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

INTERNATIONAL BIODETERIORATION BULLETIN

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

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

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

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

Reese and Levison (1952).

and in the bibliography:


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NEWS AND COMMENTS

In an attempt to reduce the hazards to aircraft caused by birds on several of their airfields in Britain the United States Air Force has recently awarded a contract worth in the region of £24,000 to a new company called Long Wings. This company has been set up by two falconry enthusiasts from Northamptonshire, David Horsfield and Tony Creswell to provide 60 falcons for use on six U.S. air bases, mostly in Eastern England. The falcons have proved an effective deterrent against the many birds which frequent the airfields. As well as the sixty birds which are worth about £40 each the undertaking will require a team of eight assistants and six vehicles. [Abstracted from the Sunday Times.]

Two new pesticidal products have been introduced by Rentokil. A cockroach bait consisting of a paste of starches, oils and sugars containing 0.125% of the stomach poison Chlordecone is intended for use either where dusts and sprays are ineffective or undesirable or as a persistent supplement to other control measures. A lethal dose is normally accumulated after a few days of feeding and maximum kill is claimed after about a week.

A new lindane-based contact dust for mouse control is also announced. This has been introduced as an alternative to warfarin to which mice are becoming increasingly resistant and to DDT. The dust is picked up on the feet or fur of the mice and is swallowed during grooming. The 50% lindane dust is claimed to be more effective than either 1% warfarin or 50% DDT dust, a complete kill of the test population being achieved after three days compared with an incomplete kill even after twelve days for the warfarin and DDT.

Both these new products have been cleared for use by trained operators under the Pesticides Safety Precautions Scheme. Further information can be obtained from Rentokil Laboratories Ltd., Products Division, Felcourt, East Grinstead, Sussex, England.

Birds strikes are causing trouble for the RB211, the Rolls-Royce engine for Lockheed’s Tristar airbus. Rolls-Royce are developing jet engine fan blades from the carbon fibre composite material known as Hyfil. Problems with rain and grit erosion and bird impact were foreseen when development started and because of this test were carried out using Hyfil blades in Conwy engines in B.O.A.C. VC 10 airliners. In addition development of an alternative, titanium blade for the RB211 was undertaken.

Initial tests showed that unprotected glass-reinforced Hyfil blades did not stand up sufficiently well to rain and grit erosion. This was counteracted by nickel-plating the leading edge of the blades, but this was inadequate to withstand bird impact. This in turn was overcome by introducing steel laminates into the leading edge which gave good protection against bird impact but introduced fatigue problems which had not been present without the steel reinforcement. Modifications to the blades have resulted in considerable improvements and intensive development is still continuing. [Abstracted from The Times 23 April, 1970.]

The so-called “super rats” of Mid-Wales are still causing concern. These form one of the world’s largest concentrations of warfarin-resistant rats and in spite of extensive measures to contain the population they are gradually spreading to neighbouring areas. The Control area, 1,000 square miles centred on Welshpool, was ringed with heavy doses of zinc phosphide in 1967 soon after the population of resistant rats was discovered. In this way the Ministry of Agriculture hoped to prevent the rats spreading from this rural area to the industrial West Midlands. Spread of the rats has been slowed down from about three miles to one mile per year but it has not been stopped and there are reports of the rats being found outside the control area. Effective containment of the rats was hampered by the restrictions imposed during the foot-and-mouth epidemic of 1967-68 which prevented rodent control officers visiting farms and by the fact that many farmers have been reluctant to use alternatives to warfarin because of the danger to farm animals. One alternative that was initially used with some success in 1968 was Racumin 57 but the rats have since been found to have developed resistance to this as well. The development of a rodenticide that will be effective against these rats without endangering domestic animals or people is still awaited. [Abstracted from the Sunday Times.]

A new method of storing surplus rice has been developed in Japan by the Nutritional Chemical Laboratory of the Department of Agriculture, University of Kyoto, led by Professor Hisateru Uitsuda. The rice is stored at the bottom of lakes in vinyl chloride tanks each holding one ton of unhulled rice. The initial experiment was carried out at Lake Biwa in Central Japan which is about 40 miles long and 14 miles wide and from 100 to 300 feet deep. The lake bottom provides a constant low temperature environment, equivalent to keeping the rice in refrigerated storage. Other possible storage sites which are now under investigation as providing a constant temperature environment are disused mine shafts. [Abstracted from the Sunday Times.]

At present being developed at the Upjohn Co. of Kalamazoo, Michigan, U.S.A. is a chemical which causes permanent sterility in male rats while leaving them vigorous and sexually competitive. Only a single small dose needs to be taken by the rat to be effective unlike the normal anticoagulant control agents. The chemical is 3-chloro-1,2-propanediol, a member of the chlorohydrin group, and causes lesions in the male rat’s reproductive tract, blocking the passage of the sperm and effectively preventing fertilisation of the female. Mating with the sterilised males causes false pregnancy in the females which prevents them from mating with further, possibly normal, males. The sterilant is
fairly specific and does not act on mice or rabbits and only temporarily on guinea pigs, rats and monkeys. It is also stated to be relatively non-toxic to other forms of wildlife, domestic animals and man. [Abstracted from Chemical and Engineering News, 15th September 1969.]

The opportunity will be provided to demonstrate the products, processes and apparatus offered by exhibiting firms and organisations for sale, licensing, adaptation, development to meet specific requirements or negotiation of agency, franchise and other forms of joint venture agreements and to identify from the various technologies and disciplines such findings as may be of value beyond their research context. Conference sessions are to be organised by the Institute of Physics and the Physical Society.

Participation is open to organisations and companies, from any country, concerned with scientific or technological research for commercial or industrial development and the negotiation of agency or licensing agreements.


The 14th International Congress of Entomology will be held in Canberra, Australia, from 22nd to 30th August. The Congress will be divided into 15 sections which will include Forest and Stored Products Entomology. Details may be obtained from: Courtenay N. Smithers, Secretary, 14th International Congress of Entomology, The Australian Museum, P.O. Box 4285, Sydney South, N.S.W. 2000, Australia.

The Royal Microscopical Society is organising Micro 70 for the week 14th-18th September 1970. This will include Symposia on Botanical Electron Microscopy, Microstructure of Materials and Mucosubstances, a series of special lectures and a trade exhibition of optical and electron microscopes, and will be held at Imperial College, London. Details of the Symposia and tickets for the Exhibition may be obtained from R. Pennington Esq., Executive Secretary, Royal Microscopical Society, Canterbury House, 393 Cowley Road, Oxford, OX4 2BS, England.

The Congress on Technology of Forestry Today and Tomorrow is being held in Munich on 8th-10th June, 1970 in conjunction with the International Exhibition of Forestry and Woodworking. The Exhibition will be open from 6th-14th June. Further information may be obtained from Münchener Messe- und Ausstellungsgesellschaft mbH, D-8000 München 12, Theresienhöhe 13, P.O.B. 200, Germany.

The 1st International Symposium on the Genetics of Industrial Microorganisms will be held from the 23rd-28th August, 1970 in Prague at the Technical University. The Symposium has been organised by the Institute of Microbiology of the Czechoslovak Academy of Sciences, in cooperation with the Research Institute of Antibiotics and Biotransformations. Enquiries may be addressed to The Secretary, 1st International Symposium Genetics of Industrial Microorganisms, Budějovická 1083, Praha 4-Kr, Czechoslovakia.

The 2nd World Food Congress, organised by the Food and Agriculture Organisation of the United Nations, is to be held from 16th-30th June, 1970 in The Hague. The Congress will be in two parts: the first phase will assess the current world food situation, within the framework of overall economic development, and will propose priorities for action and the second phase will discuss how to find the resources necessary to carry out that action. Further information on the Congress may be obtained from The Secretary, Second World Food Congress, F.A.O., Via delle Terme di Caracalla, 00100 Roma, Italy.

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PROTECTION OF WOOD IN THE MARINE ENVIRONMENT

P. C. Trussel1 and E. B. Gareth Jones2

Summary. The research work for 1968/1969 on the protection of wood in the marine environment, instigated by the Organisation for Economic Co-operation and Development, and conducted by various experts and different groups throughout the world, is reported.


In January, 1966 the Organisation for Economic Co-operation and Development (O.E.C.D.) appointed a committee to investigate the economic importance of the biodeterioration of wood in the sea. The matter was also considered by the Committee for Research Co-operation of O.E.C.D. who decided that an enquiry should be conducted to evaluate the losses incurred by the deterioration of marine wood and to establish the methods currently in use to prevent deterioration. This led to the establishment of an international group of experts to study the problem. The group have started a number of research projects and each year review progress in the field of marine biodeterioration. The first report appeared in 1968 (Jones, 1968c). The following account summaries the activities of members for the year April, 1968 to April, 1969.

During 1968/69 research directed to the protection of wood in the marine environment has been continued in a number of parts of the world. The Naval Facilities Engineering Command of the U.S. Department for the Navy, with assistance of the Wm. F. Clapp Laboratories, have been investigating the basic manner in which a number of marine protective treatments protect against marine borers. For this work individual groups of 3- × 4- × ½-inch coupons of southern yellow pine and Douglas fir were impregnated with high and low retentions of creosote, creosote-coal tar solution, ammoniacal copper arsenite, chromated copper arsenate, and dual treatments of creosote-chromated copper arsenate, and creosote-ammoniacal copper arsenite. The original coupons were exposed during July 1964 at Boston, Massachusetts, and at Wrightsville Beach, N.C. Three replicates of each treatment have been removed at six-month intervals and examined for borer attack and for preservative retention and composition.

In 1968, coupons represented the 48th month of this 60 month study. At Wrightsville Beach, N.C., southern yellow pine and Douglas fir coupons, treated with low retentions of creosote and creosote-coal tar solution, had been destroyed by Limonaria tripunctata, after 36 months’ exposure. Coupons treated to moderate and high retentions of both creosote and creosote-coal tar and exposed at Wrightsville Beach, N.C., suffered L. tripunctata attack but exposures are continuing. Molluscan borer (principally Bankia and rarely Martesia) attack was severe in the low level salt-treated coupons at Wrightsville Beach, but present only in the form of abortive pits in coupons which received 2 lb/ft² or more of the salt treatments. Scattered abortive pits were also present in most of the dual treated coupons. A few coupons with low retentions of creosote and of creosote-coal tar, exposed at Boston, Massachusetts, were just beginning to show signs of Limonaria lignorum attack. There has been no molluscan borer attack at Boston. While there has been some loss in preservation retention from the coupons during the 48 month period and there has been some increase in the ratio of saturated hydrocarbons to unsaturated hydrocarbons in the creosote and creosote-coal tar preservatives, these changes could not be correlated with borer attack.

In the past years, the Naval Research Laboratory of the U.S. Navy has undertaken a comprehensive programme on the fractionation of creosote and an assessment of the interaction of creosote and its fractions in the preservation of wood. Thirteen publications have been issued by Sweeney, Price, et al over the period extending from 1954 to 1960. Recently, this laboratory has examined the natural resistance of tropical woods to marine-borer attack. This work was reported at an international conference in Cannes in May 1965, (Southwell, et al). For a 14-month exposure period marine-borer resistance of 114 different woods ranged widely from complete protection to complete destruction. The perfect timber, resistant to all marine-borer species and occurring plentifully and in large sizes was not found. Only one species, Dalbergia retusa (cocobolo), was highly resistant in all environments. However, its potential as a commercial timber seems limited. Teredo healdi, which occurs in brackish waters, was found to be more destructive than the marine borers in the ocean and many of the well-

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known borer resistant woods such as ekki, greenheart and acapu, which were practically unattacked in the tropical ocean, were damaged by Teredo healdi. Several relatively unknown Panamanian woods, other than Dalbergia retusa, such as Chrysophyllum cainito and Bouteria campechiana, were resistant to marine borers over the period tested. One fast growing, low density resistant species was Cordia alliodora (laurel negro). It was noted that a few hard, heavy woods are able to resist tropical fungi for a great number of years. Sound trees were still standing after 50 years of partial inundation in Gatun Lake and were identified as Tabebuia guayacan, Swartziawé panamensis and Manilkara darianensis.

The Naval Civil Engineering Laboratory Harbour Screening Programme was begun in Hueneme Harbour in 1955 and in Pearl Harbour in 1957. No new test panels have been added at these locations since 1961 and 1963, respectively. A total of about 200 treating systems have been exposed and about 30 are still being exposed at Pearl Harbour. These systems were not all chemically different from one another because some contained the same components but in different concentration.

Over two-thirds of the systems did not preserve wooden panels as well as the standard creosote treatment. About 10% appeared to be equal as effective as creosote. Another 10-15%, however, appeared to be superior to creosote.

Very few single chemical treatments are effective marine borer deterrents. Among these are high concentrations of copper salts and some organotin compounds. Most effective treatments consist of a combination of ingredients, each of which is effective against one borer genus or family. Among the Limnoria deterrents are copper compounds, chlorinated hydrocarbons and organomercury compounds. Creosote, organotin compounds and the triphenylmethane dye malachite green are especially effective against Teredo and Bankia, and somewhat less effective against the pholad Martesia.

Marine-borer studies at the Forest Products Laboratory, Department of Forestry and Rural Development, Vancouver, B.C., were in abeyance in 1967 and 1968 owing to the absence of key personnel, but will be resumed during the summer of 1969. In the period 1966 to 1968, Bramhall at this station has published on inspection procedures for marine piling. During the demolition of the Pacific Dry Dock pier, North Vancouver, after 25 years service, the penetration and concentration of creosote in several hundred piles was assessed, the degree of marine-borer attack noted, and, as far as possible, the course of failure determined in the piles suffering damage. The data will be analysed in 1969 in an attempt to define minimum penetrations and retentions of creosote which protect marine piles.

Marine-borer operations at the British Columbia Research Council, Vancouver, Canada, during 1968 were valued at $145,712 compared to $119,166 in 1967. In 1968, sponsored support accounted for $109,441, BCRC financing $36,271. Of the total expenditure, $61,700 was for research, $84,012 for technical services. The total number of persons employed on marine borer work continuously was eight. Plans for 1969 include expansion of the monitoring studies for marine borer attack, sonic testing of marine piling, and development and possible commercial application of underwater coatings for wood. However, coatings for marine piling probably will not be applied commercially until 1970.

During 1968 the number of test sites for the monthly recording of marine borer attack was increased from 71 to 107. New sites were established at Sitka, Alaska, San Francisco and San Diego on the west coast; at four locations in Nova Scotia, one in Connecticut and ten in the Port of New York area on the east coast; and at four locations in the Port of New Orleans on the south coast. With the exception of the four sites in Nova Scotia which are experimental, all of the 107 test sites are associated with marine-borer protection systems for commercial logs, dry-docks, marine installations or for indicating the rate of ingress into harbours where this is expected as a result of pollution abatement. The system for monitoring marine borer attack is described in a paper by Walden, et al, (1967).

During 1968, three dry-docks were added to the treatment programmes so that now 17 dry-docks on the Pacific coast with a total capacity of 163,500 tons are being protected against marine borer attack. Intensity of attack is greatest in the Puget Sound area, where breeding of Bankia setacea has been continuous over the past 15 years, winter and summer.

During 1968, 9,500 piles were tested sonically for residual strength. These piles were associated with wharves, piers, and bridges located on the west, east and southern coasts of North America and in the Hawaiian Islands. This method of testing, described in a paper by Walden and Trussel, (1965) determines pile strength by the extent a sonic signal is weakened as it passes down a length of the piling.

During the year, laboratory studies were undertaken to evaluate the resistance to continuous leaching of modified creosotes prepared in the BCRC laboratories. Other test coupons were impregnated and immersed, containing added organolead compounds, supplementing those organolead impregnated coupons already in the water at test sites at Vancouver and Alameda, California. Impregnation and exposure test of compounds, that had shown earlier resistance to marine borer attack, of coupons, timbers and logs is being continued. Such impregnants included organolead compounds and formulations of zinc and chromium compounds. Immersions were also continued with various modified creosotes.

Also in 1968, a two-man team of biologist-divers undertook a comprehensive inspection of those areas of Canada's Atlantic Coast presently supporting commercial marine installations. Limnorial activity

(L. ignorum only) was observed in varying intensity along the coasts of Nova Scotia, New Brunswick, Prince Edward Island, the upper and lower Gulf of St. Lawrence coasts (Quebec) and the southern and south east coasts of Newfoundland. Teredo activity (T. navalis) was limited to the northern New Brunswick coast, Prince Edward Island, the Northumberland Strait and Cape Breton Island. A detailed report has been prepared and a shorter manuscript is to be submitted to the Journal of the Fisheries Research Board of Canada in the immediate future.

A coating was developed during 1968 which can be applied underwater to wood and sets as a firm but resilient coating. This coating is intended to be applied to standing piling and other permanent wooden installations which are already in the sea and which are subject to attack either because of increased water salinity, or because of increased oxygen levels. The coating is particularly useful for application to creosoted wood to protect it against attack by Limnoria tripunctata. With slight modification of the coating, it is equally useful for application to steel underwater, or wet or dry steel in air to protect it against corrosion. These coatings are currently undergoing performance testing; it is planned that the steel coating will be ready during 1969, the wood coating in 1970. For the application of the coating to piling, a special applicator is being constructed by a company closely associated with the BCRC in marine-borer protection work.

Biochemical studies were continued on the digestive metabolism of cellulose and its derivatives in the caecum of the marine borer, Bankia setacea. In this work the presence of the Embden-Meyerhoff pathway, the pentose cycle, and the citric acid cycle and the non-triose pathway have been demonstrated in the marine-borer caecum. Altogether twenty-five enzymes, including several key enzymes in the above pathways, were identified. An important observation has been the absence of any cellulolytic bacteria in the caecum of the marine borer. An offshoot of these studies has been the development of a quantitative spectrophotometric assay for cellobiase. Publications covering this recent work are Liu and Townsley (1968), and Liu and Walden (1969).

In Europe, Kühne has continued his studies of the natural durability of tropical timber species. The results (Kühne, 1968a) show that of three timber species frequently used for marine structures, heartwood of Basralocus (Dicorynia paraensis Benth.) and Bongoossi (Lophira procer a A. Chev.) proved to be very resistant against Limnoria. The heartwood of Demerara Greenheart (Nectandra rodiaei (Schomb.) Hook) was slightly attacked, while the surface of the sapwood test blocks was heavily riddled by Limnoria. Dr. Kühne (1968b) has prepared a document for the group, reviewing recent work on the natural durability of tropical timber to marine borers.

The Division de preservation des bois, du Centre Technique Forestier Tropical, France, has continued with its work of testing tropical timbers for natural resistance to marine borers. They are also working on the efficiency of various preservatives (Cr-Cu-As; Cr-Cu-B; Cr-Cu-F; Cr-Cu). This work is being carried out in France at the port of La Pallice (Atlantic coast) and at La Cadière and Port-Saint-Louis-du-Rhone (Mediterranean coast). In the tropics, sites are situated at Abidjan (Ivory Coast), Douala (Camerones) and Mahatsora and Fort-Dauphin (Madagascar). The results have been published by Fougerousse (1967) and Fougerousse and Deschamps (1968).

The Timber Research and Development Association has completed an experimental trial on the effectiveness of organo-tin preservatives against marine borers. Twelve formulations have been tested over a period of 7 years. Each formulation was applied to six beech blocks (150 x 80 x 50 mm). A report on this work will be published shortly.

TRDA have continued with trials in co-operation with the British Wood Preservation Association involving preservative screening trials at Shoreham (Eades, 1966) and preservative practical trials at Poole (Anon, 1969).

Natural durability trials have been in progress for 18 years at Shoreham harbour. This trial, which will be completed in September, 1969, has involved testing 95 timber species for their resistance to marine borers.

Hol and Drift (1968) prepared a literature survey concerning the preservation of wood for use in a marine environment and in cooling towers, with organic solvent type preservatives. There are only two references listed for 1967/1968.

During the year 1968/1969 two important symposia were held at which papers on marine biodeterioration were presented. The first was the 2nd International Congress on Marine Corrosion and Fouling held at Athens. Papers of interest were delivered by Nair, Deschamps, Nagabushanan and BalaSubramanayan, Ranvindr, Unlilikosmar Nair and Pillai. Southampton was the venue of the 1st International Biodeterioration Symposium. Becker and Haderlie in their papers dealt with marine borers and two papers on marine fungi were presented by Meyers and Jones.

The role of marine fungi in the breakdown of wood in the sea also comes within the framework of the groups activities. Papers published by Cavaliere (1968), Hughes (1968), Johnson (1968), Kirk (1969b), Kohlmeier (1968a, b, c, d) and Tubaki (1968, 1969) are largely taxonomic, while those of Kirk (1969a), Jones (1968a, b; 1969) and Meyers (1968a) deal with the ecology of marine fungi.

Dr. S. P. Meyers continues his work on the degradative activities of filamentous marine fungi. This work shows that certain marine fungi cause striking weight losses (50% within 3 weeks) of a cellulose substrate concurrent with fungal growth. Noteworthy differences among taxa are seen, with intensive degradative activity noted in the neutral to alkaline pH range. These analyses support previous and current field evidence on fungal infestation of wood, and document
the significance of these organisms, especially the *Lulworthia floridana* group, in the early colonization of submerged wood.

Physiological and ecological work on marine fungi continues at Portsmouth College of Technology. Jones, Byrne and Alderman (1968) have presented data on the salinity requirements of marine and terrestrial fungi. They show that the Ascomycetes and aquatic Phycycomycetes may be sensitive to changes in salinity, while the Fungi Imperfecti and Mucoraceous fungi are more tolerant to such changes. Experimental work has commenced on the tolerance of marine fungi to various preservatives as well as their ability to breakdown cellulose and wood.

References


The utilisation for growth and the degradation of p-hydroxybenzoate esters by bacteria.

THE UTILISATION FOR GROWTH AND THE DEGRADATION OF P-HYDROXYBENZOATE ESTERS BY BACTERIA

E. G. Beveridge and A. Hart

Summary. The aliphatic esters of p-hydroxybenzoic acid were utilized as carbon sources for growth by only one of eight bacteria known to degrade other aromatic compounds. However, suspensions of six of these bacteria grown on media containing succinate or succinate and ester metabolized the esters with varying ability in the respirometer. Numerous bacteria which utilized the esters as carbon sources for growth were isolated from natural habitats.

L'utilisation pour la croissance a la dégradation des esters de p-hydroxybenzoate par les bactéries. Les esters aliphatiques de l’acide p-hydroxybenzoïque ont été utilisés comme sources de carbone pour la croissance d’une seule bactérie parmi les huit connues pour décomposer les autres composés aromatiques. Cependant, des suspensions de six d’entre ces bactéries cultivées sur des milieux contenant des succinates ou des succinates et des esters, métabolisait les esters selon des degrés variables dans le respiromètre. De nombreuses bactéries qui utilisaient les esters comme sources de carbone pour leur croissance ont été isolées de leurs habitats naturels.

Introduction

The lower members of the p-hydroxybenzoate aliphatic ester series are used to protect various pharmaceutical and cosmetic formulations against microbial spoilage. Reports of microbial degradation of these esters are few although p-hydroxybenzoic acid itself is known to be metabolised by many micro-organisms. Sokoloski, Chidester and Honeywell (1962) detected the hydrolysis of methyl p-hydroxybenzoate (0.2% w/v) by Cladosporium resinae in a contaminated pharmaceutical suspension. An antimicrobial vehicle for the preparation of eye-drops which contained methyl and n-propyl p-hydroxybenzoates (0.023 and 0.011% w/v respectively) supported the growth of Pseudomonas aeruginosa NCTC 7244 with degradation of the esters (Hugo and Foster, 1964). Beveridge and Hart (1969) and Beveridge and Arter (1969) reported briefly a similar utilisation of these and other esters at previously recommended preserved concentrations by a variety of bacteria from natural habitats although growth in general was poor.

Experimental Methods

Esters of p-hydroxybenzoic acid were incorporated (0.01% w/v) as sole carbon sources in a simple mineral salts medium (Beveridge and Tall, 1969) containing either succinate alone (0.5% w/v) or succinate (0.5% w/v) and methyl or ethyl p-hydroxybenzoate (0.01% w/v) as carbon sources. Higher concentrations of esters resulted generally in poor yields of cells. The cells were collected in quarter strength Ringer’s solution, washed 3 times and the final suspensions adjusted to contain the equivalent of 12 mg dry wt of cells/ml.

Bacteria for manometric studies were grown for 18h at 30° on a solid mineral salts medium (Beveridge and Tall, 1969) containing either succinate alone (0.5% w/v) or succinate (0.5% w/v) and methyl or ethyl p-hydroxybenzoate (0.01% w/v) as carbon sources. Higher concentrations of esters resulted generally in poor yields of cells. The cells were collected in quarter strength Ringer’s solution, washed 3 times and the final suspensions adjusted to contain the equivalent of 12 mg dry wt of cells/ml.

Manometric studies were performed with constant volume respirometers and single sidearm flasks (Umbret, Burris and Stauffer, 1964). Each flask contained 0.5 ml of 100 mM phosphate buffer (pH 7.0) and 1.0 ml of ester solution (1.0 mM) in the main compartment, 0.2 ml of KOH (20% w/v) solution in the centre well and cell suspension (0.5 ml) in the sidearm. The gas phase was air and the bath temperature 30°. O₂ represents the oxygen uptake in the presence of ester after subtraction of endogenous uptake, the units being μl O₂ uptake (STP)/mg dry wt cells/h.

Where active metabolism of the ester occurred the QO₂ was calculated for the period of metabolism, otherwise it was calculated from the overall uptake during the experiment. After 10h incubation the flask contents were examined at 295nm (after removal of cells and dilution with 0.1M NaOH) to assess any metabolism of the esters.

Results and Discussion

Ps. aeruginosa NCTC 7244 utilized the methyl, ethyl, n-propyl, iso propyl, n-butyl, iso butyl, sec butyl, tert butyl, n-amyl and iso amyl esters of p-hydroxybenzoic acid as sole carbon sources for growth in the mineral salts medium. Growth was sparse, and with the methyl ester it was extremely poor. The amyl esters were utilized at 0.005% w/v but higher concentrations were inhibitory. None of the bacteria

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(listed in Table 1) known to degrade other aromatic compounds showed growth in media containing the methyl, ethyl, n-propyl or n-butyl esters. Failure to grow was not due to ester toxicity since all the bacteria grew when asparagine (0.1% w/v) was also included in the media.

Suspensions of bacterium NCIB 8250 grown with succinate alone avidly oxidised the esters in the respirometer after short, but well defined lag periods. Inclusion of methyl or ethyl p-hydroxybenzoate in the growth medium resulted in suspensions adapted to ester metabolism, the ethyl ester proving a slightly more efficient inducer than the methyl ester under these conditions. The oxygen uptake increased with the size of the substrate molecule (Table 1 and Fig. 1). Spectrophotometric examination of flask contents after the oxygen uptake had returned to that of the endogenous uptake revealed complete disappearance of the esters.

Suspensions of the other bacteria metabolized the esters in the respirometer with varying abilities (Table 1). In general the rates of oxidation were too low to determine with clarity whether succinate grown cells exhibited a lag phase before metabolism, or whether cells grown in the presence of the esters were adapted to ester metabolism. Ps. aeruginosa NCTC 7244, displayed only a meagre performance in the respirometer despite its ability to utilize the esters for growth. Pseudomonas desmolyticum NCIB 8859 oxidized slowly methyl and ethyl p-hydroxybenzoates with the accumulation of a metabolite having a greenish colour and a distinctive ultraviolet spectrum (Fig 2). With some bacteria O₂ uptakes greater than the normal endogenous uptake, were observed although spectrophotometric examination did not indicate ester metabolism and it is suggested that in these cases the esters had, in fact, stimulated endogenous activity. Those bacteria which metabolized the esters in the respirometer, although failing to utilize them for growth,

<table>
<thead>
<tr>
<th>Organism</th>
<th>Substrate (I μ mole)</th>
<th>Qₒ₂</th>
<th>Metabolism* (percent)</th>
<th>Qₒ₂</th>
<th>Metabolism* (percent)</th>
<th>Qₒ₂</th>
<th>Metabolism* (percent)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ps. aeruginosa NCTC 7244</td>
<td>a</td>
<td>3.5</td>
<td>16</td>
<td>0</td>
<td>0</td>
<td>1.0</td>
<td>9</td>
</tr>
<tr>
<td></td>
<td>b</td>
<td>2.4</td>
<td>30</td>
<td>0</td>
<td>0</td>
<td>1.2</td>
<td>14</td>
</tr>
<tr>
<td>Pseudomonas sp. NCIB 8858</td>
<td>a</td>
<td>3.0</td>
<td>36</td>
<td>3.4</td>
<td>36</td>
<td>3.7</td>
<td>17</td>
</tr>
<tr>
<td></td>
<td>b</td>
<td>3.4</td>
<td>44</td>
<td>3.3</td>
<td>27</td>
<td>3.8</td>
<td>17</td>
</tr>
<tr>
<td>Ps. desmolyticum NCIB 8859</td>
<td>a</td>
<td>3.0</td>
<td>71</td>
<td>3.4</td>
<td>60</td>
<td>4.2</td>
<td>64</td>
</tr>
<tr>
<td></td>
<td>b</td>
<td>5.1</td>
<td>84</td>
<td>3.6</td>
<td>62</td>
<td>5.5</td>
<td>73</td>
</tr>
<tr>
<td>Ps. fluorescens NCIB 8249</td>
<td>a</td>
<td>2.8</td>
<td>0</td>
<td>3.4</td>
<td>0</td>
<td>3.7</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>b</td>
<td>3.4</td>
<td>0</td>
<td>3.3</td>
<td>0</td>
<td>3.8</td>
<td>0</td>
</tr>
<tr>
<td>Ps. fluorescens NCIB 8251</td>
<td>a</td>
<td>0.8</td>
<td>0</td>
<td>1.7</td>
<td>0</td>
<td>1.4</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>b</td>
<td>1.5</td>
<td>0</td>
<td>1.7</td>
<td>0</td>
<td>2.0</td>
<td>0</td>
</tr>
<tr>
<td>Ps. aeruginosa NCIB 8704</td>
<td>a</td>
<td>1.1</td>
<td>0</td>
<td>0.9</td>
<td>8</td>
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<td>14</td>
</tr>
<tr>
<td></td>
<td>b</td>
<td>1.2</td>
<td>0</td>
<td>0.9</td>
<td>7</td>
<td>1.5</td>
<td>22</td>
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<tr>
<td>Ps. fluorescens NCIB 8248</td>
<td>a</td>
<td>3.0</td>
<td>0</td>
<td>3.1</td>
<td>0</td>
<td>3.3</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>b</td>
<td>2.8</td>
<td>0</td>
<td>3.2</td>
<td>0</td>
<td>3.0</td>
<td>12</td>
</tr>
<tr>
<td>bacterium NCIB 8250</td>
<td>a</td>
<td>8.2</td>
<td>100</td>
<td>9.2</td>
<td>100</td>
<td>8.5</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td>b</td>
<td>12.8</td>
<td>100</td>
<td>10.9</td>
<td>100</td>
<td>15.6</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td>c</td>
<td>16.8</td>
<td>100</td>
<td>20.7</td>
<td>100</td>
<td>22.6</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td>d</td>
<td>14.5</td>
<td>100</td>
<td>11.7</td>
<td>100</td>
<td>13.6</td>
<td>100</td>
</tr>
</tbody>
</table>

a, b, c, d, methyl, ethyl, n-propyl and n-butyl p-hydroxybenzoates respectively.

*assessed at 295nm.
The Utilisation for Growth and the Degradation of p-Hydroxybenzoate Esters by Bacteria.

Figure 1
Metabolism of p-hydroxybenzoate esters in the respirometer by bacterium NCIB 8250. Oxidation of 1 umole ester by cells grown on: a, succinate alone; b, succinate and ethyl p-hydroxybenzoate: O---O methyl p-hydroxybenzoate; ×-× ethyl p-hydroxybenzoate; □-□ n-propyl p-hydroxybenzoate; ●-● n-butyl p-hydroxybenzoate. O₂ uptakes are corrected for endogenous uptake.

Figure 2
UV spectrum of metabolite accumulating during the oxidation of methyl p-hydroxybenzoate in the respirometer by *P. desmolyticum* NCIB 8859. --- metabolite; ----- methyl p-hydroxybenzoate; solvent, 0.1M NaOH.
The utilisation for growth and the degradation of p-hydroxybenzoate esters by bacteria.

Table 2 The utilisation of p-hydroxybenzoate esters by bacteria

<table>
<thead>
<tr>
<th>Source of inoculum</th>
<th>Methyl P-hydroxybenzoate (0.01% w/v)</th>
<th>Ethyl P-hydroxybenzoate (0.01% w/v)</th>
<th>n-Propyl P-hydroxybenzoate (0.01% w/v)</th>
<th>n-Butyl P-hydroxybenzoate (0.01% w/v)</th>
<th>n-Amyl P-hydroxybenzoate (0.005% w/v)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Soil extract</td>
<td>Alcaligenes sp.*</td>
<td>Ps. fluorescens</td>
<td>Alcaligenes sp. Bacillus sp.</td>
<td>Alcaligenes sp. Ps. aeuriginosa Pseudomonas sp.</td>
<td>Alcaligenes sp.†</td>
</tr>
<tr>
<td>Drain water (1)</td>
<td>Aeromonas sp. Alcaligenes sp.</td>
<td>Ps. alcaligenes Ps. fluorescens Ps. acitivorans group</td>
<td>Ps. aeuriginosa* group</td>
<td>Ps. acidivorans* Ps. aeruginosa group</td>
<td>Alcaligenes sp.</td>
</tr>
<tr>
<td>Drain water (2)</td>
<td>Ps. aeuriginosa Ps. fragi</td>
<td>Ps. aeuriginosa Pseudomonas sp. Aeromonas sp.</td>
<td>Ps. aeuriginosa Ps. fragi</td>
<td>Ps. fragi Pseudomonas sp.</td>
<td>NT</td>
</tr>
<tr>
<td>River water</td>
<td>Alcaligenes sp. Ps. fluorescens</td>
<td>Ps. fragi Acinetobacter sp.</td>
<td>Alcaligenes sp. Actinobacillus sp.</td>
<td>Alcaligenes sp.</td>
<td>Alcaligenes sp.</td>
</tr>
<tr>
<td>Coke works effluent</td>
<td>Ps. alcaligenes group Ps. aeuriginosa Enterobacter sp.</td>
<td>Ps. alcaligenes group</td>
<td>Alcaligenes sp. Actinobacillus sp.</td>
<td>Ps. acidivorans group Alcaligenes sp.</td>
<td>Alcaligenes sp.</td>
</tr>
<tr>
<td>Ditch water</td>
<td>Ps. aeuriginosa Ps. fragi</td>
<td>Ps. fragi Pseudomonas sp.</td>
<td>Ps. fragi</td>
<td>Ps. aeuriginosa Ps. fragi</td>
<td>NT</td>
</tr>
</tbody>
</table>

*0.005% (w/v) substrate; † 0.0025% (w/v) substrate; NT not tested.

must produce metabolites which are unable to enter successfully their biosynthetic or energy yielding pathways.

All the habitats examined contained a variety of bacteria which grew readily in media containing the esters as sole carbon sources. Members of the Pseudomonas and Alcaligenes families were particularly prevalent (Table 2). To some extent the organisms isolated varied with the particular ester used as the growth substrate.

These findings suggest that the p-hydroxybenzoate esters are not unduly resistant to microbial degradation. Since the brief reports of Beveridge and Hart (1969) and Beveridge (1969) also showed metabolism to occur, although poorly, at concentrations used for preservation of aqueous solutions, the possibility of degradation should be considered when selecting the esters as preservatives. The high incidence of ester-degrading Pseudomonads with their nutritional simplicity and bio-chemical versatility in attacking many ingredients of pharmaceutical and cosmetic formulations might also be worthy of note.

We are grateful to the Medical Research Council for a Grant in Aid and one of us (A. H.) thanks Sunderland Education Authority for a Research Assistantship.

References


GROWTH OF THERMOPHILIC FUNGI ON OXIDATION PRODUCTS OF POLYETHYLENE

J. Mills and H. O. W. Eggins

Summary. Thermophilic microorganisms have been isolated from samples of self-heating refuse, rotting straw and pasture land soil. The isolated thermophilic fungi have been grown individually on the partial oxidation products of polyethylene at 40°C and 50°C and the growth pattern of Aspergillus fumigatus on these products has been demonstrated at different pH values.

La croissance des champignons thermophiles sur les produits de l’oxydation de polyéthylène. Des microorganismes thermophiles ont été isolés d’échantillons de détritus fermentés, de paille pourrisante et de sol provenant de paturages. Les champignons thermophiles ainsi isolés ont été cultivés individuellement sur les produits de l’oxydation partielle de polyéthylène à 40°C et à 50°C et les courbes de croissance d’Aspergillus fumigatus sur ces produits ont été notées à des valeurs différentes de pH.

Introduction

From a survey of the considerable amount of literature available today on the biodeterioration and biodegradation of synthetic “plastic” materials (Mills, 1969 unpublished report) it would appear that the evidence indicates that the polymeric constituents of the major large scale produced plastics (polyethylene, polypropylene, P.V.C. etc.) are extremely resistant to microbiological degradation. Biodegradable plastics, in fact, would not be desirable in situations where the immunity of plastics from attack provides one of their major advantages; however the need for biodegradable packaging plastics is increasing, especially when the waste from these materials must be disposed of by tipping or composting.

There are many problems associated with the production of a biodegradable plastic; it must be cheap to produce and fabricate, have comparable physical properties, but more importantly it must not be susceptible to degradation during its commercial life. Only when it arrives at the disposal stage, where certain parameters of temperature, pH and moisture are controllable, should it then become degradable.

An alternative approach would be to pretreat existing non-degradable waste plastic, at the refuse stage, to render the inert polymeric constituents more susceptible to biodegradation upon tipping or composting. One method of pretreating polyethylene-type plastics is to partially oxidise the plastic, breaking down the high molecular weight polymers into smaller molecules with the introduction of oxygen-containing groupings into the products (Whittaker and Forsyth, 1946).

This paper reports the utilisation of low molecular weight carboxylic acids, produced upon partial oxidation of polyethylene, for the growth of thermophilic fungi commonly found in refuse and composting systems.

Materials and Methods

A sample of town refuse, collected 14 days after dumping, and which was vigorously self-heating at the time of collection was taken from a municipal refuse dump near Birmingham, England. Small pieces of this sample, together with small pieces of rotting straw chaff and soil from a pasture land known to contain thermophilic fungi (Eggins and Malik, 1969.) were plated out on Eggins and Pugh (E. & P.) cellulose and glucose agars (Eggins and Pugh, 1962) using the methods of Warcup (1950) and Waksman (1916), and incubated at 50°C in high humidity incubators. Developing thermophiles were isolated and maintained in pure culture on cellulose, glucose and Yp Ss (Cooney & Emerson, 1964) agars.

Results

The results indicate the wide distribution of the thermophilic fungi and actinomycetes in nature.

The composting refuse samples when plated out on E. & P. cellulose and glucose agars yielded Chaetomium thermophile, Humicola inoletus, Humicola grisea and Mucor pusillus within the first two days of incubation, whilst Malbranchea pulchella var. sulphurea, Talaromyces dupontii and Aspergillus fumigatus were isolated from the glucose agar after four days of incubation. The original sample of refuse was collected 14 days after tipping and was probably in the “plateau” phase of self heating (Chang and Hudson, 1967). Several thermophilic actinomycetes were also isolated especially from the rotting straw chaff. Therm actinomycetes vulgaris, an organism widely implicated in farmer’s lung disease (Cross, 1968; Festenstein et al., 1970).

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1966; Pepys et al., 1963.) was characterised by its white colonies bearing single spores on both aerial and substrate hyphae and by its high resistance to the antibiotic novobiocin in half strength nutrient agar (Cross, 1968.) A cellulolytic actinomycete was isolated from the rotting straw chaff and from the pasture land soil. This organism produced good clearing of the ball-milled cellulose in the E. and P. cellulose agar at 50°C.

Preparation of the oxidation products of Polyethylene.

The low molecular weight oxidation products were prepared by boiling 10 g. I.C.I. “Alkathene” polyethylene (molecular weight range 10,000 to 50,000) with 200 ml. of 60% nitric acid for 24 hours. The reaction was carried out in a round bottom flask fitted with a double water-cooled condenser and a device for the safe elimination of fumes. This method is reported to produce dicarboxylic acids of a mean molecular weight of 250 containing a small proportion of combined nitrogen in the form of nitro-groups (Whittaker and Forsyth, 1946.) The acids were precipitated by diluting the resultant solution with distilled water and appeared as waxy droplets. The emulsion was then shaken vigorously in a separating funnel with large quantities of diethyl ether to extract the acids. The ether layers were separated off, washed with distilled water to remove traces of nitric acid and reduced in volume in a rotary evaporator at 30°C under vacuum. The resultant waxy acids were then converted to their potassium salts by adding 250 ml of 0.2 N potassium hydroxide.

Growth Studies

Growth studies were carried out in 250 ml. Erlenmeyer flasks each containing 50 ml. of medium. The growth medium was an Eggins and Pugh mineral salts solution (Eggins and Pugh, 1962) containing 1% (v/v) of the carboxylic acids solution. The pH of the medium was adjusted to pH 6.4 with 0.1 N potassium hydroxide or hydrochloric acid. Controls consisted of E. and P. mineral salts solution at pH 6.4. All flasks were autoclaved at 115°C for 20 minutes. Inocula consisted of mycelia cut with a sterile 2 m.m. diameter nichrome wire loop. Ten of the above isolated thermophilic fungi were used and the flasks were incubated at 40°C and 50°C for 19 days. Two replicates were made at each temperature and after incubation the mycelia were filtered off, washed with boiling distilled water and dried to constant weight in a hot air oven at 85°C.

Results

The mean mycelial dry weight of the individual thermophilic fungi tested at 40°C and at 50°C are indicated in Figures 1. and 2.

The results indicate that all the thermophilic fungi tested can utilise the oxidation products of polyethylene for growth to some extent. Malbranchea pulchella, Mucor pusillus, Cephalosporium and Sporotrichum thermophile produced good growth at 40°C with a maximum mycelial dry weight of 35 mg. for one flask of M. pulchella. At 50°C only M. pulchella showed good growth with a maximum dry weight of 23 mg. being recorded. Fungi producing better growth at 50°C than at 40°C were H. lanuginosa, H. grisea and

Key to Figures 1 and 2.

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Figure 2

Key to Figures 1 and 2.

---

Figure 3 *Aspergillus fumigatus* 40°C incubation temperature.

Talaromyces dupontii. Chaetomium thermophile produced little growth at either temperature. No growth was recorded for any of the fungi tested in the controls of mineral salts solution at 40°C and 50°C.

Growth of A. fumigatus at different pH values.

It was noted that growth of Aspergillus fumigatus was quite good at both temperatures and that sporulation was clearly visible in all flasks. Since this fungus is widely distributed in soils and in self heating and composting systems it was decided to test its growth on the oxidation products at different pH values.

It has been reported that the utilisation of monocarboxylic acids by Cunninghamella echinulata Thaxter was dependent upon the chain length of the acid and upon the pH of the nutrient medium (Lewis and Johnson, 1967). In this study the pH of the E. and P. nutrient salts medium containing 1% (v/v) carboxylic acids solution was adjusted in value from pH 3 to pH 9 with 0.1 N potassium hydroxide or hydrochloric acid. Controls consisted of flasks of E. and P. nutrient salts adjusted in value from pH 3 to pH 9. All flasks were autoclaved at 115°C for 20 minutes and inocula consisted of mycelia cut with a 2 mm. diameter nichrome wire loop and of one 2 mm. diameter loopful of spore suspension in distilled water. The flasks were incubated for 19 days at 40°C, and growth was recorded as dry weight of filtered mycelium produced by mycelial inocula and by spore inocula. Results are indicated in Figure 3.

Results

The results indicate that growth of A. fumigatus is better at neutral and slightly alkaline pH values than at lower acidic pH values. Spore germination was not inhibited by the carboxylic acids at any pH value tested and mycelial dry weight produced by the spore inocula was roughly equivalent to that produced by the mycelial inocula, except at pH 9 when spore germination was depressed. No growth was recorded in any of the control flasks of nutrient salts alone.

Discussion

It would appear that oxidation products of polyethylene can be used by thermophilic fungi as a source of carbon for growth under various conditions of temperature and pH.

Concern is growing about the increasing amounts of packaging plastics and polythene film ending up in town refuse (Abrahams, 1969; Davis, 1969; "Threat from plastics", The Times, September 10th, 1969.). It appears that some sort of pre-treatment of the plastics in refuse, though not necessarily nitric acid digestion, is needed to render the inert polymeric constituents of the plastics more susceptible to biodegradation under thermophilic (composting) conditions. This study has shown that oxidation products of polyethylene can be biodegraded by individual organisms commonly found in composting refuse.

Acknowledgement

We would like to thank the National Research Development Corporation, Victoria Street, London S.W.1, for financial assistance and generous support of this project but wish to make it clear that the opinions stated here are those of the authors and not necessarily of the N.R.D.C.

References


Results

The thermophilic microorganisms isolated from the three sources are listed in Table I.

<table>
<thead>
<tr>
<th>Organism</th>
<th>Refuse</th>
<th>Pasture Land</th>
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<tr>
<td>Aspergillus fumigatus</td>
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<td>+</td>
</tr>
<tr>
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<td>+</td>
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<td>Humicola lanuginosa</td>
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<tr>
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<td>+</td>
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<tr>
<td>Mucor pusillus</td>
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<td>+</td>
<td>+</td>
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<tr>
<td>Malbranchea pulchella var. sulphurea</td>
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<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Sporotrichum thermophile</td>
<td>-</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Cephalosporium sp.</td>
<td>-</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Thermoactinomyces vulgaris</td>
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<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Streptomyces thermoviolaceus var. pingens</td>
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<td>-</td>
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<td>Cellulolytic Actinomycete</td>
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<td>+</td>
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<tr>
<td>Streptomyces sp.</td>
<td>+</td>
<td>-</td>
<td>+</td>
</tr>
</tbody>
</table>

**Table I.**

+  Microorganisms isolated
-  Microorganisms not isolated
A BIODETERIORATION APPRAISAL OF SILICONES


Summary. Various silicones were microbiologically screened for biodegradability toxicity and hydrophobicity and proved to be inert and stable in all respects to the applied cultural conditions. These water-repellents were examined for any natural preservative qualities of model woods and found to be ineffective.

The evaluation of a polymeric material for possible applications is a complex task. Selection of potential preservatives requires a realisation of a chemical's suitability as regards its chemical, physical, economic and biological attributes. The following work is concerned with the biological aspects of silicones from which it is hoped that specific application areas can be determined for further detailed research.

A biological evaluation demands such considerations as the chemical's resistance to biodeterioration or detoxification, its toxicity or inhibitory limits against microorganisms and humans and its constancy of properties when compounded with other chemicals. Only after such research has vindicated a chemical can its value in some aspect of biodeterioration be determined.

Silicones are organo-polysiloxanes with chemical properties resembling both organic polymers and mineral silicates. Their molecular configuration is characterised by alternate silicon and oxygen atoms with hydrocarbon radicals directly attached to the silicon atom. They have a variety of unique chemical and physical properties, as reflected in their numerous technological applications in such forms as fluids, lubricants, greases, elastomers and resins.

For biological systems the silicone's chemical inactivity, stable physical qualities and remarkable physiological inertness (Levin, 1958) have already made them important. Their weakness is high cost (Hardy, 1947) but this could be lessened in a biodeterioration system where low chemical concentrations are used to preserve large masses of economically important materials and where such chemicals are becoming more selective.

Therefore, the potential of silicones warrants a microbiological assessment.

Assessment of Biodegradability

The first report on silicones (Greathouse, Wessel and Shirk, 1951) stated that silicone resins and rubbers were resistant to fungal decay.

Silicone varnishes were found to be fungus resistant (Glazer, 1954) because of their low percentage of organic material, their water-repellency and their good film continuity.

By treating textiles with polyalkyl hydrosiloxanes alkyl acyloxysilones and polyalkyl siloxanolates of certain metals in conjunction with impregnation by copper and chromium salts has resulted in water-repellant finishes which are resistant to weathering, chemical cleaning and biological attack (Voronkov and Kalugin, 1959).

When the vulnerability of polymers to deterioration by microorganisms was listed (Hueck, 1960) it was found that silicones have good resistance.

Later work by Olson, Langston and Rainey (1962) showed the silicones increase the resistance to microbiological attack of cotton fabrics without radically altering the properties of the material.

Cellulose textiles are described as being made water repellent, weather, flame and rot resistant in a patent (Bullock and Welch, 1962) when treated with polyvinyl chloride polymers, zirconium acetate and methyl hydro polysiloxane.

It has been shown (Ross, 1963) that a silicone rubber containing a dibutyl tin laurate catalyst supports fungal growth after one week of incubation at 29°C and 95% humidity. A surface coating of copper-8-quinolino sine suppressed fungal growth, as did p-chloro-m-xyleneo1 over 2% concentration. Similar results, however, were obtained using polyurethane instead of silicone.

1Biodeterioration Information Centre, University of Aston in Birmingham, 80, Coleshill Street, Birmingham 4, England.

(Copy received February 1970.)

R.T.V. 102 and 108 silicone rubber was attached by Calderon and Staffeld (1965) to glass slides and then buried in various soils for up to 107 days at 30°C and 95% R.H. Both rubbers were similarly colonized. Streptomyces were particularly prevalent in sandy soil and included *Streptomyces albus*, *S. globisporus*, *S. rochei*, *S. novaevetaearea* and *S. acidophilus*. Fungi involved were *Fusarium* spp., *Aspergillus fumigatus*, *Spondylocadum* sp., *Cunninghamella echinulata*, *Phoma pigmentivora* and *Chaetomella* sp. It was concluded that silicone rubber is resistant to fungal and bacterial degradation but allows surface growth. The streptomycetes, however, appeared to utilise the silicone under certain conditions and in a private communication it was stated that the acetate catalyst probably acts as the nutrient.

When silicone rubber was tested (Muraoka, 1966) as an electrical insulation in a deep marine environment, it was found to perform satisfactorily for twelve months and then insulation resistance declined rapidly. This was caused by slight bacterial attack and water absorption.

A patent (Dow Corning Corporation, 1966) claims that a sealing preparation containing organopolysiloxanes and phenylmercuric carboxylic acids is resistant to fungi.

A review by Fessenden and Fessenden (1967) of the biological properties of silicone compounds mentions that MacDiarmid and Brown (1965) found a *Pseudomonas* sp. was capable of using phenylsilane and toluene as a nutrient.

Therefore, it appears that past literature indicates that pure silicones are stable towards microbiological decay and that any preparation appears capable of supporting surface growth. To confirm these impressions a series of investigations were undertaken whereby different silicones were subjected to varying environmental conditions.

**Methods and Results**

Three methods for culturing microorganisms that might possibly degrade silicones were used.

1. **Isolation from Naturally Enriched Soils.**

Soil that had been accidently splashed with silicones was obtained from near the Midland Silicones Plant, Barry, Wales. Soil particles were inoculated using Warcup's (1950) method onto glucose and glucose plus silicone agars. This agar, and all nutrients used in these investigations, was composed of the mineral salts used for Eggins and Pugh cellulose agar (1962); glucose was added as a 4% concentration and methyl silicone fluid was vigorously shaken into a just molten agar in the concentration of 2.5 mls. per 10 mls. of medium. Incubation of pour plates was at 25°C. for twenty days.

Isolated fungi were *Aspergillus fumigatus*, *A. niger*, *A. repens* and *Geotrichum candidum*. Since all these fungi grew on the non-silicone agar as well as the silicone ones and no observable changes in the silicone were seen it appears that the fungi are incidental colonizers.

**Fungal Species**

| 0.9cs | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| 1.0cs | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| 3.0cs | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| 20cs | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |

<table>
<thead>
<tr>
<th>+ GLUCOSE</th>
<th>+ GLUCOSE</th>
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</thead>
<tbody>
<tr>
<td>0.65cs</td>
<td>+</td>
</tr>
<tr>
<td>3.0cs</td>
<td>+</td>
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<tr>
<td>20cs</td>
<td>+</td>
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</tbody>
</table>

Table 1 Isolated organisms from percolated soils given a mineral salts solution and various methyl silicone fluids as the enrichment.

2. Isolation from Percolated Soils.

Thirty two percolators according to the design of Sharp and Taylor (1968) were constructed and operated at 25°C, for thirty to forty days with a mineral salts solution as the cycling nutrient and various methyl silicone fluids as the soil enrichment. Afterwards soil particles were inoculated onto the surface of various agars. The isolated organisms for each kind of isolation medium are shown in Table 1.

Another thirty nine percolators were similarly set up and percolated for periods up to twenty seven days. The cycling nutrient, however, was given a 4% glucose additive. The results are seen in Table 2.

### Table 2: Isolated Organisms from Percolated Soils

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<thead>
<tr>
<th></th>
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</thead>
<tbody>
<tr>
<td>+2.5mls silicone per 10mls agar.</td>
<td>- GLUCOSE</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
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<tr>
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<td>+</td>
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</tr>
<tr>
<td></td>
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</tr>
<tr>
<td></td>
<td>3.0cs</td>
<td>+</td>
<td>+</td>
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<td>+</td>
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<td>+</td>
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</tr>
<tr>
<td>+ GLUCOSE</td>
<td>0.65cs</td>
<td>+</td>
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</tbody>
</table>

Table 2: Isolated organisms from percolated soils given a mineral salts solution containing 4% glucose and various methyl silicone fluids as the enrichment.

Finally twenty more percolators were installed with ten having glucose and the ten others being without, but this time no silicone was incorporated into the soil. The percolators were left for twenty days at 25°C, before the usual isolation procedure. The results appear as Table 3.

It was concluded that the environment circumscribed by the percolation allows the growth of common soil organisms irrespective of the presence of silicones. The silicones must also be non-inhibitory. There is no effect on growth due to the differing degrees of silicone polymerization (reflected by their viscosities).

Glucose in the isolation plates increased the species isolated and their mycelial growth. Glucose within the percolators decreases the number of species isolated and this is probably due to the thorough percolation of staling substances or toxicants produced in high glucose conditions. Cellulolytic organisms tended to appear when there was no extra carbon source present.

Thus the elective culture of common, highly sporing soil fungi without any concomitant physical change of the silicones means that the intended silicone degradation proved to be abortive.

3. Isolation from Perfused Soils.

In order to enrich a substrate with silicones and supply ancillary nutrients in a gradual manner the perfusion system of Eggins, Malik and Sharp (1968) was employed.

Various methyl silicone fluids were mixed with soil and held in a test tube through which a perfusing glass sleeving was passed. This glass sleeving passed a mineral salts, nutrient solution along by capillarity from a reservoir to the soil and then away to a tailpiece held in a dry atmosphere.

Incubation was at 25°C, for 140 days.

Afterwards soil particles next to the perfusion sleeving were plated onto various agars. After incubation fungal growths were identified as:

- Aspergillus fumigatus
- Aspergillus niger
- Trichoderma viride
- Fusarium sp.
- Penicillum sp.
- Zygomyces moellendorfii
- Eurotium verrucaria
- Chaetomium globosum
- Cercospora sp.
- Mucor sp.
- Bacteria
- Ciliates
- Nematodes

Nematodes and soil ciliates were also found. Again no silicone degradation was witnessed.

**Fungal Species**

<table>
<thead>
<tr>
<th>Eggins and Pugh mineral salts +2-5mls silicone per 10 mls agar</th>
<th>Aspergillus fumigatus</th>
<th>Aspergillus niger</th>
<th>Trichoderma viride</th>
<th>Fusarium sp</th>
<th>Penicillium sp</th>
<th>Eurotium vermiforme</th>
<th>Paecilomyces variotii</th>
<th>Zygorhydnchus moelleri</th>
<th>Micor sp</th>
<th>Absidia glauca</th>
<th>Pseudopsis sp</th>
<th>Nematodes</th>
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</thead>
<tbody>
<tr>
<td>Silicone viscosities</td>
<td></td>
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<td></td>
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</tr>
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<td>30cs</td>
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<td>+</td>
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<td>+</td>
<td>+</td>
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</tr>
<tr>
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<td></td>
<td></td>
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<tr>
<td>+GLUCOSE</td>
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<td></td>
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<td>+</td>
<td></td>
<td></td>
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</tr>
</tbody>
</table>

Table 3 Isolated organisms from percolated soils in which some were given 4% glucose and some were left untreated. A mineral salts solution was cycled but no silicones were supplied.

**Assessment of Toxicity**

History

Much has been written on the toxicity of silicones to mammals but there is little information pertaining to microorganisms.

It has been found (Speier, 1952) that bis (hydroxyphenyl) silanes formed with hexamethylenetetramine thermosetting resins are active bacteriocides. Thus 0.025 wt.% is more effective than 0.125% phenol towards *Staphylococcus aureus*.

Organosilymethyl thiocyanates (Midland Silicones, 1957) were found to be useful for modifying silicone resins and also as mould inhibitors.

When a number of microorganisms were grown on several medicinal silicone rubbers (Riley and Winner, 1960) it was found that the rubber's toxicity was low.

A report from Bailey and Pike (1960) describes organosilicon compounds containing an organosubstituted sulfonamido group attached to the silicon atom to be useful insecticides and fungicides.

A silicone-substituted penicillin has been shown to have similar activity to benzyl penicillin (Voronkina, Strukov and Shostakovsku, 1964).

To clarify the position as regards the toxicity of pure silicones towards fungi the following investigation was made.

**Methods and Results**

Thirty eight randomly selected fungal species representing all the taxonomic classes were inoculated onto petri dishes containing various silicone agars (8 ml. of viscosities 0.65 centistokes (cs.), 1.0, and 5 cs. of methyl silicone fluid together with 50 cs., 125 cs., and two 500 cs. with different specific gravities of phenyl methyl silicone fluid per 100 ml. of medium).

After two or four days incubation at 25°C, depending on the rate of hyphal growth, the cross-diameters of the fungal colonies were measured. Size of growths on pure glucose media acted as a standard.

No inhibition or stimulation resulted and therefore the degree of silicone polymerization was of no significance. Examination of the mycelia showed normal pycnidia, perithecia, sclerotia, chlamydospores, etc., and normal hyphal ramifications with occasional penetration through silicone droplets within the medium.

**Assessment of Hydrophobic Attraction**

History

Dow Corning's "D.C. 200" silicone caused *Mycobacterium tuberculosis* var. *hominis* to adhere tenaciously to a silicone film spread over a glass surface (Fisher, 1954). In the presence of polyoxyethylene sorbitan monoleate ("Tween 80") the hydrophobic environment was destroyed and the bacterium became unattached.

Methods and Results
To see whether or not silicones provide an hydrophobic environment favourable for hyphal growth, the previous experiment was repeated.

Only 30 fungal species were inoculated and 0.02% (volume to volume of medium) to "Tween 80" was introduced into the agars causing a much greater dispersal of finer silicone droplets.

It was found that similar sized growths occurred as before showing no reaction to the "Tween 80" or the silicone. There was normal fungal morphology and commensurate hyphal branching in relation to the redistributed silicone as before.

Assessment of feasibility for preventing deterioration

History
A report from Hoffmann (1964) suggests that a fungicidal wash containing a silicone compound controls mould-invested paintwork. The silicone renders the surface water-repellent and at the same time fixes the fungicide onto the surface.

A preservative treatment for electrical cables consisting of a polyester varnish, trimethyl siloxane, ethyl acetate and pentachlorophenol has been described (Vincent, 1966).

Midland Silicones (1966) hold a patent that describes certain chemical products from alkyl/chloro-silanes which, on hydrolysis, gives a solid material that may be incorporated into cements, paper etc., to improve water resistance or added to a biocide to regulate the release of active ingredients.

Alkoxy silanes have been shown (Stroganov, Mbotov, Kolosova and Kadena 1968) to be toxic to phytoplankton without affecting zooplankton or molluscs and so could be used to regulate the algal concentration developing in lakes and reservoirs.

The silicone properties so far gleaned as important to biodeterioration problems include being biodegradable, water immiscible, inert biologically, having low surface tension and a low amount of organic material that might be nutritive. An investigation was therefore undertaken to test the suitability of these properties to prevent the decay of a model substrate.

<table>
<thead>
<tr>
<th>No.</th>
<th>Treatment</th>
<th>pH on wood</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>M.S. 200/50 Dimethylpolysiloxane</td>
<td>4.2</td>
</tr>
<tr>
<td>2</td>
<td>M.S. 200/1000 Dimethylpolysiloxane</td>
<td>4.2</td>
</tr>
<tr>
<td>3</td>
<td>M.S. 510/50 Phenylmethylosiloxane</td>
<td>3.8</td>
</tr>
<tr>
<td>4</td>
<td>M.S. 710 Phenylmethylpolysiloxane</td>
<td>3.8</td>
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<tr>
<td>5</td>
<td>M.S. 1107—SiH active polysiloxane</td>
<td>3.8</td>
</tr>
<tr>
<td>6</td>
<td>Water-repellent for glass and ceramic</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>Dri-Sil 29, 49% solution of active resin in xylene</td>
<td>3.4</td>
</tr>
<tr>
<td>8</td>
<td>Dri-Sil 29 + a catalyst</td>
<td>4.2</td>
</tr>
<tr>
<td>9</td>
<td>Dri-Sil 29 + an emulsifier</td>
<td>4.0</td>
</tr>
<tr>
<td>10</td>
<td>R.T.V. (room temperature curing) rubber</td>
<td>4.0</td>
</tr>
<tr>
<td>11</td>
<td>M.S. 2202 a 50% solution of air-drying silicone water-repellent in white spirit</td>
<td>4.2</td>
</tr>
<tr>
<td>12</td>
<td>Methyltrichlorosilane</td>
<td>3.0</td>
</tr>
<tr>
<td>13</td>
<td>Trimethylchlorosilane</td>
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</tr>
<tr>
<td>14</td>
<td>Mixed chlorosilanes</td>
<td>2.8</td>
</tr>
</tbody>
</table>

Table 4 The various silicone treatments applied to a beechwood surface.
Methods and Results

The substrate adopted was a beechwood veneer. Silicones are often cited as suitable water repellents for wood and there is a description by Hyde (1967) of various polysiloxane resins curable at room temperature which give a wood finish. No determination, however, has been made to find out whether these silicones prevent the growth of wood fungi.

The test techniques adopted were a perfusion system (Eggins, Malik and Sharp, 1968) used in conjunction with a strength test method (Sharp and Eggins 1968). Three types of preservative action were considered using 13 types of silicone treatment and these are summarised in table 4.

13 veneers were set up for each treatment having their silicone coating on the upper surface only and being perfused with a mineral salt solution on their lower surface. The veneers were inoculated with pasture land soil from near Clent, Worcestershire, England, and incubated at 25°C for 39 days. One veneer of each treatment was sacrificed every 3 days.

The percentage frequencies of isolated fungi were found (see table 5) and the strength loss of each veneer determined (see graphs of results).

These results show that the silicones and their high acidities have not hindered the growth of species nor have they each elected their own mycoflora. Since the number of isolated species is normal but the loss of strength is less rapid than usually expected, it appears that the silicone has slowed down the quantity of fungal growth.

Apart from the mixed chlorosilanes there is some decay with each treatment. The chlorosilane acids partially hydrolysed the wood cellulose from the onset, so precluding its use as a preservative, and thus prevented any loss of strength occurring until there had been extensive incubation.

The greatest decay was associated with those treatments having least effect on the wood, namely, the three Dri Sil 29 preparations and the surface rubber film coatings.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Trichoderma viride</th>
<th>Humicola grisea</th>
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Table 5 Percentage frequencies of Isolation for the fungi found on the silicone treated woods.

The lowest viscosity dimethyl fluids allowed more deterioration than the others and the dimethyl fluids gave less protection than the phenylmethyl fluids. This suggests that greater protection is afforded by supplying large amounts of high viscosity silicones that are able to disperse nearest to the cellulose but this then counters economic considerations.

Discussion

The silicones examined in this work have been shown to be resistant to deterioration by common fungi, to possess no biocidal properties and when applied to a substrate to allow the colonization of fungi provided suitable growth conditions are present. When these characteristics are considered in conjunction with their other properties it should lead to new applications.

Their low surface tension could be used to aid protective penetration. Highly permeable films could coat a substrate and still allow gaseous exchange to continue. Their water-repellency could delay deterioration of materials or detoxification of biocides by denial of water to microorganisms. They could be used as inert media for culturing organisms used in biochemical processes.

Each suggested usage requires its own separate evaluation and each illustrates the need for a greater understanding of the specialised biological properties of silicones.

Acknowledgement

Dr. R. F. Sharp wishes to acknowledge the generous assistance given by Midland Silicones Limited, Barry, Glamorgan for this research.

References


Hardy, D. V. N. (1947) Organosilicon compounds and their industrial development. Endeavour. 6, 29-35.


Graphs of strength results
Graphs of strength losses of the beechwoods after the silicone treatments.
BOOK REVIEWS

CHEMICAL MICROBIOLOGY

A. H. Rose


It is not at all surprising that this work has already reached a second edition. The first edition, which appeared in 1965, had to be reprinted in 1967 and now this revised version brings topics up to date, as shown by some of the literature references. Professor Rose is a prolific writer but not a verbose one, and his presentation makes easy reading. This work is not meant for beginners, however, and it is assumed that the reader has some basic knowledge of biochemistry as well as of microbiology.

In his introductory chapter the author emphasises the difference in behaviour of organisms resulting from their growth in pure culture as opposed to their functioning in a natural environment. Those who have worked, for example, with cellulose-decomposers, sulphate-reducers, nitrogen-oxidisers or even with anaerobic rumen flora will remember the difference in behaviour of organisms resulting from the way in which they are isolated. The author also stresses the importance of understanding the nature of the reaction in question, in order to be able to follow the course of the reaction in the laboratory. This is by C. E. Taylor, C.P.H.L., Colindale, on Complement Fixation. The nature of the reaction involved in briefly discussed, followed by notes on the reagents required for the test, their preparation and handling, and the actual performance of the test by long or short fixation methods. A list of 12 antigens commonly used for virus CFT's is given; the Wassermann test and LCM is dealt with in seven lines, followed by notes on the conjugate, (2) staining methods, and (3) fluorescence microscopy. Under each heading details of the component methodology are given logically and clearly, without any padding, and adequately supported by six illustrations (pp. 20, refs. 18). Chapter 3, by D. W. G. Busby of the National Institute for Medical Research, covers Freeze Drying Methods. This forms an excellent introduction to the practicalities involved and the range of materials which may be subjected to this process. Small but essential details such as 'teasing' out plugs are illustrated, (pp. 18, refs. 41).

Chapter 4 is concerned with the Assay of Vitamins and Amino Acids by S. A. Price of Vitamins Ltd. Part 1 is related to basic methodology for tube and agar diffusion assays; and Part 2 gives procedures for assaying riboflavin, nicotinic acid, vitamin B 12 , and total and available methionine. The quasi-Latin square template is admirably presented, (pp. 29, ref. 67). Chapter 5 deals with Analysis of the Bacterial Cell by A. C. Baird-Parker and R.C.S. Woodruffe of Unilever Research. Although a very difficult subject to condense down to the purely technological level,
the authors have made this essay both concise and practical. Their warnings on the possible variations in the accuracy of spectrophotometers is particularly welcome, as is also the simplified presentation of electrophoresis techniques (pp. 39, ref. 52). Chapter 6 is on Standardization of Biological Products by Microbiological and Serological Methods by P. B. Stokes of Pfizer Ltd. This chapter is, in fact, restricted to immunological products, i.e., vaccines and antitoxins. Notes are given on bacterial, virus and Rickettsial vaccine preparation, and on bacterial toxoids and antitoxins. Despite the broad field surveyed, a considerable amount of technical information is conveyed by dint of strict economy of words and vigorous direct writing (pp. 24, ref. 5).

Chapter 7 refers purely to Gel Diffusion and Immunelectrophoresis methods by W. D. Brighton of C.P.H.L. At the technological level some may find this short paper a trifle wordy, nevertheless it repays reading and gives some useful references to special staining methods used by French workers like Uriel (pp. 15, ref. 14). Chapter 8 gives data on technical methods relating to Yeasts and Assexual Fungi as applied to the Brewing and Antibi tic Industries, by M. Richards of the Brewing Research Foundation, Nutfield. The traditional taxonomic techniques do not differentiate between brewing strains, so some details of other methods are given such as those concerned with giant colonies (Saccharomyces cerevisiae) and flocculation. Useful information appears to be given more at the level of the technological 'why' rather than the actual 'do', the latter being taken rather for granted (pp. 16, ref. 39). Chapter 9 is on Bacteriophage Typing of Staphylococci by Elizabeth Asheshov, again from C.P.H.L., Colindale. A very readable paper but somewhat more academically slanted than most of the other papers in this book. An excellent photograph, however, puts over the main practical story (pp. 11, ref. 16). Chapter 10 represents Recent Advances in the Bacteriological Examination of Water by N. P. Burman of the Metropolitan Water Board. This paper might be taken as a model of its kind, concentrating mainly on practical details such as when and why this method or that does or does not work, and what to do about it, but, at the same time sufficiently laced with other relevant information for it all to make sense. Under every heading, the author reveals as deep an interest in the minutiae of techniques in his field as would a surgeon or other master craftsman. A pleasure to read, the title is self-explanatory, (pp. 27, refs. 47). And finally, a paper on Serological Methods in Mycology by A. C. Proctor of the London School of Hygiene and Tropical Medicine. Following several pages of somewhat chatty introduction the author sets down in a very practical fashion the details of the methods now being used in this interesting field, (pp. 13, ref. 17).

A book of this kind is not intended to be read straight through, but rather one to be kept on the laboratory shelf for reference should the need arise. Yet, reviewing such an omnibus volume, it is fascinating to observe the subtle differences in the writing of those who are primarily technologists, those who are primarily academicians, and that very rare avis, the man who mastered technology first and the related academic disciplines later. This book is unique inasmuch as it presents work from all three types of author, and apart from its undoubted technological value, it is well worthy of study in terms of writing technique, approach, style, content and sense of communication at the 'doing' level.

A. H. Walters

FUSARIUM: A PICTORIAL GUIDE TO THE IDENTIFICATION OF FUSARIUM SPECIES

T. A. Toussoun and Paul E. Nelson.

(The Pennsylvania State University Press, 51 pp., 17 plates (2 coloured), [1968] Price $5.95)

The authors are both former students of Professor Snyder and the late Professor Hansen and the book is dedicated to them and to the perpetuation of their nine species system for the classification of the genus Fusarium. Both Professor Snyder and Professor Hansen were basically Plant Pathologists and their system was devised to help Plant Pathologists identify Fusarium species associated with plant disease. There is little doubt that economically Fusarium species have their greatest impact when causing plant disease; Panama disease of banana, Trachemycosis of coffee, Snow mold of cereals are examples. The first of these is caused by specific strains of Fusarium oxysporum and this species is a major wilt producer in a very wide range of economically important plants. Fusarium species also occur as rather minor pathogens of humans and animals. With the possible exception of F. solani, Fusarium species are seldom responsible for the deterioration of manufactured products. They are, however, often important in the deterioration of stored agricultural products. In grain their economic effect is two fold. They may under suitably moist conditions cause direct destruction of the grain and Fusarium equiseti, F. semitectum or F. culmorum are often found associated with these conditions. Alternatively, they may produce toxic by-products, during a comparatively minor infection, that have serious effects when the grain is used in animal feed. Quite recently F. graminearum has been found to produce an abortifacient which causes abortion and other uterine disorders when the infected grain is fed to cattle.

Using Toussoun and Nelson's guide, all the above species of Fusarium are found under F. roseum. Similarly another Fusarium common in storage is F. coeruleum which causes powdery rot of potatoes. This species is placed under F. solani. Fusarium poae, F. chlamydosporum and F. sporotrichoides a further group of important toxin producers in stored grain are placed in F. tricinctum.

The nine species system of classification is based mainly on the shape of the macroconidia. Subsidiary characters include the presence or absence of the microconidia and/or chlamydospores. Other characters such as colony morphology and pigmentation are said to be of no value in species delimitation although two colour plates are included showing these characteri-
stics. Growth rate is not mentioned although it does readily separate some of the strains of 'roseum' which show an overlap of the macroconidial morphology with strains of 'episphearia'.

The book outlines good basic techniques for culture work and formulae are included for standard, natural and selective media. Maintenance of Fusarium cultures in sterile soil is recommended.

Although a brief key to the species is included, the illustrations are the principle feature of the guide. There are 15 half tone plates with 4 or more photomicrographs on each plate. In addition there are two colour plates, 16 G could not possibly belong to F. roseum and presumably the lettering should run in alphabetical order.

The taking of photographs of Fusarium species with their thin-walled hyaline spores is rather difficult and these photomicrographs are excellent.

Only about half the 51 pages contain printed matter or photographs so there is ample room for the addition of notes or references by the users.

In general the book is a useful introduction to the morphological range of spore morphology found in Fusarium. It will not help the worker on deterioration of stored products to any great extent.

C. Booth

FOOD POISONING AND FOOD HYGIENE
Betty C. Hobbs (additional chapters by L. Kluth),

Over the past twenty years, anyone interested in food in the UK will certainly have listened one or more times to Dr. Betty Hobbs lecturing on food poisoning and food hygiene, a subject which she has made completely her own. During the years following World War II in UK this topic was far from popular, quite the contrary, yet, undaunted—indeed she loves a challenge—Dr. Hobbs sallied forth from the laboratory into the streets and there, in time, brewed up a magic recipe for her lectures which has gradually overcome all lay inertia. She has tirelessly stumped the country addressing every conceivable type of audience, treating them to a unique melange of bacteriology (her own and that of other inominate pioneers), home economics and practical feminine commonsense—all mixed up together and spiced with just a judicious touch of dire warning according to the taste and level of the audience. This missionary work gave her immense experience in learning to put the message across, and in 1953 this culminated in the appearance of the first edition of "Food Poisoning and Food Hygiene". To state that from the moment of its publication the book sold like hot cakes might be to coin a phrase, but suffice it to say that, like its author, it was the first of its kind and, as such, found wide acceptance in Public Health and food circles in this country and abroad. Countless doctors, Health Inspectors and bacteriologists and others called upon suddenly to talk upon the subject have delivered themselves almost verbatim from its pages. Nevertheless, even now, nobody can quite put the story across like Dr. Hobbs in person, who has, once again, demonstrated the truth of the adage, "If you want to learn a subject, teach it", to which, in her case, might be added, "—to all and sundry".

Time marches on and now a second edition of this famous little book, affectionately known as "Mrs. Beeton's Bacteriology" has appeared. In the intervening fifteen years the missionary work has continued and great changes have taken place in the UK in relation to the attitude of the food trade and the British public to what is still euphemistically called "food hygiene". Indeed, it is still so elusive that the term is not defined until on the last but two pages of the book as "The means to obtain clean and safe food through all stages of production to the consumer". Although food hygiene has now become respectable—even popular for sales appeal—this second edition clearly shows that the basic causes of food poisoning and the measures needed for its prevention remain essentially unaltered, which re-emphasize the classic concepts enunciated in the first edition. While containing nothing basically new, much more up-to-date data is now presented, and, on the statistical side, it is interesting to note that between 1957 and 1966 the number of recorded incidents of food poisoning in England/Wales fell from 7071 to 3744, nevertheless, each incident may mean one case only or comprise hundreds of cases. It is estimated that 3000 or more cases of food poisoning still occur annually in England/Wales and there may be many others not reported. Thus, over the last 20 years (there were 7000 recorded cases in 1949) the incidence of food poisoning seems to have remained fairly static which indicates (a) that 'eye' appeal is not necessarily good food hygiene, and (b) a continued need for public, institutional and food trade education, which more than justifies this 2nd. edition.

The contribution of three chapters by Mr. L. Kluth on kitchen design and equipment, control of infestation and legislation are very useful. No detailed references to other relevant scientific work in this field are given.

A. Harry Walters

LOW TEMPERATURE BIOLOGY OF FOODSTUFFS
Edited by John Hawthorn and E. J. Rolfe
[Recent Advances in Food Sciences, Vol. 4] xii + 458 pp. Price £6. 0. 0.)

This volume, which as been published as the fourth volume of Recent Advances in Food Science, forms the Proceedings of the NATO advanced study institute on the subject, held at the University of Strathclyde in September 1966. The standards of production—one is interested to note that the book was printed in Hungary—and the quality of the material presented are fully up to the standards which have been set by
An interesting foreword by Dr. Bate-Smith, traces the historical development of the subject back to the founding of the Food Investigation Board in 1917, and the subsequent establishment of the Low Temperature Station, where so much of the work, both basic and applied, on refrigeration of foodstuffs has been done. This is followed by twenty-three chapters, each of which deals in detail with some particular aspect of the preservation of food materials at temperatures near or below freezing, or with the scientific principles involved. Each chapter is essentially a review article on its own topic, and each has its own independent bibliography, being therefore of use on its own. Nevertheless, the fact that a group of twenty three such reviews, all written by acknowledged experts in their field, are brought together in a single volume, greatly enhances their value. The first four chapters deal with more basic aspects of ice structure and formation while of the remainder, seven are concerned with various problems of fruit and vegetables, six with foods of animal origin such as meat, fish and eggs, three treat microbiological matters such as psychrophilic microorganisms and the effects of low temperature on food microflora and three are of a more general nature.

The present reviewer naturally found those chapters dealing with vegetable materials of the greatest interest, especially Dr. Fidler’s most comprehensive survey of ‘chilling damage’—the much misunderstood and little-known effects associated with the breakdown of fruit and vegetables at temperatures near, but definitely above, the freezing point—as these are closest to his own field of activity. The quality of the various chapters is, however, consistently high, and all are useful to specialists in the different fields.

The organizing committee and the editors are to be complimented both on the arrangement of such a useful and productive meeting, and for the production of such a good permanent record of it. It is a pity though, that the book did not appear rather sooner: in spite of the date, it does not appear to have been generally available until 1969, nearly three years after the meeting it records. Nevertheless, it serves well, as one of the organizers expresses it, to identify the extent to which scientific knowledge of low temperature biology is being applied in current industrial practice, and should assist in maintaining to two-way dialogue between theory and practice that is essential for further advance.

D. G. Coursey

YAMS
D. G. Coursey
(Longmans, Green, London, 1967
xiv + 230 pp., illus. Price £3. 3. 0.)

It is very enterprising of the author and publisher to produce this excellent book on the nature, origins, cultivation and utilization of the useful members of the family Dioscoreaceae, which is the eleventh volume in the Tropical Agriculture Series under the editorship of Mr. D. Rhind. The edible yams usually receive sparse treatment in publications on tropical crops, and Mr. Coursey is to be congratulated on filling this gap so ably.

The world production of yams is estimated at 20-25 million tons per annum, of which at least two-thirds is produced in the eastern part of West Africa, with

MODERN CEREAL CHEMISTRY
D. W. Kent-Jones and A. J. Amos

Previous editions of ‘Modern Cereal Chemistry’, which was first published in 1924, have been widely recognised as valuable and comprehensive treatises on the science and technology of cereals and cereal products. In the new edition of this book the arrangement of the material is similar to that of the previous edition which was published in 1957. The text has been revised and brought up to date and some of the content of the earlier edition omitted to make room for accounts of recent developments. Over 1000 literature references are given and in general these are sufficient to enable the reader to follow up a particular line of enquiry. A new and welcome feature is the inclusion of references at the end of each chapter rather than at the end of the book.

The volume is mainly concerned with the science and technology of milling and baking and with analytical procedures for cereals, the largest chapter of over 100 pages being devoted to the latter aspect. Two of the 18 chapters in the book are concerned entirely with biological subjects. One of these chapters, contributed by Dr. E. E. Turtle and Dr. J. A. Freeman, discusses the infestation of cereals by insects and mites. This chapter has been considerably revised and includes sections on insect pests of cereals, the identification of insects and the control of insect pests by physical and mechanical methods and by contact insecticides and fumigants. The microbiology of cereals, including the microbial spoilage of bread, is the subject of another chapter of the book. Elsewhere, only three pages are devoted to the bacteriological examination of wheat and flour.

As is inevitable in a work of this kind, the quality and depth of treatment is variable. Nevertheless, it can be stated without reservation that the authors have succeeded in their object of producing a book which is of practical use to cereal chemists, millers and bakers and to others concerned with the use of cereals. The latest edition of ‘Modern Cereal Chemistry’ will be regarded, as were its predecessors, both as an essential work of reference and as a valuable laboratory manual by all those concerned with the chemistry and technology of cereals. It will be of rather less value to those concerned mainly with the biodeterioration of cereals.

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Nigeria accounting for about half the global production. Malaysia and adjacent areas produce about five million tons per year and yams are grown to a more limited extent in the Caribbean. In the yam areas of West Africa, they provide the staple carbohydrate food, with a daily ration of 0.5-1.0 kg per day. They play an important part in the socio-religious life of local peoples. The production of yams requires a great deal of labour and there is a tendency for them to be replaced by sweet potatoes and cassava.

Yams are stored for several months between harvests and also to provide 'seed' yams for planting. The basic requirements of yam storage differ fundamentally from those of temperate root crops. There is no danger of frost, and they are normally stored throughout the dry season, which is usually the hottest time of the year. Adequate ventilation is essential. They are usually stored on a shaded vertical wooden framework, to which individual tubers are tied, and which may be protected from termite attack.

Normal losses in weight during the first three months of storage are about 10-15 per cent, increasing to 30 per cent or more after six months. The main loss is due to respiration at the high prevailing temperatures of about 30°C. The other main cause of loss is by storage rots. Although little information is available on the organisms concerned, 12 spp. have been identified, of which the most important appear to be spp. of Botryodiplodia, Fusarium and Rosellinia. Mechanical damage during harvesting hastens rottion of the tubers, as does pre-harvest pests such as yam beetles and nematodes. Yams are sometimes stored in the form of flour, which is subject to insect attack.

Tubers of wild yams exhibit dormancy, which ensures the survival of the plants from one growing season to the next. Cultivated yams are grown as annuals. Ethylene chlorhydryrin has been effective in breaking the dormancy for growing the crop out of season. Sprouting has been inhibited by the use of methyl alpha-napthyl acetate (MENA).

Cold storage of yams at temperatures below 15°C results in chilling. There is an increasing demand for yams in Britain by people of African and West Indian origin, and some 6,000 tons are imported annually, mainly from Nigeria. In order to reduce deterioration, it is essential that the period in transit should be less than two weeks.

This review has been confined largely to the storage of yams, but this constitutes only a small proportion of the book. There is much of interest on all aspects of yams and their cultivation. The chapter on the origins and subsequent distribution of the edible yams is particularly fascinating, a subject to which Mr. Coursey has given much careful thought. The sapogenins from the wild yams used in the manufacture of oral contraceptives are briefly mentioned. The book is well-written and well-produced, with some good plates and very full references. It is likely to provide the standard work for many years.

J. W. Purseglove

PESTS OF COFFEE
R. H. Le Pelley


Dr. Le Pelley is to be congratulated on his comprehensive and detailed treatment of the subject of coffee pests. As is to be expected, few of the pests are of direct interest to those concerned with post-harvest problems; this review is restricted to these pests only.

The biology, natural enemies and control of Araecerus fasciculatus is covered in two pages and within this space Dr. Le Pelley has presented a lot of useful detail. Specific control recommendations are not given but mention is made of a variety of fumigants and one contact insecticide which have been used for its control. The Coffee Berry Borer, Hypothenemus hampei, essentially a field pest which carries over into, and damages, stored coffee, is dealt with in very much more detail (25 pages). The biology is well covered and the descriptions of natural enemies and attempts at biological control are particularly comprehensive, as also is the section on pre-harvest control. Some interesting observations are quoted regarding novel methods of controlling the pest in coffee after harvest.

P. E. Wheatley

KURZGEFASTE GESCHICHTE DER TIERISCHEN SCHÄDLINGE, DER SCHÄDLINGSKUNDE UND DEN SCHÄDLINGSBEKÄMPFUNG.

H. Kemper

Duncker & Humblot, Berlin, 1968. 382 pp. + 139 fig., Price DM. 78.)

The book consists of three parts. The first informs of the history of noxious animals. Men have made favourable conditions for many pests, for example in the preparation of food, the storing of seed, wood, wool and other organic material, favourable microclimate, breeding-places, and spreading by ship, railway and aircraft. Pests also depend on economy, circumstances in life, and the engagement of men. The importance of pests changes often: lice, fleas, houseflies, case-bearing clothes moth, silverfish, larder beetle, yellow mealworm and many other insects are today rarer than in preceding centuries. The original biotopes of parasites and house insects and the native countries of many pests are discussed. In the following special part the history of many pests (Eriocheir sinensis, locusts, lice, fleas, roaches, termites in Germany, Quadraspidiotus perniciosus, Viteus vitifoli, Adelgidae, Vespidae, stored product insects and plant pests, flies, mites, birds, mice, rats and so on) is reported. In the second part the author informs of the sciences of pests from the ancient cultures of the Near East up to the present day, paying particular attention to the evolution of the investigation of their biology up to the beginning of 19th century, annexed to a history of helminthology

J. W. Price

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and nematology. The third part contains a history of the deterioration by pests. There are four periods: (1) from the origin of man to the 19th century, characterized by helplessness and stupid resignation, (2) the last two thirds of the preceding century and the first ten years of this century, wherein pest control is the subject of scientific research of biologists and other naturalists, (3) World War I, teaching by scarcity of food and clothes to acknowledge the importance of deterioration by pests for the people, of establishing experimental stations, and developing the most active pesticides by chemical industries, (4) the time after 1939; the discovery of the insecticidal effect of DDT, the development of synthetic insecticides with selective effects and harmlessness for men and live-stock. There is also information about biological control, insecticide legislation, organisation of pest control and history of pest control operators or exterminators. Also a list of bibliographical dates of the late investigators of pests with many portraits is given. Numerous quotations and figures from rare antique books turn this work into a valuable documentation. A list of 356 references and an index finish this book which is equally important for zoologists, entomologists, parasitologists, phytopathologists, exterminators, teachers and students.

H. Weidner (Hamburg)

SPECIFICATIONS FOR PESTICIDES USED IN PUBLIC HEALTH


In these days of overpoweringly aggressive advertising it is somewhat unusual for one to come across any publication in which the title and subtitle on the jacket underestimate the contents, but such is my main criticism of the otherwise excellent book which is simply subtitled "Insecticides—Rodenticides—Molluscicides—Repellents—Methods".

A commercial publisher would have been quick to indicate that, besides giving the chemical and physical specifications of the pesticides in common use throughout the world, this volume also gives packaging, handling precautions and marking specifications. It also gives the approved procedures for analysis and determination of chemical and physical properties of these substances, together with references to appropriate supplementary tests and where necessary, the antidote, and medical treatment indicated in cases of poisoning. Sampling procedures are described and in addition to the chemical and physical tests which are described there are specifications of toxicity tests for rodenticides and for larvicidal oils used against mosquitoes.

The common names, trade names and chemical names of pesticides are listed in Annex 1 and finally the "composition of the Fifteenth WHO Expert Committee on Insecticides" responsible for the revision of this edition is given in Annex 2.

In the insecticides section the technical products specifications include DDT, HCH, Lindane, Dieldrin, Pyrethrum, Diazinon, Malathion, Parathion, Trichlorfon, Parathion-methyl, Fenthion and Dichlorvos.

The specifications are also given for the water-dispersible powders, emulsion concentrates and dusting powder and larvicidal oils derived from these products.

The rodenticides include Coumachlor, Warfarin and Pindone, and the Molluscicides include Copper sulphate, Pentachlorophenol, 2', 5-Dichloro-4-nitosalicylanilide ethanolamine salt and N-Triphenylmethylmorpholine. The only specification under "Repellents" section is for Deet (N, N-diethyl-m-toluamide).

The publication may have been compiled primarily for the use of Public Health Authorities, nevertheless, the pesticides with which it deals are also widely used in the field of prevention of biodeterioration of materials such as timber, textiles, furs, foodstuffs, stored products and even plastics and electrical insulating materials (which can suffer attack by boring insects). Therefore this book ought to be in the library or laboratory of every manufacturer and formulator of preservatives and protectants for these materials.

The Fifteenth WHO Expert Committee, (or should it be Committee of Experts?) should be congratulated on the contents of the revised edition, for the book is well worth the money for the analytical methods alone, but a less modest presentation is both required and deserved.

A. O. Lloyd

SOIL BIOLOGY & BIOCHEMISTRY

Vol. 1 No. 2, April 1969

Edited by E. W. Russell

(Pergamon Press, Oxford, England.)

In the present state of the publication explosion, new periodicals appear frequently. This may seem inconvenient to those who still hope to be able to keep up with the research frontier in their wider fields of interest. It is nowadays, nevertheless, a necessity to produce more specialized journals in order to keep the more general ones within a reasonable size.

The present journal, "Soil Biology & Biochemistry", whose first copy was published in April 1969 has as its aims to act as an organ of research on soil organisms, their biochemical activities and their influence on the soil environment and plant growth. Professor E. W. Russell, in a foreword to the first copy stresses the importance of this kind of research in connection with modern farming technique. Extensive use of technical equipment, heavy doses of fertilizers and application of chemicals for pest and predator control require a solid background knowledge of the soil and the processes going on in soil.
Seeming thus to be intended for research workers in agriculture and admitting that it is difficult to evaluate a journal from its first copy, Soil Biology & Biochemistry should also be considered as a valuable addition to the libraries of institutions that are not directly concerned with agriculture.

Thus, it ought to be of great interest to at least some research workers in the field of biodeterioration, since a number of deteriogenic microorganisms are autochthonic in soil.

The present copy contains eleven full-length articles and two short communications. Of the full-length articles, one by K. H. Domsch and W. Gams: “Variability and potential of a soil fungus population to decompose pectin, xylan and carboxymethyl-cellulose” is of direct interest from a biodeterioration point of view. But also the survey article by A. D. McLaren on radiation sterilization of soil and of J. J. Skujins and A. D. McLaren on assay of urease activity in soils using 14-C-labelled urea should be of considerable methodological value. The same can be said for the short communication by P. J. Harris on errors in direct counts of soil microorganisms due to bacteria in agar powders.

The journal is published quarterly, and the annual subscription rate is £12 (U.S. $30), with additional private subscription rates of £5. This seems to give a fair amount of information for the price.

Bergen, January 7, 1970

Jostein Goksøyr

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ADDENDUM

A Method for the Convenient Preparation of Artificial Gas Mixtures in Closed Containers.


In the further use of the method described in the above paper an additional source of error has been discovered, and one which is potentially very serious at low \( O_2 \) tensions.

This error may arise from the functioning of some types of pressure reducing controls fitted to gas cylinders. In the United Kingdom cylinders have an integral on/off valve, and a pressure reducing regulator is screwed onto the top of the cylinder, to control the outlet pressure. A needle valve is often fitted to the regulator to give fine adjustment of the rate of flow, and it is this needle valve which, if worn, may lead to errors in mixtures prepared as recommended in the above paper.

The shank of the needle valve is surrounded by a fibre washer (more recently teflon) to provide a gas seal. It this seal becomes worn then gas can escape round this shank, and more important, if the cylinder is connected to an evacuated vessel, air may enter by this route. Thus oxygen free nitrogen, or any gas of known composition may be adulterated with air.

To detect such leakage a simple test is performed from time to time. The main cylinder valve is closed as tightly as possible to ensure that no gas can enter the system from the cylinder. With the needle valve open, the cylinder is connected to a pump and manometer and the system evacuated back to the main cylinder valve. Now if the vacuum is maintained when the pump is disconnected, the valve is leak-free—a falling vacuum indicates a leak.

If it should prove difficult to find a leak-free needle valve, then this part can be dispensed with. A glass stop-cock will give perfectly satisfactory fine control (though because it is greased it should not be used directly on the cylinder head). Alternatively a special leak-free fine control valve is marketed by B.O.C. designed to prevent leakage of toxic gases, but equally capable of preventing leakage into the system as described above.

J. H. Walsh
KERATIN AND CHITIN (Cont), LEATHER, LIGNIN, METALS

GRAY, T. R. G. & BAXBY, P.
Chitin decomposition of soil. II. The ecology of chitinoclastic micro-organisms in forest soil. [Fungi. Bacteria. Actinomycetes.]

LEATHER

ORLITA, A.
Biodeterioration in the leather industry. [Fungi.]

ANDERSEN, J. E.; HERWIG, G. L. & MOFFITT, R. B.
Heap leaching at Rum Jungle. [Copper. Thiothrix.]

LOBL, M.
The choice of materials for gas mains and modifications of existing systems. [Soil. Corrosion. Resistance.]

IVERSON, W. P.
Corrosion of iron and formation of iron phosphide by Desulfovibrio desulfuricans.

TOPE, S. K.; SANYAL, B. & NANDA, J. N.

GUPTA, S. K.; SANYAL, B. & NANDA, J. N.
Underground corrosion: Pt. II - Methods of protection.
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