used in the laboratory test the cement paste might well contain sufficient calcium carbonate to render the material more closely akin to limestone, hence explaining their similarity in performance.

6 Conclusions

The quaternary ammonium biocide BAC was effective in reducing and in some cases eliminating algal growth on a range of natural stone and CLS mortar test blocks under laboratory conditions. It was also able to prevent or retard recolonisation for the duration of the experiment. The biocide was most effective on Carrara marble and certain limestones at 1 per cent and least effective on the two sandstones.

Comparison of results from laboratory experiments using the moist vermiculite-bed technique with parallel field trials is needed to establish the correlation necessary as the basis for predicting field performance.

References


Specialised bibliographies are produced from the Biodeterioration Information Centre’s document collection from 1965 as listed in the bibliographic journals Biodeterioration Research Titles (B.R.T.) and Waste Materials Biodeterioration (W.M.B.).

Bibliographies may be updated by use of B.R.T. and W.M.B. or purchase of new editions of existing titles. Copies of papers listed may be purchased from the Biodeterioration Information Centre.

Following are Bibliographies currently available — all currently updated:

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ROLE OF SOME MESOPHILIC SOIL ORGANISMS IN RICE STUBBLE DEGRADATION

S.K. Chatterjee and B. Nandi

Summary

Rice stubbles were found to be degraded in laboratory conditions by some fungi and Streptomyces chibaeus isolated from soil closely associated with the stubbles. Degradation of holocellulose and lignin, the major constituents of the stubbles, were mainly responsible for the loss in dry weight of the stubbles. A mixture of all the test organisms degraded stubbles and its major components at the highest rate.

Individually, Geotrichum candidum was found to be the best degrader and this was sequentially followed by Oospora lactis and S. chibaeusis. The degradation of the stubbles by the organisms took place optimally at 30°C. The optimum temperature for the production of cellulases and lignin-decomposing enzymes by the organisms was also found to be 30°C.

Introduction

In India, along with the production of 130 million tonnes of crops about 100 million tonnes of stubbles remain in the field after harvest. As rice is a major crop much of this residue is rice stubble. Stubble is usually burnt off before cultivation of the next crop but many countries are now putting emphasis on converting stubble into humus to improve soil conditions.

Our earlier studies (unpublished) identified mesophilic organisms as efficient degraders of rice stubble in the field. There have been more reports of biodegradation by thermophilic soil organisms than by mesophiles but Trigiano et al. (1979) reported the importance of mesophilic organisms in the formation of wheat compost. Our work indicated that an initial burst of the population of efficient degraders in natural soil was required for rapid degradation of plant residues in soil. This is obviously an important step in utilising the agrowastes effectively, particularly in a country like India where intervals between crops have been reduced to only one or two months.

In this present study the species which were efficient degraders in the field were tested for stubble degradation in the laboratory. Since rice stubble mainly consists of holocellulose (cellulose + hemi cellulose) and lignin, its degradation depends greatly on the rate of utilisation of these major constituents through the activities of cellulases and lignin degrading enzymes like phenol oxidases produced by the microorganisms. Kirk (1971) reported phenol oxidases to be a part of the enzyme

Table 1
Degradation of rice stubble: loss of holocellulose and lignin caused by some individual and mixed soil microorganisms after 60 days.

<table>
<thead>
<tr>
<th>Organisms</th>
<th>Degradation of rice stubble (%)</th>
<th>Loss of holocellulose (%)</th>
<th>Loss of lignin (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Penicillium cyaneum</em> (Bainier and Sartory) Biourge</td>
<td>22.0 (0.98)</td>
<td>38.62 (0.74)</td>
<td>8.08 (0.26)</td>
</tr>
<tr>
<td><em>Penicillium waksmani</em> Zaleski</td>
<td>31.30 (0.75)</td>
<td>45.95 (1.13)</td>
<td>11.20 (0.39)</td>
</tr>
<tr>
<td><em>Aspergillus wentii</em> Wehmer</td>
<td>26.50 (1.15)</td>
<td>41.76 (0.41)</td>
<td>6.19 (0.24)</td>
</tr>
<tr>
<td><em>Aspergillus niger</em> Van Tieghem</td>
<td>29.0 (0.64)</td>
<td>44.13 (0.68)</td>
<td>13.55 (0.24)</td>
</tr>
<tr>
<td><em>Oospora lactis</em> (Fresenius) Lindau</td>
<td>32.50 (1.04)</td>
<td>48.73 (0.37)</td>
<td>12.53 (0.20)</td>
</tr>
<tr>
<td><em>Thielavia terricola</em> (Gilman and Abbott) Emmons</td>
<td>28.80 (0.81)</td>
<td>41.60 (0.40)</td>
<td>10.19 (0.26)</td>
</tr>
<tr>
<td><em>Geotrichum candidum</em> Link</td>
<td>36.20 (1.10)</td>
<td>47.83 (0.34)</td>
<td>18.15 (0.42)</td>
</tr>
<tr>
<td>Fungi mixed</td>
<td>40.0 (0.69)</td>
<td>58.90 (0.58)</td>
<td>28.12 (0.20)</td>
</tr>
<tr>
<td><em>Streptomyces chibaensis</em> Suzuki, Nakamura, Okuma and Tomiyama.</td>
<td>33.0 (0.58)</td>
<td>51.67 (0.64)</td>
<td>12.09 (0.38)</td>
</tr>
<tr>
<td>Fungi + <em>Streptomyces</em> sp.</td>
<td>41.50 (0.64)</td>
<td>61.98 (0.33)</td>
<td>29.30 (0.19)</td>
</tr>
</tbody>
</table>

In fresh stubble: holocellulose, 61%; Lignin, 25.53%.
Each result is the mean of three replicates (± standard deviation)

Complex for complete decomposition of lignin. The relative efficacies of some mesophilic organisms in rice stubble degradation, its holocellulose and lignin constituents, and the optimum temperature for stubble degradation as well as for enzyme production are reported.

Materials and methods

Rice stubbles included stem (about 15 cm) and root system in the ratio of about 3:1. For the present experiment, the stubbles were uprooted from the field immediately after harvest by loosening the soil. They were then washed in running water, sun dried for two days and cut into small pieces (about 2.5 cm). Finally, the pieces were dried at 65°C in a hot air oven to constant weight.

Populations of microorganisms associated with rice stubbles in fields around Burdwan, West Bengal, were isolated from soil by the dilution plate technique. The numerically dominant species after 45 days of crop harvest were selected as the principal degraders (Table 1).

Results were based on at least three replicates of each set.

Relative efficacies of pure and mixed cultures in stubble degradation

Dried pieces of stubble (10 g) and distilled water (50 ml) were taken in flasks (250 ml) plugged and autoclaved for 1 h at 121°C. Spore suspension (2 ml) of more or less equal potency (1.2 x 10^4 spores/ml) of the
organisms (except for Thielavia terricola where a suspension of mycelial bits was used as inoculum in absence of asexual spores) was inoculated separately to each flask and thoroughly mixed. For mixed cultures, the density of the inoculum was also kept identical as far as possible. Parallel sets were inoculated with either a mixture of all fungi or all fungi plus Streptomyces sp. The inoculated flasks were then incubated at 30°C for 60 days and shaken occasionally. Partially degraded stubbles were taken from flasks after 60 days, dried at 65°C to constant weight and the loss in dry weight was used for estimating the extent of degradation.

Loss of holocellulose and lignin

Loss of holocellulose and lignin was estimated from the partially degraded stubbles after 60 days of incubation. Isolation and quantitation of holocellulose in stubbles were done following TAPPI standard (1954) and Cowling (1961). Residual holocellulose was obtained by successive pre-extraction of powdered stubble tissue (100 mg) with ethanol: benzene, ethanol and hot water to remove extraneous substances, followed by a succession of chlorination and monoethanol-amine treatment to remove lignin. The holocellulose preparation was then washed several times in ethanol and ethyl ether, kept at 35°C for at least 2 h to remove ether and finally dried at 65°C to constant weight.

Lignin content was estimated following Saeman et al. (1954) by removing the total carbohydrates from powdered stubble tissue (0.4 g) through hydrolysis with 72.01 % (w/v) ice cold H₂SO₄. The mixture was kept for 1 h under constant agitation. The acid strength of the mixture was then diluted with water to 4%, autoclaved at 121°C for 1 h, cooled, filtered through a tared Alundum crucible and washed several times with distilled water to render the lignin residue acid-free. It was then air dried at 65°C to constant weight to calculate the amount of lignin.

Determination of optimum temperature for stubble degradation

The individual organisms which degraded stubbles considerably (about 29.0%) along with a mixture of all fungi and Streptomyces sp. in the earlier experiment, were used for this purpose (Table 2). Dried pieces of stubbles (10 g) and distilled water (50 ml) were taken in flasks (250 ml), sterilized, inoculated as before and kept at 20°, 25°, 30° and 35°C for 15 days. Extraction and assay of polyphenol oxidase was made following mainly Bateman (1962). Control and partially degraded stubbles were homogenized separately with a pinch of sand in phosphate buffer (10 ml) of 0.1 M of pH 7.0 at 0°C. After filtering and centrifuging the filtrate at 3000 G, the supernatant was collected in tubes and kept in an ice bath until used.

The enzyme extract (1 ml) and distilled water (4 ml) was mixed and adjusted to zero absorbance of a colorimeter. 1 ml of catechol solution (0.8 mg/ml) was added and mixed to it. The enzyme was measured immediately in terms of its activity through change in absorbance per min (ΔA/min) at 490 nm. A set of heat killed extract at 100°C for 10 minutes, showed no change, indicating complete inactivation of the enzyme.

For peroxidase extraction and assay, partially degraded stubbles (4 g) from parallel sets were used following mainly Bateman (1962). These stubbles along with control were ground separately in cold distilled water (20 ml) in a mortar at 0°C. After filtration and centrifuging the supernatant was used for enzyme assay.

Pyrogallol reagent was prepared by mixing 0.5 M pyrogallol solution (10 ml) and 0.66 M phosphate buffer (12.5 ml) and the volume made up to 100 ml with distilled water. 5 ml of this reagent freshly prepared and 1 ml of enzyme extract were mixed in a colorimeter tube and the mixture was immediately adjusted to zero absorbance of a colorimeter. 1% H₂O₂ solution (0.5 ml) was added and thoroughly mixed to initiate the reaction. Enzyme activity was recorded on the basis of change in absorbance per minute (ΔA/min) at 430 nm immediately after the addition of substrate. Similarly, non enzymatic oxidation was maintained at different times by heating the extract at 100°C for 10 minutes. The activity was always measured, zero indicating its complete inactivation by heat treatment.

The control sets (without inoculum) for polyphenol oxidase and peroxidase production, might not be important. These sets always showed zero enzyme activity indicating absence of any enzyme.

Cellulase synthesis

Cultures were grown in modified Czapek-Dox broth by substituting glucose with 1% cellulose powder and adding peptone (0.1%). The nutrient solution (50 ml) in flasks (250 ml) was inoculated either with individual organisms which were found to degrade ligno-

Table 2
Effect of temperature on degradation (expressed by the loss in dry weight) of rice stubble by some individual and mixed soil microorganisms after 60 days

<table>
<thead>
<tr>
<th>Organisms</th>
<th>Temperature:</th>
<th>Percentage of degradation</th>
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<tr>
<td></td>
<td>20°C</td>
<td>25°C</td>
</tr>
<tr>
<td><em>Pencillium waksmani</em> Zaleski</td>
<td>18.80 (0.85)</td>
<td>25.75 (0.55)</td>
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<tr>
<td><em>Aspergillus niger</em> van Tieghem</td>
<td>14.95 (0.53)</td>
<td>24.50 (0.41)</td>
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<tr>
<td><em>Oospora lactis</em> (Fresenius) Lindau</td>
<td>24.65 (0.89)</td>
<td>29.0 (0.40)</td>
</tr>
<tr>
<td><em>Geotrichum candidum</em> Link</td>
<td>24.50 (0.85)</td>
<td>31.10 (0.47)</td>
</tr>
<tr>
<td><em>Streptomyces chibaensis</em> Suzuki, Nakamura, Okuma and Tomiyama</td>
<td>14.50 (0.49)</td>
<td>22.00 (0.58)</td>
</tr>
<tr>
<td>Mixed fungi + <em>Streptomyces</em> sp.</td>
<td>21.40 (0.26)</td>
<td>28.90 (0.65)</td>
</tr>
</tbody>
</table>

Each result is the mean of three replicates (± standard deviation)

Results and Discussion

Relative efficacies in stubble degradation

The mixture of all test organisms degraded the stubble most efficiently (Table 1). Parkhe and Shinde (1978) also reported highest degrading efficiency by the mixture of their test organisms. Individually, *Geotrichum candidum* proved to be the best, followed by *Streptomyces chibaensis* and *Oospora lactis*. These organisms degraded lignocellulosic material in rice stubble more efficiently (32.50 - 36.20%) in 60 days than the most efficient thermophiles isolated from soil by Jain et al. (1979), which degraded lignocellulosic material (25-29%) in wheat straw within the same period.

Loss of holocellulose and lignin

The mixture of all test organisms was found to be the best degrader of holocellulose and lignin (Table 1) which could possibly be due to synergistic activity of the organisms. Similar synergism in the cellulolytic activity of mixed cultures of fungi was recorded by Stewart and Walsh (1972). Individually, the most efficient degrader of holocellulose was found to be *S. chibaensis*, followed by *O. lactis* and *G. candidum*. Lignin degradation by individual organisms was effected most efficiently by *G. candidum*, followed by *Aspergillus niger* and *O. lactis*. This corroborated the report by Campbell (1952) that most fungi which were lignolytic were also cellulolytic. Reports on holocellulose degradation by saprophytic microorganisms are rather rare and most works relate to the degradation of cellulose. Haider and Domsch (1969) and Martin and Haider (1969) reported degradation of cellulose (33-66%) and lignin (33-55%) in wheat straw by microscopic fungi. The present investigators reported that *Aspergillus wentii*, *Streptomyces* spp. and *Penicillium* sp., isolated from
Fig. 1. Effect of temperature on polyphenol oxidase synthesis by some individual and mixed mesophilic microorganisms.

Fig. 2. Effect of temperature on peroxidase synthesis by some individual and mixed mesophilic microorganisms.

Fig. 3. Effect of temperature on cellulase synthesis by some individual and mixed mesophilic microorganisms.
soil associated with wheat stubble, degraded holocellulose and lignin efficiently (Chatterjee and Nandi, 1981).

Optimum temperature for stubble degradation

All the test organisms showed considerable degradation at all the tested temperatures with the optimum at 30°C (Table 2). The sequence of the organisms as regards their efficiency of stubble degradation has already been stated. The fact that the best performance of the organisms occurred at 30°C confirmed their mesophilic nature.

Polyphenol oxidase production

Polyphenol oxidase production by the organisms was recorded at all the tested temperatures (Fig. 2) with the highest by the mixture of fungi and *Streptomyces* sp. It was also highest at 30°C, the temperature which was optimum for stubble degradation. Among the individual organisms maximum activity was observed in *G. candidum* which also proved to be the most active individual organism in lignin degradation. Thus, a close correlation between lignin degradation and polyphenol oxidase at 30°C was shown.

Peroxidase production

Peroxidase synthesis was also evident at all the tested temperatures, the maximum being at 30°C (Fig. 3). At this temperature the mixture of fungi plus *Streptomyces* sp. and *A. niger* proved to be the two highest producers of peroxidase followed sequentially by *Penicillium waksmani*, *S. chibaensis*, and *G. candidum*. Thus, although the optimum temperature for peroxidase production corresponded with the optimum for stubble degradation, the organisms involved did not always show any correlation. The mixture of fungi plus *Streptomyces* sp., for example, showed a much higher rate of lignin degradation than *A. niger*, although their peroxidase production remained equal. Thus, it was quite evident that peroxidase was not the only enzyme involved in lignin degradation but formed a part of the enzyme complex, supporting Kirk (1971).

Cellulase synthesis

At all tested temperatures, considerable cellulase synthesis by the organisms was evident (Fig. 1) with the optimum at 30°C in all cases. This observation of maximum enzyme synthesis by the organisms corroborated their optimum efficiency in holocellulose and stubble degradation at 30°C. The most efficient cellulase producer was the mixture of fungi plus *Streptomyces* sp. followed by *G. candidum*. Production of a cellulase system by *G. candidum* was reported by Tikunova *et al.* (1980) who separated C-enzyme, endo-B-glucanases and cellobiase by ion exchange chromatography.

Thus, although *S. chibaensis*, and *O. lactis* were found to be efficient holocellulose degraders of the stubble, they showed relatively low FPD (filter paper degrading) ability. This indicated that the organisms required some specific substances for good production of cellulase, which were available when grown on stubble. Jain *et al.* (1979) also observed that some of their experimental fungi although degrading cellulose in wheat straw quite efficiently, showed no FPD activity when cellulose was provided as sole carbon source in submerged culture.

The degradation of the stubble and its major constituents (cellulose and lignin) with relation to enzyme production indicated the soil microorganisms to be the principal degraders. Mixture of these organisms increased significantly their efficiency of degradation. Products from degraded rice stubble in soil have been shown to promote growth of crop plants (unpub. data) revealing thereby the role of the microorganisms in biodegradation and soil productivity.

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CORRECTION

The Editor apologises to readers and to the authors of ‘Microfouling on Metal Surfaces Exposed to Seawater’ by Sharon G. Berk, Ralph Mitchell, Ronald J. Bobbie, Janet S. Nickels and David C. White which was published in Volume 17 (2), Summer 1981.

Unfortunately several lines of text were omitted together with one sub-heading. Adjacent is the correct version of column 1 of page 30 of that article.

There is also an omission from the legend of figure 5, page 36 where the scale bars: A, B, C and E should read = 10 μ (the number 10 has been omitted).

organisms living in the sea (Baier, 1980). The organic layer is believed to attract microbes (Young and Mitchell, 1973). The adsorbed layer of biopolymer, which consists of proteins, polysaccharides and lipids, has a lower surface tension than the metal surface which it covers and thus increases the adhesion of the organisms. Free swimming bacteria are believed to be attracted to the higher nutrient concentrations of the film and attach reversibly. Shortly thereafter irreversible attachment and the secretion of acidic polysaccharide extracellular polymeric films begins (Marshall, 1976). Different surfaces have been reported to select fouling populations with different morphologies (Marsalek et al., 1979).

Effect of the Metallic Surface Texture on the Morphology of the Microfouling Organisms

One-inch square metal sheets were exposed to the sea at Nova University Ocean Sciences Center, Fort Lauderdale, Florida, two meters below high, and 0.5 meters below the low, tide level, and collected after 6, 10 and 15 days. Half the samples were sandblasted before immersion to test for effects of surface texture. The specimens were fixed in glutaeraldehyde dehydrated in acetone, and critical point dried, prior to scanning electron microscopy. The surface metallic texture apparently had little effect on the rates of microfouling. Comparison of rough and smooth surfaces of copper, stainless steel, and aluminium, is shown in Fig. 1. At lower magnification (not shown) little difference between the morphology of the microorganisms on the rough and smooth surfaces of the three metallic surfaces could be detected.

Effect of the Metallic Surfaces on the Rate of Colonization

Copper surfaces foul more slowly than aluminium, titanium or stainless steel and at 15 days bacteria are the only organisms visible (Figure 1A). The fouling of stainless steel illustrates a progression with time showing bacteria (6 days), stalked algae and chains of diatoms (10 days) and protozoa (15 days). Aluminium surface corroded very rapidly and was covered by aggregates of bacteria with an occasional flagellate protozoan visible within 6 days (Figure 2). Longer exposure allowed development of stalked algae and sessile protozoa which are themselves coated with bacteria (Figure 3). The copper surface induced bacteria to form an extracellular film which can be seen peeling off the metallic surface (Figure 4). The peeling may be a fixation artefact.

Titanium fouls most rapidly of the metals tested and forms a diverse microbial community (Figure 5). The film contains microorganisms that do not appear on other metals.

Microfouling in Flowing Seawater Systems

Scanning electron microscopy shows that the microfouling film which forms on the surface of 5052 aluminium or titanium after 29 days’ exposure to flowing seawater at Panama City, Florida, has a complex morphology (Figure 6). The resistance to heat transfer for the aluminium and titanium pipes was 5.8 x 10⁻⁴ units when not fouled but increased to 8.0 x 10⁻⁴ units after this time.*
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BOOK REVIEWS

SOIL BIOCHEMISTRY.

Volume 5


The editors of this volume have not attempted to attain a single theme, but have deliberately chosen a wide range of subjects, and have selected a globally widespread group of contributors: 3 from Australia, 5 from Canada, 3 from New Zealand, 1 from Sweden, 2 from U.K. and 4 from U.S.A.

The three longest chapters are potentially of direct interest for workers in biodeterioration. Chapter 1 covers naturally occurring resistant organic materials, mainly humic and non-humic compounds. There is consideration of the turnover of C compounds; plant and animal residues; radio-Carbon dating and the problems involved, modelling of organic turnover; the formation and chemistry of various organic complexes in the soil. Chapter 6 is concerned with petroleum components in the soil, and deals with their biochemistry and microbiology. The range and diversity of the micro-organisms which can use crude and refined petroleum oils is considered; and aspects of the influence of crude oil, oily wastes and hydrocarbons has been examined. The decomposition pathways for various oil fractions, rates of decomposition, organisms involved (generic names only), the range of substrates and their fate in the soil are dealt with. Chapter 10 deals with the measurement and turnover of microbial biomass using different methods, which range from direct microscopy to analysis for special biomass constituents such as ATP, muramic acid and nucleic acids.

The other seven chapters cover methods for in-situ analysis of organic matter in soil; the oxidative coupling of aromatics by enzymes, which is important when considering the disposal of synthetic waste materials; urea transformations, the properties of urease and the importance of urease inhibitors when urea is used as a N fertilizer; quantitative assessment and turnover of amino sugars; nitrogen fixation and denitrification, where methods for measurement, and the organisms involved are considered; and the presence and toxicity of heavy metals, and their role in nitrogen metabolism. These chapters contain material which is at least of indirect interest in biodeterioration, and which is directly relevant for soil biologists, soil chemists and biochemists.

The volume is a useful addition to the series which is concerned with Soils and the Environment.

G.J.F. PUGH

MANAGING LIVESTOCK WASTES


Living on a densely populated and intensively farmed island it is difficult to comprehend why there is a need in the USA, with its vast areas of sparsely populated land, to even consider livestock wastes as a problem. However the first chapter of this book soon reveals that all is not well in a discussion of the scope of the problems with relevant statistics. This incorporates a section on management concerns and the pollution potential of a total animal population of 3867 x 10^6. Having read the first chapter it is obvious that the contents relate to the American experience of waste management and that one can only extrapolate to the European situation. Two chapters follow on sanitary microbiology (the use of micro-organisms in the purification of water and treatment of wastes) and the fundamentals of applied microbiology. These two chapters briefly describe the role of micro-organisms in the treatment of waste water and its purification. Sanitary microbiology is probably a more apt blanket term to cover both chapters: the title 'fundamentals of applied microbiology' being rather over-ambitious. The remaining chapters are on the whole of a non-biological nature relating to the characteristics of animal wastes, their collection, storage, transport and separation. A section is devoted to methods of waste treatment by use of stabilisation ponds, activated sludge processes, anaerobic digestion, composting, drying and incineration. The final section concerns itself with utilisation of wastes, overall planning of a waste handling system and the all-important financial considerations.

The preface states that the book is aimed at agricultural engineering students and this reviewer would whole-heartedly agree. Its use to workers in biology is in its description of handling systems and the practicalities in operating them. Workers investigating agricultural waste treatment using novel microbial systems should be aware of how their systems fit in with the total management of wastes. Although geared toward the engineer it should be easily read and understood by the biologist, there being a large number of line drawings and diagrams to supplement the text throughout.

K.J. SEAL
CARING FOR BOOKS AND DOCUMENTS

A.D. Baynes-Cope


Dr Baynes-Cope has admirably distilled his considerable wisdom on this subject into a single slim volume without loss of clarity or authority. Primarily intended for the non-conservator, the book is especially appropriate to the needs of librarians, book collectors, and others having the preservation (as distinct from conservation) of books and documents in their charge. It concentrates largely on the reasons for deterioration and what may be done to prevent it, rather than specifying remedial treatments, although advice is given on the small-scale fumigation of material for insect and microbiological attack.

Ideal storage conditions (13-18°C and 55-65% R.H.) are discussed in the context of what might be possible where air-conditioning cannot be achieved. The materials which may be encountered in books and documents are reviewed and potential sources of their deterioration set out in some detail under the headings ‘Physical and mechanical damage’, ‘Biological enemies’, and ‘Chemical enemies’. Recommendations for prevention of deterioration are not complex and Baynes-Cope gives appropriate weight to the importance of building maintenance and good housekeeping in storage areas for books and documents. He points out the provision of free air circulation as being the most important single climatic factor for the safe storage of books. Provision of a moderate and stable climate is also to be recommended.

There is little in this book to which one can take exception and it should be well received.

G.S. LEARMONTH

SMITH'S INTRODUCTION TO INDUSTRIAL MYCOLOGY

A.H.S. Onions, D. Allsopp and H.O.W. Eggins


‘An Introduction to Industrial Mycology’ by Mr. George Smith was first published in 1938 and after six editions this successful work has been revised and updated by A.H.S. Onions, D. Allsopp and H.O.W. Eggins. The aim of this book is to assist non-specialists in industry to identify fungi. This has been achieved with notable success in the first 270 pages which are devoted to descriptions of fungi, line drawings and photographs. Fungi are notoriously difficult to photograph under the microscope and the plates in this book are as good as any that can be found elsewhere. The chapter on the Zygomycotina has improved illustrations but covers the same somewhat restricted range as earlier editions. It would have been improved by the inclusion of representatives of the Choanephoraceae and Cunninghamellaceae. The Ascomycotina have been expanded to provide a compact but useful account of this very diverse group. The Yeasts have been completely rewritten by Dr. R.R. Davenport to give an excellent account supported by tables and photographs which should place the identification of the common yeasts well within the readers grasp. The Coelomycetes deserve rather more attention but unfortunately this edition is as unhelpful as earlier ones when dealing with this group. There are important pycnidial species other than Phoma; likely candidates would include Botryodiplodia; Diplodia and Pyrenoehaeta.

The number of Hyphomycete genera described has been increased from 25 to 32 and accompanied by much improved photographs and drawings. The key to these genera has been much improved and the reader will find it accurate and friendly, in that predictable misinterpretations by the novice user will still lead to the correct identification. Two chapters (100 pages) are devoted to Aspergillus and Penicillium respectively, these are much improved on the original versions. Many will find this book well worth having for these chapters alone.

The final third of this text is devoted to industrial aspects of fungal ecology, spoilage exploitation and control. Some of these chapters are largely unchanged and it would have been better if the authors had been more ruthless in their approach. Some sections offer invaluable information and practical advice but others are rather dated. One criticism relates to the recommended use of propylene oxide for cold sterilization without reference to safety precautions and the highly explosive properties of this material. Recent developments in the use of electron beam sterilization should have been included in the section on sterilization. The section devoted to biochemical studies should have included stirred tank fermenters. However, there is still a lot of good material.

In conclusion, I predict that this book will be heavily used in the laboratory for the identification of fungi. In this role it will give instruction and confidence to the novice and be a support to the experienced mycologist. It is well worth the price of £37.50.

R.N. SMITH
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Wood rotting fungi, culture using waste tea leaves

Vermiculite bed test method for algicides

Yam tubers damaged by Botryodiplodia theobromae

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Special bibliographies on specific aspects of biodeterioration and biodegradation are produced from the document collection from 1965 and updated regularly. See the advertisement in this issue.

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Three quarterly journals are published:

1. International Biodeterioration Bulletin (IBB) A scientific journal for publication of original works, including reviews and book reviews on all aspects of biodeterioration and biodegradation. Also contains the Biodeterioration Society Newsletter and short abstracts of papers presented at meetings of the Society in Great Britain and Ireland.

2. Biodeterioration Research Titles (BRT) A bibliographic journal which presents, in classified form, references to published literature on all aspects of biodeterioration and biodegradation. About 2000 references per annum.

3. Waste Materials Biodegradation Research Titles (WMB) A bibliographic journal similar to BRT dealing with all aspects of the biological treatment of solid and liquid wastes and the biodegradation of waste materials in nature. About 1800 references per annum.

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