A combined primary and abstract journal covering the fields of biodeterioration and biodegradation-biotechnology.
INTERNATIONAL BIODETERIORATION

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International Biodeterioration replaces the International Biodeterioration Bulletin, Biodeterioration Research Titles and Waste Materials Biodegradation Research Titles previously produced by the former Biodeterioration Centre at the University of Aston-in-Birmingham.

International Biodeterioration is a unique quarterly journal of news, reviews, original papers and abstracts covering the whole field of biodeterioration and biodegradation.

Items for the News and papers sections, as well as items for abstracting, should be addressed to the Editor-in-Chief.

Biodeterioration Centre
The Biodeterioration Centre at the Commonwealth Mycological Institute provides most of the services provided by the Aston Centre (which closed in December 1983). These include information services (online and using the extensive reprint collection now transferred to CMI from Aston), specialist bibliographies, confidential investigations and consultancy, short-courses, and the microbial testing of materials to British and foreign standards.

For further information of the activities of the Centre contact its Director, D. Allsopp Ph.D., M.I.Biol., M.I.Inf.Sci.

The Centre is a part of the CMI Culture Collection and Industrial Services Division which supply a wide range of services to industry.

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[Note: Prices are subject to change without notice]
This issue is dedicated to the memory of Thomas Alan Oxley (1910-1983), Visiting Professor to the Biodeterioration Centre, University of Aston 1970-1983, and sometime Editor of the International Biodeterioration Bulletin.

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Introduction to Authors

Contributions from anywhere in the world are welcome for consideration for inclusion in *International Biodeterioration*. Papers should be submitted in duplicate to the Editor-in-Chief. For the style of presentation see the latest issue of the journal. All original papers and reviews are independently refereed prior to acceptance for inclusion.

In order to minimise delays in publication, proofs are not normally sent to authors for correction but checked by the Editor-in-Chief.

Authors receive two copies of the part of the journal in which their paper appears free of charge. No reprints are supplied, but authors are authorized to reproduce copies of their own papers for personal distribution.

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EDITORIAL

This issue marks the twentieth year of the publication of the International Biodeterioration Bulletin, which I founded in 1965 at the Birmingham College of Advanced Technology, later to become the University of Aston in Birmingham, England; soon after this the bibliographic journal Biodeterioration Research Titles was published, to be followed by Waste Materials Biodegradation Research Titles.

During this time, the circulation of these journals has grown steadily, thanks to the support of authors and subscribers, and the stirring editorial work in recent years of Professor Tom Oxley, who died last year and to whom this issue is dedicated; an obituary appeared in the previous issue of this journal. Until recently, the journals were owned by the University of Aston which ensured that editorial control and overall policy remained very much in the hands of those actively concerned in biodeterioration, working at Aston's Biodeterioration Centre.

Recently, the 30% cut in Government funding experienced by Aston University had profound effects on the Biodeterioration Centre's work at Aston, especially in drastically reducing the numbers of research students that the Centre was permitted to have, whilst the academic situation of the Centre within the University became uncertain. Clearly this would have had effects on the future of the biodeterioration journals. Fortunately, at this time, Mr Anthony Johnston, the then Director of the Commonwealth Mycological Institute at Kew, asked me to co-operate in the production of an Abstract journal in biodeterioration. This publication is the result of that approach: the combining of the International Biodeterioration Bulletin (IBB) and International Biodeterioration Abstracts (IBA) (replacing both Biodeterioration Research Titles and Waste Materials Biodegradation Research Titles)—as two parts of one publication, International Biodeterioration, of which this is the first issue.

In 1982 I decided to leave Aston (my resignation having effect from July 1984) to concentrate on a range of interests including my Company, Bioquest Ltd, with my colleagues Dr Dennis Allsopp and Dr Ken Seal at present as fellow directors. Last year Aston decided on closure of the Centre and transferred ownership of the journals to me, whilst I transferred them to Bioquest Ltd. and continued arranging for the Commonwealth Agricultural Bureaux (CAB) to act as publishers of International Biodeterioration, the CAB being the parent body of the Commonwealth Mycological Institute (CMI). Dr Allsopp was appointed as Industrial Mycologist at the CMI later that year and I was delighted to arrange for the transfer of the Biodeterioration Centre and its unique literature collection to the CMI, where I have been appointed Senior Consultant to the Centre. CMI's involvement in biodeterioration was increased in the early 1960's by John Elphick's activities and continued subsequently by Dr A. H. S. Onions. Dr Allsopp as the new Director of the Biodeterioration Centre at CMI will continue that tradition.

International Biodeterioration will continue to maintain the International Biodeterioration Bulletin as a primary journal publishing original papers on biodeterioration and biodegradation, whilst International Biodeterioration Abstracts will normally have about 700 abstracts; this first issue contains fewer than will be normal owing to computer selection-date procedures used for producing this section of the journal.

Photocopy and individual search services can still be obtained through CMI, but in addition on-line retrospective search facilities on the abstracts published will also now be available on the CAB ABSTRACTS database via Lockheed 'DIALOG' and other database hosts; CMI will be pleased to quote costs for carrying out such searches. Technical enquiry services will be augmented by manual methods using the previous journals, bibliographies, and the Centre's document collection.

I am delighted at the new arrangements for the International Biodeterioration journals of which I have been Editor-in-Chief from the beginning, as their ownership in Bioquest Ltd will ensure continuing direction by those directly involved in biodeterioration, whilst publication by the CAB will improve world-wide sales and the provision of abstracts from CAB's information network will meet with wide approval.

The News section is much expanded and I am sure will prove of interest. I am very anxious that it should be truly international in context and I hope very much that contributions will be submitted for inclusion—length is immaterial—for which small payments can be made. In the IBB itself more review articles will be published and I welcome receiving suggestions.

Please write to me at the Commonwealth Mycological Institute over any editorial matters which relate to International Biodeterioration.

H. O. W. Eggins
Editor-in-Chief

21 March 1984
The Biodeterioration Society

The aims of the Biodeterioration Society are to promote the science and technology of biodeterioration and its prevention and biodegradation of economic importance.

It is an international society, open to all with a scientific, technological, practical or commercial interest in biodeterioration and biodegradation. At present the membership comprises more than 240 members from 27 countries.

If you wish to join the Society please contact the Secretary:
Mrs J. M. Maw
Biological Sciences
Hatfield Polytechnic
Hatfield P.O. Box 109
Herts AL10 9AB
UK.

For U.K. members: Membership of the Society is initially £4.50 per year on joining, then £4.50 per year if payment is made by standing order. For payment other than by standing order the subscription is £5.50 per year due on 1st April each year. Details required to pay your subscription to the Biodeterioration Society by standing order are: The Biodeterioration Society Bank is: The National Westminster Bank PLC, 2 Market Street, Penistone, Sheffield, UK; Sorting Code 54-21-34; Account 02939185.

For Overseas Members: Membership of the Society is £10 for three years or £3.50 per year.

All members of the Society pay reduced registration fees at the Society's scientific meetings and are entitled to a reduced subscription rate to the International Biodeterioration provided they are a member of an organisation that is already subscribing at the full rate.

Sixth International Biodeterioration Symposium
5-10 August 1984
The George Washington University, Washington DC, USA.

This symposium is being co-sponsored, on behalf of the Biodeterioration Society, by the Department of Forensic Sciences of The George Washington University and by the Department of Biology of the Virginia Commonwealth University.

The George Washington University is in downtown Washington and immediately adjacent areas are the White House, the World Bank, the National Academy of Sciences and the John F. Kennedy Center for the Performing Arts.

Accommodation will be in University halls or at local Hotels.

Registration Fees:
Paid by 1st April, 1984 U.S. $175.00
Paid after 1st April, 1984 U.S. $200.00

Registered (PAID) participants will be sent at a later date a final registration form from George Washington University for accommodation and social events. Participants who register after 1st April, 1984 cannot be guaranteed accommodation. Registration fees should be paid in U.S. dollars with cheques made to the Sixth International Biodeterioration Symposium.

Students will be charged Registration Fees at half rate, but will not receive a copy of the Proceedings. Full Registration details can be obtained from:
Dr Charles E. O'Rear,
Biodeterioration Symposium Registrar,
Department of Forensic Sciences,
George Washington University,
Washington, DC 20052, USA.
Telephone: 202-676 7319
The Scientific Program will include the following topics:
General Biodeterioration—Techniques, Methods and Specific Environments
Chemical Control of Biodeterioration by Means of Biocides, Biotats and Preservatives
Biodeterioration of Structural Materials including Woods, Metals, Stone, Plastics and Related Materials
Biodeterioration of Fuels, Lubricants, Petroleum Products, Pharmaceuticals, Cosmetics and Xenobiotic Substances

Organisms as Biodeteriogens, including Plants, Micro-Organisms, Insects, Birds, Rodents and Marine Organisms
Biodeterioration of Hydrocarbon Spillages, Solid Municipal Wastes, Agricultural Wastes, Biocides and Industrial Effluents
Biodeterioration of Pre- and Post-Harvest Cereals and Agricultural Products, including Production of Mycotoxins
Biodeterioration of Polymers and related Materials
Biodeterioration in Museums, Galleries, Libraries and Archives
Biodeterioration of Forensic Science Evidence and Materials
There will be both pre-planned lectures and offered papers. Further details may be obtained from:
Dr Gerald C. Llewellyn,
Biodeterioration Symposium Program Organizer.
Department of Biology, 816 Park Avenue, Virginia Commonwealth University, Richmond, Virginia 23284, USA.
Telephone: 304-257 1562

Standing Committee for International Biodeterioration Symposia (SCIBS)

Dr Howard Eggins has been appointed Chairman of this Committee by the Council of the Biodeterioration Society; this was necessitated by the sad death of the previous Chairman, Professor T. A. Oxley.

Network of Resource Centres in the Caribbean Region for Applied Microbiology and Bioengineering

The Biotechnology and Bioengineering Guatemala Microbial Resource Center (MIRGEN) functions within the Biotechnology Group of the Applied Research Division of the Central American Research Institute for Industry (ICAITI) involving Costa Rica, El Salvador, Guatemala, Honduras and Nicaragua.
This MIRCEN is composed of a Network of Resource Centres in Central and Latin America and Caribbean Regions who are affiliates and provide scientific and technological experience.

Carlos Rolz is the MIRCEN Director and Roberto de Leon Project Leader. There have been three recent Workshops:

**Solid Substrate Fermentations** which concerned, amongst other topics, the upgrading of agricultural wastes, and the production of animal feed-stuffs, and the growth of *Agaricus* sp. and *Pleurotus* sp. The proceedings in Spanish (US $15, including air mail delivery).

**Advances in Anaerobic Digestion** described the principles of anaerobic digestion, the characteristics of new designs, and then specific descriptions of the treatment of various agricultural, industrial and municipal wastes. Proceedings in English (US $15, including air mail delivery).

**Advances in Ethanol Production** covered a range of topics including economics, purification, the usefulness of various substrates and purified cellulose from steam-exploded wood. Proceedings in press.

The address of ICATII is: Avenida La Reforme 4-47, Zona 10, Guatemala, C.A.

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**International Biodeterioration Research Group (IBRG)**

The Autumn Meeting of the Group was held at the Biodeterioration Centre at Aston in Birmingham, England. Apart from the meetings of the individual Working Groups, this was the occasion for the Annual Plenary Meeting of IBRG that approved the move of the Secretariat to the Commonwealth Mycological Institute (CMI). The Officers and addresses are as follows:

**President:** Dr J. M. Shewan
**Chairman:** Dr A. G. Shewan
**Treasurer:** Dr A. F. Bravery
**Secretary-General:** Dr D. Allsopp, Commonwealth Mycological Institute, Ferry Lane, Kew, Surrey, TW9 3AF, UK.
**Telephone:** 01-940-4086

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  School of Natural Sciences,
  The Hatfield Polytechnic, P.O.
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**Industrial Problems Working Group Technical Secretary:** Mrs C. Allsopp, CMI

**Metal Working Fluids Working Group Technical Secretary:** Dr K. J. Scal,

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**Microbial Corrosion Working Group Technical Secretary:** Dr Christine Gaylarde,

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**Paints Working Group Technical Secretary:** Mr M. Greenhalgh,

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**Taxonomy and Ecology Working Group Technical Secretary:** Dr Glyn Morton,

Division of Biology,
Preston Polytechnic,
Corporation Street,
Preston, Lancs,
PR4 1RA, UK.
**Telephone:** 0772 22141

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**Dr James Shewan**

Dr James Shewan was awarded an Honorary LLD by the University of Aberdeen in July 1983. He had been at the Torry Research Station from 1935 to his retirement in 1974, latterly as a Senior Principal Scientific Officer; from 1959 to 1974 Dr Shewan was Curator of the National Collections of Industrial and Marine Bacteria, which has now been taken over by the University of Aberdeen.

The International Biodeterioration Research Group are especially indebted to Dr Shewan because of his work in 1962 on the British National Co-ordinating Committee on Biodeterioration, and then as Chairman and now President of the Group. Enthusiasm and generosity have been obvious characteristics of Jim Shewan’s many activities in microbiology and biodeterioration and his unfailing encouragement of the work of the Biodeterioration Centre from its inception at Aston and during his time as Chairman of its Consultative Council were much appreciated.

Many will remember Dr Shewan’s superb work as Chairman of the Organizing Committee of the 5th International Biodeterioration Symposium held at Aberdeen in 1981.

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**Chemicals as Biocides—Applications and Markets**

On 24 November, 1983, the Biodeterioration Society held a joint one day meeting with the Industrial Marketing Research Association at the Café Royal, London, under the Chairmanship of the Society’s President, Dr R. Neil Smith. The following are synopses of the papers presented:

1. **Chemicals as Biocides for Food and Dairy Processing Plants**, R. Hamblin (Diversey (SB) Ltd). The need for hygiene in the Food and Dairy industries becomes greater every year. Sterility of process plants and in some cases, of finished product has always been necessary to ensure both public safety and product integrity. However, the extensive publicity which may now be given to food poisoning outbreaks derived from commercially prepared processed food, and the great emphasis which must be given to product quality in an increasingly competitive market, mean that the need for hygiene in the Food and Dairy industries is greater than ever before.

The tonnage of chemical disinfectants used in food processing the EEG in 1979 was 11/182 tonnes and it is projected to show a 36% increase during the 10 years up to 1989.
The chemicals which are used in the Food and Dairy Processing industries for their disinfectant properties may be broadly divided into nine groups: Caustic Alkalis, Amphoteric compounds, Aldehydes, Biguanides, Oxygen-releasing compounds, Chlorine-releasing compounds, Iodophors, Acidic disinfectants and Quaternary Ammonium compounds.

(2) Safety Legislation and Marketing Restraints, A. G. Wilkie (Health and Safety Executive). In the UK we tend to have general legislation dealing with the safety of products and their labelling, whereas specific arrangements particularly in the pesticide/biocide field rest on non-legislative agreements such as the Pesticides Safety Precautions Scheme.

Legal freedom exists therefore to market biocides in most areas, subject only to the requirement of the Consumer Protection Act or Section 6 (4) of the Health and Safety at Work Act. These broadly require that the product be safe for its intended purpose and the latter specifically requires that the product be tested in order that adequate information is available. A recent enactment "The Notification of New Substances Regulations 1982" requires notification of a broad range of physico-chemical and toxicological properties before marketing any new substance not subject to hazard appraisal in specific areas of use, e.g., medicines, food additives and pesticides.

Regional Water Authorities also constitute another large layer of control when biocides can find their way into water courses and sewage systems but being autonomous bodies they exercise their own prerogatives as far as effluent standards or prohibitions are concerned.

New Regulations are expected to be published at the end of the year which will extend the current Dangerous Substances Regulations which require specified hazard labelling of some 1400 chemicals. These labelling regulations constitute a restraint to marketing but only so far as a dangerous product must be appropriately labelled.

(3) Chemicals as Biocides in the Hospital/Medical Field, G. R. Lloyd (Sterling Ltd). There are three major areas for which biocides are used in hospitals: Surface disinfection, Instrument disinfection and skin disinfection.

Surface disinfection refers to walls, floors, trolleys, benches here by necessity there is a need for a highly active biocidal agent. In common use are hypochlorite solutions, phenolic compounds and alcohols.

Products based on dialkyl ethers are commonly used for instrument disinfectants as they have a wide spectrum of activity and are compatible with the materials of construction.

Skin disinfectants must above all be of low toxicity. Alcohols, iodine and phenolic compounds are all used although the major share of the market for general skin disinfection is held by chlorhexidine based products.

(4) The Status of Biocides in Water Treatment, E. Stafford (Nalfloc Ltd). In industrial water treatment, biocides form a key element of any cooling water treatment programme. Biocides are necessary for the control of the growth of microorganisms, which left to multiply would inhibit heat transfer by fouling heat exchange equipment and, additionally, cause more rapid corrosion of the fabric of the plant. The application of biocides in cooling water treatment represents by far the largest portion of the Industrial Water treatment applications. All plants from the largest refinery or petrochemical complex to small air conditioning plants will be using some form of microbiological control. The total market is estimated to be some 6000-8000 tonnes per annum of which sodium hypochlorite or chloramine represents some 90%, the balance being in the form of proprietary biocides.

(5) Timber Preservatives, B. Richardson (Penarth Research International Ltd). Wood preservation is one of the largest markets for biocides, a market that is still developing in both size and complexity in most countries. The selection of an appropriate wood preservation system depends upon the type of wood to be treated and the service conditions that it will encounter, particularly the attacking organism. Preservation must involve the use of a chemical supplied at a sufficient retention to ensure adequate toxicity, although it is also necessary to ensure adequate penetration as moisture content cycling may result in the development of splits which will penetrate a superficial treatment. Generally impregnation techniques are used which involve the treatment of the wood under pressure and vacuum cycles in a closed cylinder.

The "Remedial Treatment" sector of the wood preservation industry has developed only over the last 50 years, and involves treatments applied in situ in buildings, usually when deterioration is suspected or has been found; application is normally by low pressure spray, although with injection into larger timbers which are considered to represent special risks.

Coal tar, a product of the distillation of coal, was originally used as a wood preservative, but the lighter cresote fraction was adopted following the introduction of pressure impregnation as its lower viscosity improved penetration. Water-borne preservatives consist of solutions of salts, generally selected because they possess an active ion. Commercially the most important preservatives today are copper-chromium-arsenic multi-salt preservatives, formulated from salts, oxides or acids.

The most promising developments in recent years are concerned with nitrogen compounds, particularly certain amines and quaternary ammonium compounds.

(6) Food Preservation, P. Goodenough (Long Ashton Research Centre). The act of converting plant or animal tissue into a transportable food product invariably involves death of the tissue. Unfortunately the tissue is rarely consumed immediately and preservation is needed to prevent degradation during the time before consumption.

The pressure exerted by groups concerned that food preservatives should only be added in minimal quantities and be free of any suspicion of causing harm will militate against the few chemicals we have at present.

Traditional preservatives used are: Salt and sucrose which in a concentrated form within foodstuffs
exert a desiccating effect upon spoilage organisms. Chemicals in a complex mixture which are toxic to microorganisms and include woodsmoke, spices, herbs and essential oils. Sulphur dioxide is permitted in 52 kinds of food. The mechanisms of sulphur dioxide action is still uncertain but this very reactive molecule will bind to many organic molecules. Benzoic acid or one of its derivatives is permitted in 24 kinds of food. Sorbic acid is an unsaturated fatty acid which inhibits fungal growth. This compound is useful in protein products such as meat and cheese and has no known harmful effects.

Nitrites were originally used in the curing of ham and bacon. Unfortunately the nitrites are rather toxic and combine with haemoglobin to form methaemoglobin in which oxygen is no longer available to the tissues.

As well as preservatives found in general use in the food industry, various other flavour and colouring additives are used but these are progressively being investigated for their toxicological properties.

(7) Petrochemical Products and Metal Working Products, E. C. Hill (University College, Cardiff). There is a long established history of using anti-microbial chemicals in petroleum products, particularly those destined to be dilutes with water for use in the metalworking industry.

For many years the growth of the market has been in general steady and has reflected an increased awareness of the cost of microbial problems. Compatibility of biocide and petroleum product is always of prime importance and we can thus anticipate that the antimicrobials used may change when product formulations change.

The biological activity of the chemicals used must be good against Gram negative bacteria, sulphate reducing bacteria and in special cases against fungi and yeasts. There is no magic cure-all biocide.

(8) Paints and Antifouling Agents

Part I. Paints, W. R. Springer (Paint Research Association). The paint industry's microbiological problems are of a sporadic and intermittent nature but, when they have occurred, both bacteria and fungi have been implicated as causative agents. Avoiding microbial problems requires a multilevel control strategy, in which the early use of effective chemical biocides is a very significant part.

Commercial biocidal products intended for emulsion paint preservation and/or equipment sterilisation generally have good antibacterial action, good water phase solubility, and are produced in a readily dispersible form. The Paint RA carried out a survey in 1980 and published a "Guide to Preservatives for Waterborne Paints". This identified 97 companies around the world supplying 130 products for preservation of aqueous paints.

Commercial biocidal products intended for paint film protection generally have good antifungal action and low water solubility. In 1979 the Paint RA carried out a survey and published a "Guide to Paint Film Fungicides". This identifies 51 companies around the world supplying 145 products for paint film protection.

Part 2. Antifouling Agents, A. O. Christie (International Paint PLC). The growth of plants and animals on the underwater surfaces of ships is well known and documented. It has been estimated that the annual cost of roughness and fouling to the British Navy alone is about £400 million. The cost of cleaning offshore structures in the North Sea for relief of structural loading problems and for inspection purposes was £3 million in 1977 and forecast as £17 million in 1985. Hence there is considerable demand of efficient preventative measures. The requirements for a new antifouling biocide are a broad spectrum of toxicity, sea water solubility at a level less than 50 ppm, a safe level of mammalian toxicity, no bio-magnification in marine ecosystems, long term stability in paint and acceptable cost. Mixtures of selective biocides still offer avenues for further development.

The key to fouling control by toxic systems is that of controlled biocide release from the surface being protected. In the operational ship a complete break with traditional technology has been achieved with the introduction of 'Self Polishing Copolymer' systems. These rely on the flow of water past the ship to provide a polishing action which allows a fresh antifouling surface to be maintained by continuous and controlled removal of the surface layer.

News from Sweden

In Stockholm a symposium was held on the 8-9 November, 1983, on "Oil and Applied Microbiology". This meeting was organized by the Royal Swedish Academy of Engineering Sciences (IVA).

The purpose of the symposium was to inform Swedish technical directors as well as scientists about the research going on in the field of petroleum microbiology. On the one hand it was the positive uses of microorganisms for oil clean-up or desulphurization and on the other hand the negative effects when storing petroleum products in rock caverns that were discussed.

Six scientists from the USA and the UK had been invited to present papers. The program was divided into two parts. One day with presentation of papers followed by a day of round table discussions.

The program for the symposium part was as follows:

Opening: Prof. H. G. Forsberg, IVA.

Microbial degradation of hydrocarbons (land and sea)—a survey: Prof. R. Atlas, University of Louisville, USA.

Desulphurization of oil and coal: Prof. W. R. Finnterry, University of Georgia, USA.

Fundamental aspects of the genetics and biochemistry of hydrocarbon oxidation by bacteria: Dr P. Williams, University of North Wales, UK.

Genetics of hydrocarbon degrading micro-organisms related to oil service industry: Prof. A. Chakrabarty, University of Illinois, USA.

Biotechnical oil cleaning: Prof. E. Rosenberg, Tel Aviv University, Israel.

Microbiological aspects of oil storage: R. Rolley, National Defence Research Institute, Umeå, Sweden.

An industrial view on microbial degradation of hydrocarbons: Dr R. J. Watkinson, Shell Bioscience Lab., Sittingborne, UK.
A report from the meeting will be prepared and can be obtained from the Royal Swedish Academy of Engineering Science, Grev Turegatan 10, Box 5073, S-102 42 Stockholm, Sweden.

Roger Roffey
Umeå

Swiss Activities in Biodeterioration

Biodeterioration as the "undesired" part of biodegradation involves many different Swiss Federal Offices as well as research and testing laboratories and, of course, many different industries.

Whilst the Bundesamt für Gesundheitswesen in Berne covers mainly aspects of toxicity for men of preservatives and residues of preservatives, the Bundesamt für Umweltschutz (Berne) is engaged in ecotoxicological problems. Swiss federal research laboratories are working mainly in three different areas: (1) agronomy and forestry (many research stations); (2) water protection and solid's recycling (EAWAG in Dübendorf); (3) materials testing and research (EMPA in Dübendorf and St Gallen).

Besides many EN-standards for the timber industry, testing of wood preservatives, and some Swiss test methods for the textile industry (testing against moth, beetles, fungus and bacteria and two standards for culturing microorganisms for microbiological testing) there are only two further Swiss standards existing for testing against biodeterioration: plastic foils and bituminous materials against root penetration and influence of microorganisms (soil burial test).

All these mentioned standards are methods which were registered by the Swiss standardizing organization after evaluation by the EMPA, in collaboration with interested industries.

The main problem in the actual biodeterioration research is the increased weight which is to be given to residues and the contamination of the environment. Many preservatives are nowadays critically observed and the industry has to develop alternatives.

From our point of view, some very real problems of biodeterioration to be mentioned are:

- growth control (algae, fungi) on façades, e.g. in connection with thermal insulation.
- growth control in connection with destruction of emulsions and other water based systems.
- resistance of textiles against microorganisms.
- microbiostatic finishes on textiles.
- efficacy of the new generation of insecticidal wool preservatives.
- prolonging the durability of wood constructions and timber by wood preservatives and/or wood preservation by constructional details.

Paul Raschle
EMPA, CH-9001 St. Gallen

National Corrosion Co-ordination Centre

In an effort to reduce the losses through corrosion to the UK economy and to maximise the use of available resources, the Department of Industry has initiated a scheme to establish a national centre to coordinate research on specific corrosion control topics. The operation will be controlled by the Head of the National Corrosion Service Mr J. A. Bernie, aided by an Advisory Panel from the UK corrosion community.

Clubs will be set up on separate themes, with a Manager selected to direct each activity. The scheme aims to be truly national, and Managers will be appointed from staff at corrosion centres and consultancy organizations throughout the UK.

The management costs will be met by funding from the Department of Industry through the Materials, Chemicals and Vehicles Requirements Board (MCVRB).

Activity areas which could sustain interest will be identified, and Managers will survey relevant industries to develop Research and Development programmes to attack common corrosion problems. Funding of the research work will be on a collaborative basis with government support available for projects approved by the MCVRB.

Contracts for the work will be placed at UK centres which can demonstrate proven specialization in the designated activities.

It is envisaged that up to five clubs will be formed in the first year of operation and the following activity areas have already been identified:

- microbial corrosion;
- high-temperature corrosion.

If you have an interest in these fields, or would like to learn more of the overall operation, contact John Bernie (National Corrosion Service, National Physical Laboratory, Teddington, Middlesex, TW11 0LW, UK; tel. 01-977 3222 ext. 4174).

Microbial Corrosion Club

Corrosion induced by bacteria usually emphasizes the role of the anaerobic organisms known as the sulphate-reducing bacteria. The most serious and widespread problems are in water and effluent systems and in soils and sediments. However there is now evidence to show that the effects of mixed-culture systems are becoming more prevalent and these have been associated with unexpected corrosion problems.

Major failures of stainless-steel plant have occurred after hydrotesting with potable water of an acceptable quality in the presence of both anaerobic and aerobic bacteria. A research programme is being formulated to identify the role of mixed bacterial systems, and industrial support for this work is currently required.

To help industry to become more aware of these problems and to develop remedial measures the National Corrosion Co-ordination Centre (see above) has established a Microbial Corrosion Club. Current interests relate to:

hydrotesting of stainless-steel pipelines;
a standards approach to biocide testing to ensure that test methods relate to environmental and operating conditions;
reappraisal of the reliability of results of soil-testing procedures with a view to improving predictability.
All projects are being carried out on a collaborative basis between industry and government. If you have suffered from any of these problems, or wish to know more about this scheme, contact Mr. A. K. Tiller (National Corrosion Service, National Physical Laboratory, Teddington, Middlesex, TW11 0LW; tel. 01-977 3222 ext. 4172).

In introductory papers A. K. Tiller dealt with Electrochemical Aspects of Microbial Corrosion with particular reference to the role of sulphate reducing bacteria in the postulated 'classical' or 'alternative' mechanisms of corrosion, and J. W. Hopton gave a general survey of the influence of physical and nutritional conditions on microbial growth which included studies of mixed culture systems and growth of organisms in extreme environments. R. Watkinson presented a paper on Hydrocarbon Degradation which covered specifically the interaction of micro-organisms with oil and oil products and the influence of environmental conditions on that interaction. In a contribution on Sulphate-Reducing Bacteria: Their Role in Corrosion, J. A. Hardy discussed the particular significance of ferrous sulphide films generated by sulphate-reducing bacteria in the microbial corrosion process. Papers on Corrosion and Fouling Problems during Oil and Oil Product Transportation by A. Galloway and Light Fuel Oils by D. Houghton were concerned with the involvement of micro-organisms in the corrosion which can occur in ships carrying cargoes of coal, sulphur or crude oil and in the fuel supply lines and fuel tanks of gas turbine driven ships, respectively.

P. D. Gilbert's paper on Concrete Corrosion dealt with the role of micro-organisms in the intricate chemical conversions which occur when concrete deteriorates. In a paper entitled Microbial Ecology and its Significance, E. C. Hill pointed out that the oil/water environment is an aggregate of many mini-environments and not a single environment and preventive and remedial measures against corrosion must be instituted with this in mind. R. A. King in a paper on Corrosion Hazard Assessment dealt with the variety of corrosion problems facing the practising engineer and showed how simplified routine check systems backed by ranking tables signifying degree or severity could alert operators to sources of potential problems.

The proceedings will be published by the Institute of Petroleum.

J. W. Hopton

Seventh International Conference on the Global Impacts of Applied Microbiology
Helsinki
12-16 August 1985

The conference will focus on the contributions that applied microbiology and biotechnology can bring to the economy and welfare of the developed and developing countries. The conference will, therefore, help to strengthen efforts to combat world hunger and to promote the conservation, distribution and environmentally sound management of available natural resources in relation to overall human and social development. It is desirable that participants should include, besides microbiologists, representatives of governmental bodies, administrators of universities and technical institutions as well as representatives of industrial firms engaged in the utilization of microbiologically-based technologies.

Major areas to be considered at the 1985 conference will be (1) Food and Agriculture, (2) Man, the Environment, Wastes and Industries, (3) Medical Microbiology, (4) Sustainable Development—Social and Cultural Aspects of Applied Microbiology, (5) Bioinformatics, (6) Basic Microbiology, and (7) Microbiological Resource Centres (MIRCENS).

Requests for further information should be sent to: Professor H. G. Gyllenberg or B. O. Fabricius (Department of Microbiology, University of Helsinki, SF-00710 Helsinki 71, Finland).
THE ROLE OF SULPHATE-REDUCING AND SULPHUR-OXIDIZING BACTERIA IN THE LOCALIZED CORROSION OF IRON-BASE ALLOYS — A REVIEW

Gustavo CRAGNOLINO and Olli H. TUOVINEN

Abstract: Chemical and microbiological interactions of inorganic sulphur compounds, which result in the formation of various aggressive sulphoanions such as sulphide, thiosulphate, and tetrathionate are reviewed with emphasis on the catalytic role played in these transformations by exposed metal surfaces and traces of metal ions in solution. The effects of these sulphur anions on the electrochemical conditions leading to pitting, crevice corrosion and stress corrosion cracking of iron-base alloys in chloride- and non-chloride-containing environments are also reviewed, as an appropriate background for discussing the role of sulphate-reducing and sulphur-oxidizing bacteria in localized corrosion processes.

Introduction

Examination of the literature on corrosion induced by bacteria indicates that localized corrosion can occur. Especially the presence of sulphate-reducing bacteria in corroded pipelines and other industrial installations has been reported. The chemistry of this type of corrosion can involve a number of entities, particularly metastable sulphur compounds. This review discusses the various metabolic pathways associated with sulphur-oxidizing thiobacilli and sulphate-reducing bacteria. Both groups of microorganisms are ubiquitous in soils, sediments, and waters and their ability to utilize sulphur compounds is well documented. Metastable sulphur species, such as thiosulphate and polythionates are involved in both the oxidative and reductive biological transformations. These compounds are known to interact with each other, often generating or involving ionic species that are extremely aggressive towards iron, carbon steels and stainless steels at ambient temperatures. Possible effects of thiosulphate, tetrathionate, hydrogen sulphide, and other inorganic compounds of sulphur are discussed in relation to pitting corrosion, crevice corrosion, and stress corrosion cracks. In the latter case, attention is drawn to the selective chemistry which will encourage this type of corrosion at ambient temperature, particularly where the environment is conducive to bacterial growth and biofouling. Although no case histories have so far been reported on microbiologically induced stress corrosion cracking, the potential for these problems to occur should not be overlooked.

Chemical Interactions of Inorganic Compounds of Sulphur

Several excellent reviews have been published on the chemistry of metastable ionic sulphur species (Schmidt 1972, Schmidt & Siebert 1973, Nriagu & Hem 1978). Particular emphasis has been placed on inorganic sulphur compounds regarding the environmental cycling and the methods of their determination (Szekeres 1974, Granat et al. 1976, Brown 1982). Though the role of sulphur anions has been recognized in laboratory studies of pitting and stress corrosion cracking, these compounds have received relatively little attention in corrosion related studies of installation failures.

Some of the inorganic sulphur compounds are poorly characterized and defined. An example is colloidal sulphur prepared by sulphidolysis of tetrathionate. The sulphur thus produced is extremely metastable and probably a complex mixture of $S_6$, polythionates, and polysulphides. Even the relatively stable $S_6$ ring structure is susceptible to nucleophilic attack by a variety of reductants including sulphide and cyanide. According to the potential-pH diagram for the sulphur-water system at 25°C (Valensi et al. 1974), all sulphur species between the oxidation numbers of $-2$ (sulphide) and $+6$ (sulphate) are thermodynamically metastable with the exception of elemental sulphur. Thiosulphate ($S_2O_3^{2-}$) and polythionates ($S_xO_{2x^-}; x = 3, 4, 5, 6$) all tend to decompose in aqueous solutions. A simplified Pourbaix diagram for some of these metastable sulphur ions is shown in Fig. 1 (Valensi 1973) in which the stability domains of $S$, dissolved $H_2S$, $HS^-$, and $S^{2-}$ are also included. A complete series of polysulphides can coexist according to the following equilibrium:

$$2S_x^{2-} = S_{x+1}^{2-} + S_{x-1}^{2-}$$

but they would appear in the diagram only at a higher total sulphur concentration than that presented in Fig. 1, $S_2^{2-}$ being the predominant species. The diagram yields the potentials and pH values at which disproportionation reactions occur. For example, thiosulphate is decomposed in acid solutions as follows (Johnston & McAmish 1973):

$$S_2O_3^{2-} + H^+ \rightarrow S \text{ (colloidal)} + HSO_3^-$$

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2Department of Microbiology, The Ohio State University, Columbus, Ohio 43210, USA.
Fig. 1. Potential-pH metastable equilibrium diagram for the system $S$-$H_2O$ at 25°C and 1 atm. Dithionite ($S_2O_3^{2-}$), dithionate ($S_2O_4^{2-}$), trithionate ($S_3O_6^{2-}$), and sulphate ($SO_4^{2-}$) are not included. Total concentration = 0.2 g $S$/$H_2O$ (Valensi 1973). With permission of CEBELCOR.

but neither one of the products is stable. Colloidal sulphur may readily be transformed to polysulphides through a nucleophilic attack of the $S_8$ ring by $HS^-$ or $S^-$ particularly in neutral and alkaline solutions (Schmidt & Siebert 1973).

Although the potential-pH diagram for the thermodynamically stable species of the sulphur-water system (Valensi et al. 1974) indicates that sulphate can be reduced to sulphur or sulphide in aqueous solutions, the reduction of sulphate is a highly irreversible process. Therefore, sulphate is electrochemically inactive even at very low potentials. On the other hand, sulphate can be easily reduced to polythionates, thiosulphate, and sulphide by anaerobic bacteria.

The chemistry of metastable sulphur compounds is extremely complex as illustrated by the following examples. Polythionates are able to react with sulphite (Blasius & Münch 1972, Wagner & Schreier 1978, Tuovinen 1978):

$$S_4O_6^{2-} + SO_3^{2-} \rightarrow S_2O_3^{2-} + S_2O_4^{2-}$$

$$S_4O_6^{2-} + (x-3)SO_3^{2-} \rightarrow S_2O_6^{2-} + (x-3)S_2O_3^{2-}$$

In alkaline solutions polythionates are hydrolyzed:

$$S_4O_6^{2-} + OH^- \rightarrow S_2O_3^{2-} + S^0 + HSO_4^-$$

They are also powerful oxidizing agents for sulphide:

$$S_4O_6^{2-} + S^{2-} \rightarrow 2S_2O_5^{2-} + S^0$$

$$S_4O_6^{2-} + S^{2-} + SO_3^{2-} \rightarrow 3S_2O_3^{2-}$$

Oxidants such as Fe(III) and MnO$_4^-$ are able to oxidize thiosulphate to tetrathionate in a manner similar to that by iodine:

$$2S_2O_3^{2-} + I_2 \rightarrow S_4O_6^{2-} + 2I^-$$

Ferrous, ferric, manganous and manganic ions, and other transition metal ions (CuII, NiII) are known to have catalytic effects on sulphur transformations such as polythionate and thiosulphate degradation. Their reduced forms (Fe$^{2+}$, Mn$^{2+}$) can also be oxidized by aerobic micro-organisms. Thus, in oxygenated environments not only the metastable sulphur species as discussed in the following section, but also some transition metal cations are biologically active.

It should be emphasized that sulphide-covered surfaces and metal cations produced by the dissolution of iron base alloys can catalyze chemical transformations of sulphur compounds without the direct involvement of sulphate-reducing or sulphur-oxidizing microorganisms. In industrial installations, and even in synthetic solutions, trace level contamination by metal ions is to be expected and therefore, the distribution profiles of reactive sulphur species need to be determined in order to predict the chemical pathways and to identify the species responsible for localized corrosion processes.
Microbiological Reactions of Inorganic Compounds of Sulphur

Aerobic sulphur-oxidizing thiobacilli

Bacteria capable of deriving energy for growth from the oxidation of inorganic sulphur compounds are ubiquitous in the nature. Thiobacilli are the best-known group of sulphur-oxidizers and they have been implicated in microbiological corrosion phenomena because of their ability to produce sulphuric acid (Table 1). Several oxidation reactions have been presented by Roy & Trudinger (1970) and Kuenen & Tuovinen (1981).

Table 1. Oxidation reactions of thiobacilli.

<table>
<thead>
<tr>
<th>Reaction</th>
<th>Products</th>
</tr>
</thead>
<tbody>
<tr>
<td>( \text{S}_2\text{O}_3^{2-} + 2\text{O}_2 + \text{H}_2\text{O} \rightarrow 2\text{SO}_4^{2-} + 2\text{H}^+ )</td>
<td></td>
</tr>
<tr>
<td>( \text{S} + 3\text{O}_2 + 2\text{H}_2\text{O} \rightarrow 2\text{SO}_4^{2-} + 4\text{H}^+ )</td>
<td></td>
</tr>
<tr>
<td>( 4\text{S}_2\text{O}_3^{2-} + \text{O}_2 + 2\text{H}_2\text{O} \rightarrow 2\text{S}_2\text{O}_4^{2-} + 4\text{OH}^- )</td>
<td></td>
</tr>
<tr>
<td>( 2\text{S}_2\text{O}_3^{2-} + 7\text{O}_2 + 6\text{H}_2\text{O} \rightarrow 8\text{SO}_4^{2-} + 12\text{H}^+ )</td>
<td></td>
</tr>
<tr>
<td>( 2\text{SCN}^- + 4\text{O}_2 + 4\text{H}_2\text{O} \rightarrow 2\text{SO}_4^{2-} + 2\text{CO}_2 + 2\text{NH}_3 )</td>
<td></td>
</tr>
<tr>
<td>( \text{H}_2\text{S} + 2\text{O}_2 \rightarrow \text{SO}_4^{2-} + 2\text{H}^+ )</td>
<td></td>
</tr>
<tr>
<td>( 2\text{H}_2\text{S} + \text{O}_2 \rightarrow 2\text{S} + 2\text{H}_2\text{O} )</td>
<td></td>
</tr>
<tr>
<td>( 2\text{S}_2\text{O}_3^{2-} + 4\text{O}_2 + 4\text{H}_2\text{O} \rightarrow 5\text{SO}_4^{2-} + 6\text{H}^+ )</td>
<td></td>
</tr>
<tr>
<td>( 5\text{H}_2\text{S} + 8\text{NO}_2^- \rightarrow 5\text{SO}_4^{2-} + 4\text{N}_2 + 4\text{H}_2\text{O} )</td>
<td></td>
</tr>
<tr>
<td>( 5\text{S} + 6\text{NO}_3^- + 2\text{H}_2\text{O} \rightarrow 5\text{SO}_4^{2-} + 3\text{N}_2 + 4\text{H}^+ )</td>
<td></td>
</tr>
<tr>
<td>( 5\text{S}_2\text{O}_3^{2-} + 8\text{NO}_3^- + \text{H}_2\text{O} \rightarrow 10\text{SO}_4^{2-} + 4\text{N}_2 + 2\text{H}^+ )</td>
<td></td>
</tr>
</tbody>
</table>

As the reactions in Table 1 indicate, many inorganic sulphur compounds are susceptible to microbiological oxidation. In the biological pathway of oxidation, the various oxidative steps are mediated by specific enzymes with coupling to an electron transport system where oxygen is reduced to water as the terminal reaction:

\[ \frac{1}{2}\text{O}_2 + 2\text{H}^+ + 2\text{e}^- \rightarrow \text{H}_2\text{O} \]

Fig. 2 outlines the various steps involved in the biological oxidation of sulphide, elemental sulphur, and sulpho-oxyanions. The current knowledge on sulphur oxidation pathways in thiobacilli has been discussed by Kelly (1982). Little information is available on the microbiological oxidation of polythionates other than \( \text{S}_4\text{O}_6^{2-} \).

![Fig. 2. A schematic, non-stoichiometric presentation of the microbiological oxidation of inorganic sulphur compounds. Several enzymatic reactions need to be characterized, especially those associated with the oxidation of sulphide, polysulphide, and elemental sulphur. Several other pathways have been presented in the literature (Kelly 1982). APS = Adenosine 5'-phosphosulphate.]

The role of thiobacilli in producing sulphur oxyanions as metastable intermediates has not been associated with aerobic corrosion problems of microbiological origin. These intermediates do not persist in oxidative, bacteria-containing environments. However, some intermediates such as \( \text{S}_2\text{O}_3^{2-} \) may accumulate until the substrate is virtually completely oxidized, but the ensuing oxidation leads to the formation of sulphuric acid (Murphy et al. 1972, Tuovinen & Kelly 1974). Sulphur may accumulate as a result of \( \text{S}_2\text{O}_3^{2-} \) disproportionation by thiobacilli if a stress factor is imposed on the bacteria; e.g. inadequate aeration, toxic metal ions, or excessive acidity (Tuovinen 1973, Tuovinen & Kelly 1974).

Some thiobacilli, namely \( \text{T. denitrificans} \) and \( \text{T. thioparus} \), are able to oxidize sulphur compounds under anaerobic conditions if sufficient nitrate or nitrite is present to substitute for oxygen as an electron acceptor as illustrated in Fig. 3. The sequential oxidative pathway of sulphur is coupled via specific enzymes and electron carriers to the reduction of nitrate as shown in Fig. 4.
In biological systems electrons have a higher affinity towards oxygen as compared with other electron acceptors (e.g., NO\textsubscript{3}^{-}, SO\textsubscript{4}^{2-}) and therefore denitrification usually occurs only in the absence of O\textsubscript{2}. This has been demonstrated by several techniques, including a gradual transition from aerobic to anaerobic conditions; the concentration of nitrate did not decrease during thiosulphate oxidation by \textit{T. denitrificans} until oxygen was depleted below the level of detection (Justin & Kelly 1977).

Thiobacilli comprise a rather heterogeneous group of bacteria some of which have only a few common characteristics (Kuenen & Tuovinen 1981). All thiobacilli are able to oxidize some inorganic compounds of sulphur. For example, \textit{T. acidophilus}, an acid-tolerant bacterium, can grow with elemental sulphur as a substrate, but not with thiosulphate according to its original description (Guay & Silver 1975). Such a limited capacity to oxidize sulphur compounds has also been reported for a few other thiobacilli and may indicate either the lack of certain enzymes of the sulphur metabolism or the lack of appropriate conditions for testing the microbiological oxidizability of sulphur compounds.

The oxidation rates of sulphur by thiobacilli vary depending on the particular sulphur compound. Both freshwater and marine strains of thiobacilli have been isolated and characterized from a variety of sources. The marine strains have an obligate requirement of chloride ion (as NaCl) in the growth medium (Tilton \textit{et al.} 1967, Adair & Gundersen 1969) and therefore, their activities are not hindered in high-chloride (0.5 M) environments. In contrast, the freshwater isolates of thiobacilli are inhibited at high chloride concentrations.

Thermophilic sulphur-oxidizing thiobacilli have been described which tolerate temperatures up to 55-60°C (Brierley \textit{et al.} 1980). With the exception of one disputable and poorly characterized isolate, none of the thiobacilli form spores. Therefore, these bacteria are relatively more sensitive than the spore-forming sulphate-reducers to chemical disinfection agents and heat treatment.

Some thiobacilli require a low pH environment; these include \textit{T. thiooxidans}, \textit{T. ferrooxidans}, \textit{T. kabobis}, and \textit{T. acidophilus}. It is not uncommon to find these acidophilic thiobacilli associated with sulphur waste piles and acid mine drainage effluents where the biological production of sulphuric acid is difficult to curtail.

\textbf{Anaerobic sulphate-reducing bacteria}

Sulphate-reducing bacteria have often been detected in corrosion deposits and their catalytic effect on accelerating the corrosion of cast iron and steels has been demonstrated in many laboratory studies. These bacteria are obligately anaerobic and become inactivated upon exposure to air (oxygen). The inhibition by aerobiosis may reverse upon resumption of anaerobic conditions.

The microbiological reduction of sulphate is a respiratory activity (Thauer & Badziong 1980) in which sulphate substitutes for oxygen as the terminal electron acceptor (Fig. 5). Intermediates of the microbiological sulphate reduction are indicated in Fig. 6. All intermediates of the reductive pathway are metastable and susceptible to microbiological oxidation under suitable conditions.

Both thiosulphate and tetrathionate can be detected in transient concentrations in culture solutions. The enzymes mediating the intermediate reactions vary in different sulphate-reducing bacteria. Work published during recent years indicates that sulphate reducers represent a heterogeneous bacterial group capable of using many different acids and
sugars as substrates (Postgate 1979). New descriptions include Desulfobacter (Widdel & Pfennig 1981), Desulfomonas (Moore et al. 1976), and Desulfurocococcus (Zillig et al. 1982) whose carbon metabolism seems to be distinctly different from that of Desulfovibrio and the spore-forming Desulfotomaculum. The suitability of organic compounds for supporting growth of sulphate reducers is an important consideration since these bacteria are not able to satisfy their carbon requirement by the fixation of carbon dioxide. In marine systems, for example, it is the concentration of organic substrate rather than that of sulphate that may be a limiting factor for their development.

Elemental sulphur is not an intermediate in the biological reduction of sulphate to sulphide. Colloidal sulphur and elemental sulphur \( (S_0) \) can both be reduced by some species (Pfennig & Biebl 1976, Biebl & Pfennig 1977, Fauque et al. 1979) including sulphur-respiring anaerobic archaebacteria (Zillig et al. 1983a, b), but this activity has not been well characterized.

Both freshwater and marine species have been described in the literature (Postgate 1979). Thermophilic sulphate and sulphur reducers have also been isolated from thermally influenced environments (e.g. hot springs, pipe lines, sewage digestors).

Biological sulphate reduction can also be coupled to the oxidation of \( H_2 \):

\[
4H_2 + SO_4^{2-} + 2H^+ \rightarrow H_2S + 4H_2O
\]

The role of \( H_2 \) as a reductant for sulphate reduction has evoked interesting ideas about the significance of the hydrogen oxidation system in the surface events of microbiological corrosion. Hydrogenase, the enzyme mediating the electron transfer, is found in some sulphate-reducing micro-organisms (e.g., Desulfovibrio). The reaction can be expressed with the following equations:

**Hydrogenase:**

\[
H_2 = 2H^+ + 2e^-
\]

**Hydrogenase coupled with the reduction of cytochrome \( c_5 \):**

\[
H_2 + \text{cytochrome } c_5 \text{ (oxid) } \rightarrow \text{cytochrome } c_5 \text{ (red) } + 2H^+
\]

Many sulphate-reducing bacteria appear to have at least two hydrogenase enzymes, one cytoplasmic and the other located in the periplasmic space of the cell wall (Le Gall et al. 1982). The presence of hydrogenase in sulphate-reducers has lent support to the cathodic depolarization hypothesis which will be discussed in a later section of this paper.

None of the sulphate-reducers are known to tolerate low pH values (pH < 3-4) for prolonged periods. It would not be surprising, however, to detect these bacteria in low pH environments because corrosion deposits and other precipitates may exhibit pH gradients, thereby rendering the interior more suitable for sulphate-reducers as opposed to the low pH of the bulk solution.

In laboratory cultures the redox potential needs to be lowered to \( < -100 \text{ mV}_{\text{SHE}} \) before the sulphate-reducers are able to grow. Ascorbic acid, sulphide, and thioglycollate can be used to poise the redox potential. In corrosion deposits the interior redox potential is typically low and provides the reducing environment for these bacteria, even if the bulk solution is oxygenated as is the case for tubercles in water distribution systems (Tuovinen et al. 1980, Tuovinen & Hau 1982).
Effect of Sulphur Compounds on Localized Corrosion

It is well known that some sulphur compounds, such as H$_2$S and SO$_2$, as well as their respective anions enhance the dissolution rate of iron and steels in acidic solutions. Iofa (1970, 1980) suggested that the catalytic effect of sulphide ions on the anodic reaction can be explained by the formation of intermediate adsorbed sulphide species as opposed to hydroxide species as follows:

\[
\begin{align*}
\text{Fe} + \text{HS}^- &= \text{Fe(}\text{HS}^-\text{)}_{\text{ads}} \\
\text{Fe(}\text{HS}^-\text{)}_{\text{ads}} &\rightarrow \text{Fe(}\text{HS}_2\text{)}_{\text{ads}} + e^- \\
\text{Fe(}\text{HS}_2\text{)}_{\text{ads}} &\rightarrow \text{FeHS}^+ + e^- \\
\text{FeHS}^+ + \text{H}_3\text{O}^+ &\rightarrow \text{Fe}^2+ + \text{H}_2\text{S} + \text{H}_2\text{O}
\end{align*}
\]

The acceleration of the cathodic reaction in the presence of H$_2$S has been explained by the following equations (Iofa 1970, 1980):

\[
\begin{align*}
\text{Fe} + \text{HS}^- &= \text{Fe(}\text{HS}^-\text{)}_{\text{ads}} \\
\text{Fe(}\text{HS}^-\text{)}_{\text{ads}} + \text{H}_3\text{O}^+ &\rightarrow \text{Fe(}\text{H-S-H}\text{)}_{\text{ads}} + \text{H}_2\text{O} \\
\text{Fe(}\text{H-S-H}\text{)}_{\text{ads}} + e^- &\rightarrow \text{Fe(}\text{HS}^-\text{)}_{\text{ads}} + \text{H}_{\text{ads}}
\end{align*}
\]

in which the catalytic active species Fe(\text{H-S-H})_{ads} is readily reduced to adsorbed H atoms. More detailed discussions on the effect of sulphide concentration, pH and other environmental variables on the general corrosion of iron and carbon steel have been presented (Sgúry 1976, Foroulis 1980). The anodic behaviour in alkaline sulphide solutions was investigated by Shoesmith et al. (1978a, b), taking into consideration the nucleation and growth of iron sulphides (i.e. mackinawite) accompanied by the formation of sulphur, polysulphides and eventually thiosulphate. More recently the anodic behaviour has been interpreted (Salvarezza et al. 1982) in terms of competitive adsorption of H$_2$O, OH$,^{-}$, and HS$.^{-}$. Processes like the formation of mackinawite, development of pitting, or passivation of the metal surface by an oxide layer are suggested to depend on the HS$^{-}$/OH$^{-}$ concentration ratio.

Even in the case of more corrosion resistant alloys, such as stainless steels, the anodic dissolution rate in acidic solutions can be enhanced by several orders of magnitude in the presence of H$_2$S or SO$_2$, as reported by several authors (Herbsleb & Schwenk 1966, Greene & Wilde 1970, Crolet et al. 1976, Masuo et al. 1978). Relatively low concentrations of either species have a pronounced effect on the anodic behaviour, shifting the corrosion potential to more negative values and the passivation potential to more positive values, thereby enlarging the active range as illustrated in Fig. 7 for the case of SO$_2$ additions. While the effect of sulphur compounds on uniform corrosion of steels and stainless steels has been extensively studied due to their widespread applications in the chemical and petrochemical industries, the role of metastable sulphur compounds in promoting or accelerating localized corrosion processes has not been properly recognized in many circumstances. Therefore, we will briefly review the influence of a variety of sulphur species on three types of localized corrosion phenomena: pitting, crevice corrosion and stress corrosion cracking which are usually identified as the main cause of failure in the use of steels, particularly stainless steels, and other corrosion resistant alloys in industrial installations. At the same time the advantages of using electrochemical techniques for studying localized corrosion processes will be emphasized.

![Fig. 7. Anodic polarization curves of Type 304 stainless steel in 5% H$_2$SO$_4$ solution showing the influence of SO$_2$ addition to nitrogen gas used for deaeration (curve 2 was obtained after a cathodic pretreatment for 10 min. at -0.7 V_scce) (Masuo et al. 1978). With permission of the Japan Society of Corrosion Engineers.](image-url)
Pitting corrosion

It is commonly accepted that for a variety of metal/solution systems, in which the metal surface is covered by a protective passive layer, pitting occurs only above a certain critical potential, which can be measured by anodic polarization of the metal or alloy in the test environment. Using this technique as well as other electrochemical methods (Pessall & Liu 1971, Smialowska & Czachor 1974) pitting corrosion of stainless steels in chloride solutions has been extensively investigated (Smialowska 1974, Hisamatsu 1976, Okamoto & Shibata 1978). The pitting potential decreases with increasing chloride concentration. Different anions, e.g., OH\(^{-}\), NO\(_3\)\(^{-}\), ClO\(_4\)\(^{-}\), and SO\(_4^{2-}\), inhibit pitting as evaluated from the increasing values of the pitting potentials in the presence of increasing concentrations of the inhibiting anions.

Except for sulphate no studies were available until recently on the effect of sulphur compounds on the pitting potential of austenitic stainless steels. The effect of sulphate should be clearly distinguished from that of other sulphur species. Leckie & Uhlig (1966) found that sulphate concentrations ranging from 0·0125 to 0·15 M monotonically increased the pitting potential of Type 304 stainless steel in 0·1 M NaCl. Competitive electromigration of SO\(_4^{2-}\) (passivating anion) with respect to Cl\(^{-}\) (aggressive anion) was suggested as a reasonable explanation for that inhibiting effect (Galvele 1976). On the other hand, Newman et al. (1982a) demonstrated that sulphur species such as H\(_2\)S, S\(_2\)O\(_3^{2-}\), S\(_2\)O\(_3^{3-}\) and SCN\(^{-}\) within certain concentrations ranges decrease the pitting potential of Type 304 stainless steel in neutral or slightly acidic solutions containing 0·25 M NaCl. The behaviour is clearly illustrated in Fig. 8. It seems that additions of S\(_2\)O\(_3^{2-}\) ranging from 0·01 M to 0·02 M lowered the pitting potential by more than 300 mV, while additions of more than 0·5 M inhibit pitting. SCN\(^{-}\) showed similar but less marked effects, while increasing Na\(_2\)S additions up to 0·1 M (present as H\(_2\)S and HS\(^{-}\) at neutral pH) caused a monotonic decrease in the pitting potential. The effect of S\(_2\)O\(_3^{2-}\) is similar to that of S\(_2\)O\(_3^{3-}\), but the minimum value of the pitting potential is displaced to higher concentrations. Newman et al. (1982a) noted that pits formed in the presence of sulphide, thiosulphate or tetrathionate contained metal sulphide as a black deposit on the pit bottom. Since thiosulphate alone did not promote pitting, Newman et al. (1982a) suggested that the main role of the active sulphur species is to impede the repassivation of the bare metal surface following chloride induced film breakdown, thereby enhancing the dissolution via the presence of adsorbed sulphide or sulphur.

![Fig. 8. Pitting potential data for Type 304 stainless steel in 0.25 M NaCl with additions of sulphur compounds. The dotted line at 260 mV represents the pitting potential with no additions. Pitting potentials are shown for Na\(_2\)S\(_2\)O\(_3\) (■) and Na\(_2\)SO\(_3\) (▲) with no pH adjustment and for the following with 5·05 < pH < 6·3: Na\(_2\)S\(_2\)O\(_3\) (■), KSCN (◇), H\(_2\) (○). Pitting potentials indicated by "s" were measured by using a scratching technique (Newman et al. 1982a). With permission of the National Association of Corrosion Engineers.](image-url)
Fig. 9. Pitting potentials of Type 316 (O), 304 (□), and 430 (△) stainless steels in 0.017 M NaCl solution containing increasing concentrations (0 to 20 ml/l) of a sulphurous solution containing 30 1 SO₂/1 H₂O (Luffkin 1973). With permission of the Anti-Corrosion Methods and Materials.

given in Fig. 9 cannot be directly compared with those provided by Newman et al. (1982a) because different potential scanning rates were used in both studies. It is known that pitting potentials for stainless steels are extremely dependent on the potential scanning rate employed for anodic polarization. Luffkin (1973) observed that the enhanced effect of sulphur compounds on the pitting corrosion in chloride solutions was not confined to SO₂-containing solutions. A decrease in the pitting potential was found in solutions containing either Na₂S₂O₃, Na₂S₄O₆, or Na₂S₂O₅. Luffkin (1973) suggested that the reduction of SO₂ or HSO₃⁻ leads to the formation of metastable species, such as S₂O₄²⁻, S₀₂⁻ or even S⁻ and S²⁻ that may be responsible for the decrease in the pitting potential. A significant decrease in the pitting potential of Type 304 stainless steel in acidified (pH < 3) 3:5% NaCl solutions was also observed by Masuo et al. (1978) when Na₂S (7:3 ppm) was added to the solution. A similar effect was noted when nitrogen gas contaminated with up to 1% SO₂ was bubbled into the solution. The lowering of the pitting potential was attributed (Masuo et al. 1978) to the specific action of H₂S which was formed by reduction of SO₂ combined with the decrease in pH resulting from the addition of SO₂.

Herbsleb (1982) studied the effect of H₂S and SO₂ on the pitting potential for a series of Cr-Ni and Cr-Ni-Mo stainless steels exposed to 1 M NaCl solution. These results are summarized in Fig. 10, where the pitting potentials in N₂-deaerated chloride solutions are compared with those measured in chloride solutions saturated with either SO₂ (pH 0:7) or H₂S (pH 3:96). Pitting potentials are plotted as a function of an efficacy factor W(%) = Cr(%) + 3:3 (Mo(%) in order to rank the pitting resistance of the various alloys. For all alloys studied, H₂S induced a larger decrease of the pitting potential than SO₂, but both sulphur species were conducive to pitting corrosion at lower potentials than those for plain chloride solutions. The favourable effect of increasing Mo content in plain chloride solutions was significantly attenuated in the presence of H₂S or SO₂. Only the alloy with the highest Mo content showed a pitting potential in SO₂-containing solutions higher than that in plain chloride solution (Fig. 10). Hersleb (1982) discussed redox and
disproportionation reactions of SO₂ and its anions in aqueous solutions, but he concluded that it is difficult to identify the specific species responsible for the stimulation of pitting.

Except for SO₄²⁻, most of the metastable sulphur oxyanions, as well as H₂S and its related anions (HS⁻), decrease the pitting potential of stainless steel in chloride solutions. The effect seems to be due to a delayed repassivation of pit initiation sites in the presence of adsorbed hydrosulphide species formed by the reduction of sulphur oxyanions.

Crevice corrosion

Pitting and crevice corrosion are considered (Rozenfeld 1974, Sedriks 1979, Ijsseling 1980) closely related phenomena, having the same propagation mechanism particularly in the case of stainless steel in chloride solutions. The main difference between these corrosion processes is exhibited during the initiation stage. Crevice corrosion occurs within crevices or other shielded areas where a stagnant solution is present, whereas pitting takes place on smooth metal surfaces. A wide variety of macro-organisms (i.e., barnacles, algae) can lead to the formation of crevices as a consequence of marine fouling. Micro-organisms that grow in a coherent colony or mass of 'slime' on damp or immersed metal usually lead to the establishment of occluded cells.

Crevice corrosion has been extensively studied on corrosion resistant alloys exposed to chloride solutions. However, the effect of sulphur species has not been investigated, even though sulphur compounds are prevalent in polluted sea water. Electrochemical techniques (Rozenfeld 1974, Ijsseling 1980) have lead to considerable progress in the understanding of the mechanisms of crevice corrosion. Differential aeration seems to be the initial stage in the development of crevice corrosion but a sustained attack is only possible as a consequence of local acidification produced by hydrolysis of metal cations within the crevice. Coupled to this process a local build-up of chloride anions is required for maintaining electroneutrality, but the generation of an environment rich in chloride and metal ions induces a further decrease in the pH leading to enhanced anodic dissolution.

The propagation of the attack within the crevice area can be in the form of general corrosion or localized pitting. Oldfield & Sutton (1978a,b) presented a detailed mathematical model, in which the various stages of crevice corrosion are simulated, taking into consideration several factors involved. Four stages were distinguished, namely: (a) deoxygenation, (b) increase of salt and hydrogen ion concentration, (c) depassivation, and (d) propagation. Based on the pitting studies summarized in the previous section, it seems that the presence of metastable sulphur oxyanions or sulphide may decrease the incubation time associated to the generation of an aggressive solution within the crevice responsible of the depassivation stage.

Stress corrosion cracking

Stress corrosion cracking (SCC) is an insidious form of metal failure because its occurrence is difficult to predict. The presence of a tensile stress (applied and/or residual) and the existence of a susceptible metallurgical microstructure, coupled with the simultaneous action of a specific environment are the requirements for the occurrence of SCC. Although the environmental requirements are highly specific, the list of environments identified as causing cracking for a given alloy continues to grow with time. Apart from the well known effect of H₂S on causing hydrogen embrittlement of high strength steels, a variety of sulphur species are able to induce intergranular stress corrosion cracking (IGSCC) of sensitized austenitic stainless steels at ambient temperature (Cragnolino & Macdonald 1982). In the early 1950s intergranular cracking of catalytic reformers used in the petroleum industry was observed. Dravnieks & Samans (1957) demonstrated that polythionic acids, formed by the reaction of oxygen and water with an iron sulphide scale, were the species responsible for cracking. Since then "polythionic acid cracking" of sensitized austenitic stainless steel has been extensively studied. The subject has been reviewed recently (Cragnolino & Macdonald 1982), covering the effect of pH, solution composition, potential, sensitization, and alloy composition, as well as the influence of stress on the cracking behaviour. The following reaction has been proposed (Brophy 1974) to account for the interaction between iron sulphide and aerated water:

\[ 8\text{FeS} + 11\text{O}_2 + 2\text{H}_2\text{O} \rightarrow 4\text{Fe}_2\text{O}_3 + 2\text{H}_2\text{S}_4\text{O}_6 \]

in which, for simplicity, only the formation of tetrathionic acid is indicated. However, it was found recently (Horowitz 1983) that the oxidation of FeS in oxygenated water should be expressed by the equation:

\[ \text{FeS} + 3/4\text{O}_2 \rightarrow 1/2\text{Fe}_2\text{O}_3 + \text{S} \]

in accordance to the yields for the various reaction products given in Table 2. In addition, tetrathionate and thiosulphate were detected polarographically as soluble reaction products. The remaining sulphur species was considered to be sulphate, which is polarographically inactive.

Intergranular cracking has also been observed at room temperature in water saturated with SO₂ (Piech 1964), H₂S (Ryabchenkov & Nikiforova 1962, Heller & Prescott 1965), in aqueous solutions of Na₂S₂O₃ (Isaacs et al. 1982, Dhwale et al. 1982, Newman et al. 1982c), and KSCN (Isaacs 1980). As an example, Fig. 11 shows the elongation to failure as a measure of SCC susceptibility, plotted against the sulphur concentration for sensitized Type 304 stainless steel tested under slow straining conditions in air saturated 0.21 M boric acid solution containing various concentrations of either Na₂S₂O₃ or Na₂S₂O₆. The data show that a threshold concentration exists for both sulphur oxyanions, below
Table 2. Products of FeS-O₂ reaction
(Horowitz 1983)*.

<table>
<thead>
<tr>
<th>Substance</th>
<th>Concentration (mM)</th>
<th>% Yield</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fe (in Fe₂O₃)</td>
<td>In solid</td>
<td>89-1</td>
</tr>
<tr>
<td>Elemental S</td>
<td>In solid</td>
<td>81-1</td>
</tr>
<tr>
<td>Soluble Fe⁺⁺</td>
<td>16-7</td>
<td>10-1</td>
</tr>
<tr>
<td>S₂O₄²⁻</td>
<td>0·6</td>
<td>0-7</td>
</tr>
<tr>
<td>S₄O₆²⁻</td>
<td>0·9</td>
<td>2-1</td>
</tr>
<tr>
<td>S⁰</td>
<td>1·4</td>
<td>0-8</td>
</tr>
</tbody>
</table>

* 5 moles FeS suspended in 30 cc water

** 38% Ferric ion, 62% Ferrous

Identified and roughly estimated by differential pulse polarography

which IGSCC was not observed. The threshold concentration is an order of magnitude lower in thiosulphate than in tetrathionate solutions, but it is also evident that very low concentrations of either anion were sufficient to induce severe IGSCC. The effect of potential on the cracking susceptibility in thiosulphate-containing solution is depicted in Fig. 12. The potential range of maximum susceptibility corresponds to the corrosion potential measured in air saturated solutions.

Sulphate is not able to induce IGSCC (Cragnolino & Macdonald 1982), whereas the interaction of SO₄ or H₂S with oxygenated water in the presence of suspended iron sulphide or an iron base surface covered with a sulphide layer leads to the formation of tetrathionic acid (Ahmad et al. 1981).

A detailed discussion of the cracking mechanism is beyond the scope of this review. However, it should be noted that some authors (Cragnolino & Macdonald 1982, Dhawale et al. 1982, Newman et al. 1982c) have claimed that the main role of the metastable sulphur oxyanions is to release atomic sulphur by a disproportionation reaction to the acidified crack tip, thereby enhancing anodic dissolution of the chromium depleted grain boundaries and therefore the propagation of intergranular cracks according to the film-rupture mechanism for SCC. The thermodynamic basis of this interpretation can be visualized on the composite potential-pH diagram shown in Fig. 13. In this diagram only the metastable oxyanions of sulphur are included with the stability field for Fe₂S₇. Potential ranges for severe IGSCC in polythionic and thiosulphate solutions are included on the basis that there is no essential distinction between either types of environments aside from pH effects. The correlation with the stability domain for Fe²⁺ + S is apparent and therefore, the formation of atomic sulphur may be important in promoting intergranular cracking. Chemiadsorbed sulphur enhances significantly the rate of active dissolution of Fe and Ni in acidified solutions (Lacombe 1962, Oudar & Marcus 1979), inhibiting the passivation for sulphur coverages slightly lower than a complete monolayer. Even wet
elemental sulphur produces a significant increase in the corrosion rate of iron and mild steel (Farrer & Wormwell 1953, Macdonald et al. 1978).

The above data indicate that metastable sulphur oxyanions may induce severe IGSCC on sensitized stainless steel, even at very low concentrations and in almost neutral solutions. The effect is by no means confined to stainless steels, since nickel base alloys such as Incoloy 800 and Inconel 600, heat treated under conditions leading to carbide precipitation and concurrent chromium depletion, are also extremely susceptible to IGSCC in tetrathionate and thiosulphate solution (Scarberry et al. 1976, Cowan & Gordon 1978, Lee et al. 1981, Newman et al. 1982b). With the exception of sulphate, which is electrochemically inert even at very low potentials, almost all metastable sulphur oxyanions or compounds are able to induce cracking under appropriate conditions. However, the complexity of the sulphur compound chemistry makes difficult to establish unequivocally the nature of the specific species responsible for cracking.

**General remarks**

From the previous discussion it is apparent that a variety of sulphur compounds are able to accelerate or promote localized corrosion of corrosion resistant materials (stainless steels and nickel base alloys) in chloride- and non-chloride containing environments. The role of these compounds seems to be associated with their ability, in many cases via the formation of other sulphur species by redox or disproportionation reactions, to delay repassivation of bare metal surfaces in competition with oxygen-containing passivating species. In the case of pitting corrosion it seems that their effect can be exercised only in the presence of an aggressive anion such as chloride, which is able to induce film breakdown. Although there are no data to ascertain this hypothesis, a similar consideration may be valid for crevice corrosion. On the other hand, the presence of chloride anions is not required under sustained stress, indicating that the localized mechanical breakdown of a protective film is the unique requirement for the initiation of intergranular cracks in materials possessing a pre-existing active path (chromium depletion along grain boundaries). In the case of transgranular cracking of quench-annealed austenitic stainless steels in chloride-containing environments, the possible synergistic effect of sulphur compounds should be investigated.

**Effect of Sulphate-Reducing and Sulphur-Oxidizing Bacteria on Localized Corrosion**

The effects of sulphate-reducing and sulphur-oxidizing bacteria on the corrosion of ferrous materials have been studied extensively. Very useful reviews have been published during the last decade (Miller & Tiller 1970, Booth 1971, Miller...
1981, Iverson 1972, 1974, 1981). However, most of the available information as well as the discussion of the mechanisms involved are confined to the corrosion behavior of cast iron and carbon steels. Historically, these materials were used in buried pipeline constructions under conditions promoting the growth of the aforementioned bacteria. With the development of the chemical, petrochemical, and pulp and paper industries, more corrosion-resistant alloys were required. As a consequence, many failures have been reported in the literature in which sulphate-reducing bacteria were implicated in the localized corrosion of stainless steels and nickel-base alloys. Several examples can be cited. Kobrin (1976) reported intense microbial activity, including sulphate reduction, associated with extensive pitting of nickel and nickel-base alloys such as Monel 400 and Hastelloy B in heat exchangers cooled by river water. Tatnall (1981a) described severe crevice corrosion of Type 304 stainless steel in and around gasketed joints in a cooling tower system which was fed with river water; the effluent contained a high concentration of chloride and microorganisms such as iron bacteria and sulphate-reducing bacteria (Desulfotomaculum). Crevice corrosion was always observed in the presence of bulky deposits. Another case, in which Type 304 stainless steel was involved (Tatnall 1981a), revealed the development of deep pits under voluminous, mound-like deposits on an air distribution pipe located inside a waste water treatment tank and covered by sludge in which high counts of sulphate-reducing and iron bacteria were determined. A pump propeller and a screen, both made of Type 304 stainless steel, showed severe localized corrosion induced by bacteria in the clarifier of a paper mill closed water system carrying "white water" (Tatnall 1981a). The presence of Desulfotomaculum and Desulfotomaculum was suspected under slime deposits. Tatnall (1981a) also described two cases of pitting of galvanized steel in a cooling tower basin. High counts of both aerobic sulphur-oxidizing thiobacilli and anaerobic sulphate-reducers were observed.

In a review of corrosion problems in the pulp and paper industry, Chakrapani & Czyzewski (1978) discussed the occurrence of localized corrosion in the form of pits under slime and fibrous deposits on Type 304 and Type 316 stainless steels used in the fabrication of head boxes employed in the paper making stages. They attributed the damage to sulphate-reducing bacteria, present in an environment of pH 4.9 containing 140 ppm SO\textsubscript{4}\textsuperscript{2-} and 8 ppm Cl\textsuperscript{-}, because energy dispersive X-ray analysis of the deposits found inside pits revealed the presence of metallic sulphides. Charlton (1978) has also shown the occurrence of pitting on Type 316 stainless steel used as liner in a paper machine head box, attributing to sulphate-reducing bacteria the localized corrosion, but no other details were provided. Soimajärvi et al. (1978) have conclusively proved the presence of sulphate-reducing bacteria belonging to the genus Desulfotomaculum in paper machine waters and in plugged perforations of a suction roll used in the paper-making stage. Thus, there is no doubt that sulphate-reducers are present in many circumstances leading to localized corrosion in diverse industrial installations. Experimental observations have focused particularly on sulphate-reducing microorganisms; however, in many failure cases extremely heterogeneous microbial populations, including both aerobes and anaerobes, are likely to be present but rarely determined. It should also be noted that the mere presence of sulphate-reducing bacteria, determined mostly by enumeration of viable cells, is not a sufficient demonstration of the causative relationship with respect to the corrosion problem. An environment may support relatively high numbers of sulphate-reducers but their in-situ activity, which could be expressed by sulphate reduction, sulphide production, hydrogen uptake, or organic carbon utilization as a function of time, has never been determined in field studies of corrosion problems. Laboratory studies provide little insight into this relationship because they employ high nutrient concentrations under non-steady conditions. In the field, sulphate-reducers are influenced by numerous factors, including competition for nutrients and synergistic interactions, and thus their enumeration cannot be used as a reliable measure of their activity. Except for the case of buried pipelines made of cast iron and carbon steels (Miller 1981), very little is known about corrosion problems of other alloys attributable to thiobacilli. Since sulphuric acid is the main product of the activity of these bacteria, it seems that more acid-resistant alloys, such as stainless steels, are not so adversely affected. However, since these bacteria are able to form metastable sulphur species in both aerobic and anaerobic environments, their role in localized corrosion phenomena deserves to be further investigated. A recent study with T. thioparum indicates that, in addition to sulphuric acid formation, other metabolic products (which were not identified) may have an accelerating effect on the corrosion of a low alloy steel (Baru et al. 1982).

Sulphate-reducing bacteria: proposed mechanisms

The role of sulphate-reducing bacteria in inducing or accelerating electrochemical corrosion processes has been interpreted on the basis of different mechanisms, which can be conveniently classified as follows:

1. Stimulation of the cathodic reaction in the absence of oxygen by sulphate-reducing bacteria, either directly by removal of atomic hydrogen or indirectly by the formation of iron sulphides or hydrogen sulphide.

2. Acceleration of the anodic reaction by the action of sulphide ions or other sulphur species produced by the sulphate-reducing bacteria.

3. A combination of both effects.

Von Wolzogen Kühn & van der Vlugt (1934) provided an explanation for the underground corrosion of cast iron by the sulphate-reducing bacteria in electrochemical terms according to the so-called cathodic depolarization theory. They proposed that the bacteria could remove hydrogen from a cathodic area on the iron surface by the hydrogenase enzyme coupled to the reduction of sulphate to sulphide. This causes the depolarization of iron, thereby enhancing its
dissolution. The overall mechanism has been usually described as follows:

\[
\begin{align*}
\text{Anodic reaction} & \quad 4\text{Fe} \rightarrow 4\text{Fe}^{2+} + 8e^- \\
\text{Dissociation of water} & \quad 8\text{H}_2\text{O} = 8\text{H}^+ + 8\text{OH}^- \\
\text{Cathodic reaction} & \quad 8\text{H}^+ + 8e^- \rightarrow 8\text{H} \\
\text{Cathodic depolarization by bacteria} & \quad \text{SO}_4^{2-} + 8\text{H} \rightarrow \text{S}^{2-} + 4\text{H}_2\text{O} \\
\text{Corrosion products formation} & \quad \text{Fe}^{2+} + \text{S}^{2-} \rightarrow \text{FeS} \\
& \quad 3\text{Fe}^{2+} + 6\text{OH}^- \rightarrow 3\text{Fe(OH)}_2 \\
\text{Overall reaction} & \quad 4\text{Fe} + \text{SO}_4^{2-} + 4\text{H}_2\text{O} \rightarrow 3\text{Fe(OH)}_2 + \text{FeS} + 2\text{OH}^- \\
\end{align*}
\]

The cathodic depolarization step was based on the findings of Stephenson & Stickland (1931) who first suggested the biological activation of H and termed the enzyme hydrogenase. It should be noted, however, that the charge transfer step for the cathodic reaction leads to the formation of adsorbed hydrogen atoms:

\[
\text{M} + \text{H}^+ + \text{e}^- \rightarrow \text{M-H}_{\text{ads}} \\
\]

Therefore, it is by no means clear, as pointed out by Miller (1981), how an intact bacterium can remove adsorbed hydrogen atoms from the metal surface as distinct from the uptake of molecular hydrogen dissolved in water. Furthermore, the two alternative steps for completion of the hydrogen evolution reaction are (1) the chemical recombination

\[
\text{M-H}_{\text{ads}} + \text{M-H}_{\text{ads}} \rightarrow \text{H}_2 + 2\text{M} \\
\]

and (2) the electrochemical desorption

\[
\text{M-H}_{\text{ads}} + \text{H}^+ + \text{e}^- \rightarrow \text{H}_2 + \text{M} \\
\]

For transition metals there are conflicting views about the rate determining step (Bockris & Reddy 1973, Subramanyan 1981). In addition, the presence of SH\(^{-}\) modifies the path of the charge transfer reaction. It is doubtful that the bacterial uptake of H\(_2\), as related to the hydrogenase activity, would accelerate these reactions because the desorption of molecular H\(_2\) does not control the reaction rate.

Many years later, Horvath & Solti (1959) and Booth & Tiller (1960) used polarization curves as a measure of cathodic depolarization of iron in culture media inoculated with different species of Desulfotomaculum. Booth & Tiller (1960) found that only D. vulgaris, a hydrogenase-positive species, caused a marked decrease in cathodic polarization, while Desulfotomaculum orientis, a hydrogenase-negative sulphate-reducer, did not affect the cathodic polarization curve. Both organisms induced the formation of a partially protective film of iron sulphide after one week exposure. At that time these results were interpreted (Horvath & Solti 1959, Booth & Tiller 1960) as a confirmation of the cathodic depolarization theory, even though the corrosion rates obtained were significantly lower than those measured in the field. Booth et al. (1965) found later on that the semiprotective sulphide films became detached after 20-30 weeks exposure to bacterial action and the corrosion rates increased significantly, even in the case of D. orientis. The direct correlation between corrosion and hydrogenase activity, which is one of the basic assumptions of the cathodic depolarization theory, became doubtful. Booth et al. (1967) also observed that the addition of high Fe\(^{2+}\) concentrations to the culture medium gave rise to very high corrosion rates, comparable to those measured in the field, because ferrous iron reacted with sulphide produced by bacterial action and thus prevented the formation of a semi-protective sulphide film. Under such conditions a completely unprotective and loose mass of corrosion products, consisting of iron sulphide and ferrous carbonate, was formed. Booth et al. (1968) were able to demonstrate that chemically-produced suspensions of ferrous sulphide added to 1% NaCl solutions caused considerable cathodic depolarization of mild steel. It was shown (King & Wakerley 1973, King et al. 1973a, b) that different iron sulphides can lead to accelerated corrosion of mild steel, indicating that the action of sulphate-reducing bacteria is exercised through the formation of fresh iron sulphides. The properties of different iron sulphides (mackinawite, pyrrhotite, greigite, marcasite, etc.) and their corrosive effects were reviewed by Smith & Miller (1975). On the basis of work conducted at the Corrosion and Protection Centre, UMIST (UK), Miller (1981) suggested that all iron sulphides are cathodic towards iron. Recognizing the fact that in bacteria-free systems they do not act as permanent cathodes, he claimed that the role of bacteria could be either to "regenerate" (or depolarize) the iron sulphide enabling it to remain cathodic, to produce "fresh" iron sulphide by their growth reaction or even to bring fresh iron sulphides surfaces constantly into contact with the steel by cell movement.

A different point of view is held by Costello (1974). He measured cathodic polarization curves of mild steel in cultures of D. vulgaris and two other strains of the genus Desulfotomaculum at pH 5.5 and compared the results with those obtained in the presence of 0.01 M H\(_2\)S at the same pH. He concluded that cathodic depolarization in cultures of sulphate-reducing bacteria may be attributed to the cathodic activity of dissolved hydrogen sulphide produced by the microorganisms. The specific role of biogenic hydrogen sulphide was clearly demonstrated by Togano et al. (1975). The authors measured corrosion rates of mild steel as a function of time by linear polarization methods, accompanied by simultaneous measurements of the corrosion potential, concentration and rate of formation of H\(_2\)S, and viable numbers...
of sulphate-reducing bacteria. A correlation was established indicating that the corrosion rate was proportional to the instantaneous concentration of H₂S produced by the bacteria, although the influence of H₂S became complicated because of the formation of sulphide films. Togano et al. (1975) also claimed that the accelerating effect of the H₂S was greater on the anodic reaction than on the cathodic reaction. The same opinion was expressed in the early 1950s by Wanklyn & Spruit (1952). There is no doubt, as noted in the section devoted to the effect of sulphur compounds on a localized corrosion, that H₂S can accelerate both the anodic and cathodic reaction. The complexity of the systems associated with the growth of sulphate-reducers makes it more difficult, compared with sterile environments, to define the precise role of H₂S in the corrosion kinetics. The participation of other metastable sulphur species in the acceleration of the anodic reaction cannot be excluded. Iverson (1981, 1983) suggested, on the basis of experiments in which high corrosion rates were observed, that in addition to hydrogen sulphide sulphate-reducing bacteria produce a highly corrosive substance, possibly a soluble compound containing phosphorus, which enhances the dissolution of iron under anaerobic conditions at a neutral pH. He emphasized that enhanced corrosion can be expected only when the substance comes in contact with iron before sulphide film formation takes place. Otherwise corrosion is stifled, although the subsequent breakdown of the film could result in a further increase of the corrosion rate.

On the other hand, Schaschl (1980) showed that elemental sulphur, dissolved in the presence of sulphide ions, promotes the accelerated corrosion of mild steel in contaminated brines by a concentration cell mechanism similar to that of differential aeration. He claimed that dissolved sulphur acts as a cathodic reactant, indicating that bacteria may provide the shielding action needed to promote concentration cell action. Bates (1981) reinterpreted the action of sulphur, proposing that polysulphides (Sₓ²⁻) are the cathodic reactants.

Until now, we have considered the role of the sulphate-reducing bacteria independently of the morphological development of the attack. In the case of localized corrosion, a basic requirement is the physical separation of anodic and cathodic sites. Uniform or general corrosion takes place when such physical separation does not occur. A condition frequently found for the localization of anodic sites arises from the existence of areas of the metal surface occluded by some means and hence less oxygenated than others. All types of microbes can colonize surfaces and produce a mass of "slime", thereby establishing a differential aeration cell. In such cases active growth of the micro-organisms decreases the concentration of oxygen to very low levels. Even the death of the organisms in the interior of the colony will not inhibit the development of the electrochemical cell because a physical barrier prevents the ingress of oxygen. Such anaerobic conditions are conducive to the development of sulphate-reducing bacteria. In the case of cast iron the preferential dissolution of iron leads to the well known phenomenon of graphitization. For carbon steels, the attack manifests itself in the form of pitting, but due to the relative poor protective properties of the passive film the attack tends to be shallow, but extended over a large area. In more corrosion resistant alloys covered by protective films, pits are usually small in diameter, but can be extremely deep. In this context, it is appropriate to indicate the concern (Tatnall 1981b) that the replacement of carbon steels by stainless steel may lead to even greater corrosion problems if the mechanisms of the bacterial action are not well understood. The use of appropriate electrochemical methods, coupled with a careful characterization of the type of bacteria and evaluation of the effect of their metabolic products can lead to a better understanding of the mechanisms involved.

As an example of the type of approach that seems to be fruitful in the study of localized corrosion processes, we can mention the studies of Salvarezza & Videla (1980). They studied the effect of sulphate-reducing bacteria on the anodic corrosion rate of AISI 1020 steel in artificial sea water contaminated with sulphate-reducing bacteria for various incubation periods. 96 hr of incubation (total sulphides 10⁻⁴ M, pH 7.8, redox potential −510 mV) (O); 72 hr of incubation (total sulphides 14 × 10⁻⁴ M, pH 7.5, redox potential −500 mV) (Δ); 240 hr of incubation (total sulphides 8 × 10⁻⁴ M, pH 7.2, redox potential −510 mV) (D) (Salvarezza & Videla 1980). With permission of the National Association of Corrosion Engineers.

![Fig. 14. Potentiostatic polarization curves for AISI 1020 steel in artificial sea water contaminated with sulphate-reducing bacteria for various incubation periods.](image1)

![Fig. 15. Potentiostatic polarization curves for AISI 1020 steel in artificial sea water contaminated with sulphate-reducing bacteria.](image2)
behave of mild steel in deaerated artificial sea water using potentiostatic polarization methods. A significant decrease in the pitting potential (>150 mV) was found when artificial sea water was contaminated with cultures of sulphate-reducing bacteria (*Desulfovibrio*). Fig. 14 shows the effect of various incubation times on the polarization curves, revealing that the decrease in the pitting potential is associated with the bacterial formation of sulphide. This relationship is further illustrated in Fig. 15, where the anodic behavior in the presence of bacteria is compared with that observed in a sterile environment containing an equivalent concentration of sulphide. Salvarezza & Videla (1980) observed the development of pits in the samples, but the attack seemed to be shallow and extended over large areas.

In the case of stainless steels, as mentioned in the section devoted to the effect of sulphur compounds on localized corrosion, a significant decrease in the pitting potential with respect to that in plain chloride solutions can also be expected because of the action of biogenic hydrogen sulphide or other sulphur species produced by the sulphate-reducing bacteria. The development of crevice corrosion in the presence of bacteria may be also explainable in terms of the effects exercised by their metabolic products. On the other hand, no cases of stress corrosion cracking in relation to sulphate-reducers have been reported, but indirect evidence indicates that their metabolic products may also induce cracking failures under appropriate stress conditions.

**Concluding Remarks**

A survey conducted in the UK (Wakerley 1979) indicated that microbiological corrosion problems are widely distributed in the industry. In many cases there is no precise documentation on the chemical or microbiological characterization of failure cases. A causative relationship has not clearly emerged from the examination of corrosion failures in the industry, but is well established in laboratory studies. In future studies it would be helpful to analyze ionic sulphur species present in the corrosive environment because there is no doubt about their aggressive role in the stimulation of corrosion. In the laboratory almost any living (and dead) micro-organisms populations can at least partially attach on metal surfaces, and by extrapolating this phenomenon to industrial failures it is not surprising that diverse micro-organisms are found in corroded specimens. In view of the ubiquity of micro-organisms in the nature at ambient temperatures, it seems that we cannot rely on qualitative descriptions of micro-organisms detected in failure cases. In future endeavours, microbial activities should be estimated together with the chemical speciation of major elements in order to better understand the dynamic character of microbiologically induced corrosion problems. In many cases, however, the presence of micro-organisms determined, for example, by enumeration of viable cells serves to indicate potential problems. Perhaps even more importantly, microbial counts indicate the efficiency of protection measures (e.g. disinfection) adopted to curtail the microbiologically mediated deterioration of installations. More intensive surveillance programmes are warranted in industries that use materials susceptible to corrosion. It is obvious that better predictions and effective counter-measures can be offered once the microbiological and chemical conditions and interactions are better elucidated.

A grant from the Ohio Air Quality Development (O.H.T.) in support of the work is gratefully acknowledged.

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The Role of sulphate-reducing and sulphur-oxidizing bacteria


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GROWTH OF SCYTALIDIUM ACIDOPHILUM ON DEFINED MEDIA, WHEY AND ACID SULPHITE WASTE

J. D. MILLER\textsuperscript{1}, K. C. IVARSON\textsuperscript{1} and W. M. KAEPPNER\textsuperscript{2}

Abstract: Scytalidium acidophilum is an acidophilic hyphomycete that has potential as a source of single cell protein. The growth of \textit{S. acidophilum} in defined media was investigated at pH values from 0.5 to 8.0 at 10, 20 and 30°C. The ability of the fungus to degrade whey and pulp mill waste sulphite liquor was also studied. The optimal pH for growth by dry weight and economic coefficient was approximately 5, with good growth from pH 1 to 6. \textit{S. acidophilum} grew very slowly on whey, consuming most of the lactose after 60 d incubation. After a period of adaptation, \textit{S. acidophilum}, grew well on waste sulphite liquor. A yeast-like growth of \textit{S. acidophilum} is described.

Introduction

Starkey & Waksman (1943) reported a fungus later described by Sigler and Carmichael (1974) as \textit{Scytalidium acidophilum} that can grow under acidic conditions (pH 0). \textit{S. acidophilum} has been suggested as a useful fungus for single cell protein production because acid-hydrolyzed substrates do not need to be neutralized, and fermentation under acid conditions virtually eliminates contamination by undesirable micro-organisms (Boa & Leduy 1982\textsuperscript{1}). It has been shown that this fungus can grow on a wide range of hexoses, pentoses and uronic acids (Ivarson & Morita 1982). Peat hydrolysate, newspaper and waste paper have been tested as carbon sources for this fungus (Boa & Leduy 1982, Ivarson & Morita 1982). The fungal protein thus obtained has been found satisfactory for an animal feed supplement in preliminary tests (Sibbald & Ivarson, unpubl.).

Further testing of this organism with other waste substrates requires an examination of the growth of this fungus under different conditions. This paper reports on the growth of \textit{S. acidophilum} at 10\textdegree, 20\textdegree and 30\textdegree C from pH 0.5 to 8.0. Because large quantities of whey (Freyssinet & Nigon 1980) and sulphite liquor (Gold \textit{et al}. 1981) are disposed of without treatment, the ability of \textit{S. acidophilum} to degrade these industrial wastes was also investigated.

\textsuperscript{1}We have examined the fungus used by Boa and Leduy and it is \textit{S. acidophilum}.

Sigler & Carmichael (1974) separated \textit{S. acidophilum} from the type species of the genus, \textit{S. lignicola} Pesante on the basis of its growth at low pH (cf. Gould \textit{et al}. 1974). We report also on the effect of pH on the growth of \textit{S. lignicola} in comparison to \textit{S. acidophilum}. In addition, a yeast-like stage of \textit{S. acidophilum} which is induced by some culture conditions, is described.

Materials and Methods

pH—Temperature tests

Inoculum was prepared according to the method of Sguros \textit{et al}. (1962). Erlenmeyer flasks (250 mL) containing 50 mL of Fries medium (Booth 1971) with 10 g/L glucose and adjusted to pH 3 with concentrated HCl were inoculated with pieces of an agar culture of \textit{S. acidophilum} (ATCC 26774) or \textit{S. lignicola} (DAOM 58080) and incubated at 30°C. After 7 days, the cultures were (individually) homogenized, centrifuged for 20 min at 13,000 g and resuspended in sterile water, all under aseptic conditions. The concentration of the mycelium was adjusted to 10 mg/mL on a dry weight basis.

Triplicate tests were also carried out in 250 mL Erlenmeyer flasks containing 50 mL of Fries medium adjusted to the required pH with 2N HCl. Glucose (10 g/L) was added to the media after pH adjustment and samples were retained for glucose analysis. Flasks were inoculated with 1 mL each of the suspended hyphae (10 mg/50 mL) and incubated for 5 days at 30°C, 6 days at 20°C and 10 days at 10°C on a rotary shaker at 125 rpm.

At the end of the incubation period, the contents were filtered through 0.45 μm HA Millipore filters and the dry weight of mycelium was determined. A small amount of the mycelium (approximately 200 mg) was then removed from each filter and analysed for nitrogen by the Microkjeldahl method. Samples of the aqueous phase were analysed in duplicate for glucose by the Shafer-Somogyi method (AOAC 31-052-31-053, 13th Ed., 1980).

Larger scale experiments were conducted in a modified medium reported by Ivarson and Morita (1982): glucose (14 g/L), MgCO\textsubscript{3} (5 g/L), ZnSO\textsubscript{4}·7H\textsubscript{2}O, (600 mg/L, the final concentrations in the growth medium added to the trace element solution). After adjusting the pH to 0.5, 1.0 and 2.0 with H\textsubscript{2}SO\textsubscript{4}, 1 L portions of the media were dispensed into 5 L Roux bottles and inoculated with 0.5 g (dry weight) of freshly harvested mycelium grown for 2 days at pH 0.5 and 30°C in the above medium. The bottles were placed in water baths adjusted to provide culture temperatures of 20°C and 30°C, and the cultures aerated (15 000 mL/L/min) with H\textsubscript{2}O-saturated filtered air. All tests were done in duplicate. At 24 h intervals the mycelium were harvested by filtration through Whatman # 3 filter paper, washed and the dry weight determined. The residual glucose in the filtrate and the nitrogen content of the dried hyphae were determined as noted above.

Cottage cheese whey powder was obtained from a local dairy (75% lactose, 17% protein, 1% fat). The whey powder was reconstituted with distilled water to give a lactose concentration of 3% (w/v) and adjusted to pH 0.5 with H\textsubscript{2}SO\textsubscript{4} or to pH 1.4 with 1 N HCl. After autoclaving at 112°C for 15 min, 0.5 g/L (NH\textsubscript{4})\textsubscript{2}SO\textsubscript{4}, 0.5 g/L MgSO\textsubscript{4}·7H\textsubscript{2}O, 0.5 g/L KH\textsubscript{2}PO\textsubscript{4} and trace elements (Ivarson and Morita, 1982) were added as sterile solutions. Portions (1 L) were dispensed into 5 L Roux bottles, inoculated, aerated and incubated at 30°C as described above for up to 60 d.

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For fermentation studies Canadian International Paper Company (Hawkesbury, Ontario) supplied the sulphite liquor that was derived from hardwood (pH 1.8). Trace amounts of Ca, Fe, Mn, Mg, K and Na were present in the liquor and its caloric value was 1 Kcal/Kg. Prior to fermentation, the free SO₂ content was reduced to ca. 0.5% by boiling. Water was added to bring the liquor back to the original volume. This solution is referred to as stripped sulphite liquor. The sulphite liquor experiments were carried out in a 2 L Multigen Fermentor (New Brunswick Scientific) adapted for continuous culture (fermentation volume 750 mL, 30°C, 280 mL/L/min air). The fermentation was initiated by inoculating the liquor with 3 g (dry weight) of inoculum prepared as described for the whey experiments, and allowed to proceed batchwise until good growth was observed at which time the continuous feed was started (20 mL/h). Twice daily, several drops of antifoam (Dow Corning) was added and any hyphae on the walls of the vessel were scraped into the medium. Each fermentation run was 2 L. The inoculum consisted of hyphae from the previous run. Details are given in Table 2.

1 solids 15.4%; S 1.04%; NH₃ 0.5%; OCH₃ 1.6%; hexoses 1.6%; pentoses 2.7%.

![Graph](image-url)  
Fig. 1. Growth of S. acidophilum and S. lignicola on defined media at different pH's and temperatures.

- S. lignicola 30°C, 5 day incubation
- S. acidophilum 30°C, 5 day incubation
- S. acidophilum 20°C, 6 day incubation
n = 3, s.d. of dry weights 1% of means.

<table>
<thead>
<tr>
<th>pH</th>
<th>S. lignicola</th>
<th>S. acidophilum</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>economic coefficient</td>
<td>% N</td>
</tr>
<tr>
<td>0.5</td>
<td>nd</td>
<td>nd</td>
</tr>
<tr>
<td>1.0</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>2.0</td>
<td>5.43</td>
<td>58.5</td>
</tr>
<tr>
<td>3.0</td>
<td>5.35</td>
<td>45.5</td>
</tr>
<tr>
<td>4.0</td>
<td>5.78</td>
<td>45.1</td>
</tr>
<tr>
<td>4.0</td>
<td>5.77</td>
<td>46.2</td>
</tr>
<tr>
<td>6.0</td>
<td>5.76</td>
<td>49.1</td>
</tr>
<tr>
<td>7.0</td>
<td>5.85</td>
<td>48.3</td>
</tr>
<tr>
<td>8.0</td>
<td>5.51</td>
<td>+</td>
</tr>
<tr>
<td>9.0</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>10.0</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>11.0</td>
<td>-</td>
<td>+</td>
</tr>
</tbody>
</table>

*note: standard deviations of these data 1% of the mean n = 2 or 3.*

nd = not determined
- = insufficient sample
+ = 100% of glucose not used
Results and Discussion

The growth of *Scytalidium acidophilum* at pH 0.5 to 8.0 at 20° and 30°C is shown on Fig. 1. In the defined medium used, the fungus grew well between pH 1 and pH 6 with steep declines in the dry weight produced above and below these values. This pattern was consistent at both 20° and 30°C, although significantly more growth occurred at 20°C (P = 0.01) albeit over a longer incubation period. Growth at 10°C was limited and the results are omitted. The optimum pH for growth (by dry weight and economic coefficient, Fig. 1, Table 1) was ca. 3 at 50°C and somewhat higher at 20°C. The shapes of the pH—dry weight curves at both temperatures are typical for fungi growing in defined media (Griffin 1981). The nitrogen content of the mycelium of *S. acidophilum* also varied according to the pH and temperature (Table 1). The economic coefficients at both temperatures are relatively high in this medium (cf. Cochrane, 1958). Results obtained in the large scale batch fermentations at pH 0.5, 1.0 and 2.0 were similar to those in the small scale fermentations with regard to nitrogen content of the hyphae, economic coefficients and relative dry weights and are not reported. In the large scale experiments however, the growth rate was approximately double that of the small scale, probably because of increased aeration.

These results are in contrast to those obtained by Gould *et al.* (1974) with *S. acidophilum* ATCC 26772. The pH—dry weight curve published by these authors was determined by growing the fungus in Difco nutrient broth and shows an optimum initial pH of ca. 2 with a decline in growth from pH 2 to pH 8. It might have been expected that the medium used by Gould *et al.* (1974) would produce a "flattened" pH—dry weight curve since it has been reported that the growth of fungi at non-optimal pH values in complex media (like nutrient broth) is enhanced compared to defined media (Griffin 1981). The pH—dry weight curve of *S. lignicola* was distinct from that of *S. acidophilum* (Fig. 1). *S. lignicola* growing well over the range of pH 3-9. However, it could also be that there is a difference between the two isolates. Another possibility is that more nutrients were made available from the Difco nutrient broth by hydrolysis at the low pH values.

The pH data have relevance to the use of this organism as single cell protein. Since *S. acidophilum* can grow over a range of pH values, the substrate may need to be acidified only to the degree required to avoid contamination of the substrate. Similarly, the protein and possibly other characteristics of the hyphae can be altered by substrate pH and fermentation temperature.

The fermentation of whey at pH 0.5 was very slow. Economic coefficients were relatively high (52-54%), but 50 to 60 days were required for the fungus to consume 98% of the lactose. Growth in whey at pH 1.4 was noticeably faster, however after 10-14 days the *Scytalidium* became yeast-like and the experiments were stopped.

The yeast-like stage of *S. acidophilum* (Fig. 3, 4, 5) was first observed when *S. acidophilum* was cultured on autoclaved whey of pH 1.4. In the larger scale fermentation system described above, the fungus predominantly appears as individual yeast-like cells. On agar media, in addition to the yeast-like cells, a branching-budding structure is seen (Fig. 3). The factors that induce the change in morphology are not completely known. However, the yeast-like phase is favoured by more alkaline pH values (<pH 1.4) and specific carbon sources. The *Scytalidium* phase is easily induced from an inoculum of the yeast phase and *vice versa*. The growth of the yeast-like phase on galactose agar is shown in Fig. 4. When the yeast-like phase is plated on xylose agar (salts and trace elements as described for the larger scale fermentation) the *Scytalidium* state appears (Fig. 5). The yeast-like phase has the ability to ferment alcohol, and this is the subject of further study.

When the fermentor was charged with undiluted sulphite liquor, fungal growth and sugar utilization were low in 3-4 week fermentations. Similar results were initially obtained when the liquor was diluted with water 3:2. Upon lowering the ratio to 1:1, rates of growth and sugar consumption increased. This led us to design a system whereby after several runs at the 1:1 dilution, the liquor concentration was increased stepwise. The results (Table 2) show that at all concentrations, ca. 85% of the sugars were consumed. The nitrogen content of the hyphae and the economic coefficient showed no appreciable change. It is possible that a longer retention time would result in greater sugar utilization. The results suggest that this fungus may have a value in lowering the BOD of sulphite liquor while producing a valuable by-product.

<table>
<thead>
<tr>
<th>Dilution²</th>
<th>pH</th>
<th>No. of runs</th>
<th>Dry wt. of mycelia (g/2L)</th>
<th>% N in mycelia</th>
<th>% sugars consumed³</th>
</tr>
</thead>
<tbody>
<tr>
<td>1:1</td>
<td>1-96</td>
<td>3</td>
<td>17.3 ± 0.8⁴</td>
<td>5.8 ± 0.1</td>
<td>84.8 ± 3.3</td>
</tr>
<tr>
<td>3:2</td>
<td>1-92</td>
<td>3</td>
<td>20.2 ± 2.2</td>
<td>5.1 ± 0.1</td>
<td>87.7 ± 0.5</td>
</tr>
<tr>
<td>7:3</td>
<td>1-88</td>
<td>3</td>
<td>22.1 ± 1.1</td>
<td>5.0 ± 0.2</td>
<td>83.0 ± 3.5</td>
</tr>
<tr>
<td>6:1</td>
<td>1-84</td>
<td>2</td>
<td>18.8 ± 1.7</td>
<td>5.3 ± 0.1</td>
<td>81.3 ± 3.9</td>
</tr>
</tbody>
</table>

¹Total vol/run = 2L; at flow rate of 20 ml/hr.
²Ratio of liquor to distilled H₂O.
³Sugar content of unfermented liquor = 85 g/2L.
⁴mean ± standard deviation.
Fig. 2. Hyphae, arthroconidia and sclerobia of Systidium acidophilum (X ca. 700).

Fig. 3. Yeast-like state of S. acidophilum showing budding-branching structure and individual yeast-like cells (Nomarski interference contrast (X ca. 700)).

Fig. 4. Yeast-like state of S. acidophilum plated on galactose agar (X ca. 0.06).

Fig. 5. Yeast-like state of S. acidophilum plated on xylose agar reverting to the Systidium state at ca. 10-14 days (X ca. 700).

Note: All magnifications refer to the actual size of the enclosed photographs.
It should be noted that, although other species of the form genus *Scytalidium* have been found to have a pycnidial phase, *Hendersonula* (Ellis 1976, Punithalingam & Waterston 1970) when cultures of *S. acidophilum* were grown on twigs of spruce, fir and birch (which had been boiled in water) for 60 days at 25°C and no pycnidia were observed (cf. Natrass 1933). Cultures were also grown on potato carrot agar and corn meal agar at pH 1 and 2-27 for 30 days at 23°C under UV and white light according to the method of Campbell (1974). No pycnidia were observed, but there was an increase in the number of sclerotia (Sigler & Carmichael, 1974, on potato carrot agar at pH 2-7 compared to other treatments (Fig. 2).

**References**


THE EFFECT OF MACERATION ON FILAMENTOUS ALGAE USED FOR THE TESTING OF ALGICIDAL COMPOUNDS

L. H. G. MORTON^, M. L. LOVELL^ and A. F. MITCHELL^

Abstract: An account is presented of the effect of maceration on the growth on solid media of two filamentous test algae known to foul paint surfaces. Maceration was found to have little effect upon algal viability, showing that macerates of filamentous algae may be used in algicidal assay procedures. An assay procedure using filter paper discs impregnated with Nuodex 87 is outlined.

Introduction

This work was undertaken to study the effect of maceration upon the subsequent re-growth of filamentous algae employed in the testing or screening of algicidal compounds or of paint films containing such compounds. It was felt that since uniform or standard inocula of test organisms is a desirable feature in such tests, information on maceration and its effect on the viability of filamentous test organisms is of considerable importance. Two filamentous algae were chosen for this investigation namely, a species of Trentepohlia supplied by Durham Chemicals Ltd, and a culture of Oscillatoria tenuis supplied by ICI ‘Organics’ Division at Blackley. Both of these organisms are cited as filamentous algae which foul terrestrial substrates including paint films (Wec & Lee 1980, Grant 1982).

Materials and Methods

Liquid shake cultures of the test organisms were grown in Difco Algal Broth whilst stock cultures were maintained on this same medium containing 2% agar. The apparatus for undertaking shake cultures was a Gallenkamp orbital incubator set at 30°C and operating at 140 rpm with three 30 W fluorescent strip lights as the source of illumination. Shake cultures were carried out in 250 cm^3 flasks containing 100 cm^3 of algal broth. Algal growth on solid medium was maintained by placing cultures under a bank of fluorescent strip lights.

Methods employed for macerating or breaking up the filamentous algae were as follows:

(i) vigorous shaking of liquid cultures either by hand or by using a FISON'S WM/250/F WHIRLIMIXER. These methods were used for Trentepohlia.

(ii) stirring of liquid cultures of Oscillatoria for 30 minutes using a magnetic flea and a RODWELL MONOTHERM stirrer set at speed setting 10.

(iii) homogenization of Oscillatoria using a POTTER ELVEJEM type homogenizer with a 25 cm tube.

The maceration or separation procedures were followed by inoculation of aliquots of macerated material onto the surface of the agar medium, such cultures were illuminated and assessed for viability by observing their growth or lawn formation on the agar medium. The effect of Nuodex 87 (Durham Chemicals Ltd) upon the viability of the test organisms was assessed in the following manner: Filter paper discs measuring 0.5 cm in diameter were impregnated with aqueous solutions of 2.5 and 5.0% Nuodex 87, after removing surplus biocide, by blotting, the discs were used in three separate viability tests.

Viability test 1

0.1 cm^3 of macerated material was surface spread onto agar medium. The plates were prepared and filter paper discs impregnated with 5.0% Nuodex 87 were placed onto three of them. The plates were inverted and placed under the lights for 14 days. Three plates were used as controls containing discs without biocide. Further plates containing macerated material alone were prepared at daily intervals using the initial macerated material in order to assess the effect of time elapsed after maceration on the growth of algal lawns.

Viability test 2

From an established lawn of Trentepohlia growing on agar medium a suspension of the organism was prepared by adding sterile growth medium to the culture and scraping the surface with a flamed loop. Some of this suspension was spotted onto the surface of a plate of agar medium. The drops each of 0.025 cm^3 volume were arranged in an hexagonal pattern and allowed to dry. Filter paper discs containing 2.5 and 5.0% Nuodex 87 were placed 2 mm from the algal drops. Controls consisted of unimpregnated discs. The plates were illuminated for 14 days. This test procedure was repeated with a macerate of Oscillatoria (Fig. 1).

Viability test 3

Using the Trentepohlia and Oscillatoria suspensions from test 2, bands of each macerated material measuring 1 x 7 cm were carefully inoculated onto the surface of separate agar plates and allowed to dry. Impregnated discs and controls were placed on either side of each band 2 mm from its margin. The plates were inverted and illuminated as before (Fig. 1).

Assessment of inhibition of growth

Inhibition of algal growth where it occurred was assessed by measuring the distance from the edge of the disc to the boundary between living and dead algae, which was in all cases quite distinct. The criteria for viability was pigmentation (Morton 1979).

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**Results**

Table 1(a) Shows the inhibition zones produced when discs impregnated with 5·0% Nuodex 87 were placed onto agar plates spread with macerated *Trentepohlia* and *Oscillatoria* cultures. *Oscillatoria* appears to be significantly more susceptible to the biocide than *Trentepohlia*.

Table 1(b) Shows the results of the drop test technique where discs impregnated with 2·5 and 5·0% Nuodex 87 were placed in close proximity to drops of algae macerate. The test shows that *Oscillatoria* appears to be significantly more susceptible to the biocide than *Trentepohlia* at both concentrations employed.

Table 1(c) Shows the results of the band inoculum technique. In the case of *Trentepohlia* an increase in the concentration of biocide has produced a significantly greater inhibition of algal growth. In the case of *Oscillatoria* the concentration of biocide provided by the close proximity of the discs prevented growth of this organism.

**Table 1. The effect of Nuodex 87 on the growth of the Test Algae**

(a) The Spread Plate Technique

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Trentepohlia</th>
<th>Oscillatoria</th>
<th>t ratio</th>
<th>level</th>
</tr>
</thead>
<tbody>
<tr>
<td>5%</td>
<td>22·0</td>
<td>33·0</td>
<td>6·32</td>
<td>&lt;0·2%</td>
</tr>
</tbody>
</table>

Results are expressed as the mean value of 6 replicates.
The effect of maceration on filamentous algae....

(b) The Drop Plate Technique

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Trentepohlia</th>
<th>Oscillatoria</th>
<th>t ratio</th>
<th>level</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.5%</td>
<td>2.6</td>
<td>5.4</td>
<td>6.00</td>
<td>&lt;0.2%</td>
</tr>
<tr>
<td>5.0%</td>
<td>4.3</td>
<td>6.8</td>
<td>7.02</td>
<td>&lt;0.2%</td>
</tr>
</tbody>
</table>

Results are expressed as the mean value of 8 replicates.

(c) The Band Plate Technique

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Trentepohlia</th>
<th>Oscillatoria</th>
<th>t ratio</th>
<th>level</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.5%</td>
<td>4.6</td>
<td>*</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>3.0%</td>
<td>6.4</td>
<td>*</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

Results are expressed as the mean value of 8 replicates.

In all cases the control values were zero.

Discussion

Material plated out daily following initial maceration showed no apparent loss of viability over four days. The criterion used to assess viability, that is, algal growth on agar indicates that breaking up filaments with minimal disruption of cells does not seem to affect their ability to grow once more. The growth of Oscillatoria on agar was as expected filamentous. Trentepohlia, however grew as scattered clumps of cells. This type of growth is documented fully in Chapman (1962).

The technique described using impregnated filter paper discs to assess algicide activity can be successfully employed with macerated material of the two test algae. Leaching from paint films can also be demonstrated using the type of test procedure outlined in viability test 1 (Greenhalgh pers. comm.). However, this work was undertaken primarily to assess the effect of maceration on algal viability rather than to design new test methodology. Methods for the evaluation of algicidal biocides are available in Springle (1975), Morton (1979), Morton & Peel (1980), Grant (1982), Grant & Bravery (1981a,b).

References


THE INTERACTION BETWEEN TRI-N-BUTYL Tin OXIDE AND AGAR GEL AS RELATED TO A FUNGUS INHIBITION ASSAY

R. J. ORSLER and G. E. HOLLAND

Abstract: Experiments have been carried out to test the ability of tri-n-butyl tin oxide (TnBTO) to diffuse through agar gel, particularly in relation to the validity of a petri dish fungal bio-assay technique. It is concluded that TnBTO does not diffuse through agar gel to a measurable extent, nor is it deposited from the atmosphere on to the agar surface, although the aerial concentration itself may be important. The use of this bio-assay technique as a means of obtaining a quantitative assessment of the fungicide present is not recommended.

Introduction

Scheffer & Graham (1973) proposed the use of a simple fungus inhibition bioassay as an indicator of the effectiveness of residual pentachlorophenol (PCP) in spray treated cedar poles. This was further investigated by Scheffer & Lew (1976) who extended the work to include various water-soluble formulations. They concluded that the assay response could be correlated with the retention of PCP in the wood and that the method showed promise for indexing retentions of ammoniacal copper arsenate and surprisingly, for the highly fixed copper/chromium/arsenic preservatives.

The test involves placing a sample of treated wood at a fixed distance from a decay fungus growing on malt agar in a petri dish. After incubation for a set period, the extent to which the growth of the fungus across the plate is inhibited by the presence of the sample is used as an indication of the protection afforded by the preservative within the sample.

The simplicity and speed of this test attracted attention amongst mycologists since it promised to be a suitable method for screening the toxicity of candidate fungicides to specific organisms, and to allow the acquisition of some quantitative assessment of effectiveness without recourse to chemical analysis. However, Scheffer and Lew were quite clear that their results were not intended to be used to estimate preservative retentions. Nevertheless, some workers have used this type of test as a substitute for chemical analysis (Ingley 1982).

Henshaw et al. (1978) attempted to use a similar method to assess the effectiveness of tributyltin oxide (TnBTO) as a wood preservative, but concluded that the inhibition-type test could not be used, as yet, to predict the degree of protection given by TnBTO residues in weathered wood. The ability to carry out a rapid bioassay on preservative-treated timber would be useful to that part of the current programme of this laboratory concerned with the long-term performance of the organic solvent type wood preservatives. Consequently it was felt appropriate to examine in some detail the likely distribution of wood preservatives within the petri dish in an attempt to define the mechanism whereby the “zone of inhibition” is set up. Central to this issue is the way in which those preservatives having little or no solubility in water, and small but significant vapour pressures, react. Amongst these TnBTO is of considerable importance, and as a consequence it was chosen as the preservative to be investigated. Since diffusion is commonly considered to be the most likely mechanism, in the following study experiments 1 and 2 are concerned principally with liquid diffusion, and experiments 3 and 4 with vapour diffusion.

Experimental

In each of experiments 1 to 3 described below, very large excesses of TnBTO were used, far greater than the concentrations normally used for biocidal experimentation. This was done in order to maximise movement of TnBTO by physical processes. It was considered that if diffusion did not occur to a measurable extent with these high concentrations it would be unlikely to occur from the smaller amounts and lower concentrations found in treated wood samples.

Experiment 1

50 ml of hot (liquid) 2 per cent malt agar was treated with 0.5 ml TnBTO dissolved in a little ethanol and thoroughly mixed. The resultant white, opaque, emulsion was equally divided between two petri dishes (90 mm diameter dishes were used throughout this work) and allowed to set. Disks were cut from the congealed mass using a 14 mm diam cork borer.

Nine petri dishes, each containing 20 ml of 3 per cent malt agar gel, were sub-divided into three sets of three. For one set, filter papers were cut to fit across the entire surface of the agar except for a central hole coinciding with the shape of a TnBTO/agar disc; for the second set cellophane sheets were similarly used; the third set was unmodified. A single disc of TnBTO/agar was placed on the surface at the centre of the agar and the whole batch of nine dishes, with lids on, were stored in a dark cupboard at room temperature.

After one week, two weeks, and four weeks respectively, one dish from each set was removed for analysis. The TnBTO/agar disc, and the covering, if any, were removed and discarded. Three analytical samples were taken from the gel within each plate, a central disc cut with a cork borer 15 mm in diameter to include the zone covered by the TnBTO/agar disc, and an inner and outer portion of the remaining annulus were separated by a circular cut midway between the inner and outer circumferences (Fig. 1). Each analytical sample was held gently in a pair of forceps and sprayed with n-hexane from a wash bottle to remove any TnBTO on the surface. The sample was then extracted with 50 ml of 0.5 per cent HCl/EtOH by refluxing for 10 minutes. After filtering, the resultant solution was submitted for polarographic analysis as described in PRL Analytical Method Sheet No. 14 (Anon 1981).

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A further three petri dishes were prepared, again containing 20 ml of 3 per cent malt agar gel and with a disc of TmBTO/agar placed at the centre of each agar surface. They were covered and stored in a dark cupboard, as before, for one week and then analysed. However, for these samples the gel was not washed with n-hexane prior to the analysis.

**Experiment 2**

Two lengths of glass tubing (internal diam 22 mm) held vertically, were each closed at the bottom end by a rubber bung, and hot (liquid) 2 per cent malt agar poured in to form a column 20 cm high. After setting, a small well was cut into the top of each column and filled with TmBTO. A second rubber bung was placed in the top of each tube and they were then allowed to stand undisturbed, at room temperature, column A for 18 days and column B for 21 days. At the end of this period the TmBTO was removed from the wells with a pipette and the tops of the agar columns rinsed with n-hexane. Removal of the bottom bung allowed the columns to slide from the tubes so that samples could be taken. The general plan of sampling collection may be seen in Fig. 2. A diffuse zone of white opaque agar extended from the well into the gel in all directions, the bulk of which was included in sample 2. Sample 1 comprised the clear outer zone from the vicinity of the well. For column A samples 3 to 5 represented consecutive 5 mm thick discs cut from below the well. For column B, sample 3 was cut to remove all the opaque zone before the 5 mm discs of samples 4 to 6 were taken. Similar discs were cut from the bottom of each column as blanks.

All samples were washed, extracted, and analysed polarographically as described in experiment 1.

**Experiment 3**

Three filter papers, each cut to fit within a petri dish, were marked out in sections—centre, inner and outer, corresponding with the agar subdivision used in experiment 1 and shown in figure 1. Having been dampened with water, to ensure they remained flat, each filter paper was placed in a petri dish and a small plastic cup (approximately 6 mm diam) positioned at its centre. This cup contained TmBTO made into a slurry with cellulose powder to avoid spillage. The dishes were fitted with lids and the completed assemblies placed in a dark cupboard for one week at room temperature.

A similar set of three petri dishes containing dampened filter paper were prepared, and discs of TmBTO treated agar (as prepared in experiment 1) placed centrally in each one. These were also covered and then stored in a cupboard for one week.

After storage, all filter papers were cut into the previously marked sections and the individual pieces extracted with 10 ml 0.5 per cent HCl/EtOH. These solutions were submitted to polarographic analysis as described in experiment 1, although the ion exchange step was deemed unnecessary and omitted.
The interaction between tri-n-butylin oxide and agar gel..

Experiment 4

The "clearance-zone test" employed by Henshaw et al. (1978) was used in a modified form so that the wood block and the agar gel were not in direct contact. The petri dish containing the agar gel and fungal implant was inverted and placed in the upturned lid which held the treated wood block. A single petri dish was involved, in which the fungus was challenged with a Scots pine sapwood block treated by vacuum impregnation with a 0.4% w/w TnBTO solution in 60-80 petroleum spirit. The area of inhibition was measured after two weeks incubation at 22°C.

Results and Discussion

The solubility of TnBTO in water has been determined as 100-120 µg/ml⁻¹. This value can be assumed for agar gel, since such a gel is essentially equivalent to water alone in its solvent properties. It follows that TnBTO diffusing into agar gel cannot result in concentrations higher than its solubility value. Any recorded values in excess of this must be the result of other transfer mechanisms.

![Table 1: µg ml⁻¹ TnBTO in agar samples. No washing with n-hexane](image)

The values presented in Table 1 for the analysis of the "centre" portion of the unwashed plates demonstrate that the TnBTO/agar disc deposited a large amount of TnBTO on to this area during one week. The TnBTO/agar disc was essentially a solid suspension of TnBTO droplets in an agar gel, and this was observed to shrink during the week, presumably due either to syneresis or the evaporation of water. Such a process would lead to a proportion of the TnBTO being expressed from the gel and deposited on the surface directly beneath and possibly to the side of the disc. Should some of this TnBTO have subsequently diffused into the plate's gel to any significant degree, it would have done so in all directions from the source of TnBTO. Under these circumstances the "inner" portions should contain TnBTO, but from the analyses recorded they did not (although one sample contained a trace quantity). This indicates, in general, that diffusion does not take place.

The results from experiment 1 (Table 2), in which petri dishes were left for one, two, and four weeks, suggest that the attempt to wash the TnBTO from the surface of the agar gel was not completely successful, for the majority of the "centre" samples contained more TnBTO than would be expected from diffusion alone (i.e. more than 100-120 µg ml⁻¹).

![Table 2: µg ml⁻¹ TnBTO in agar samples. Samples washed with n-hexane before analysis](image)

Four of the nine "inner" samples were found to contain TnBTO, although two returned levels that were lower than the limit of detection recommended for the analytical method used. However, if diffusion was taking place in the system under study, TnBTO should have been detected in all these samples, especially those analysed after four weeks, and it would seem more rational to ascribe the occasional presence of TnBTO to contamination from an unspecified source.

No significant differences are apparent when comparing the no cover/filter paper/cellophane groups. These were primarily intended to reveal what might occur through the transfer of TnBTO by volatilisation and condensation processes. However, from the analytical results obtained after four weeks it would appear that the mass transfer of TnBTO by any means associated with the system under study is highly unlikely (see also the discussion on experiment 3).

The results from the experiment involving agar gel columns are presented in Fig. 2. The first column to be analysed was column A, where the high TnBTO contents of the samples including or closely associated with the white opaque
zone greatly exceeded 100-120 µg ml\(^{-1}\), indicating that the presence of TnBTO must be due to the free, rather than the dissolved, compound. It appeared probable that the white opaque zone was similar in composition to the TnBTO/agar discs and had been formed by the migration of TnBTO into the many tiny fissures in the walls of the well, these fissures constituting the inevitable damage that occurred when the well was cut out of the column. These results were confirmed by the subsequent analysis of column B. The 39 µg ml\(^{-1}\) of TnBTO recorded for the sample less than 5 mm away from the bottom of the white opaque zone in column A may indicate that some diffusion has taken place, although this was not confirmed by the analysis of column B.

Experiment 3 was used to investigate the possibility of TnBTO transfer in the gas phase. Should sufficient TnBTO volatilise from a central source and diffuse outwards through the air space, condensation from the air may supply a measurable quantity of TnBTO to the surrounding surface. Since the experiment was concerned only with surface deposits, filter papers were used instead of agar to allow a shortened analytical procedure to be employed. When the source was TnBTO in a small plastic cup, placed centrally on a filter paper and left in a petri dish for one week, the resultant analyses showed that no TnBTO could be detected in the “centre”, “inner” and “outer” portions of the filter paper. Clearly it appeared that no significant quantity of TnBTO was deposited on to the surrounding surface even though TnBTO vapour was almost certainly present in the air space. The modified repeat of this experiment, using TnBTO/agar discs instead of plastic cups confirmed that no measurable transfer of TnBTO took place via the vapour phase (see table 3). In addition, it can be seen that while TnBTO was expressed on to the filter paper directly beneath the discs, no lateral movement appears to have taken place through the matrix of the filter paper.

Table 3. µg ml\(^{-1}\) TnBTO in filter paper samples. TnBTO/agar discs as TnBTO source

<table>
<thead>
<tr>
<th>Sample</th>
<th>Dish number</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1</td>
</tr>
<tr>
<td>Centre</td>
<td>324</td>
</tr>
<tr>
<td>Inner</td>
<td>0</td>
</tr>
<tr>
<td>Outer</td>
<td>0</td>
</tr>
</tbody>
</table>

Overall, in looking at the analytical results from all three experiments, it may be concluded that diffusion through agar gel and mass transfer via the vapour phase does not occur for TnBTO within a petri dish environment. It could be argued that this view is too simplistic and not entirely substantiated by the evidence presented, since a few samples from the diffusion experiments were found to contain TnBTO. However, the fact that these form a minority of the results obtained from a series of similar experiments prompts the assumption that they were due to artefacts rather than diffusion itself, for if diffusion was possible it would have been inevitable under such favourable conditions. In other words, if diffusion was operating the system under study its identification would have been commonplace.

In experiment 4 the treated wood block produced an area of inhibition of 21 cm\(^2\). For comparison, Henshaw et al. (1978) obtained areas of inhibition in the range 23-32 cm\(^2\) for similar wood blocks using the conventional procedure and an incubation period of three weeks. Although only a single experiment, this result suggests that for TnBTO the area of inhibition could be due solely to the aerial concentration of TnBTO vapour.

The observation that a zone of inhibition is often set up during bioassay exercises (Ingleby 1982) suggests that the fungus is affected by the aerial concentrations of TnBTO vapours rather than the presence of this chemical within or upon the agar substrate. This hypothesis is supported by the result from experiment 4. In bioassays on treated wood samples the aerial concentration of TnBTO will be dependent upon the ability of the TnBTO to evaporate from the wood surface. It has been demonstrated (Orsler & Stone 1982) that only the preservative in the outermost 2 mm of the treated timber is vulnerable to evaporative loss. It follows, therefore, that the zone of inhibition, will be due to the loading in this 2 mm zone only and not to the overall loading within the wood sample. Furthermore, the rate or extent of volatilisation will be dependent on the distribution of TnBTO within this zone. This reinforces the original statement of Scheffer & Lew (1976) that the method should not be used to estimate preservative retention in treated timber.

Conclusions

1. TnBTO does not diffuse through agar gel nor is it deposited on to the agar surface from the vapour phase within a petri dish environment.

2. The zone of inhibition observed in the simple fungal bioassay technique first proposed by Scheffer and Graham (1973) does not necessarily indicate the amount of preservative present in the test sample.

The mycological work in experiment 4 was carried out by the Biodeterioration Section, Princes Risborough Laboratory.

References

ABSTRACTS

The method described for the determination in 1 h of aerobic microorganisms in food in the range between 10^2 and 10^8 bacteria per ml resp. g operates by polarographic measurement of oxygen consumption in a tight cell filled with the liquid product to be evaluated. The correlations between microbial counts and oxygen consumption rates are given for samples of pasteurized milk and semi-liquid egg.

Preservation

See also absts. 101, 105, 110-111, 160


d-Coumaric acid was generally the most effective inhibitor tested causing more than 99.9% inhibition of Escherichia coli at 1000 µg/ml (pH 5, 48 h), Staphylococcus aureus at 500 µg/ml (pH 5, 48 h) and Bacillus cereus at 500 µg/ml (pH 7, 9 h).


The antifungal effects of 16 ground herbs and spices, 4 other plant materials, 3 commercial antifungal agents, tannic acid and 2 experimental mould inhibitors were tested against seven mycotoxin-producing moulds. Clove was the strongest antifungal spice and combinations of different levels of potassium sorbate and cloves showed an enhanced or possible synergistic inhibitory effect on growth of all seven moulds tested, indicating the possibility of using spices and commercial antifungal agents together in small amounts to obtain antimycotic activity.

Bacterial toxins

See also absts. 99, 103, 107


This report details the experimental conditions required to elicit optimal sensitization of guinea pigs to staphylococcal enterotoxin B (SEB). For analysis of SEB in food extracts, the entire assay can be accomplished within 20 min. with a sensitivity of 10-100 pg of SEB/ml of prepared food samples, and the recovery of enterotoxin from spiked food products ranged between 75 and 89% of the amount added.
Fungal toxins (mycotoxins)

See also: abs. 4, 97, 234


A short account of the trichothecene mycotoxins is given, with special reference to their possible use as weapons in war. Emphasis is given to the fungi which produce trichothecenes and their distribution, toxicity for man and animals and the detection and identification of trichothecenes.


An 8-week experiment on the effects of continuous feeding of mycotoxin graded levels to male and female broiler chickens was carried out. Mortality was similar in all groups including the control. The depression of body weight was found to be proportional to the level of ochratoxin A. Residues of ochratoxin A in livers and white and red muscles disappeared completely after 4 days when birds were fed with ochratoxin A-free feed. The accuracy of this method was confirmed using the modified method.


Examination of 23 A. tamiuri isolates, 17 of which were from groundnuts, showed that 22 produced the mycotoxin. These findings are discussed with regard to the safety of commodities contaminated by A. tamiuri.


Four toxigenic A. isolates were grown on grains (wheat, rye), whole wheat bread and malt extract agar. The ability of these moulds to form the toxins tenuazonic acid, alternariol, alternariol monomethyl ether and altenuene was determined by thin-layer chromatography. Except for tenuazonic acid, Alternaria toxins were produced on the substrates tested, the fungi showing individual differences in their toxin forming capacity. Rye and malt extract agar were the least suitable substrates.


Caffeine inhibited the growth of Aspergillus versicolor. Penicillic acid citrinum and P. urticae and decreased sterigmatocystin production by A. versicolor and patulin production by P. urticae. The effect on citrinin production by P. citrinum appeared to be limited to a delay in mycotoxin synthesis. A preliminary examination of P. urticae suggested that caffeine's anti-mycotoxigenic activity does not involve a generalized inhibition of lipid synthesis.


Recovery of ochratoxin A as ochratoxin A-O-methyl, methyl ester from chicken kidney homogenates and human plasma were quantitative following simple extraction and clean-up procedures, reaction with diazomethane and HPLC analysis. The ochratoxin A derivative was detectable at levels as low as 3 ng.


A technique is described for the simultaneous detection of deoxynivalenol, diacetoxyisercpenol, HT-2 toxin, T-2 toxin, fusarone-X and zearalenone in cereal samples. Recovery was 70-80%; relative standard deviation was 16-18%. The sensitivity of the procedure is high enough to detect 100 p.p.b. of mycotoxin in food. Several advantages of this technique. Fusarone-X toxins were detected in 15 of 23 feed samples examined during 1980-81; zearalenone in 9 samples at 0.2-7.5 mg/kg, T-2 toxin in 12 at 0.2-5.8 mg/kg, HT-2 toxin in 3 at 0.2-0.7 mg/kg, diacetoxyisercpenol at 0.5-2.1 mg/kg and deoxynivalenol in 3 at 0.2-1.3 mg/kg.


A technique is described for determination of xanthomene in grains at levels ranging from 150 to 1200 mg/kg. Xanthomene is extracted with chloroform and 0.1M phosphoric acid. An aliquot of the extract is purified by column chromatography using a commercially available silica gel cartridge. Xanthomene is then separated from the remaining interferences by HPLC with a reverse phase C8 column, and subsequently determined by absorbance detection at 405 nm. Levels of xanthomene added to grains and animal feeds at 150-1200 mg/kg averaged 82% with a coefficient of variation of 10.2%.


Using the method of Möller & Joseffson (RMVM 16, 1587) for the determination of patulin (high performance liquid chromatography of ethyl acetate extracts) non-reproducible values for patulin were found. It was observed that patulin, an alkali-sensitive γ-lactone is partially destroyed when the ethyl acetate extracts are washed with Na2CO3 solution. Neutralization of the extract prevents a degradation of the mycotoxin and stabilizes the extract. By using this modified method higher yields of patulin were found.


Differences in the effects of trace elements, amino acids and temperature on aflatoxin biosynthesis and/or growth of A. flavus and A. parasitici, including the influence of a number of factors that promote secondary over primary metabolism and are reviewed. It is suggested that the failure of some investigators to report or recognize the sp. contribute to biological control of aflatoxin contamination in maize.


Taxonomic criteria used to distinguish fungi in the Aspergillus flavus group that are known to produce aflatoxin (i.e., A. flavus, A. parasitici and A. toxicarius) are reviewed. The fungus is reviewed as primary inoculum in regions where aflatoxin contamination of agricultural produce is a recurrent problem. Sclerotium germination is sporogenic with conidia representing the disseminated infective inoculum. Dispersal of inoculum by arthropods or wind may be facilitated by strongly roughened or echinulate conidial walls. It is argued that aflatoxin, kojic acid, cyclopiazonic acid and aflatrem, all of which are toxic
metabolites of A. flavus, represent a fungal investment in chemical defences that reduce predation. Evidence is offered to support a hypothesis that the yellow-green koji moulds A. oryzae and A. sojae are vars. of A. flavus and A. parasiticus. It is also argued that A. effusus; listed elsewhere as a var. of A. oryzae, is a var. of A. flavus.


The literature on the effects of plant metabolites on the A. flavus group is considered and supplemented with data on volatile maize metabolites. Compounds present in developing maize ears tested on cultures of A. flavus included 2-heptanol, 1-hexanol, 1-heptanol, 2-nonenol, cis-3-hexen-1-ol, 3-methylbutan-1-ol, 1-hexen-3-ol, ethanol, 1-nonenol, 2-methylbutan-1-ol, geraniol, 1-propanol, 2-octanol, limonene, 8-ionone, 2-decanol, 2,4-hexadienal, trans-2-hexenal and trans,trans-2,4-decadienal. Several of the alcohols and aldehydes were inhibitory to A. flavus. The compound with the most dramatic effect on A. flavus and A. parasiticus was 8-ionone, which severely restricted growth, sporulation and aflatoxin production. It is suggested that active compounds that occur naturally may be useful in plant breeding or in chemical control of A. flavus in the field or in storage.

ANIMAL FEEDS

See also abst. 194


Consumption of kesari (Lathyris sativus) grains has been banned in India because it causes paralysis of the legs. Samples of undamaged and insect-damaged kesari seeds were examined separately for contaminating fungi and for aflatoxins by methods that are described. The incidence of both fungi (mainly Aspergillus, Penicillium and Fusarium spp.) and aflatoxins was significantly higher in insect-damaged than in undamaged seeds, and that of aflatoxins was above the tolerance level of 20 ppb, in 75% of the samples. The incidence of aflatoxins and severity of the damage was highest in the grains containing maize, wheat or oats in the United States. Journal of Economic Entomology (1983) 76 (4) 717-722 [En, 23 ref., 5 fig.] US Grain Marketing Research Laboratory, ARS, USDA, Manhattan, Kansas 66502, USA.

More than 100 strains of Tribolium castaneum (Hbst.), Rhzopertha dominica (F.), Sitophilus spp., Cryptolestes spp. and Orzyaeophilus spp. were collected from grain bins containing maize, wheat or oats on farms in 14 grain-producing states of the USA. Malathion resistance of the strains was determined by the discriminating dose technique, using impregnated filter papers. Measurable tolerance occurred in 31 of 36 strains of T. castaneum, 11 of 13 strains of R. dominica and 5 of 42 strains of Cryptolestes spp. None of 13 strains of Orzyaeophilus spp. Resistance was widespread and severe only in T. castaneum. When 15 of the T. castaneum strains were tested further, all achieved resistance levels of more than 20-fold after a single selection. Resistance in this species was largely or completely suppressed by triphenyl phosphate in all 13 strains tested.


The technique described incorporates methylene chloride and citric acid sol. extraction, cleanup on a small silica gel column and thin layer chromatography for quantitation. Various animal feeds were spiked with naturally contaminated maize at 4 different levels of aflatoxin B_1 (16-130 μg/kg). Mean recoveries were 85.9-92.8% at levels of 16.5, 32.9, 65.8 and 131.6 μg/kg. The relative standard deviation per assay was 18.6%. This method is more rapid and less involved than most previously published methods for mixed feeds.

GRAIN AND PULSES

See also absts. 7, 9, 47, 170, 173, 176-177, 180, 192

species. About 1 farm in 12 was infested with insects that can cause damage. Insecticide usage is tabulated and compared with a 1977 survey.


A study was conducted on wheat and rice grains treated with pirimiphos methyl for the control of stored-grain insect pests. A wk after spraying the residues in wheat sprayed at the doses of 0.15, 0.30 and 0.45 g ai/m² were below 0.3 p.p.m. and those in rice sprayed at 0.15 g ai/m² were below 1 p.p.m., the tolerance limits prescribed by the WHO/FAO.


Using a previously described technique [RMV 17, 1173, 1175] ochratoxin A, citrinin and zearalenone were observed in 40, 7 and 31%, respectively, of cereal samples examined during 1977-81. Aflatoxin and sterigmatocystin were not found. Citrinin and penicillenic acid were found accompanying ochratoxin A.


Four classes of microbiological quality of cereal grain are proposed, based on frequency of contamination of kernels with penicillia, aspergilli and phycymyctes. Grain lots contaminated with ochratoxin were usually of 11th and 1Vth class quality in a study of wheat and barley samples.


Toxigenic Fusarium spp were isolated from 31% of cereal grain samples. However, zearalenone was present in only 0.3% of 384 cereal samples assayed during 1979-81, at 0.2-1.2 mg/kg. Fusarium culmorum was the dominant toxigenic isolate and yielded up to 700 mg/kg zearalenone.


Histological examination of wheat kernels invaded by Aspergillus and Penicillium spp. proved penetration of fungal mycelium into subaleurone and endosperm cells through aleurone layer cells. Observation of kernels under a stereomicroscope was found to be a sensitive way to detect fungal invasion in cereal kernels.


40% of the main detectable conc. is 140-160 µg/kg when aluminium chloride sol. is used as the spray reagent and 85-110 µg/kg when Fast Violet B salt is used.


Crude metabolites of 21 of 60 fungal cultures isolated from some of the common cereals collected from different parts of India were found to be toxic. Of these toxin-producing fungi, 7% caused hepatic pathology of varying severity in mice. Serum glutamate pyruvate transmission values and blood urea N were found to be high in such experimental animals.

Barley, Oats, Rye
See also abst. 48


In a batch of barley associated with field cases of mycotoxic porcine nephropathy and containing ochratoxin A and citrinin, fungi were isolated. Viomellicin was detected in barley at approx. 1 mg/kg. This is the first report of viomellicin as a natural contaminant of foodstuffs.

Maize
See also abst. 234


The contents of protein, starch, fat, sucrose, glucose and fructose in the kernels of 22 maize genotypes were determined in laboratory studies in the USA and correlated with food utilization parameters and indices determined for developing examples of Sitophilus oryzae (L.) reared on each genotype. The larval period was shorter and pupae were heavier on grain with high glucose contents. Increased levels of protein reduced the amount of food consumed and increased the food utilization efficiency. Larvae reared on pelleted germless kernels required longer to reach pupation and had lower pupal weights and food utilization efficiencies than larvae reared on pelleted whole kernels. However, mortality was similar in both treatments, indicating that nutrients found in the germ were not essential. A homogenous distribution of nutrients in the food reduced larval development time and increased food utilisation efficiency.

36 GOMEZ, L. A.; RODRIGUEZ, J. G.; PONFLEIT, C. G.; BLAKE, D. F. Relationship between some characteristics of the corn kernel pericarp and resistance to the rice weevil (Coleoptera: Curculionidae). Journal of Economic Entomology (1983) 76 (4) 797-800 [En, 9 ref.] Kentucky State University, Frankfort, KY 40601, USA.

Two resistant, 2 intermediate and 2 susceptible maize genotypes were used in studies in the USA to examine relationships between physical characteristics of the pericarp and resistance to Sitophilus oryzae (L.). A significantly higher number of eggs were found in kernels without pericarp than in undamaged kernels. No correlation was found between the number of eggs laid and thickness of the pericarp measured in the kernel tip. A paraffin coat applied to the kernel tip, the zone most preferred for oviposition, apparently did not greatly influence the number or location of eggs laid. Pericarp topography did not seem to be associated with resistance. It was thought that the oviposition behaviour of the females in the treatments used in these experiments might be explained by the presence of an oviposition stimulant in the kernels.

37 McGAUGHKEY, W. H. Compatibility of Bacillus thuringiensis and captan when used in a mixture for treating seed corn for moth control. Journal of Economic Entomology (1983) 76 (4) 897-903 [En, 10 ref.] Commodity Marketing Research Laboratory, ARS, USDA, Manhattan, Kansas 66502, USA.

Laboratory studies were carried out in the USA to determine whether mixing the fungicide captan with Bacillus thuringiensis subsp. kurstaki (Dipel) in aqueous suspension would affect the subsequent pathogenicity of the bacterium on maize seed to Plodia interpunctella (Hb.) and Ephestia...
caustella (Wilk.). When caplan at 58.6 mg a.i./ml was mixed with a spore preparation of the bacterium in aqueous suspensions, such treatment prevented spore germination but did not kill the spores. Upon dilution with water, spores that had been incubated with caplan for 4 h germinated and produced colonies on agar plates. Caplan had no effect on the pathogenicity of the bacterium to E. caustella and only a slight negative effect on that to P. interpunctella. Caplan alone was slightly toxic to both insects but did not increase the pathogenicity of B. thuringiensis in mixtures.

38 IVERIARO, M. F. Toxicity of neem seed, Azadirachta indica A. Juss., to Sitophilus oryzae (L.) in stored maize. Protection Ecology (1983) 5 (4) 353-357 [En, 7 ref.]
Department of Agricultural Extension, Ifand University, Nigeria.

In tests in Nigeria, maize grains mixed with dry ground seeds of neem (Azadirachta indica) at 0.5, 1 and 2.5 g/20 g maize were protected for 6 months in storage from damage by Sitophilus oryzae (L.). At the highest rate, neem seed prevented oviposition, at the lowest rate it markedly reduced oviposition, and at all the rates tested it completely halted post-embryonic development. Mortality among the parent weevils reached 100% within 5 days. Adult weevils placed on maize that had been treated 4 months previously with 2.5 g neem/20 g maize failed to oviposit, and 76% of them died within 10 days; when adults were placed on maize treated 4 months previously with 1 g neem seed/20 g maize, progeny emergence was delayed 4 months and very few were produced. Neem seed had no adverse effects on the viability of the maize grains and no fungus or shrinkage was observed.


Hand-harvested and hand-shelled kernels of maize inbreds and hybrids commonly grown in this area were evaluated in storage for their reactions to invasion by fungi and stored at 25°C. In all tests, fungal infection and visible mould, number of fungal propagules and ergosterol were generally high. Because the propionates and some acetates have been found effective in stored maize. Over 30 pesticides have been tested for their ability to inhibit aflatoxin production and growth of A. flavus, but only 5 have been applied to maize during the growing season 1979. The most effective pesticides to reduce aflatoxin can be reduced or prevented. The insecticide Pyrethrum [tetrachlopyrifos] when sprayed 3/4 week reduced, but did not eliminate, aflatoxin B1 accumulation in preharvest maize. Application of the neonicides Bux carbaryl, carbarlyl and Dyfonate [fonofos] reduced aflatoxin B1 levels in naturally contaminated preharvest maize kernels from 28.9 p.p.b. to 4, 1 and 2 p.p.b., respectively. The feasibility of using pesticides to suppress aflatoxin production is discussed.

42 DRAUGHON, F. A. Control or suppression of aflatoxin production with pesticides. In Aflatoxin and Aspergillus flavus in corn [edited by Diener, U.L.; Asquith, R.L.; Dickens, J.W.]. Auburn, USA; Alabama Agricultural Experiment Station (1983) 81-86 [En, 1 fig., 7 tab.]
Dep. Food Technol. Sc., Tennessee Univ. Knoxville, Tnn. 37901, USA.

Numerous antimicrobials have been studied as a means of controlling growth of Aspergillus flavus and aflatoxin production in stored maize. A number of chemicals, including pesticides, inhibit aflatoxin production in vitro, but only the propions and some acetates have been found effective in stored maize. Over 30 pesticides have been tested for their ability to inhibit aflatoxin production and growth of A. flavus, but only 5 have been applied to maize during the growing season 1979. The most effective pesticides to reduce aflatoxin can be reduced or prevented. The insecticide Pyrethrum [tetrachochlorpyrifos] when sprayed 3/4 week reduced, but did not eliminate, aflatoxin B1 accumulation in preharvest maize. Application of the neonicides Bux carbaryl, carbarlyl and Dyfonate [fonofos] reduced aflatoxin B1 levels in naturally contaminated preharvest maize kernels from 28.9 p.p.b. to 4, 1 and 2 p.p.b., respectively. The feasibility of using pesticides to suppress aflatoxin production is discussed.


From a review of past and recent literature, it is suggested that the most practical method for salvaging aflatoxin-contaminated maize is by ammoniation. A procedure is described whereby introduction of ammonia reduces aflatoxin in maize from more than 1000 p.p.b. to less than 10 p.p.b. Although toxicological studies have not yet been completed, this procedure for FDI of aflatoxin levels in maize, although toxicological studies have not yet been completed. This procedure for FDI of aflatoxin levels in maize, although toxicological studies have not yet been completed. This procedure for FDI of aflatoxin levels in maize, although toxicological studies have not yet been completed.

44 HASSAN, M. N.; SELIM, S. A. Some toxigenic fungi associated with stored corn in Egypt. Journal of the Egyptian Veterinary Medical Association (1982) 42 (4) 3-12 [En, 13 ref.]

In ten samples of corn harvested in different parts of Egypt, toxicity was determined by percentage of Pseudomonas cerealetta, pathogenicity of the bacterium to E. caustella and E. stellata, pathogenicity of the bacterium to E. caustella and E. stellata, pathogenicity of the bacterium to E. caustella and E. stellata, pathogenicity of the bacterium to E. caustella and E. stellata.

Rice
See also abats. 169, 235


A laboratory study was carried out in the USA to determine some of the characteristics of rice grains that may affect their relative resistance to attack by Sitotroga cerealella (Ol.) and Rhyzopertha dominica (F.). Imperfect hulls favoured infestation by S. cerealella, but planting date


During 1977 and 1980, losses to individuals, firms and public expenditures due to aflatoxin contamination of maize in southeastern USA amounted to approx. $200 million and $235 million, respectively. Losses at all levels in the production, marketing and utilization process are discussed and it is suggested that if recurrent aflatoxin contamination in maize cannot be eliminated or detoxified, farmers will change to other crops with less risk. The impact of these possible changes on both producers and consumers is considered.

47 DRAUGHON, F. A. Control or suppression of aflatoxin production with pesticides. In Aflatoxin and Aspergillus flavus in corn [edited by Diener, U.L.; Asquith, R.L.; Dickens, J.W.]. Auburn, USA; Alabama Agricultural Experiment Station (1983) 81-86 [En, 1 fig., 7 tab.]
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Numerous antimicrobials have been studied as a means of controlling growth of Aspergillus flavus and aflatoxin production in stored maize. A number of chemicals, including pesticides, inhibit aflatoxin production in vitro, but only the propions and some acetates have been found effective in stored maize. Over 30 pesticides have been tested for their ability to inhibit aflatoxin production and growth of A. flavus, but only 5 have been applied to maize during the growing season 1979. The most effective pesticides to reduce aflatoxin can be reduced or prevented. The insecticide Pyrethrum [tetrachochlorpyrifos] when sprayed 3/4 week reduced, but did not eliminate, aflatoxin B1 accumulation in preharvest maize. Application of the neonicides Bux carbaryl, carbarlyl and Dyfonate [fonofos] reduced aflatoxin B1 levels in naturally contaminated preharvest maize kernels from 28.9 p.p.b. to 4, 1 and 2 p.p.b., respectively. The feasibility of using pesticides to suppress aflatoxin production is discussed.
did not. Wild and domesticated strains of the moth varied with regard to fecundity, but the relative resistance of varieties to 
infestation varied. This diversity in the central vascular bundle in the 
abscission scar affected the ability of the moth to penetrate grains with intact hulls. S. cerealella larvae penetrated through the vascular bundle of the susceptible variety 
Vista but not through that of the resistant CI 12273.

46 SINGARAVAVEL, K.; RAI, S. A. Changes in parboiled 
rice caused by improper drying and microbial infection. 
1 tab.] Paddy Processing Res. Cent., Tamil Nadu Agric. 
Univ., Tiruvurur, India.

When parboiled rice was not dried for 7 days due to humid weather moulds increased to 2.45 X 10^6/g from 6 X 10^6/g and bacteria increased to 75.9 X 10^9/g from 5.1 X 10^9/g observed initially. Both milled rice and head 
meal yielded decreased and the resultant bran contained less oil. 
Breakdown changes were induced which increased the levels of 
sugars, amino acids and polyphenols in grains. This might 
cause kernel discoloration in the associated heat development 
during infection.

47 TANAKA, T.; OKAZAKI, N.; KITANI, M. [Comparison of 
growth and enzyme production between A. oryzae and 
Rhizopus sp. Growth of mould on uncooked grain] Hakko-kogaku 
Brewing, Tokyo, Japan. From Report of the Research 
Institute of Brewing No. 155, 100.

48 TANAKA, T.; OKAZAKI, N. [Growth of mould on 
uncooked grain.] Hakko-kogaku (1982) 60, 11-17 [Ja, en, 6 
From Report of the Research Institute of Brewing No. 135, 93.

Aspergillus and Rhizopus grow well on non-steamed grains (rice, 
barley and wheat bran). The specific growth rate of A. oryzae on non-steamed rice grain was the same as 
on uncooked rice grain, while that of Rhizopus was decreased remarkably by steaming the rice grain. The decrease was 
assessed to the heat denaturation of protein in rice grain.

The specific growth rate of A. oryzae decreased when the wetness 
content of non-steamed rice grain was less than 0.45 (g-H_2O/g-initial dry matter), while the enzyme 
production (a-amylase, glucoamylase, acid protease and acid 
carboxypeptidase) was best at a water content of 0.4.

Wheat, Buckwheat

See also abs. 48, 178-179

49 SINHA, R. N. Effects of stored-product beetle 
infestation on fat acidity, seed germination, and microflora of 
Station, Winnipeg, Manitoba, R3T 2M9, Canada.

The keeping quality of wheat grain, with or without 
dockage, infested with Oryzaephilus surinamensis (L) and 
Cryptolestes ferrugineus (Steph.) at 30°C and 70% RH was 
determined over a 20-week period in studies in Canada. The 
quality assessment criteria used were the changes in fat 
acidity values, seed germination, germ and endosperm 
damage, and fungal and bacterial infection. Heavy 
infestation (650-800 adults per 130 g) by both insects in 
Wheat with the dockage treatment resulted in significantly 
higher levels of infection by Penicillium and bacteria, which 
led to greater loss in seed germination than in uninfested 
Wheat with dockage. Fat acidity values in the Wheat 
with dockage treatment increased significantly only with 
infestations of O. surinamensis, which produced significantly 
more adults and larvae than C. ferrugineus. Infestation by 
insects resulted in a reduced level of seed infection by fungi of 
the Aspergillus flavus group. Damage to seed germ by 
C. ferrugineus exceeded damage by O. surinamensis; both 
species damaged the germ more than the endosperm.

50 LOSCHIAVO, S. R. Distribution of the rusty grain 
beetle (Coleoptera: Curculidae) in columns of wheat stored 
dry or with localized moisture content. Journal of Economic 
Entomology (1983) 76 (4) 881-884 [En, 17 ref., 3 fig.] 
Research Station, Agriculture Canada, Winnipeg, Manitoba, 
R3T 2M9, Canada.

Adults of Cryptolestes ferrugineus (Steph.) were found 
in laboratory studies in Canada to move downwards after 
being placed at the top of a column of uncooked wheat 
with 13 or 13.4% moisture content. During the 
first 3 days, most beetles were near the top of the column, 
but, after 3 days, most were found near the bottom. The 
number near the bottom increased with time. Struggle 
distribution in the column at the bottom was stabilised by the 3rd to 5th day. Conversely, from 80 to 92% of the beetles 
aggregated at the top or middle zones of the column when 
Wheat with 16-17% moisture content was placed only in these zones. Beetle distribution in the moist zones became 
stable within 3 days.

51 KEEVER, D. W. Distribution patterns of lesser grain 
borer (Coleoptera: Bostriochidae) in towers of wheat, and 
effects of the presence of the granary weevil (Coleoptera: 
492-495 [En, 8 ref., 1 fig.] Stored-Product Insects 
Research and Development Laboratory, ARS, USDA, 
Savannah, Georgia 31403, USA.

In studies in the USA, adults of Rhysopertha dominica (F.) and Sitophilus granarius (L.) were introduced 
to the tops of 6 towers (1.1-2.6 m high) filled with wheat 
grain samples taken 2, 3, and 4 months after harvest. For 8 weeks, when the R strain had produced 5 times as 
many larvae as the S strain, the R strain had produced 5 times as 
many larvae as the S strain. Damage to seed germ by 
S. granarius apparently affected the distribution patterns of adults of the R strain, but observations differences in the distribution patterns of adults and 
immature examples of R. dominica.

52 BARKER, P. S. Comparison of two pelletized 
formulations of aluminum phosphide for the control of adults 
and eggs of the rusty grain beetle (Coleoptera: Curculidae). 
Journal of Economic Entomology (1983) 76 (3) 599-600 
[En, 13 ref., 1 fig.] Research Station, Agriculture Canada, 
Winnipeg, Manitoba, R3T 2M9, Canada.

Laboratory studies were carried out in Canada to determine the 
effectiveness of German and Brazilian formulations of 
alfonuminium phosphide (which release phosphine gas) for 
the control of adults and eggs of Cryptolestes ferrugineus (Steph.) in wheat grain in 326-kg drums. Gas concentrations 
were monitored. The results showed that the Brazilian 
formulation evolved phosphine slightly more rapidly than the 
German formulation but that the two formulations were 
equally effective.

53 WHITE, N. D. G. Effect of continuous exposure of 
malathion-resistant red flour beetles (Coleoptera: 
Tenebrionidae) to malathion-treated wheat or wheat exposed to 
treated surfaces. Journal of Economic Entomology (1983) 76 
657-661 [En, 20 ref.] Research Station, Agriculture Canada, 
Winnipeg, Manitoba, R3T 2M9, Canada.

Adults of susceptible (S) and malathion-resistant (R) 
strains of Tribolium castaneum (Hbst.) were exposed 
continually in laboratory studies in Canada to wheat grain 
treated with malathion dust or liquid at 8 p.m. or to grain 
3 cm deep above plywood panels treated with 0.05 mg/cm^2 
malathion. Controls consisted of S and R strains of insects 
exposed to untreated grain. After 1, 2, 4, and 8 weeks 
storage at 21°C, the results for each treatment were 
analyzed for comparison of insect numbers, seed germination, moisture 
content, seed damage and seed-borne microflora. Virtually all 
(> 98%) adults in the untreated controls survived for 
8 weeks, when the R strain had produced 5 times as 
amany larvae as the S strain. Few susceptible insects in the 
panel-grain treatment in each period were exposed to the 
control-grain treatments survived. Insects of the R strain in both the pane 
dust treatments had about 30% mortality by the end of 
the 1st week; this remained constant for 8 weeks. Mortality 
adults of R-strain adults in the liquid treatment was 48, 84 and 
90% after 1, 2 and 4 weeks, respectively; few larvae were 
observed. There was negligible seed damage in all treatments 
by 8 weeks of storage; moisture content in all the treatments 
remained between 16 and 17% and was similar throughout 
the study. Seed germination declined by about 10% in the 
malathion-liquid treatments. The fungal group of Aspergillus flavus was up to 9 times as 
great on malathion-treated seed as on untreated seed 
probably because the presence of living insects inhibited 
fungal growth. It was thought that malathion applied to 
gean as a liquid drip during grain movement could offer
54 MIAU, L. S.; MULLA, M. S. Effects of insect growth regulators on the germination of stored wheat. *Protection Ecology* (1983) 5 (4) 369-373 [En, 14 ref.] Department of Entomology, California University, Riverside, CA 92521, USA.

The effects of the insect growth regulators triflumuron (BAY SIR-8514), diflubenzuron and methoprene on the germination of stored wheat were studied at various intervals after the grain had been treated with aqueous solutions at rates to give 1, 5 or 10 p.p.m. Grain viability was significantly reduced by all the treatments when it was assessed 1 week and 1 month after treatment. Losses in germination were significantly higher than the allowable limit of 7%. Evaluations of organic solvents for their impact on grain viability showed that 70% ethanol was the most active compound inhibiting germination, followed by acetone (analytical grade), benzene, acetic acid (solvent grade) and hexane.


Of 25 Fusarium isolates from moulded wheat, 3 F. graminearum [Gibberella zeae] isolated produced vomitoxin (deoxynivalenol) and zearealenone; the respective amounts of these mycotoxins were 70, 160, 600 and 400 and 250 mg/kg for deoxynivalenol and 600, 400 and 250 mg/kg for zearealenone. A technique for estimation of deoxynivalenol in cereals involving high pressure liquid chromatography is described. The detection limit was 20 µg/kg, compared with a detection limit of 100 µg/kg for deoxynivalenol by thin layer chromatography.


The movement of fumigants was studied by gas chromatography on columns of wheat coupled directly to a detector. The fumigants were methyl bromide, monochloro dibromomethane, carbon tetrachloride, chloropicrin and ethylene dibromide. Their chromatographic behaviour was the same with carbon dioxide as carrier gas as with air or nitrogen. Improved distribution of fumigants added with carbon dioxide in silos is attributed to other factors and not to changes in the dynamics of adsorption of fumigant to wheat.


Sterilized wheat grains were inoculated with Aspergillus and Penicillium and treated with Bavistin (2-methoxy-carbamoyl)benzimidazole) [carbendazim] and Bavistin + TMTD (tetramethyl thiuram disulphide) [thiram]. The mixture of fungicides gave 100% protection for one year while carbendazim alone protected the grain fully for up to 30 days.

Pulses

See also abstr. 168


Applications of 5 chemically differing oils (groundnut oil, coconut oil, mustard oil, a mineral oil and a polyethylene glycol) at dosages of 5.0 ml of oil/kg of seed in laboratory studies in the USA effectively protected stored cowpeas (*Vigna unguiculata*) from attack by Callosobruchus maculatus (F.). Oils caused high mortality of eggs and larvae on the surface but had no effect on individuals that successfully entered the seed. A lack of oil-specific activity indicated that the protective property was physical rather than chemical. After 30 days of storage, treated seeds were more deterrent to ovipositing females than were newly treated seeds, but the stored seeds were less toxic to eggs and larvae. It was thought, therefore, that oil application could be useful for only a limited period of time.


The powdered kernel of neem seeds (*Azadirachta indica*) was added to stored seeds of cowpea (*Vigna unguiculata*) in the laboratory in Nigeria which were then kept in open glass jars at ambient temperatures for 8 months. Treatment at 0.5 parts per 100 parts of seed was effective in controlling *Callosobruchus maculatus* (F.) for up to 4 months, but thereafter considerable damage occurred. Treatment at 1.0 and 2.0 parts per 100 parts of seed was effective for 8 months; the number of hulled seeds per 500 grains averaged 32 and 18, respectively, as compared with 445 for no treatment.


All of 22 cowpea lines were susceptible to inoculation with *A. parasiticus* (NRRL 3145) and subsequent aflatoxin production. The amounts of aflatoxin produced ranged between 466.6 µg/kg-1,806 µg/kg for aflatoxin B (B, + B,) and 20.8-827 µg/kg of seed for aflatoxin G (G, + G). It is suggested that there is partial resistance to aflatoxin production in some cowpea lines and that further selection and breeding could provide cowpea cultivars resistant to *A. parasiticus* infection and aflatoxin production.


When greengram (*Vigna radiata* (Linn.) Wilczek) and chickpea (*Cicer arietinum* Linn.) were converted into dal (split, without seedcoat), or flour, cooked whole or as dal or made into fried pancakes, there was a great reduction in the residues of phosphate. Fumigant residues had some effect on sensory characteristics of some cooked products.

**FLOUR AND BAKERY PRODUCTS**

See also abstr. 9

Flour

See also abstr. 179

Bakery products


Investigations were carried out on naturally mouldy bread causally associated with a mycotoxicosis characterized by lathyral gastroenteritis in poultry, rabbits, and dogs. A sample of mouldy bread was found contaminated by 80 mg of ochratoxin A and 9.6 mg of ochratoxin B per kg of dry bread and colonized by *Aspergillus ochraceus* Ochratoxins were determined by high-performance liquid chromatography. Their identification was performed by spectroanalytical data as well as by derivative preparation. Analysis of the same sample for aflatoxins, patulin, penicillic acid, and citrinin gave negative results. This is the first instance of the natural occurrence of ochratoxin B in bread; moreover, for the first time ochratoxin B was found in feedstuffs in Italy. The mycotoxicological risks associated with the indiscriminate use of waste urban foods as feedstuffs are discussed.
SUGAR AND CONFECTIONERY PRODUCTS

Sugar

The role of Leuconostoc, Lactobacillus and Bacillus spp, in sugar losses, particularly where the conventional milling train is replaced by or supplemented with a diffuser, in which cane juice is lached from the cane by warm water, is discussed. Using an ion chromatograph and microscopic techniques it was shown that sucrose losses in a diffuser in a Qd., Australia, mill were due to fermentation by Lactobacillus sp. The problem was solved quickly by installing an extra heater at an appropriate position in the juice stream.

OILSEEDS, NUTS, VEGETABLE OILS

Oilseeds

Aflatoxins were not produced by Aspergillus flavus SRRC-1000 on unautoclaved soybean meal. Addition of zinc (as ZnSO4) to autoclaved meal inhibited aflatoxin production and supplementation with sodium phytate relieved this inhibition. Addition of sodium phytate alone promoted production. When cottonseed meal was treated to release native phytate into the meal from phytate-sequestering globoids, aflatoxin production increased. However, the largest production on cottonseed meal occurred upon dialysis of the meal without releasing phytate, implying removal of a small mol. wt. inhibitor.


Groundnut meals containing an av. of 250 µg/kg aflatoxin B1 were processed in a twin-screw extruder in the presence of 0-2.5% ammonium hydroxide. The aflatoxin B1 content determined after extrusion was reduced by 23-66% in the absence, and by 40-87% in the presence of ammonium hydroxide. When non-extruded or extruded groundnut meals were incubated with proteolytic enzymes at acid and/or alkaline pH, and at 37 or 50°C, the aflatoxin B1 content determined by extraction from the dried enzyme-treated meals was found to be lower than that of the initial meals. The authors suggest that extrusion could either cause destruction of aflatoxin molecules or their binding to groundnut meal constituents.


The toxic and mutagenic effects of γ-irradiated groundnut meal contaminated with aflatoxin B1 were studied in Salmonella typhimurium str. TM 677, using forward mutation to 8-azaguanine resistance. After treatment with 5-10 Mrad γ-radiation, the contaminated groundnut meal lost its toxic and mutagenic properties. Irradiation at 0.1-1 Mrad removed 75-100% of the toxicity but not mutagenicity.

Vegetable oils

See also abst. 171

COFFEE, COCOA AND TEA

Cocoa

Fungi of the A. flavus group were detected in seeds from 4 of 5 areas, but none of the 104 isolates tested produced aflatoxins.

TOBACCO

See also abst. 166

SPICES


Seeds of two spices Coriandrum sativum and Foeniculum vulgare were dressed separately with essential oil of Cedrus deodara as well as with five synthetic fungicides. Treated seeds were stored in polythene bags for 12 months. Mycological analysis showed that the oil was more effective than the synthetic fungicides.


The technique described consists of extraction of the paprika sample, then chemical purification is followed by a capillary gas-chromatographic analysis, after recovery and determination of the scatter of results. Zearalenone content was determined in paprika samples having higher than av. mould counts. No zearalenone was found in samples examined in 1980-81.

BEVERAGES

See also abst. 154


Aflatoxins were detected in none of the 86 beer samples and 88 samples of raw materials and by-products of brewing examined.

FRUIT AND VEGETABLES

See also abst. 14

71 SPIFTLER, G. H.; COUEY, H. M. Methyl bromide fumigation treatments of fruits infected by the Mediterranean fruit fly (Diptera: Tephritidae). Journal of Economic Entomology (1983) 76 (3) 547-550 [En, 9 ref., 1 fig.] Stored Products Insect Research Laboratory, ARS, USDA, Fresno, California 93727, USA.

Laboratory tests were carried out in California to determine the survival of Ceratitis capitata (Wied.) in cherries, nectarines, pawpaws, peaches, pears and plums that had received various treatments with the fumigant methyl bromide without a subsequent cold treatment. The treatments were 48 g/m3 for 2 h, 48 g/m3 for 3 h and 32 g/m3 for 4 h; all fumigations were made at 21°C and normal atmospheric pressure. Fumigation chambers were aerated for 2 h immediately after the treatments. Probit 9 security (P less
than 0.05 for 99.997% mortality) was given by all 3 treatments.

A reverse phase HPLC method for the determination of alternaric acid and its metabolites was described. The method was applied to 50 commercial products and about 20 mouldy fruit samples analysed by this technique, 2 mouldy apple samples contained 160 p.p.b. alternariol and 250 p.p.b. alternariol monomethyl ether, respectively.

Pome fruit

A. alternata stem decay occurs in fruit treated with benzimidazole postharvest drench for control of Penicillium expansum and subsequently stored for prolonged periods. The fungus favours the incidence of A. alternata, a slower growing and less competitive fungus than P. expansum at the osmotic potential (~22 bars) and temp. (c. 0°C) conditions of postharvest storage. Benzimidazoles, prochloraz and triadimefon in combinations with benomyl effectively controlled both diseases on fruit treated and kept for 6-7 months in cold storage. Best results were obtained with [000 µg/ml prochloraz + 500 µg/ml benomyl]. Reduction of linear growth of A. alternata on PDA amended with prochloraz was not a useful method of determining the most effective conc. for disease control; 1 µg/ml inhibited mycelial growth but 1000 µg/ml was required for control.


The fungus, which was isolated from dump-tank water at a large apple storage and packing facility and which is not usually associated with apple diseases, caused a brown, firm rot in wound- inoculated apples stored for 1 wk at 20°C.

Tropical and Subtropical fruit
See also abs.ts. 167, 192

The effect on Ephestia cautella (Wlk.) of feeding exclusively on irradiated dried dates was investigated in Iraq, in order to determine whether irradiation was a practical means of controlling the pest. The criteria used were the average numbers of larvae and pupae produced from fertilised eggs 30 days after incubation; percentage of adult survival; sex ratio; average number of spermatothoraces per surviving female; infestation mating frequency; average number of eggs per female; average hatch rate; and mating frequency, fecundity and fertility of the offspring of insects that had fed on irradiated dates. According to these criteria, irradiation of dates at 50 or 100 krad had no significant adverse effect either on the generation feeding on them or on their progeny.


Imazalil was applied in water or water-based resin sol. wax, using a non-recovery spray application to oranges revolving on horsehair brushes saturated with the treatment sol. Applications in water resulted in higher residues than comparable cones. Applied in water wax and residues from water treatments were also enhanced by increased time on the brushes. Residues were not enhanced by degreening fruit with ethylene before fungicide application. Injured rind contained higher residues of imazalil than uninjured tissue and residues on fruit washed after treatment were only slightly reduced. Higher cones of imazalil were required in water than in water applications for control of infection of posttreatment injuries and sporulation of P. digitatum.


In vitro dosage responses for 3 isolates of P. digitatum and guazatine indicated an approx. 10-fold shift in tolerance when compared with wild-type strs. BD values for resistant strs. were approx. 0.5 µg/ml compared to 0.05 µg/ml for wild-type strains. The resistant isolates on selective media containing carbenzadine showed that they were also resistant to benzimidazole fungicides. In vivo tests on oranges inoculated with strs. previously characterized in vitro confirmed resistance to guazatine and benomyl. A combined treatment of these fungicides at 400 µg/ml and 500 µg/ml respectively, which normally gives protection against decay, failed to provide adequate mould control. Growth and pathogenicity of the strs. resistant in these tests were indistinguishable from those of wild-type strs.

78 WILLS, R. B. H.; MULHOLLAND, E. E.; BROWN, B. L. Notes on two new cultures of guava fruit for measuring decay Tropical Agriculture (Trinidad) (1983) 60 (3) 175-178 [En, 10 ref., 4 tab.] Sch. Food Technol., Univ. New South Wales, Kensington, NS, 2033, Australia.

Guava fruit (cv. 1050 and 'GA 11-56') stored at 20°C had a storage life of approximately one week. Cool storage in the temperature range 0-10°C extended the post harvest life for approximately two weeks. The main changes which limited the storage life were the development of rot and degradation of flesh tissue. Storage at 0°C considerably reduced rotting but there was pulp injury at this temperature. The optimum storage temperature is 5°C.


Alternaria alternata, the causal organism of black spot disease in mango, Mangifera indica, penetrates the fruit through lenticels. After infection in the orchard, the hyphae remain latent until postharvest ripening of the fruit, then develop intercellularly. Assessment of the latent infections during postharvest ripening and storage on mango, and the effect of this latent infection on the infected surface of fruit set until harvest. Protectant fungicide sprays begun after fruit set decreased the latent infection surface from fruit set until harvest. Protactant fungicide sprays began after fruit set decreased the latent infected surface and resulted in a significant reduction in the incidence of black spot disease during storage. A significant correlation coefficient was found in latent infected surface of mature mango fruits by Alternaria in the field and the incidence of black spot disease in storage.


Total soluble ascorbic acid, acidity, total phenols, sucrose, glucose and fructose, and several amino acids decreased considerably in infected fruit in storage, while ascorbic acid increased. Alanine was not detected in healthy fruit but appeared in traces in infected fruit.


Isolates of Colletotrichum gloeosporioides [Clonemera cinnaga], Dipodia mali, theobromae and Phomopsis citri [Diasporie citri] showed resistance to benomyl. Cross-resistance of the three fungi was shown between benomyl, thidiazoxide and thiophane-methyl fungicides but not to imazalil and CGA-64251 in vitro tests. CGA-64251 was the most effective fungicide tested for control of postharvest decay of Tommy Atkins and Keitt mangos.
82 SLABAUGH, W. R.; GROVE, M. D. Postharvest diseases of bananas and their control. *Plant Disease* (1982) 66 (8) 746-748. Ref., 10 ref., 7 fig. [Univ. Arkansas, Monticello, Ark., USA.] Crown rot is the most serious postharvest problem in commercial bananas. Other postharvest diseases are described and the fungi implicated are listed. Control measures are discussed.


Guava pulp was stored in 5 kg white PVC containers with 500, 750 and 1000 p.p.m. SO2 at room temperature. Fungi detected included *Aspergillus niger*, *Alternaria sp.*, *Canadila sp.*, *Helminthosporium sp.* and *Saccharomyces sp.* For shorter periods of storage (up to 60 days), 500 p.p.m. SO2 is sufficient to check the deterioration. For longer periods of storage, 1000 p.p.m. SO2 is required.


Cultural extracts of the fungus were injected intra-abdominally into white mice at 0.5, 0.25 and 0.1 ml. At medium high, such extracts caused death from the 3rd day, while recovery was possible with the min. dose. On feeding mice with grains contaminated with a month-old culture of the fungus grown on wheat grains, death occurred on the 3rd-7th day with highly toxic and on the 7th-10th day with toxic stress. With weakly toxic stress, there was loss of appetite, co-ordination and weight and tremor.

Melon, Pumpkin, etc.


Postharvest treatment of mature ripening cantaloupe (*Cucumis melo var. reticulatus*) with a dip containing both benomyl and guazatine controlled the disease complex responsible for market wastage in Australia. Naturally inoculated melons treated with 250 mg/l of benomyl and 200 mg/l of guazatine were still marketable after holding at 25°C for 1 wk after harvest.

Dried fruit


Four experiments in Victoria, Australia, with malathion and chlorpyrifos on mature, harvested and harvested sultana grapes provided information on the efficiency of application and the rate of loss of the insecticides through the phase of preharvest (when the fruits are attacked by species of *Drosophila* and *Carphophilus*) to processing (when they are attacked by *Ephestia figulella* Cost.) *Carphophilus* (syn. *Drosophila interpunctella* (Hb.). Unharvested bunches retained 3.5-8.1% of the active ingredient, whereas harvested bunches laid out to dry retained 23%. Insecticide decay was greatest immediately after application, probably due to volatilisation of unbound chemical, but the rate of loss was accelerated by the use of the alkaline drying oils which stimulated hydrolytic degradation. Drying oils are conventionally applied to hasten drying. The residue on fruit at the end of drying was generally higher than that on the fresh fruit because of the concentrating effect of dehydration. Processing and storage did not alter the residues, probably because the chemical migrated into the fruit and became bound to inert compounds.

Potato


A rapid HPLC (high-performance liquid chromatography) method for determining teucumene dissolved on individual potato tubers was developed to compare the distribution of the chemical achieved by different methods of application. Disease and weight loss in store were too small to show effects of differing distribution but possible advantages of methods of achieving more uniform distribution are discussed.

Green and salad vegetables


There was a gradual decrease to a total loss of acetic acid at 25°C in fruits infected by *Fusarium oxysporum* and *F. equiseti* with increase in incubation period. In infected fruits stored at 30°C for 8 days, a total loss of acid was also recorded. There were fluctuations in the losses of reducing sugars and pectin. Guay, stored for 8 days, at different temp, while a gradual decrease in the amount of sugars was recorded in infected fruits at 25°C during storage for 14 days.

Legumes

See also abst. 231


Of 26 seed samples of urad (*Vigna mungo*) and mung (*V. radiata*) tested, 8 and 11 respectively contained aflatoxin B1 or B2 and B1 and aflatoxins G1 and G2 were not detected.

FISH AND SEAFOOD

91 LANNINGLOUWE, M.; FINNE, G.; HANNA, M. O.; NICKELSON, R. II; VANDERZANT, C. Microbiological and chemical changes during storage of swordfish (*Xiphias gladius*) steaks in retail packages containing CO2-enriched atmospheres. *Journal of Food Protection* (1982) 45 (13) 1197-1203 [En, 32 ref., 6 fig., 3 tab.] Texas Agric. Exp. Sta., Texas A & M Univ., College Station, Texas, USA.

Retail packaging of swordfish steaks in modified atmospheres containing CO2 was shown to be effective in extending the shelf-life. This extension was the result of bacterial inhibition and subsequent reduction in bacterial metabolites. The degree of inhibition of the bacteria was proportional to the CO2 concentration and was higher in atmospheres of CO2:N2 as compared to atmospheres of CO2:O2.


Fish was processed, packaged under carbon dioxide and refrigerated. Stability of the fish under this modified atmosphere preservation (MAP) system was compared to that of fish stored conventionally. Use of the MAP system resulted in a 45 to 55% increase in stability.

Initial total counts were in the order of 10^6/g rising to 10^7/g after 7 weeks storage. Initially the incidence of yeasts was low (10^3/g) but after 9 weeks of storage these were no longer detected. Homofermentative Lactobacillus spp (g) increased during the keeping period reaching 84% of the total flora after 12 weeks storage. No correlation was observed between sensory scores and total microbial counts. Total plate counts appear to have a minor significance in quality assessment of vacuum packed smoked herring fillets.


The effects of different packing regimes on the spoilage characteristics of chill-stored sausages (Pecten alba) were investigated. Sausages were packed aerobically in vacuum packs and in vacuum packs with an inoculum of lactobacilli and the microbial flora examined after several days storage at 4°C. Vibrio spp. dominated the initial flora (approx. 10^5 cfu/g) and were still present in the vacuum-packed sausages after 13 days' storage (approx. 10^7 cfu/g) and the aerobic pack microbial counts were higher (approx. 10^5 cfu/g, 95% of which were Aleromona spp. With the exception of lactobacilli and spore formers in the vacuum pack, other organisms were not suppressed and spoilage proceeded at a rate similar to that in the aerobic vacuum packs as judged by microbiological and raw odour assessment.

MEAT


Sausages, with NaNO_2 added at 156 or 300 p.p.m., were inoculated with A. parasiticus and incubated at 2, 26 and 37°C (± 1°C). Sabouraud’s glucose agar with 50, 156, 200 or 500 p.p.m. NaNO_2 was inoculated with A. parasiticus and incubated at approx. 24°C. More fungal growth was observed with high amounts of added NaNO_2 after 4 days of storage, but before that time greatest fungal growth occurred with low amounts of nitrite. Aflatoxin production increased as nitrite levels decreased in sausage. The max. aflatoxin level reached was 0.8 p.p.m. at 37°C and 1.7 p.p.m. at 26°C.


Fungi were found in 28 of 32 samples of raw material for cheeste (salami type sausages), Cladosporium and Penicillium spp. predominating. Fusarium sp. was found in 2 samples and Aspergillus candidus and A. flavus in 1. Of 40 samples of chorizo 11 were sterile, 23 had less than 100 colonies and only 3 more than 100. C. sp. occurred in 17 (48%), while Penicillium, Fusarium and Mucor spp. occurred in isolated samples. In 1 sample there were 24,000 colonies/g of Wallenda icothopha. Of 13 samples of raw material for sausages 4 contained C. 3 P. and 1 each A. flavus and A. candidus. All 28 samples of Frankfurt sausage were negative. Aflatoxins B_1, B_2, G_1, G_2 and zearalenone were not found in any sample of any material.


Aspergillus flavus NRRL-6549 and NRRL-6550 were tested for their ability to produce aflatoxins, but only NRRL-6549 produced detectable amounts of B_1, summer sausages prepared and inoculated with NRRL-6549 were smoked and held at 10 or 30°C for 3 or 6 wk at low or high humidities. Mold growth occurred on both inoculated and uninoculated sausages, the amount increasing with time, temperature and humidity. Smoking delayed but did not prevent mould growth. At 3 wk, aflatoxins were detected only in unsmoked inoculated sausages held at 30°C and high relative humidity. After 6 wk, B_1 was found only in unsmoked inoculated sausages held at 10°C and high humidity, but at 30°C was present at 2.6-6.6 µg/kg in all inoculated samples at both low and high humidities.


Antioxidants, esters of p-hydroxybenzoic acid and gallic acid, and related phenolic compounds were evaluated for their activity against growth and toxin production of C. botulinum types A and B in comminuted pork. 8-Hydroxyquinoline at a concentration of 200 p.p.m. or in combination with sodium nitrite (40 p.p.m.) inhibited the growth and toxin production of C. botulinum on comminuted pork for 60 days at 27°C. 8-Hydroxyquinoline was more active in inhibition of growth and toxin production of C. botulinum in comminuted pork than in unsmoked and inoculated sausages held at 10°C and high humidity, but at 30°C was present at 2.6-6.6 µg/kg in all inoculated samples at both low and high humidities.


Irradiation with 0.5 Mrad prevented spoilage of uninoculated bacon by virtue of reducing the aerobic plate count to less than 1/g; irradiation with 1 Mrad sterilized bacon. Bacon inoculated with C. botulinum spores (2/g) swelled and became toxic; development of swelling and toxicity was delayed by incorporation of NaNO_2 (40 µg/g) or by irradiation with 0.5 Mrad. Irradiation with 1.5 Mrad prevented swelling and toxicity of bacon inoculated with 2 spores/g.


The numbers of psychrotrophic bacteria in prerigor ground pork were slightly greater than in postrigor ground pork throughout an 11-day storage period at 2°C. Freezing both types of samples and subsequent thawing before storage at 2°C reduced the initial psychrotrophic counts but the freezing effects did not persist beyond 8 days of storage. Differences were not sufficiently great to limit the use of prerigor grinding of pork.


Compared with meat systems containing nitrite or nitrite plus supplemental iron compounds, CO was not antibotulinul. NO treated meats did swell slower, but nitrite was also found in these systems. Addition of ferric chloride or p-hydroxyquinoline decreased the amount of nitrite, but samples containing nitrite plus EDTA or denatured nitrosylated myoglobin swelled slower.

Lactobacillus spp. comprised at least 44% of the total in both atmospheres. 


At 0.125%, all mono- and dimethyl and ethyl esters of fumaric acid tested were equal or superior to 120 p.p.m. nitrite in inhibiting toxin formation in cans incubated at 30°C for 8 weeks.


At 25°C, growth of E. coli and S. typhimurium on beef was influenced by type of tissue, pH, gaseous atmosphere and physiological state of inoculating cells. Results suggest that during cooling of hot-boned meat growth of E. coli and Salmonella is more likely on fatty tissue or muscle of high pH than on lean tissues of low pH.

105 KEMP, J. D.; LANGLOIS, B. E.; FOX, J. D. Effect of potassium sorbate and vacuum packing on the quality and microflora of dry-cured intact hams. Counts within all vacuum shelf life parameters when stored in the latent temperature controls than in either nonvacuum or vacuum packaged products. More low oxygen-barrier film.


After 3 months, and 0.91% at 20°C.


The limiting water activity (a,) for the growth of S. aureus sealed in cans at an oxygen conc. of 5.5% was 0.87 at 37°C and 0.91% at 20°C. Enterotoxin A production was detected for population levels more than 10^6 CFU/g bacon and were 100 ng/g bacon at 37°C and 16 ng/g bacon at 20°C.


Swedish meat balls with sauce were to establish shelf life parameters when stored in the latent temperature zone (-2°C to 4°C). Both coliforms, yeast and mold count showed no increase in count during the storage period of 1 month. Total plate count showed an increase from 10^6 to 10^7 (20°C) and 10^6 to 10^7 (30°C). There was no significant difference in taste panel scores for acceptability comparing freshly prepared samples with those stored 1 or 2 weeks.


The value of microbial indicators for assessing the microbiological safety, sanitary quality and rate of spoilage of meat and poultry products, is discussed. The article concentrates on the use of microbial indicators for these products as they leave commercial manufacturing plants and as they move through domestic or international commerce.


Residual nitrite was lost from chub-packed luncheon meat during storage through both chemical breakdown and microbial consumption. The relative importance of these mechanisms in this pasteurized product was determined by the speed of development of the spoilage microflora, which is influenced by storage conditions. Nitrite did not inhibit the aerobic growth of either Bacillus or Streptococcus species associated with spoilage but did inhibit the anaerobic growth of B. spp. This bacteriostatic effect of residual nitrite in anaerobic conditions will decrease during storage as nitrite level falls and oxygen penetrates the chub pack. Nitrite-mediated bacteriostasis does not obviate the need for refrigerated storage but does afford a real, if ephemeral, safeguard against spoilage occurring during short periods of temperature abuse.


A group to 550 ppm. (550 µg/ml) of nisin in combination with 60 p.p.m. (60 µg/ml) of nitrite failed to prevent overgrowth of C. botulinum spores in pork slurry adjusted to pH 5.8. Reducing the pH enhanced nisin activity. Proteolytic and nonproteolytic type B spores were equally resistant to nisin.


A group of 8 heterofermentative L. sp. Isolated from raw vacuum-packaged, as well as SO2-treated, minced beef, in the course of shelf-life studies on this product. The incorporation of these brand in a new sp., L. divergens, is suggested.


Lactic acid conc. correlated with oleogranule spoilage of refrigerated coarsely ground beef stored in casings with low oxygen permeability. Lactic acid increased with time in all samples, as did the bacterial counts and % of Gram-positive organisms in the total microflora, the latter representing an increase in the lactic acid-producing bacteria. With samples evaluated by a sensory panel, lactic acid and levels were found to correlate inversely with odor acceptability.

POULTRY AND EGGS

See also arts. 7, 109


In 1979 a survey of selected chicken eviscerating plants was conducted to determine the levels of coliforms, Escherichia coli and Salmonella spp. on eviscerated chickens under current manufacturing practices. A comparison was made of the data from this survey and one conducted in 1979 with general bacterial criteria in 1979 of chicken carcasses and chiller water were lower than in 1969.

115 TERENCE, M. W.; STOLOFF, L.; YOUNG, K.; WYATT, R. D.; MILLER, B. L. Aflatoxicol and aflatoxins B,

Hens were fed an aflatoxin B₁-contaminated feed (8 μg/g) for 7 days. Aflatoxol, aflatoxin B₁ or both were found in eggs and tissues (kidneys, liver, muscle, blood and ova). Aflatoxin B₁ (0.04-0.1 ng/g) was found only in the kidneys. In eggs, the levels of aflatoxol and aflatoxin B₁ (0.02-0.2 ng/g) increased steadily for 4 or 5 days, then decreased after B₁ withdrawal. At 7 days after withdrawal, only trace amounts of aflatoxol (0.01 ng/g) remained in eggs. All tissues, except blood, from hens sacrificed immediately after aflatoxin withdrawal contained aflatoxol (0.04-0.4 ng/g) or aflatoxicol and B₁ (0.04-0.8 ng/g). The aflatoxicol (0.03-0.11 ng/g) was the only aflatoxin detected in milk; aflatoxin B₁ was the only aflatoxin in blood. Seven days after aflatoxin withdrawal, B₁ (0.08 ng/g) was found in 1 of 9 livers and aflatoxicol (0.01-0.04 ng/g) in 8 of 9 muscles analyzed.


Three methods for C. jejuni detection on freshly processed poultry were evaluated. All three sampling techniques were essentially equivalent for fresh carcasses but when samples were stored frozen for 7 to 10 days to simulate transport conditions from sampling locations to laboratory, the incidence of detection was significantly reduced. Use of cryoprotective agents was an effective method to preserve samples during frozen storage.


Some screening methods were applied at factory level. Depending on the topological circumstances all tested methods seemed to be applicable and would also be useful in other food processing establishments.

**DAIRY PRODUCTS**


Aflatoxin M₁ was stable to freezing in both ice-cream and orange sherbet made with naturally contaminated milk. The aflatoxin M₁ content of ice-cream and sherbet remained stable throughout 8 months of frozen storage. There was an apparent increase of aflatoxin M₁ after sherbet mix was prepared from milk.


Streptococci were found to grow better in raw milk with redox potential similar to that inside the udder than staphylococci. In these redox conditions in active milk containing about 10⁶ colony forming units/ml, both streptococci and staphylococci survive but only some strains grow. In aerobic conditions active milk inhibited the growth of streptococci more than that of staphylococci.


The thin layer chromatographic technique described enables detection of aflatoxin M₁ at 0.04 μg/g in milk and at 0.5 μg/kg in powdered milk. Using the technique, of 142 raw milk samples and 70 powdered milk samples examined only 2 were found to contain aflatoxin M₁. All these samples were powdered milk samples that were detected.


When artificially and naturally contaminated milk was heated at 64 and 86°C, the aflatoxin M₁ content was unaffected even after 2 h of treatment. Aflatoxin M₁ was also stable in naturally contaminated milk heated at 100°C for 2 h. Overnight refrigerated storage of heated milk had no effect on stability or recovery of aflatoxin M₁. Aflatoxin M₁ in Melville's buffer at pH 2, 4, and 6.6 was stable for 4 days after heating. The pH of the buffer was raised from 100°C to 10°C for 2 h. Overnight refrigerated storage of heated milk had no effect on stability or recovery of aflatoxin M₁.


Campylobacter jejuni was isolated from raw milk by a method that can routinely detect <1 organism/ml. This procedure was used in a survey of 195 separate farms and showed a 1.5% incidence of C. jejuni in milk from bulk tanks.

**BUTTER**


The number of contaminating bacteria in salted butter was reduced during storage irrespective of storage temp. (-12, 2, 8 and 12°C) and pH in butter serum. In unsalted butter the reduction rate decreased with increasing temp. In unsalted sweet cream butter the number of coliform bacteria increased with increasing storage temp. above 0°C. The number of coliform bacteria was reduced during storage regardless of storage temp. and type of butter. Lower storage temp. and salt had a profound inhibitory effect on yeast.

**Cheese**


Cheeses were prepared from milk naturally contaminated with aflatoxin M₁ and then were stored at 4°C and at -23 ± 6°C for 2 months. There was a 2.84-fold increase of aflatoxin M₁ in curd of queso blanco cheese over the amount present in milk from which the cheese was made. The aflatoxin M₁ content of the cheese varied during refrigerated and frozen storage, but aflatoxin M₁ was present near initial levels at the end of storage. Bakers' cheese was prepared with and without added rennet. More aflatoxin M₁ was found in cheese and whey than in milk from which cheese was made. Aflatoxin M₁ tended to be concentrated in curd. Cheeses made without rennet had greater enrichment (4.24-fold increase over that found in milk) than those made with rennet (2.97-fold increase over that found in milk).

**ANIMAL PROTEIN**


The transformation of hairs of the muskrat *Ondatra* bissethi (Humm.) is described and illustrated from laboratory studies in France. Larvae fed on hairs from black rabbits grew faster than those fed on hairs from white...
rabbits, although both diets had the same energy content. Relative humidity was found to be important in the utilisation and rearing of different species; a dry environment was best for the digestion and development of *T. biselliella* on muskrat hairs, while a damp one was more suitable for *Tinea pellionella* (L.). The digestibility coefficient in tineids was found to be 32–78% and the assimilation coefficient 51–83%; these high values resembled those in carnivorous rather than phytophagous insects.

**PLANT FIBRES**


The biodegradation of wood tannin was tested with 6 yeast strains isolated from decaying bark samples. The degradation of the condensed tannin was assigned in flasks and a 2 1 jar fermenter by analysis of the residual fermentation broth using TLC analysis. *C. tropicalis* (Y65) mostly degraded the leucoanthocyanin groups with liberation of catechin. These results show for the first time the capacity and a 2 l Jar fermenter by analysis of the residual environment was best for the digestion and development of those in carnivorous rather than phytophagous insects.

127 Tygai, B. K.; Pandey, P. C.; Rehill, P. S.; Sen-Sarma, P. K. *Termites-fungi interactions* III. Laboratory testing of *Diplodia pinea* attacked woodchips to *Microcerotermes bessonii* Snyder (Isoperma: Termitidae). *Annals of Entomology* (1983) 1 (1) 43-44 [En, 2 ref., 1 pl.] Directorate of Biological Research, Forest Research Institute, Dehra Dun, Uttar Pradesh 249006, India

In laboratory studies in India on interactions between the termite *Microcerotermes bessonii* Snyder and the stain fungus *Diplodia pinea* in blocks of wood and malt-agar, the fungus acted as an attractant for workers, but only when it was alive.


Pencil streak, affecting *E. papuana* with pale-coloured wood, is a reaction between the living tree and wound induced chemical compounds. Pencil streak is produced by ambrosia beetles. The nature, development and significance of the condition, the relationship of decay to pencil streak in living trees and the nature of stains in eucalypt logs are discussed. Some other work on the stains and decays of *E. papuana* and *G. mollis* is reported. Culture media and tests for fungi are listed and cultural characteristics of logs basidiomycetes isolated from *E. papuana* are tabulated. The effects of this disease on timber strength are discussed.

**TANCHER**

**PLANT FIBRES**


A study was made of the behaviour of 65 mould cultures, isolated from particle boards, towards 8 different resin adhesives: (a) phenol resorcinol formaldehyde; (b) urea melamine formaldehyde; (c) UF; (d) isocyanurate; and (e) PF resins. The cultures were generally, strongly inhibited by (a) and 2 of the PF resins. Resin (b) did not inhibit the fungi and it could not be utilized by them. Only a few of the fungi were capable of utilizing (c) and (d), but about one third of the fungi were able to utilize the other two PF resins as a carbon source.


Decay of samples of pine sapwood by (a) *Phellinus igniarius*, (b) *Coniophora puteana*, or (c) *Gloeophyllum sepiarium* was monitored. After 16 wk, the following losses were observed: timber attacked (a) 20%, (b) 7%, and (c) 2%; ether-extractables (a) 86%, (b) 72%, (c) 72%; cellulose (a) 15%, (b) 63%, (c) 40%; lignin (a) 20, (b) 4, (c) 12; percent (%) (a) 21, (b) 68, (c) 35. Degree of polymerization of cellulose was reduced from initial values of 700 to 2200 by (a) after 4 wk, and to 700 by (b) after 4 wk. Chemical changes were also recorded in pine stumpwood solid timber or chips stored (without visible fungal attack) for 2 yr. Less alteration in composition was observed in chips stored in the open than in solid wood or chips stored under cover.


The conc. of viable spores determined with an Andersen-sampler varied between 1 and 10 000 (10 m–3) during storage and the total spore conc., measured with a Casella-sampler varied between 1000 and 1 000 000 (103 m–3) depending on the time of storage and the quality of chips. The total spore conc. of viable spores consisted mainly of mesophilic and thermotolerant genera, but also great numbers of thermophilic spores of fungi and actinomycetes were observed occasionally. When handling the chips the most common organisms encountered were *Stereomycos spp.*, *Thermotactinomycoses vulgaris*, *Humicola sp.*, *Aspergillus fumigatus*, *Penicillium* and *Trichoderma spp.* and *A. niger*. Precautions to be taken when handling fuel chips to keep exposure to mould dust to a minimum are discussed.


In connection with using wood chips for fuel in heating buildings, a number of people in Sweden were taken ill with a respiratory allergy similar to farmer's lung. It is suggested that the disease is caused by airborne fungal particles (spores and hyphae) which are inhaled when working with infected wood chips. The occurrence of the disease in the halls and kitchens was studied in 64 buildings heated by chips. In the chip storage rooms of 10 of the 64 buildings examined, more than 500 fungal colonies were recorded before disturbing the chips. After disturbance the number of buildings with more than 500 colonies increased to 28. In the halls of three of the buildings and in the kitchens of two, more than 500 fungal colonies were recorded.


The effect of cold air drying on fungus growth and matter loss during the storage of chipped log residuals was studied in 10 small buildings and 6 large halls of three of the buildings and in the kitchens of two buildings, Sweden.

The average dry matter loss was lowest in the silos which were stored for 8½ months. The average dry matter loss was highest in the silos which were stored for 15 months. The dry matter loss in the silos which were stored for 8½ months was reduced with reduced drying time. The total fungal particle size was reduced by 1.3 X 109 kg TS (dry matter) at the start of the study. During the storage period the total fungal particle size was reduced by 1.5 X 109 kg TS (dry matter). The storage time reduction of 6 times was registered for the total visible fungal spores which grew in the material which dried the shortest time. The greatest increase, 870 times, was noted in the undried material.


The change in moisture content, dry matter loss, ash content, heat value and total fungal particles in chipped logging residues, during one year’s storage was studied. It was shown that the pile’s average moisture content increases with increasing storage time. Matter losses are entirely dependent on the material’s moisture content at the time the pile is laid. The total number of viable fungal particles increased during the first two storage months on an average for the section by 1000 times. After 4 months the increase was 8000 times. The last 4 months the total of the viable fungal particles decreased somewhat.

Insect attack
See also abst. 155

136 Don-Pedro, K. N. Natural resistance of some Nigerian timber species to Amitermes evanescer Silvestri (Isoptera). Recherches en Zoologie Afrique (1983) 97 (3) 647-652 [En, 5 ref.] Department of Biological Sciences, Lagos University, Nigeria.

The natural resistance of the timber of 4 species of trees in Nigeria to attack by Amitermes evanescer Silvestri was tested in the laboratory under choice and forced-feeding conditions. Under both conditions, Nesogordonia papaverifera was the least resistant to termite attack, followed by Azellia sp. teak (Tectonia grandis) and Gossweilerodendron balsamiferum in that order. The rate of survival of the termites over a period of 8 weeks under choice feeding conditions using a sawdust matrix was uniform, and significantly higher than for forced feeding.

137 Miller, L. R.; Paton, R. Cryptomeria in mangroves in the Northern Territory (Isoptera: Kalotermitidae). Journal of the Australian Entomological Society (1983) 22 (3) 189-190 [En, 8 ref., 1 fig.] Division of Forest Research, CSIRO, Private Bag 44, Winnellie, Northern Territory 0789, Australia.

Neotermitinae insularis (Wlk.), Cryptomeria domestica (Hav.) and C. secundus (Hill) were found attacking mangroves in the Darwin region of the Northern Territory of Australia. C. domestica has been found only in mangroves in the Northern Territory, but in northern Queensland it can be a destructive pest of timber. It is suggested that there may be 2 strains of this termite species in Australia, a possibly native one in the mangroves near Darwin and an introduced one infesting buildings in northern Queensland.


Cricophalus [Arhopalus] syriacus, Rhagium inquisitor und Ergates faber were studied.

Marine borer attack
See also abst. 161


Examples of the species Lyrodus sp. (probably L. takanothomisii Rhoch) were found in sunken driftwood collected at low tide from the head of Ladysmith Harbour, British Columbia. It is suggested that this wood-boring mollusc could have been introduced from Japan more than 20 years earlier with infested wooden boxes used for packing immature oysters for culture in Canada.

Preservation
See also absts. 192, 232


One to eight beech or pine sapwood samples, treated with graded concentrations of Cu-Cr or coal tar creosote, were incubated in one test vessel. Toxic limit varied with number of samples tested; the greatest wt. loss was observed with one sample per vessel. Wt. loss decreased with increasing number of samples, even when some of the samples in the vessel were untreated.


The analysis by GC of PCP in various formulations of preservative (incorporating fuel oil as solvent) can be more accurately measured by prior separation of PCP from the solvent, suppression of the phenol function of the PCP and/or the use of appropriate stationary phases to separate the different preservatives in a mixture.


Tests were made in southern Minnesota on several kinds of millwork joints of Douglas fir heartwood and southern pine sapwood treated with PCP in oil or as a grease-like matrix, and then exposed out of doors for 18 to 20 years. All treatments proved highly effective in protecting Douglas fir joints from decay. For the more susceptible pine joints, PCP floated from a brush into the joint surfaces prior to assembly proved as effective as a water-repellent formulation that had been tested previously on the same site. Certain PCP solutions and grease formulations also proved to be effective when applied after assembly.


An analytical method is described in which the Cu, Cr and As ions present in a CCA mixture are successively observed with one sample per vessel. Wt. loss decreased with increasing number of samples, even when some of the samples in the vessel were untreated.


Nine air dried sapwood blocks prepared from red pine (Pinus resinosa), ponderosa pine, southern yellow pine (mainly P. palustris) and hem-fir (a commercial mixture of western hemlock and amabilis fir) were pressure treated with
PLASTIC POLYMERS


The use of isothiazolone chemicals as preservatives for the bulk storage of latices is described.

PHARMACEUTICALS AND COSMETICS


The majority of dermatological preparations are prepared under aseptic conditions and packed into metal tubes, for many reasons the most suitable form of packing. Serious danger arises from packing in jars which predominates in the cosmetics field but is also still preferred in clinics and hospital dispensaries. Control of the raw materials is a prerequisite for ensuring that no specific pathogens and other so-called risk germs, usually gram-negative bacteria, are present. Under these circumstances and if the products are packed into tubes or aerosol cans the use of preservatives can often be dispensed with. Only a small number of preservatives are available for these products. Even products containing preservatives are not 100% guaranteed against microbial contamination.


Chemical-physical properties and some microbiological and toxicological aspects of this antimicrobial agent for use in non-ensulted cosmetics are described.

METALS

See also abst. 156

STONE AND CONCRETE

See also abst. 155

CONSTRUCTIONS

See also abst. 129

All 9 mineral-based insulating materials tested were colonized by wood-destroying basidiomycetes, introduced from a malt agar medium, and in most cases the fungi penetrated them. Only the UF foams were markedly affected (by shrinkage) by the presence of the fungi.

Packaging


The presence of microorganisms from the tree to the cork stopper is confirmed. These microorganisms are capable of degrading certain cork constituents with the formation of volatile metabolites that are able to contaminate wine on contact with the cork. While this is not the only mechanism, it is certainly one cause of "gouts de bouchon" or corky flavour of wines.

Instruments and Equipment

See also abst. 159

Structures and Vehicles


Malathion and pirimiphos-methyl were applied to plywood at 0.125-500 g/m² in the laboratory in Canada to determine their effectiveness in controlling adults of Prostephanus truncatus (Horn) in empty storage structures for grain. The plywood was stored at 21°C and was bioassayed over a period of 16 weeks. Concrete blocks were sub-divided into 2 parts and one section was covered with a thin layer of maize dust. Both dust-free and dust-covered concrete were treated with malathion or pirimiphos-methyl at 0.5-2.0 g/m². The concrete was stored at 21°C and bioassayed with P. truncatus over a period of 8 weeks. The residual toxicity of both insecticides was greater on dust-covered than on dust-free concrete. Malathion was less effective than pirimiphos-methyl, especially on concrete.

Transport systems

See also absts. 183-186


Corrosion failure was associated with constant bacterial load and sulphide in the crude oil and produced water. The bacterial load included a variety of anaerobic and aerobic/facultative bacteria which acted in concert to produce sulphide, giving rise to a "cascade of sulphide generation". A total of 256 isolates from the crude oil were tested for ability to reduce oxidized sulphur compounds to sulphide. Five groups of bacteria existed in the crude oil system, based on their ability to reduce sulphur compounds with different oxidation states. A synergistic relationship exists whereby intermediate products of reductive activities of each group form the substrate for subsequent action by other groups until sulphide is produced.

FOULING

Aquatic surface fouling - marine and freshwater


Trichlorfum chloride was microencapsulated and added to a Vinyl Resin base paint as a dry powder, in an attempt to produce a product that would extend the underwater antifouling performance of organometallic biocides in marine coatings. The formulation was shown to be effective in submerged panel tests, although the mechanism for sustained release is unclear.


Ten fouling bacterial isolates were characterised. Employing poly styrene dishes, a novel microfouling assay was developed, based on the extraction and fluorometric determination of DNA. The assay was rapid, enabled the detection of as little as 0.15 µg of DNA/dish (approx. 5 000 cells/mm²), and showed good agreement with a direct count assay.


The growth of microbial populations during in situ leaching is believed to be one of the causes of flow path plugging in the ore body, which results in decreased uranium production. Leach sol, and solid samples from well casings and submersible pumps were collected from an in situ mining operation experiencing plugging problems. Bacillus sp., Micrococcus sp., pseudomonads and xanthomonaids were isolated from these samples at 10⁶ CFU/ml. A mixed culture of these organisms was inoculated into a uranium core specimen in the laboratory to assess the role of microbes in the plugging problem. A one-third decrease in permeability was effected in 16 days. Hydrogen peroxide (0.2 g/l) killed the microorganisms in the core and alleviated the plugging problem.

BIODETERIORATION ORGANISMS

See also absts. 108-109

Bacteria

See also absts. 46, 63, 91-94, 98-104, 106-107, 110-114, 117, 119, 122-123, 151, 156, 159, 207, 223, 227

Actinomycetes

See also abst. 154

Fungi

See also absts. 1, 4, 18, 21, 46-48, 53, 57, 68, 79, 81-83, 86, 89, 96, 126-127, 133-135, 153-154, 207


Fungal isolates from various foodstuffs were investigated for their sensitivity to heat and subsequent tolerance to sorbic acid (SA) and butylated hydroxyanisole (BHA). Wide variations in sensitivity were noted. M. oryzae was most sensitive to BHA while Trichoderma sp. was most tolerant to SA. Cladosporium, Trichoderma and Fusarium sp. were increasingly sensitive to heat. Heat injury also brought about increased sensitivity to the preservatives.

Invertebrates

See also abst. 139

161 Sathakumar, L. N. A new record of marine wood-borer Lyrodus massula (Lamy) (Mollusca: Teredinidae) from Indian waters. Journal of the Timber Development

The wood-boring mollusc, L. mass (Lamy) has been recorded for the first time from Indian waters, based on two specimens collected from Pilibana near Panaji (Goa). Synonyms, diagnostic characters and distribution of this species are also included.

Insects

See also abs, 18, 23, 26-27, 35-38, 45, 49-54, 58-59, 71, 75, 87, 125, 127, 136-138, 155, 190-191, 231


In laboratory studies in the USA, untreated females of the stored-product pest Tyrophagus putrecessiae (Schr.) were crossed with both irradiated and untreated males, and the effects of the order of pairings on fecundity, egg viability, female productivity and progeny sex ratio were determined. Untreated females that were crossed with males exposed to γ-radiation at doses of 60, 80 or 100 krad laid only a few eggs during the first insemination and none producing eggs thereafter. Untreated females that were paired with irradiated males for 5 days and then with untreated males were as fecund as control females that paired only with untreated males. The eggs laid by such treated females were viable in the opposite pairing combination, females laid more eggs during the 1st 10 days after treatment than in the 2nd 10-day period. These eggs were apparently fertilized by the untreated males, since their percentage viability was very high. The results indicate that the sterile male release method is not feasible for the control of the mite.

163 BEEMAN, R. W. Inheritance and linkage of malathion resistance in the red flour beetle. Journal of Economic Entomology (1983) 76 (4) 737-740 [En, 26 ref., 1 fig.] US Grain Marketing Research Laboratory, ARS, USDA, Manhattan, KS 66502, USA.

A strain of Tribolium castaneum (Hbst.) from Georgia, USA, was found in laboratory studies to have 73-fold resistance to malathion when compared with a susceptible laboratory strain and was highly cross-resistant to phenthoate (53-fold) but not to structurally dissimilar hydrocarbons or pyrethroids. Malathion resistance (Rm) was inherited as a simple autosomal gene with a linkage map units from group VI, 24.6±1.0 map units from Microphthalmic.


In laboratory studies in the USA, last-instar larvae of Amyelois transstella (Wlk.), an important pest of stored almonds, were exposed to 13 levels of low-oxygen (<1%) atmospheres, containing various combinations of oxygen and carbon-dioxide concentrations, at 27°C and 60% RH. LT50s and 95% confidence intervals were determined. The oxygen concentration was more important in reducing the kill than the carbon-dioxide concentration. The economic implications of the data on an insect control programme are summarised.

165 WHITE, N. D. G.; NOWICKI, T. W.; WATTERS, F. L. Comparison of fenitrothion and malathion for treatment of plywood and galvanized steel surfaces for control of the red flour beetle (Coleoptera: Tenebrionidae) and the rusty grain beetle (Coleoptera: Tenebrionidae). Journal of Economic Entomology (1983) 76 (4) 856-863 [En, 16 ref., 4 fig.] Research Station, Agriculture Canada, Winnipeg, Manitoba, R3T 2M9, Canada.

In laboratory studies in Canada, for plywood and galvanized steel panels were treated with sprays of fenitrothion or malathion at rates of 0.25 or 0.50 g a.i./m². The panels were stored at 25°C, and bioassay tests were carried out after 1, 2, 4, 8, 16 and 35 weeks with adults of Tribolium castaneum (Hbst.) and C. ferrugineus (Steph.), 2 of the commonest pests in Canadian granaries, using exposure periods of 1, 3 or 6 h at 30°C and 75% RH. Insect mortality observed 3 days after exposure to insecticide was considerably higher than was knockdown immediately at the end of the exposure periods. Overall, fenitrothion was significantly more toxic than fenitrothion at 0.5 g a.i./m². The type of surface did not affect the relative toxicity of the chemicals except that, with malathion at 0.50 g a.i./m², C. ferrugineus mortality was significantly higher on steel than on plywood. The uptake by both insects of fenitrothion adjacent to steel surfaces was more rapid and resulted in higher residue levels than those in seed adjacent to wood surfaces. Fenitrothion levels in reased were consistently higher than malathion levels. It was concluded that the malathion would be more toxic than fenitrothion for the treatment of empty granaries at similar rates of application under the conditions studied.

166 CHILDS, D. P.; OVERBY, J. E. Mortality of the cigarette beetle in high-carbon dioxide atmospheres. Journal of Economic Entomology (1983) 76 (3) 544-546 [En, 8 ref.] Tobacco Storage Insect Research Laboratory, ARS, USDA, Richmond, Virginia 23240, USA.

A strain of Lasioderma serricorne (F.) reared on stored tobacco was found in laboratory studies in the USA to tolerate carbon dioxide better than a strain reared on wheat flour. Overall, an atmosphere of 65% CO₂ was more toxic to eggs, larvae, pupae and adults than was 35 or 92% carbon dioxide. The pupa was the stage most tolerant of carbon dioxide (some surviving exposure for 7 days), followed by the larva, adult and egg, in that order. A few eggs were killed after 15 days, and mortality was 99.9% with 3 or more days of exposure.


The possibility of using heat to control the fig moth Ephesia cautella (Wlk.) in stored dates, as an alternative to chemical insecticides, was investigated in the laboratory in Iraq. Eggs, larvae of the 1st and 4th instars, pupae and adults were exposed to 45, 50, 55 and 60°C (at 20 or 70% RH) for as long as it took to obtain 100% mortality. The results indicated appreciable differences between the stages in susceptibility to temperature but not to humidity. The time required to obtain complete kill of the stages mentioned at 60°C and 35% RH was 6 days. For eggs, larvae of the 1st and 4th instars, 0.5 h for pupae and 0.41 h for adults, and the corresponding times for exposure to 45°C and 20% RH were 15, 18, 10 and 12 h, respectively.

168 HUSSEIN, M. H. Alorsid SR 10 as a seed protectant against the cowpea seed beetle Callousobruchus maculatus (Fab.). International Pest Control (1983) 25 (5) 140-141, 158 [En, 26 ref.] Plant Protection Department, College of Agriculture, Assuit University, Assuit, Egypt.

In laboratory tests in Egypt, seeds of broad bean (Picea faba) were dipped in aqueous solutions of methoprene (Alorsid) and exposed to ovipositing females of Callousobruchus maculatus (Fab.) (0.7, 1.4 and 2.1 mg/ml). The solutions containing more than 100 p.p.m. methoprene reduced adult emergence; the percentage reductions, as compared with no treatment, afforded by concentrations containing 800 or (in brackets) 1600 p.p.m. methoprene were 95.78 (97.27), 95.46 (97.16), 53.61 (70.89) and 24.22 (27.24) for exposure 0, 7, 14 and 21 days after treatment, respectively.


In studies in China on the protection of stored rice, it was found that when root-bark powders of Celastrus angulatus and Tripterygium wilfordii were mixed with the rice at 0.5%, or oil of neem (Azadirachta indica) was mixed in at 5 ml/kg, the population of Sitophilus oryzae (L.) and S. zeamais Motsch. was inhibited by 90%. The root-bark powder of C. angulatus had no contact
or fumigant action but inhibited the development of the eggs and young larvae. Volatile essences from *Litslea cubeba*, *Curcurbita pepo*, *Cuminum cyminum* and orange were evaluated with 0.5 ml/petri dish killed adult weevils in 3 days and completely suppressed population build-up.


Permethrin, deltamethrin and fenvalerate were applied in the laboratory in Victoria at 0.01-0.5 g/m² to blocks of concrete, galvanised iron and wood, which are commonly used in the construction of silos and other facilities for stored grain. Their effectiveness was compared with that of fenitrothion at 1.0 g/m², the currently recommended treatment. The treated surfaces were bioassayed with adults of organophosphate-resistant strains of *Rhizophagus dominica* (Fr.), *Sitophilus oryzae* (L.) and *Tribolium castaneum* (Hbst.) up to 32 weeks after treatment. Chemical assays of deposits were carried out after storage for 1 and 32 weeks. Deltamethrin at 0.05 g/m² was the most effective.

171 NARA, J. M.; BURKHOLDER, W. E. Influence of the molting cycle on the aggregation response of *Tragoderma glabrum* (Coleoptera: Dermestidae) larvae to wheat germ oil: an enhanced response after a completed moult and reached a minimum just before the next moult. Larvae that were starved in individual tubes were generally much less responsive to wheat-germ oil than those fed normally. Starved larvae that had undergone a regressive molt within the previous 2 days regained their responsiveness to the attractant. The ideal specimens for bioassay should be within 1 day of each other in age and 24 h from the previous molt. These findings have major implications for any behavioural assay for larvae of this species.


A laboratory study carried out in the USA, larvae of *Ephestia cautella* (Hbst.), which is most commonly found on wheat grains, responded to wheat-germ oil, a food attractant, for a relatively short time during the intermoult period. The response was greatest immediately after a completed molt and reached a minimum just before the next molt. Larvae that were starved in individual tubes were generally much less responsive to wheat-germ oil than those fed normally. Starved larvae that had undergone a regressive molt within the previous 2 days regained their responsiveness to the attractant. The ideal specimens for bioassay should be within 1 day of each other in age and 24 h from the previous molt. These findings have major implications for any behavioural assay for larvae of this species.


In samples from 8 bulk storages of grain in 1979, field resistance to fenitrothion in *Oryzaephilus surinamensis* (L.) was recorded for the first time in Queensland. In tests with treated grain, resistance was 53-62-fold. For the strain most resistant overall, resistance measured by 2 methods was 159- and 53-fold to fenitrothion, 48- and 32-fold to malathion, 37- and 32-fold to methomyl, 1.8- and 5.7-fold to methiocarb and 3.2- and 4.0-fold to permethrin.


Life tables were constructed to assess the relative importance of some factors causing mortality in *Tribolium confusum* medium in the laboratory and gage their response to increasing population density. Observations were made on 3 population densities (100, 400 and 800 individuals/g medium) from the egg to the adult stage, and the medium was not renewed in order to maximise predatory interactions. General patterns of larval mortality were density-dependent, averaging 42, 50 and 74% at these densities, respectively. Mortality in the first 10 days was also density-dependent, reaching a maximum of 72% at the highest density; predation by small larvae on eggs seemed to be the principal cause. The overall pattern of larval mortality was density-independent. Data on the mortality of pupae and newly emerged adults were ultimately consistent with an inversely density-dependent pattern. Apparently, only mortality in the first 10 days was capable of causing population regulation.


A new class of compounds derived from hydroquinones was synthesised in Italy and bioassayed for juvenile hormone activity in *Tenebrio molitor* L., *Tribolium confusum* Duve and *Ephestia kuehiella* Zell. (*Anagasta kuehiella*). Structure-activity relationships of the new compounds are tabulated and discussed. Two of the new compounds, 1- and 1-[5-chloro-4-pentynyl]oxybenzene (I) and 1-[5-chloro-4-penty1]oxy-4-phenoxbenzene (II) showed outstanding activity against *Tenebrio molitor* in short-term tests with field studies against *Culex pipiens L*. *Aphis pomi Deg.* (on apple), *Psylla pyri* (L.) (on pear) and *Trialeurodes vaporariorum* (Westw.) (in the greenhouse) and previously published studies on *Solenopsis invicta* Buren that compound II is of particular interest for the control of *Homoptera*, mosquitos, fire ants and stored-product pests.

176 PIERCE, R. Fumigating empty metal grain bins. *Agricultural Research, USA* (1983) 32 (2) 14 [En, 1 fig.] AR, SEA, USDA, Pocia, Illinois, USA.

A study was carried out in Kansas by J.K. Quinlan in which the 4 fumigants chloropicrin, phosphine, carbon tetrachloride with carbon disulfide, and ethylene dichloride with carbon tetrachloride were tested for the control of adults and eggs of the red flour beetle (*Tribolium castaneum* (Hbst.)) and immature stages of the rice weevil (*Sitophilus oryzae* (L.) in less than 15 days. Mortality counts of adults were immediately made after the 48-h fumigation period and larval counts 4 and 6 weeks after treatment. Chloropicrin at 16 US fl oz/bin was inadequate, but the recommended application of 32 US fl oz/bin was the most effective treatment, killing all stages of the test insects on and below the floor, while some of the insects suspended above the floor survived. An application of 2 gallons of the primary mixture also caused 100% mortality of all stages of the pests except those suspended above the floor. Phosphine was only effective when a plastic sheet was placed over the perforated floor and a 60-tablet dosing was applied, and the binary mixture was ineffective.


Experiments are described in which age-specific survivorship and fecundity of *S. oryzae* were determined over a wide range of environmental conditions. Data collected from these experiments, together with some previously published, were used to develop mathematical submodels for these processes. The models are continuous functions of time, grain moisture content and grain temperature. Rate of increase per week and percent required for calculation of the range of environmental conditions used. The results are discussed in relation to previously published work, and the potential usefulness of the submodels is discussed briefly.

178 LONGSTAFF, B. C.; DESMACHEREL, J. M. The effects of the temperature-toxicity relationships of certain

Two pesticides, pirimiphos-methyl and deltamethrin were shown to have opposite relationships with temperature. The consequence of these relationships are discussed in relation to the population growth of S. oryzae and, in particular, the use of cooling in combination with low levels of pesticide as an integrated control measure for this and other pest species.


Cryptolestes ferrugineus, Oryzus oryzae sulurinensis, Rhysophera dominica, Sitophilus granarius, S. oryzae and Tribolium castaneum were studied in cooled wheat or flour. Survival at low temperatures differed between species and, there was considerable interaction between the effects of temperature and humidity.


The effect of constant and intermittent exposures to temperatures of 40-45°C on the mortality and pupation rate of diapausing larvae was examined. Exposures at high temperature significantly hastened the termination of diapause. In general, the higher the level of mortality in the sample, or the greater the number of exposures at high temperature, the sooner pupation occurred in long days at 25°C.

Mites


A. farris of the stored grain pests Acarus siro L. and A. farris (Oudem.) were crossed in the laboratory in Poland with differing proportions of females of A. farris and A. siro. Males of both species mated continuously and rarely restricted mating to conspecific females. Overall, the higher the level of mortality in the sample, or the greater the number of exposures at high temperature, the sooner pupation occurred in long days at 25°C.

182 WANG, C. L. The infestation and control of bulb mite (Rhizoglyphus robini) on gladiolus. Journal of Agricultural Research of China (1983) 32 (1) 75-82 [En, ch, 8 ref., 1 fig.] Department of Applied Zoology, Taiwan Agricultural Research Institute, Wufeng, Taichung, Hsien 431, Taiwan.

In Taiwan, Rhizoglyphus robini Capinard$ is a serious pest of gladiolus, attacking bulbs in both the field and in storage. Field and laboratory studies in 1981-82 showed that percentage damage to the stalks to plants at the 5-leaf, 7-leaf, corm-mature and corm-harvest stages was 78.6, 39.0, 80.0 and 54.0%. The number of mites found on individual damaged corms in the field ranged from 4 to 540. Only 55.5% of corms remained undamaged after storage in the laboratory at 15-28°C and 51-87% RH for 3 months. Effective control of the mite was obtained with dip treatments with bromopropylate, benoxonimate (benzoxonate) or demeton-methyl (methyl-demeton) or fumigation for 48 h with phosgene from aluminium phosgene tablets at a rate of 1 tablet/m² at 25-38°C or 2 tablets/m² at 15-20°C.


Possible methods of reducing bird-strike hazards were suggested. Options considered include reductions in overall populations, manipulation of habitats and food sources and dispersal mechanisms including auditory and visual scaring devices. Initial reactions and avoidance procedures are also mentioned.


This paper concentrates on the increasing use of jet engines for private small aircraft (Corporate/Executive jets), bird behaviour in relation to the latest generation of large quiet jet transports, where there is less audible warning to birds, and the effects of speed in relation to bird-strike damage.


Some background information on bird strikes is given. Quantitative observations were made on the visits of different kinds of birds notably kites (Milvus migrans) and crows (Corvus splendens), and on their movements around the different parts of the primary sewage treatment plant located in the neighbourhhood of the airport at Bangalore. Suggestions are given to prevent the visits of birds to the airport area, mainly relating to the maintenance of sanitary conditions.


Identification of bird pests from 553 incidents, both military and civil, is described. The study of lapwing behaviour and bird-strikes, started in 1980, is continuing.


The habits, ethology and ecology of rats are reviewed. On the basis of the author's experiences control principles are discussed by which, under appropriate conditions, a total and lasting rat-free condition can be achieved.


Rat feeding behaviour and its effect on the efficiency of baiting was investigated. Studies on house mouse populations in farm buildings were completed. Laboratory tests and field trials of rodenticides are described and work on resistance to rodenticides is reported.

TECHNIQUES

See also abst. 2


Various kits which have been devised recently for the rapid identification of Candida spp. and other medically important yeasts [KMYM 11, 1187; 16, 2722 et paste] are based solely on the assimilation and fermentation of various sugars, and this may sometimes result in errors. Therefore the authors have devised a kit comprising a plastic box with
7 minutubes containing various media for the study of morphological (yeasts, pseudomycelium, chlamydospores and germ tubes) and physiological (urease, sensitivity to acididone cycloheximide, tetrazolium reduction, fermentation of glucose, fermentation of maltose) characteristics. This is used concomitantly with auxanogram tests for the assimilation of 15 carbon and 2 nitrogen compounds. The method permits the rapid identification of most yeasts of interest in medicine and the food industry. The characteristics of 22 spp are tabulated.

CONTROL

See also absts. 38, 54, 57, 59, 68, 79, 81-82, 86-87, 152, 157, 172-173, 173-176, 178, 188


The protection of stored products against insect pests without the use of insecticides is reviewed on the basis of published work. The topics discussed include the use of gas pheromones, juvenile hormone analogues, insect growth regulators, biological control using the predacious mite Blattisocius tarsalis, the use of feeding attractants, low temperatures, controlled atmospheres and resistant packaging.


The literature published in 1981-82 on the protection of stored products against arthropod pests is reviewed with reference to the progress made in the taxonomy and distribution of some species, problems of stored-product protection in the German Democratic Republic, German Federal Republic and other countries, the biology and ecology of some pests and the development of control measures (quarantine measures, chemical methods, fumigation and use of insecticides of plant origin).

192 Industry resigned to loss, turns to alternatives. Citrograph (1983) 69 (1) 11-13 [En]

In September 1983, the Environmental Protection Agency in the USA ordered the immediate suspension of the use of ethylene dibromide (EDB) as a soil fumigant, and set a schedule for phasing it out for other applications, including quarantine fumigation for citrus and tropical fruits. The date announced for the end of fumigation was 1 September 1984. Alternative methods for controlling fruit-flies on citrus in Florida, Texas and California are reviewed, together with the problems of finding alternatives to fumigating grain, felled logs and other products.


A mixture of 50% methyl bromide and 50% carbon dioxide by weight was tested as a space fumigant. Initial results in fumigation chambers and empty and loaded freight containers are extremely encouraging, excellent gas distribution being achieved rapidly with no fan stirring, even at low temperatures. The technique should find application where rapid gas distribution and the need to avoid contamination with liquid methyl bromide are of paramount importance.


A new N-oxime ether (CG-97967) was tested for activity in unaltered maize meal. Using inhibition of the production of respiratory CO2 as the criterion of antifungal activity, the N-oxime ether was equal or superior to propionic acid in 3 batches of maize meal. Further testing against pure cultures of fungi, yeasts and bacteria, including Aspergillus flavus, Fusarium tricinctum, Penicillium citrinum, Candida pseudotropicalis and Staphylococcus aureus, revealed that the experimental compound was unstable and was inactivated by heating, which caused an oxidative decarboxylation. It is suggested that use of N-oxime ether would be inappropriate in pelleted poultry feed.

BIODEGRADATION - GENERAL

See also abst. 233


The major products of present-day biotechnology (including those from waste) and the industries making them are indicated. The future of biotechnology is explored.

Planning and Plant Design

See also abst. 204

Hygiene and Pathogens

See also abst. 207


Ground-water recharge via wastewater application to land or via direct injection appears to be an attractive alternative to other methods of disposing of domestic waste water. However little is known about the survival of pathogenic microorganisms in ground water. A comparative study was undertaken on the decay rates of three bacterial types (S. typhimurium, E. coli and S. faecalis), an enterovirus (polioivirus type I) and a bacterial phage (F2) in ground water maintained under laboratory conditions. Except for F2 phage, all the microorganisms tested were relatively stable in ground water. Under field conditions, bacterial indicators were also found to be stable in the ground-water environment.

Analysis and Sampling

See also abst. 78

ENVIRONMENTAL POLLUTION


Some progress has been made in the genetic engineering of microorganisms for pollution control, but strain selection and mutagenesis may be more important. Biotechnology companies lack the economic incentives to concentrate research in this area. In addition, legal and ecological problems are not yet resolved.

Water pollution

See also absts. 196, 206, 227


Tastes may be produced in the water distribution system by microbiological activity which must be suppressed. The removal of odorous compounds in some Paris water treatment plants is disturbed.

Freshwater pollution

See also abst. 199

Marine pollution

See also abst. 223
LIQUID WASTE TREATMENT AND DISPOSAL


In the Netherlands the raw water, obtained from the heavily polluted river Rhine, has off-flavours which must be removed by using sophisticated and expensive treatment methods to be acceptable for drinking. Methods are reviewed and it is concluded that ozonation and activated carbon filtration are the most effective treatment processes for improvement of taste and odour.

BIOLOGICAL WASTE TREATMENT

See also absts. 195, 197, 219, 228

Composting

See also absts. 209, 211


Saccharine bagasse was composted alone (as a control), with the addition of poultry manure, and with the addition of defatted soybean meal and urea. After 90 days the compost was stabilized. The stack which contained poultry manure decomposed hemicellulose and cellulose effectively. Total nitrogen and ash were also increased tremendously compared to the stack which contained urea and defatted soybean meal. Concentrations of lignin from both stacks were not altered significantly. Although studies of the application of this compost to fields are not complete, increased crops of sugar cane are reported.


The investigation was carried out to obtain basic data and to establish proper standards for determining when compost is completed. Undigested sewage sludge cake was composted with rice hull as a bulking agent. Various fractions of carbon and nitrogen compounds, activities of various enzymes, microflora, solubility of heavy metals in extractants and the state of decomposition of filter paper and rice hull were studied.

Activated sludge treatment

See also absts. 205, 222


A large number of media were tested for cultivating M. parvicella, an organism often present in the activated sludge of oxidation ditches. Growth yields of 1.3-1.5 g/l were obtained on media containing TWEEN 80 (4 g/l), reduced N and reduced S compounds, calcium and magnesium salts, phosphate buffer, trace elements, thiamin and cyanocobalamain. The opt. temp. for growth was approx. 25°C and pH should be above 7.

Ponds, Lagoons, Oxidation ditches

See also abst. 225

Aerobic digestion

See also abst. 204


Currently many of the more traditional fermentations are only economically viable if they utilize biological wastes and agricultural surpluses as substrates. By combining biological and chemical procedures the profitability of even some superficially unpromising fermentations could be substantially enhanced. Collaborative research by microbial physiologists, geneticists and biochemical engineers is a prerequisite for marketable success in new applications of fermentative anaerobes and their enzymes.

WASTE DISPOSAL (BIOLOGICAL ASPECTS)

See also abst. 195

Land treatment and Irrigation

See also abst. 196

Biological upgrading to feedstuffs

See also absts. 210-211, 221, 224

Biological upgrading - other

See also abst. 211


Three models describing toxicity in methane fermentation systems are presented. One models response to slug additions of toxicants and two describe the effect of toxicity on bacterial kinetics. The importance of adequate biological solids retention time in maximizing process stability is demonstrated.

MUNICIPAL WASTES

See also abst. 201

INDUSTRIAL WASTES

Liquid wastes

See also absts. 206, 221, 224-225


In order to mimic incomplete and critical metabolism of chloroaromatics in the laboratory, model ecosystems of activated sludge were designed where naturally occurring microbial communities are exposed to haloraromatics. Although the total degradation of monochloro- and dichloro-aromatics as critical components of industrial sewage is feasible, the presence of polyhalogenated aromatics in very low concentrations means it will not be possible to establish special organisms in activated sludge for the elimination of polyhalogenated aromatics.

AGRICULTURAL WASTES

Poultry waste

See also abst. 200
SPECIFIC WASTE MATERIALS

See also absbs. 205, 225


Dyes are so varied in their structures that no simple scheme for their removal from domestic waters. Some will undergo biodegradation and metabolites will be formed of which some are likely to be more noxious compounds than the parent dyes. If manufacturing processes permit, it is preferable to isolate and remove the dye in a concentration prepared from styrene and α- or γ-methylol. Low highly coloured liquids to be discharged to sewage treatment works. If treatment has to be considered, that treatment can include physical, chemical, physico-chemical or biological procedures.


The survival of selected viruses in Lactobacillus- and yeast-fermented edible waste material was studied to determine the feasibility of using this material as a livestock feed ingredient. There was more rapid inactivation of viruses by the Lactobacillus fermentation at 30°C than by the yeast fermentation.


The microbial degradation of lignin in compost formed from sewage sludge and microalgal materials has been investigated with Alcaligenes sp. str. 559' and Pseudomonas sp. str. 419. All the St-MeSt codimers can be decomposed by the two strains but the MeSt homodimers are not degradable.

Cellulosic wastes

See also absbs. 200, 215


A. nidanus was found to grow and sporulate best at 37°C in continuous light and alternating light-darkness respectively. The fungus was able to cause losses in the dry weights of filter papers on incubation and made appreciable growth on carboxymethyl cellulose (CMC) and hemicellulose. The culture filtrates contained cellulases which hydrolysed filter papers and CMC to reducing sugars, and were only able to produce these enzymes in the presence of cellulose or its derivatives in the growth medium. The CM-cellulases had peak activity at pH 5.2 and at 50°C while optimal OP-activity occurred at a pH of 5.5 and at 45°C. The participatory role of A. nidanus in composting is discussed.


The cellulolytic enzyme complex displayed optimal activity at pH 5.0 and 25°C. Carboxymethyl-cellulases with a high and substitution grade, rise to poor growth and low cellulase activity. Insoluble crude cellulases such as newstrep, recycled paper, rice and flax straw were substantially solubilised at 28°C within 3-5 days of fermentation. A study of the cellulase-complex formation during the growth cycle revealed that β-glucosidase was produced mainly intracellularly in the early exponential phase, while the overall exo-1,4-β-glucosidase and endo-1,4-β-glucanase formation gradually increased during the total fermentation cycle. The mycelial protein of C. cristanum grown on crude celluloses displayed a favourable amino acid pattern, indicating its potential value as a source of single cell protein.


Commercial exploitation of straw depends on good development and marketing. Straw, horticultural compost and fuel briquettes are now being marketed but there is also interest in ethanol production and fungal degradation of straw by producing Pleurotus.


A three-stage biochemical procedure to convert β-1,4-glucopolymer (cellulose) to α-1,4-glucopolymer (starch) was studied in vitro. Cellulose was hydrolyzed to cellulose by cellulase producing cellulase which was isolated from culture filtrates of Cellulibio gilvus. The second step was the conversion of cellulase to glucose 1-phosphate by cellulase phosphorylase purified from extracts of C. gilvus. The third step, the bioconversion of glucose 1-phosphate to α-glucoselymer was carried out by α-glucan phosphorylase. The result shows that approximately 10% of cellulose could be converted to α-1,4-glucan, such as amylose, via glucose 1-phosphate.


The molecular biology of Phanerochaete chrysosporium (which is known to degrade lignin), the isolation and characterization of actinomycetes that attack lignin, and the mobilization of genes coding for degradation of low-molecular-weight lignin-related compounds from strains of Gram-negative bacteria are studied.


A radiotracer assay was developed and used to determine the characteristics of ligninocellulose degradation in wood. The assay was used to determine patterns of microbial decomposition in large logs of Douglas fir (Pseudotsuga menziesii) which have been lying in known periods of time in a stream channel of an old-growth forest.

Starch


Brief mention is made of attempts to transform recalcitrant carbohydrates derived from waste materials. The reasons for transforming carbohydrates are given. Detailed examples of carbohydrate transformations with immobilized cells are given.


T. petrophilum can synthesise either a glycolipid surfactant or a protein emulsifier depending on the substrate used. Most of the emulsifiers were used with kerosene but the emulsifier was also active when pure hydrocarbons or vegetable oil were used. The compounds synthesised were not produced to facilitate the uptake of an insoluble carbon source. The glycolipids produced were identical to the mixture isolated from T. bombicula.

Hydrocarbons, Petroleum

Detergents


Analysis was undertaken using BiAs-analysis for primary degradation, DOC-analysis for ultimate degradation and HPLC. GC, GC/MS identifications for intermediate degradation products. The bioassay was a static test procedure (EMPA-test) which, according to the OECD classification belongs to the group of "inherent biodegradability" tests. Analysis of effluents of sewage treatment plants was also undertaken to detect alkylphenol ethoxylates.

BIOCIDES

Agricultural biocides


Agglutination tests and immunofluorescence tests with antisera against 4 strs. of chloridazon-degrading bacteria revealed the serological uniformity of a group of 22 chloridazon-degrading bacterial strs. No serological relationship could be found between chloridazon-degrading bacteria and representatives of other Gram-negative bacteria. On the basis of immunofluorescence data a linkage map was worked out to represent serological relationships in the group of chloridazon-degrading strs.

PHOSPHORUS


A biological/chemical mechanism for enhanced phosphate removal in biological treatment plants was investigated. The experiments confirmed that precipitation in biofilms may lead to calcium phosphate precipitation inside the biofilms due to the increased pH created by the denitrification reaction. A mathematical model has been developed relating the phosphate to nitrate molar removal rates to the concentration of phosphate, calcium, bicarbonate, the solubility of the solid phosphate phase and the type of carbon source used for denitrification. Biofilm precipitation may be of significant practical interest, but factors such as filamentous growth on the biofilm surface or precipitation of an easily soluble amorphous calcium phosphate can also make biofilm precipitation insignificant. The precipitation effect is expected to be feasible in flocs, if a homogeneous biomatrix with a thickness of more than approx. 100 µm exist in the floc substructure.

RADIOACTIVE WASTE


Precultured Penicillium biomass can accumulate uranium from solutions of uranyl chloride. A 4 g (fresh weight) fungal mass will remove, over a 1 to 4 h period, between 70% to 90% of the U(IV) from 1 to 10 p.p.m. solutions. Accumulation is more or less the same between pH 5.5 to 7.5, and retains over 2/3 of its efficiency at pH 2.5. The data support strongly the feasibility of using fungal biomass to decontaminate transuranous wastewaters, even at high temperatures and acidic conditions.

SPECIFIC INDUSTRIES

Food industry


Waste waters discharged from heated potato juice contains a large amount of coagulated protein (decanter waste). The supernatant of heated potato juice (clarifier waste) showed more than 10 000 p.p.m. of COD and contained more than 500 p.p.m. of SO_2. Treatment of these two wastes was examined using yeasts. Saccharomyces cerevisiae var. ellipsoides and Hansenula anomala grew best and were used for further experiments to find the best conditions for treatment. The yielded solid from the decanter waste contained 63.9% crude protein.

Fermentation industry


By increasing the sludge loading rate in the activated sludge process, the upper limit of the load at which the final effluent quality and characteristics of the sludge become worse and microorganisms do not settle properly can be found. To determine the maximum COD loading, brewery crude effluents were treated under various conditions of COD loading, temperature and DO level in miniature plants, pilot plants and a full scale plant. The sludge volume index (SVI) always increased on increasing the COD loading. The COD level in the final effluent usually increased but sometimes the COD in the final effluent and the SVI of the sludge increased simultaneously.


Pseudomonas aeruginosa and Vibrio cholerae showed a strong positive chemotactic response towards rum distillery wastewaters (mostos) and a high oxygen uptake rate in the presence of this complex substrate. Rum slops stimulated only motility in Aeromonas hydrophila and Escherichia coli. The A. hydrophila and E. coli isolates were unable to oxidize mostos significantly.

Chemical industry


The quantities and amino acid composition of proteins from Chlorella vulgaris, Scenedesmus obliquus and Stichococcus bacillaris used in purification experiments on wastewater from a nitrogen fertilizer factory are given. The quantities of exogenous amino acids of the investigated algae and "ideal protein" standard of the FAO are compared. The protein from Scenedesmus and Chlorella appeared to be richer in exogenous amino acids than the FAO standard. Stichococcus bacillaris has a lower content of exogenous amino acid but nevertheless has a high content of threonine and lysine, higher than in the other investigated algae.

Pulp and Paper industry

See also abst. 213

225 VOSS, R. H. Chlorinated neutral organics in biologically treated bleached kraft mill effluents. Environmental Science and Technology (1983) 17 (9)

Extracellular laccase was induced in *Flammulina velutipes* using ferulic acid and 2,5-xylidine as inducers. Kraft lignin and lignosulfonates were degraded by p-diphenol oxidase (laccase).

**Water treatment plants**

See also abs. 198, 217, 219


Prechlorination with chloramine (unit 1) and prechlorination with free chlorine (unit 2) were equally effective in removing and controlling trihalomethane (THM) precursors in parallel pilot systems that varied only in the method of prechlorination. As expected, the THM levels in unit 1 were low and those in unit 2 were high. Control of the bacterial population (as determined by standard plate counts) in unit 1 was more consistent than that in unit 2.


Microbial populations were monitored in the influent and effluent waters and on the granular activated carbon (GAC) particles for 16 months by means of total plate counts and ATP assays. Microbial populations between the influent and effluent waters of the GAC columns generally increased logarithmically with microbial growth. The dominant genera isolated from interstitial waters and GAC particles were *Achromobacter*, *Acinetobacter*, *Aeromonas*, *Aerobacterium*, *Bacterium*, *Chromobacterium*, *Corynebacterium*, *Micrococcus*, *Micrococcus*, *Paracoccus* and *Pseudomonas*. Coliform bacteria were found in small numbers in the effluents from some of the GAC columns in the later months of the study.

**BIODEGRADATION ORGANISMS**

**Bacteria**

See also abs. 196, 202, 208, 212-213, 218, 228


Some of the systems presently available for genetic engineering in *Pseudomonas* are discussed. Their use for manipulation of its ability to degrade natural and xenobiotic aromatic compounds, some of which constitute sources of environmental pollution, are outlined.

**Actinomycetes**

See also abs. 213


This review includes discussion of the amenability to genetic manipulation of yeasts used in the biodegradation and utilization of various waste products.

**Algae and Lichens**

See also abs. 224

**REPORTS**


The research projects reviewed in this report on tropical agriculture from an international organisation in Colombia include those on the control of arthropod pests of beans (*Phaseolus vulgaris*) in the field and in store (mainly by plant resistance), cassava (*Manihot*) (by plant resistance, biological control and integrated control), rice (by plant resistance and improved agronomic practices) and pasture grasses (by plant resistance).

**CONFERENCES**


A collection of 12 papers:

*Goldstein, I. Chemical improvement of wood — a historical overview, 4-7 [7 ref.]*

*Çolé, W.A., Jr. The influence of wood anatomy and ultrastructure on fluid flow, 8-15 [18 ref., 4 pl.]*

*Thomas, R.J. The mechanism of pit aspiration. 16-23 [11 ref., 2 pl.]*

*Vihavainen, T. Fixation mechanisms of wood preservatives, 24-31 [48 ref.]*

*Rowell, R.M. Wood preservation and stabilization by chemical modification of the wood substance, 32-43. [15 ref., 2 pl.]*

*Johansson, I. Dimensional stabilization of wood products, 44-49 [20 ref.]*

*Karlivon, V. [Studies of the Institute of Wood Chemistry in the Latin SSR in the field of wood modification]. Untersuchungen des Institute für Holzchemie der Lattischen SSR auf dem Gebiet der Holzmodifizierung, 50-61 [17 ref.]*

*Chow, S. Steiner, P. Significance of chemical bonding in wood gluing, 63-65 [16 ref.]*

*Chow, S. Physical and chemical aspects of adhesion. 66-73.***

*Skafizadeh, F. The chemistry of fire retardants. 74-84 [19 ref.]*

*Banks, W.B. Factors influencing the performance of water repellents. 85-90 [13 ref., 1 pl.]*

*Miller, E.R. Chemical aspects of external coatings for softwoods. 91-98 [31 ref., 1 pl.]*

AWH


Fourteen papers give up-to-date reports from international biochemists on aspects of their work involved with biotechnology. Relevant papers are abstracted elsewhere.

Series Bulletin 279 for Southern Regional Research Project S-132

This publication consists of the manuscripts prepared for the symposium on "Aflatoxin and Aspergillus flavus in Corn" held in Atlanta, Ga. on Jan. 26-27, 1982 and includes a bibliography and subject index. Topics discussed included biology of A. flavus and A. parasiticus, epidemiology of aflatoxin formation by A. flavus, sampling and aflatoxin analysis in maize, biological and economic effects of aflatoxin and prevention and control of aflatoxin in maize. Relevant papers are abstracted elsewhere.

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