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Biodeterioration Society Newsletter

The role of dodecanoic acid in the microbiological corrosion of jet aircraft integral fuel tanks

Estela R. de Schiaparelli and Blanca R. de Meybaum

Role de l’acide dodecanoic dans la corrosion microbiologique des réservoirs des ailes de carburant pour des avions à réaction.

Einfluss der Dodekansäure in der mikrobiologischen korrosion der bensintanks von flugzeugen.

Rol des acido dodeganoico en la corosion microbiologica de tanques integrales de aviones a reaccion.

Fungal flora of pulped pepper (Capsicum frutescens) stored under various conditions

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Changes in properties of finished indian leather in store due to fungal infestation

O.P. Sharma and K.D. Sharma

Changements de propriétés du cuir indieu fini par des infestations fongiques au cours de stockage.

Veränderungen der Eigenschaften von zugerichtetem indischem leder während der Lagerung durch Pilzbefall.

Cambios en las propiedades de cueros indios en almacenamiento debido a infestacion por hongos.

A bacterial contribution to wood nitrogen

B. King, W.J. Henderson and M.E. Murphy

Contribution bacterienne à l’azote du bois.

Der Beitrag von Bakteriun zum Stickstoffgehalt des holzes.

Contribution Bacteriana en el Nitrogeno de la Madera.

Book Review

Correction

Professor Maria Bassi has asked us to point out that in the paper: The Use of Fungicides on Mold-Covered Frescoes in S. Eusebio in Pavia, Volume 16 No 2, Summer 1980, pages 45–51 incorrect information is given in Table 1, page 51.

The material Florasan (active principle Imazalil) is made by Janssen Pharmaceutica and not as indicated in the table.
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ST. PETER’S COLLEGE,
SALTLEY, BIRMINGHAM B8 3TE.

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

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

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

Illustrations must be very clearly drawn, normally larger than the size finally desired. The suggested final size should be clearly indicated but the Editor reserves the right to vary this in the interests of economy and clarity.

ACKNOWLEDGEMENTS TO SUSTAINING ORGANISATIONS

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All articles are submitted by the Editor to one or more independent referees for advice on their clarity, originality, and general suitability for publication, but the final decision whether or not to publish an article rests with the editors. If articles are rejected the substance of the referee’s report will usually be communicated to the author and in suitable cases the Editor will be pleased to help authors to improve their papers with a view to possible publication.

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

Reese E.T. and Levinson H.G. (1952)

Comparative study of the breakdown of cellulose by microorganisms.

Physiologia Plantarum 5: 354–366

or:

Darby R.T., Simmons E.G. and Wiley B.J. (1968)

A survey of fungi in a military aircraft fuel supply system.


References to books, conference proceedings, etc. should quote first the author(s) or editor(s), then the year of publication and title followed by the name of the publisher and the city in which it is published. As far as possible titles of journals should be given in full except for such abbreviations as ‘Journ.’, ‘Proc.’, ‘Trans.’ etc.

20 reprints will be sent free of charge to the first named author unless otherwise instructed. Any number (normally not more than 150) of additional reprints may be purchased if ordered sufficiently in advance. An order form and price will be sent giving about one month’s notice.
Membership

The Society continues to expand slowly but still has only a small fraction of those concerned with Biodeterioration in membership. Members are urged to make full use of the application form for membership which appears on the back page of this Newsletter.

The practice of making a charge of 50p to £1 to non-members for attendance at meetings has been re-introduced.

At the recent course on biodeterioration held at the Biodeterioration Centre, University of Aston, all sixteen of the (overseas) participants expressed interest and joined the Society. It is hoped that this will encourage more meetings outside Britain.

Winter meeting 1980

The winter meeting will be held at the Princes Risborough Laboratory of the Building Research Establishment on Thursday 11th. December. It will take the form of a one day symposium on:

“Effects of Toxic Chemicals on Microbial Ecology”

The meeting is being organized by Dr. R. Neil Smith of the Department of Biological Sciences, Hatfield Polytechnic, P.O. Box 109 Hatfield Herts. AL10 9AB, to whom offers of papers should be sent.

For local arrangements apply to Mrs Wendy Worley, Biodeterioration Section, Princes Risborough Laboratory, Building Research Establishment, Princes Risborough, Aylesbury, Bucks. HP17 9PX

5th International Symposium, Aberdeen, 1981

The scientific committee which is planning the programme for the 5th. International Symposium has decided to operate by inviting a number of distinguished scientists each to organize and chair a session on an appropriate subject. In this way the Symposium programme can be expected to reflect current interests. At the time of going to press the following have agreed to collaborate in this way:

- Allsopp (Aston University, Birmingham) Biodeterioration by Higher Plants
- Bravery (Building Research) Biodeterioration of Wood and Wood-based Products.
- Edwards (Rothamsted Experimental Station) Biodeterioration by Insects.
- Flannigan (Heriot-Watt University, Edinburgh) Biodeterioration of Cereal Grains and Agricultural Products
- Griffin (Ecological Materials Research – Brunel University) Macrobiodeterioration
- Hawkes (Aston University, Birmingham) Biodegradation of Effluents
- Hill (University of Wales, Cardiff) Biodeterioration of Petroleum and Petroleum Products
- Houghton (Portsmouth, Naval Marine Technology) Marine Biodeterioration
- Kaplan (U.S. Army Laboratories, Natick) Biodeterioration of Plastics
- Lloyd (Welwyn Garden City) Biodeterioration by Lichens, Algae and Mosses
- Paulus (Bayer AG, Krefeld, Germany) Chemical Control by Biocides, Biostats and Preservatives.
- Traxler (University of Rhode Island) Fate and Effect of Petroleum in the Environment.

Other sessions may additionally be organised on:
- Biodeterioration of Paints
- Rodents as Biodeteriogens
- Biologically caused Corrosion
- Pesticide Legislation and Regulations
- Factory Hygiene
- Fungi as Biodeteriogens
- Bacteria as Biodeteriogens.

These sessions will be organised only if appropriate papers are contributed, or if suitable willing organisers/chairmen can be found.

Professor Dr. Dr.h.c. Günter Becker

As we go to press we have learned with very great regret of the death of Dr. Becker. An obituary will appear in the next issue.
Abstracts of some papers presented at the Symposium on
The Biodeterioration of Polymeric Materials
held in connection with the Society's annual Summer Meeting at
Preston Polytechnic.
10th and 11th. July 1980

Title: Heat control of microbial colonisation of shipboard fuel systems.
Authors: E.T. Wycislík and D. Allsopp
Address: Biodeterioration Centre, University of Aston, St. Peter's College, College Road, Saltley, BIRMINGHAM, B8 3TE

ABSTRACT

The microbial flora of a water bottom sample from a shipboard diesel fuel tank, and spore suspensions of Cladosporium resinae, and a Penicillium sp. were subjected to heat at 45°, 55°, 60° and 65°C. Using a spread-plate technique, numbers of surviving organisms were determined at time intervals and inactivation rates were calculated. The results indicate that 65°C may be a suitable temperature for use in an in-line pasteurization treatment for fuel. The effect of an organo-boron compound on spores of C. resinae at 25° and 45°C was also investigated. For a given concentration of the compound, the inactivation rate of organisms increased significantly at the higher temperature.

Title: Wood nitrogen influences on toxicity of Copper Chrome Arsenic preservatives.
Author: B. King
Address: Department of Molecular and Life Sciences Dundee College of Technology, DUNDEE, DD1 1HG

ABSTRACT

Soluble nitrogenous materials concentrated at the evaporative surfaces of wood are shown to produce a reduction in toxicity of CCA preservatives in wood in soil. Such nutrients also act as attractants stimulating movement or growth of organisms and thus nitrogen transfer from soil to wood placed therein. The role of primary coloniser autolysis and re-use as nutrient by secondary colonising microorganisms is discussed with particular reference to bacteria and actinomycetes and nutrient and biomass transfer from soil to wood is proposed as a biotic explanation for failure of CCA preservatives in the field.

The publication of these abstracts by the Biodeterioration Society does not constitute publication in the usual sense. No reprints are available. Those wishing to pursue these subjects further should write directly to the authors at the addresses shown and not to the Society.
Title: Extracellular enzyme activity in wood inhabiting microfungi.
Authors: L.H.G. Morton and K.B. Manners
Address: Division of Biology, Preston Polytechnic, Corporation Street, PRESTON, PR1 2TQ

ABSTRACT

Methods available for assaying the extracellular cellulolytic, amylolytic and pectinolytic activity were reviewed.

Two new methods for assessing cellulolytic activity were described, one employing a phototransistor cell to measure the degradation of cellulose particles suspended in agar and the other employing a cone/plate viscometer to assess CMC-ase activity of crude enzyme preparations.

The results of experiments into the effect of TnBTO in ethanol upon enzyme activity showed that the enzymic degradation of particulate cellulose continued in the presence of TnBTO at concentrations up to 800 ppm, CMC-ase activity continuing at concentrations of TnBTO up to 200 ppm. Amylolytic and pectinolytic activity occurred at concentrations of TnBTO up to 100 ppm.

Title: Interaction between early wood-colonizing microfungi and bacteria
Author: D.T.M. Kelly
Address: Division of Biology, Preston Polytechnic, Corporation Street, PRESTON PR1 2TQ

ABSTRACT

When supernatants from certain fungal shake-cultures, grown in a mineral medium containing glucose as the sole carbon source, were assayed against bacteria, inhibition of bacterial growth resulted. Inhibition due to the combined influence of secondary metabolites and pH was recorded for supernatants at pH 5.0 and below, at values above pH 5.0 inhibition of bacterial growth due to the action of secondary metabolites was recorded. The duration of incubation of fungal shake cultures in media containing glucose, cellulose and sodium polypectate was varied and subsequent bacterial inhibition recorded. Incubation times at 25°C for optimum production of inhibitory secondary metabolites are recorded for cellulose and sodium polypectate.

The publication of these abstracts by the Biodeterioration Society does not constitute publication in the usual sense. No reprints are available. Those wishing to pursue these subjects further should write directly to the authors at the addresses shown and not to the Society.
FORTHCOMING CONFERENCES, MEETINGS AND COURSES

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<td>Control of Insects and Rot in Buildings Three Day Course Fee:- £75</td>
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<td>Miss Diane Poole, Building Research Establishment, Princes Risborough, AYLESBURY, Bucks. HP17 9PX. Phone: 0844 43 3101</td>
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<td>Rubber International Rubber Conference and Exhibition ‘Rubbercon ’81’</td>
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<td>Richard H. Craven, Plastics and Rubber Institute, 11, Hobart Place, London SW1W 0HL.</td>
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<td>Cereals: a Renewable Resource Registration Fee:- $195</td>
<td>Copenhagen, DENMARK.</td>
<td>Y. Pomeranz, U.S. Grain Marketing Research Laboratory, 1515 College Avenue, Manhattan, Kansas 66502, U.S.A.</td>
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<td>6–12 September 1981</td>
<td>Biodeterioration 5th International Biodeterioration Symposium Registration Fee :- £50 (to 1 April) £65 (thereafter)</td>
<td>Aberdeen, SCOTLAND.</td>
<td>Dr. J.M. Shewan, 79, Duthie Terrace, ABERDEEN, Scotland. Phone: (0224) 37363</td>
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THE ROLE OF DODECANOIC ACID IN THE MICROBIOLOGICAL CORROSION OF JET AIRCRAFT INTEGRAL FUEL TANKS

Estela R. de Schiapparelli and Blanca R. de Meybaum

Summary

Proliferation of the fungus Cladosporium resinae produces localized corrosion of the structural aluminium alloys used in integral fuel tanks of jet aircraft.

Dodecanoic acid is one of the metabolic products of the biodegradation of hydrocarbons by C. resinae.

By means of electrochemical measurements the aggressiveness of this acid on 2024 structural aluminium alloy was measured and it was shown to lower pitting potential.

Scanning electron microscopy was employed for the morphological analysis of the attack produced on samples submitted to the electrochemical tests.

Role of the acid dodecanoic in the corrosion microbiological of reservoirs d'ales de carburant pour des avions à réaction.

La proliferation du champignon C. resinae produit une corrosion localisée des alliages structuraux d’aluminium utilisés dans des réservoirs de carburant d’avions.

L’acide dodecanoïque est un des produits métaboliques de la biodégradation des hydrocarbures par C. resinae.

A travers des mesures électrochimiques on a déterminé l’agressivité de cet acide sur l’alliage structural d’aluminium 2024 et on a démontré qu’il produisait la diminution du potentiel de pincération.

Des échantillons soumis aux essais électrochimiques ont été analysés moyennant le microscope électronique à balayage, pour définir la morphologie de l’attaque produite.

Introduction

Various roles have been attributed to the microorganisms involved in the corrosion of metals and alloys. The widely reported mechanisms proposed are based on:

- metabolic production of aggressive substances, principally organic acids by hydrocarbon degradation.
- establishment of differential aeration cells by symbiotic action.
- cathodic depolarization of the hydrogen as a result of hydrogenase activity.
- provision of enzymatic redox systems which are able to accelerate anodic or cathodic reactions depending on the possibility of production of oxidase or hydrogenase respectively by the microorganisms involved.

selective elimination of atoms from the alloy by enzymatic extracellular activity.

In previous papers (Schiapparelli and Meybaum, 1977; Meybaum and Schiapparelli, 1978, 1979) we established, by means of electrochemical measurements, that metabolites of Cladosporium resinae and Pseudomonas aeruginosa are aggressive to the aluminium alloys employed in the construction of integral fuel tanks of jet aircraft. The technique introduced by us (Schiapparelli and Meybaum, 1977) in order to measure the aggressiveness of metabolites on aluminium alloys is useful because the EP determined is almost independent of the support electrolyte present. This enabled other authors to apply it to the same contaminants, in analogous conditions, obtaining similar results in other support electrolytes (Salvarezza and Videla, 1978; de Mele, Salvarezza and Videla, 1979).

1 CITEFA (Grupo Corrosion), Zufristegui y Varela, (1603) Villa Martelli Buenos Aires, Argentina.
(Received, May 1980; in final form, July 1980)
The pitting potential (Ep) decrease for increasing metabolite concentration explains the characteristic pitting attack widely reported in service conditions. Considering that metabolic products of the biodegradation of hydrocarbons include organic acids, and that laboratory cultures of the microorganisms mentioned above show pH decrease with growth (Meybaum and Schiapparelli, 1978), the acidity increase could be considered to be the cause of the aggressivity of the medium.

Schiapparelli and Videla (1978) reported that they have found a great influence of pH on the Ep of these aluminium alloys. We, however, have demonstrated (Schiapparelli and Meybaum, 1980) that a laboratory culture with a high content of metabolites did not change the Ep of the aluminium alloy 2024 T 351 in spite of being made alkaline with NH₄OH from pH4 to pH 8. We concluded that the anion concentration of one of these metabolic acids could control the Ep value.

McKenzie, Akbar and Miller (1977) found among the principal metabolites products of oxidation of hydrocarbons the organic acids citric, isocitric, isocitronic and ketoglutaric. They also stated that these acids do not show an appreciable aggressiveness towards aluminium alloys.

Siporin and Cooney (1975) determined the extracellular acids produced by Cladosporium resinae by degradation of hydrocarbons of definite length of chain. The only free fatty acid found was dodecanoic (lauric), the following other organic acids being mentioned: acetic, glicolic, glioxilic, and other unidentified organic acids which were shown not to include lactic, succinic, pyruvic, ketoglutaric, fumaric or citric.

In the present work the influence of some organic acids and of free fatty acid reported in the literature to be metabolic products of hydrocarbon degradation by Cladosporium resinae on aluminium alloy 2024 T 351 is investigated by means of Ep determinations.

Experimental

The test samples were of aluminium alloy 2024 T 351 polished up to 600 grade emery paper.

The pitting potentials were determined from the anodic potentiokinetic curves, in Bushnell Haas (1941) solution, used as support electrolyte, diluted 1:10 with distilled water. A Tacussel PKT 20 2X was employed, a potential scan of 10 mV min⁻¹ being applied with a Tacussel Servovit 9 A. The electrochemical tests were performed in a conventional Pyrex glass cell, using a platinum counter electrode and a saturated calomel electrode (sce), as reference, through a Luggin capillary.

The aqueous solutions of the cultures were filtered in order to avoid microbial adherence to the alloy. Deaeration by N₂ 99.99% bubbling was done prior to and during the polarization tests. After having determined the open circuit potential in each medium, anodic polarization was applied up to +100 mV above the pitting potential.

The aqueous media used for the electrochemical tests were:

- a. Bushnell Haas sterile solution diluted to 1:10 with distilled water, adjusted to pH = 6.0 with H₃PO₄
- b. as a, but adjusted to pH = 3.5 with acetic acid
- c. as a, but saturated in dodecanoic acid at room temperature. (The 1.26 mg. 100 ml⁻¹ which was added was not completely dissolved), pH = 5.2
- d. as c, but adjusted to pH = 8.0 with NH₄OH
- e. as c, but with addition of isocitric acid up to 1.26 mg. 100 ml⁻¹, pH = 5.0
- f. as a, but inoculated with mycelium of Cladosporium resinae isolated from water drained from a jet fuel tank which showed a high proliferation of this fungus. This culture was done in a 500 ml nile-way flask containing 300 ml of the nutrient solution and 50 ml of Jet A 1 as the only carbon source. After 5 days of incubation at 30°C the fungal growth covered the whole water-fuel interface. After 90 days incubation the pH of the aqueous phase used in the electrochemical test had dropped to 4.5
- g. as f, but adjusted to pH = 7.5 with NH₄OH
- h. as a, but inoculated with a culture of Pseudomonas aeruginosa isolated from contaminated jet fuel tanks and maintained on malt agar. The aqueous phase was tested after 90 days of incubation when its pH had decreased to 5.5
- i. Water drained from a jet aircraft fuel tank showing high proliferation of Cladosporium resinae, pH = 5.0

Each curve of Figure 1 is the mean value of triplicate runs.

A scanning electron microscope Joel JSM-U3 was used to determine the morphology of the pits and crevices formed during the electrochemical tests.

Results and Discussion

The results of the electrochemical tests are shown in the form of potentiokinetic polarization curves of 2024 T 351 aluminium alloy in figure 1.

The good correlation which has been observed between acidification of the medium and the amount of proliferation and increase in aggressiveness in laboratory cultures of Cladosporium resinae (Meybaum and Schiapparelli, 1978), could suggest that pH decrease would control the Ep value of aluminium alloys. However, comparison of curves (d), (b), and (e) of Fig. 1 shows that pH decrease caused by the addition of pure organic acids (derived from hydrocarbon biodegradation) does not modify the Ep of the 2024 alloy in the support electrolyte used. Neither the acetate nor the isocitrate ion influences the Ep of the 2024 aluminium alloy. The anion of dodecanoic acid, however, shifts the Ep to a
lower value which is pH independent. This acid was identified by Siporin and Cooney, (1975), as an extracellular metabolic product of *Cladosporium resinae*, being found in a concentration of 1.26 mg. 100 ml⁻¹ in the Bushnell Haas nutrient medium used, once the stationary phase of the culture was attained. Although, in the medium in which curve (c) of Fig. 1 was obtained the dodecanoic acid was not so soluble as in Siporin’s cultures, a high Ep decrease was observed.

It is possible that *Cladosporium resinae* could produce other substances which might be responsible for higher solubility of dodecanoic acid which would further increase the aggressiveness of media with increased proliferation. *Pseudomonas aeruginosa* growth also affects the Ep of the alloy through its production of metabolites (Fig. 1, (h)).

The shape and size of the flask used for laboratory cultures determine the area of the fuel-aqueous interface, thereby conditioning the amount of growth (Parbery, 1971); greater area gives more growth and a good correlation was observed between the amount of growth and the drop in pH of the aqueous phase of the medium (Parbery, 1968).

In previous papers (Meybaum and Schiapparelli, 1978; Schiapparelli and Meybaum, 1980) we showed that cultures of *Cladosporium resinae*, after 60 days of incubation at 30°C, gave a pH drop of the aqueous phase to 4.5 when the incubation was done in 500 ml Erlenmeyer flasks, and to pH 3.5 in 4000 ml flasks of the same shape. All the other conditions were kept constant. In the case of aircraft in which, for design reasons, the water which accumulates at the bottom of

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**Figure 1** Potentiokinetic polarization of 2024 T 351 aluminium alloy

- a) sterile support electrolyte, pH = 6.0
- b) sterile support electrolyte + acetic acid, pH = 3.5
- c) sterile support electrolyte + dodecanoic acid, pH = 5.2
- d) sterile support electrolyte + dodecanoic acid + NH₄HO₂, pH = 8.0
- e) sterile support electrolyte + dodecanoic acid + isocitric, pH = 5.0
- f) *C. resinae* metabolites, pH = 4.5
- g) *C. resinae* metabolites + NH₄HO₂, pH = 7.5
- h) *P. aeruginosa* metabolites, pH = 5.5
- i) Water drained from a contaminated jet fuel tank.
the tanks could not be appropriately drained, very low pH values might be expected. However, our determinations in the water drained from tanks exhibiting high proliferation, had never given values below pH 4 even when deep pitting was confirmed. In the medium corresponding to curve (i), the water drained from a jet fuel tank was very aggressive to 2024 alloy, and its pH was 5.0

Even when organic and free fatty acids known to be metabolites of Cladosporium resinae are present in contaminated tanks of jet aircraft, there must be other substances present, different from those involved in the well controlled conditions of the laboratory tests. This could explain the random pH values observed by us in water drained from sterile or contaminated jet fuel tanks.

The characteristic morphologies observed after anodic polarizations are shown in Figures 2, 3 and 4. The inflections observed in the curves (a) and (b) of Fig. 1 for sterile media at + 50 and 0 mV SCE, suggested the desirability of morphological examination of the samples which had been subjected to anodic polarizations. These values are close to those given by Salvarezza and VideIa (1978), in a similar support electrolyte, to be the pitting potential of Al 99.99% (but without evidence of pit nucleation) although the 2024 alloy showed a lower pitting potential than pure aluminium (See also Schiapparelli and Meybaum, 1980). The samples tested by us in the media of Fig. 1 (a) and (b) were examined by SEM when polarization had reached + 200 mV SCE and the absence of pit nucleation was verified. When the second inflection was attained, however, the pitting process suggested by the shape of the curves was verified. Figure 2 shows the appearance of the samples subjected to up to + 600 mV SCE. The appearance of pits which developed during the electrochemical tests corresponding to curves (c), (d) and (e) is shown in Figure 3, the presence of dodecanoic acid being the cause of the pitting near to - 300 mV SCE. In figure 4 the morphology shown was observed after polarization curve (i) of Figure 1 in drainage water from an aircraft fuel tank with high proliferation of C. resinae. The morphologies found in the last two figures appears to confirm that the pitting process is controlled by dodecanoic acid.

When crevices are formed on an alloy specimen, increasing current densities with potential appear some 100 mV below the pitting potential (Ep). This is

![SEM micrograph of aluminium 2024 polarized up to + 600 mV SCE in sterile Bushnell-Haas solution.](image1)

![SEM micrographs of aluminium 2024 polarized up to - 150 mV SCE in Bushnell-Haas solutions containing dodecanoic acid.](image2)
Crevice Region

Figure 4 SEM micrographs of aluminium 2024 polarized up to -150 mVsc in water drained from a jet fuel tank showing high fungal proliferation.

Atlas and Bartha (1973) determined the inhibitory action of dodecanoic acid on the biodegradation of petroleum by bacteria. Other fatty acids also inhibit certain bacteria (Galbraith et al., 1971; Haska et al., 1972, and Nieman, 1954). So the possibility, inferred from these results, that *Pseudomonas aeruginosa* also produces dodecanoic acid as well as *Cladosporium resinae*, would explain why the latter does not completely inhibit its proliferation but only slows it. In contaminated fuel tanks the population of *Pseudomonas aeruginosa* determined by us indicated a very low proliferation. In mixed cultures *Pseudomonas aeruginosa* has been reported to be the only bacterium which survived in the presence of *Cladosporium resinae* (Parbery, 1971).
Conclusions

The pitting potential of 2024 T 351 alloy is lowered by the metabolites derived from degradation by Cladosporium resinae and Pseudomonas aeruginosa of Jet A 1 fuel.

The acidity increase due to the organic acids produced by the biodegradation of hydrocarbons is not itself the cause to the Ep decrease.

The anion of dodecanoic acid, a metabolite of Cladosporium resinae, lowers the Ep of the alloy at pH values between 5.2 and 8.0 in the support electrolyte employed.

In the presence of crevice conditions on the alloy, as when fungal mats adhered, or there were discontinuities in the protective coat, etc., pitting selectively nucleates in these crevices.

Acknowledgement

The authors gratefully thank the Servicio Naval de Investigación y Desarrollo de la Armada Argentina for its financial support for this work, Dr. J.M. Casellas and Lie. D. Cabral for the isolation and preparation of pure cultures of P. aeruginosa and C. resinae respectively.

References

Atlas, R.M. and Bartha, R. (1973)
Inhibition by fatty acids of the biodegradation of petroleum.
Antonie van Leeuwenhoek Journ. of Microbiology and Serology 39: 257–271

Bushnell, L.D. and Haas, H.F. (1941)
The utilization of certain hydrocarbons by microorganisms
Journ. of Bacteriology 41: 653–673

Galbraith, H., Miller, T.B., Patton, A.M. and Thompson, K.J. (1971)
Antibacterial activity of long chain fatty acids and the reversal with Ca, Mg, ergocalciferol, and cholesterol.
Journ. Applied Bacteriology 34: 803–813

Haska, G., Noren, B., and Odham, G. (1972)
Effect of fatty acids on the activity of bacteriolytic enzymes.
Physiologica Plantarum 27: 181–194

Fungal corrosion of aircraft fuel tank alloys.
Institute of Petroleum (Technical paper) 37–50

Microbiological contaminants influencing the electrochemical behaviour of aluminium and its alloys in fuel water systems.

Meybaum, B.R. de and Schiapparelli, E.R. de (1978)
Microbiological corrosion prevention in jet fuel tanks.
Proc. 7th. International Congress on Metallic Corrosion, Brasil. 3: 1424–1430

Corrosion by microorganisms in jet aircraft fuel tanks. Part II Corrosion.
International Biodeterioration Bulletin 16(2): 31–36

Nieman, C. (1954)
Influence of trace amounts of fatty acids on the growth of microorganisms.
Bacteriological Reviews 18: 146–163

Parbery, D.G. (1968)
The role of Cladosporium resinae in the corrosion of aluminium alloys.
International Biodeterioration Bulletin 4(2): 79–81

Parbery, D.G. (1971)
Physical factors affecting growth of Amorphotheca resinae in culture
International Biodeterioration Bulletin 7(1): 5–9

Corrosion of aluminium and its alloys by microbial contaminants of jet fuels.
7th. International Congress on Metallic Corrosion, Brasil 3: 1389–1398

Schiapparelli, E.R. de and Meybaum, B.R. de (1977)
Corrosion by microorganisms in jet fuel tanks.
III Reunion Latinamericana de Electroquimica y corrosión. Argentina. 239–243

Mechanism of microbiological corrosion.
V Congreso Internacional sobre Corrosion Marina e inrustaciones, España

Siporin, C. and Cooney, J.J. (1975)
Extracellular lipids of Cladosporium (Amorphotheca) resinae grown on glucose or on n-alkanes.
Applied Microbiology 39(3): 604–609
FUNGAL FLORA OF PULPED PEPPER (Capsicum frutescens) STORED UNDER VARIOUS CONDITIONS

S.K. Ogundana¹ and E. Ajulo¹

Summary

Ripe fruits of a variety of Capsicum frutescens were ground to a pulp and subjected to various storage treatments including boiling, addition of sodium chloride, refrigeration, and various combinations of these designed to reflect current culinary practice. By means of the dilution plate method viable counts of fungi were made. The fungal isolates included Aspergillus flavus, A. niger, Alternaria (3 spp.), Epicoccum sp., Cladosporium sp., Geotrichum candidum, Gliocladium sp., Glomerella cingulata, Rhizopus spp., and Trichoderma harzianum.

Refrigeration hindered the proliferation of fungi. Storage at room temperature was generally poor. Boiling alone did not improve storage except when combined with refrigeration. Salting (addition of sodium chloride) before refrigeration further increased delay in fungal growth.

Flora fungique du Piment (Capsicum frutescens) stocké dans des conditions variées.

Des fruits mûrs d’une variété de Capsicum frutescens furent broyés en pâte et soumis à des traitements de stockage variés parmi lesquels l’ébullition, l’addition de chlorure de sodium, la réfrigération et diverses combinaisons de ces traitements pour refléter les pratiques culinaires courantes. Au moyen de la méthode de dilution en plaque on a fait des comptages viables de champignons. Les champignons isolés comprenaient Aspergillus flavus, A. niger, Alternaria (3 espèces), Epicoccum sp., Cladosporium sp., Geotrichum candidum, Gliocladium sp., Glomerella cingulata, Rhizopus sp., et Trichoderma harzianum.

La réfrigération a empêché la prolifération des champignons. Le stockage à température ambiante fut généralement médiocre. L’ébullition seule n’a pas amélioré le stockage sauf si elle est combinée avec la réfrigération. Le salage (addition de chlorure de sodium) avant réfrigération a davantage retardé la croissance fungique.

Introduction

Capsicums are small annual plants belonging to the family Solanaceae. Some species are now grown all the year round in Nigeria under irrigation. The fruits are used as condiments and spices either pulped fresh or dried and ground. Since the fresh fruits are now available all the year round, the drying of fruits has tended to diminish in importance because the fresh fruit is believed to be more pungent and hence more flavourful than the dried fruits. This was confirmed by Agboola (1973) in a survey conducted in Ibadan markets.

Experience has shown that Nigerian housewives prefer to pulp enough fresh fruit for use over a period of time. They have devised various household methods of storing fresh fruits in the pulped form, the commonest of which involves adding an arbitrary quantity of salt and boiling prior to storing at room temperature, or in the refrigerator where this is available.

Flora Fungica de la Pulpa de la Pimienta (Capsicum frutescens) Almacenada bajo varias condiciones.

Se molieron los frutos maduros de una variedad de Capsicum frutescens y fueron sometidos a varios tratamientos de almacenamiento incluyendo cocción, adición de cloruro sódico, refrigeración y varias combinaciones de estos tratamientos, para reflejar el uso habitual en la práctica culinaria. Se hizo el recuento de los hongos viables por el método de dilución en placa. Entre los hongos aislados aparecían: Aspergillus flavus, A. niger, Alternaria (3 spp.), Epicoccum sp., Cladosporium sp., Geotrichum candidum, Gliocladium sp., Glomerella cingulata, Rhizopus spp., y Trichoderma harzianum.

La refrigeración impide la proliferación de los hongos. El almacenamiento a temperatura ambiente no es eficaz. A sola cocción no mejora el almacenamiento, excepto cuando se combina con refrigeración. La adición de cloruro sódico antes de la refrigeración retarda el crecimiento de los hongos.

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many foods (Bainton and Jones, 1977; Gedek, 1977; etc.). In particular Frank (1966) reported that Aspergillus flavus produced aflatoxin on dried red pepper (cited by Scott and Kennedy, 1973).

The present study was carried out to simulate eight different storage treatments that may be used by the housewife with a view to determining the most suitable.

Materials and Methods

Five grams of ripe fresh fruits of Capsicum frutescens were reduced to a pulp with 45 ml sterile glass distilled water (GDW) to make a dilution of 1/10 from which further dilutions were made to 1/1000 which is subsequently referred to as 'pulped fruit'.

Storage treatments

Pulped fruits were variously treated as follows:

(1) FRT - fresh pulp left at room temperature
(2) FSFg - fresh pulp kept in a refrigerator
(3) BRT - boiled pulp left at room temperature
(4) BSFg - boiled pulp kept in a refrigerator
(5) BFg - boiled pulp left at room temperature
(6) FSRT - fresh pulp, plus sodium chloride and stored at room temperature
(7) BSRT - boiled pulp, plus sodium chloride and kept in a refrigerator
(8) BSFg - as in (7) above, but stored in a refrigerator

For each treatment, 2 ml of pulped fruit was added to 18 ml of sterile molten malt extract agar (MEA) contained in McCartney bottles. The contents were thoroughly mixed and poured into 80 mm diameter petri dishes, and incubated at 28°C (representing room temperature) or at 5°C (to represent the highest temperature obtainable in a low-regulated refrigerator).

In the case of boiled treatments, the pulped fruit was boiled for 20 minutes at 100°C and cooled. In treatments where salt (sodium chloride) was used, 10% by weight of salt was added to the pulped fruit or boiled pulp where boiling was involved.

The dishes were set up in quadruplicate and viable counts of fungal colonies were made twice a week for four weeks. The pH values of the remaining pulps of the various treatments were taken once a week. Another set of three samples were sterilized and kept as controls.

Salt tolerance studies

Malt extract agar media were prepared containing 0%, 5%, 10%, 15%, 20%, and 25% (w/v) sodium chloride and were inoculated with the most frequently isolated fungi from salt treated pulps. The cultures were incubated at 28°C and 5°C.

Results and Discussion

Storage treatments

The variations in the numbers of fungal colonies associated with the different storage treatments are shown in Figure 1. The counts in refrigerated treatments are shown in histograms while the lines represent those stored at room temperature. Colony counts at room temperature were significantly higher than those in the refrigerated conditions. Storage at room temperature was poor generally, and worse when the pulp was boiled before storage, as can be seen from graphs 'A' and 'B'. It is possible that by boiling, either more nutrients are released from the plant material which favour fungal growth or the reduced flora was able to proliferate more due to less competition.

When graphs 'A' and 'C' are compared the effect of adding salt is shown. Graph 'C' shows a substantial reduction in the number of fungal colonies whereas 'A' does not during the 4-week period. This result is similar to that of Agboola (1973) for the bacterial counts on stored pepper.

By comparing graphs 'B', 'C' and 'D', the effect of salting or boiling separately and combined are shown. Boiling alone did not sterilize the pulped pepper although it may have reduced the active population as the colony counts at 48 hours in BRT were less than in FRT. From graphs 'B' and 'C' salting was better than boiling alone. A comparison of 'B' and 'D' shows that salting greatly improves storage both at room temperature and in the refrigerator.

However, refrigeration kept down the number of fungal growth for several days (histograms 'C' and 'D') and therefore has advantage over room temperature storage. Addition of salt to the pulped pepper, fresh or boiled, further reduced the fungal colonies.

Organisms isolated

The species, and frequency of occurrence of the fungi isolated are shown in Table 1, whence it can be seen that A. flavus was isolated from every treatment and A. niger from all except FSFg. Several other species were isolated from only one, or a few, treatments but there was no species which was isolated from all Fg (refrigeration) and not from others, although the yeast was found in three of the four Fg treatments.

The presence of yeast is not surprising because yeast contamination in foods has been traced to condiments and spices. Temperatures of 0°C and 4°C do not protect products from incipient fermentation by yeasts unless the storage is for a short period of time only. It has also been confirmed that a high concentration of salt, refrigeration and application of other storage conditions will not wholly safeguard a food from the action of yeasts provided storage is sufficiently long (Phaff, Miller and Mrall, 1966).
Figure 1  The number of microorganisms in fresh capsicum under various storage conditions.
Fungal flora of pulped pepper (Capsicum frutescens) stored under various conditions. S.K. Ogundana and E. Ajulo

Table 1. Fungi isolated from the various storage treatments

<table>
<thead>
<tr>
<th>Storage treatment</th>
<th>Organism</th>
</tr>
</thead>
<tbody>
<tr>
<td>FRT</td>
<td>b c d f i j</td>
</tr>
<tr>
<td>FFg</td>
<td>a b c f g j</td>
</tr>
<tr>
<td>BRT</td>
<td>b c j</td>
</tr>
<tr>
<td>BFg</td>
<td>b c g k</td>
</tr>
<tr>
<td>FSRT</td>
<td>b c f k</td>
</tr>
<tr>
<td>BSRT</td>
<td>b c k</td>
</tr>
</tbody>
</table>

a = Alternaria spp.; b = Aspergillus flavus; c = A. niger; d = Cladosporium sp.; e = Epicoccum sp.; f = Geotrichum candidum; g = Gliocladium sp.; h = Glomerella cingulata; i = Rhizopus spp.; j = Trichoderma harzianum; k = yeast.

For meaning of treatment symbols see text.

Salt tolerance studies

The degree of salt tolerance was studied in fungi most frequently isolated from salt-treated samples because they were presumed to have halophilic properties, and it was desired to ascertain what level of salt concentration would be needed to prevent their growth. From the results shown in Table 2 the growth of the fungi at each salt concentration was lower at 5°C than at room temperature. The result, however, may mean that with some microorganisms the effect of salt is further complicated by an optimum temperature effect. The fact that these same organisms grew well at 28°C is an indication that the poor growth at 5°C was not an effect of the salt but of temperature.

Table 2

Salt tolerance of fungi isolated from salt treated samples

<table>
<thead>
<tr>
<th>Organism</th>
<th>NaCl (% w/v)</th>
<th>Incubation Temperature</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
<td>5</td>
</tr>
<tr>
<td>Aspergillus flavus</td>
<td>++</td>
<td>++</td>
</tr>
<tr>
<td>A. niger</td>
<td>+++</td>
<td>++</td>
</tr>
<tr>
<td>Geotrichum candidum</td>
<td>+++</td>
<td>++</td>
</tr>
<tr>
<td>Trichoderma harzianum</td>
<td>+++</td>
<td>+++</td>
</tr>
</tbody>
</table>

For meaning of treatment symbols see text.

pH determinations

The initial pH values of the various treatments and the subsequent weekly values for 4 weeks are given in Table 3. There were no initial differences between the sterilized and the unsterilized portions of each of the same treatments but the addition of salt tended to lower the pH values which might have accounted for the better preservation of the pulps. Although the pH values of the various treatments did not change appreciably over the 4-week period, those of salt-treated samples remained constantly lower than the non-salted counterparts. The pulps remained fresh throughout with no apparent change in colour except a colour change from orange to yellowish brown or brown in the contaminated samples.

Table 3

The pH of pulp in different storage treatments

<table>
<thead>
<tr>
<th>Storage treatments</th>
<th>Day 1</th>
<th>Week 1</th>
<th>Week 2</th>
<th>Week 3</th>
<th>Week 4</th>
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<tbody>
<tr>
<td></td>
<td>a</td>
<td>b</td>
<td>a</td>
<td>b</td>
<td>a</td>
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<tr>
<td>FRT</td>
<td>5.8</td>
<td>5.8</td>
<td>6.0</td>
<td>5.6</td>
<td>6.0</td>
</tr>
<tr>
<td>FFg</td>
<td>5.8</td>
<td>5.8</td>
<td>5.4</td>
<td>5.8</td>
<td>5.4</td>
</tr>
<tr>
<td>BRT</td>
<td>5.6</td>
<td>5.6</td>
<td>5.6</td>
<td>5.4</td>
<td>5.6</td>
</tr>
<tr>
<td>BFg</td>
<td>5.6</td>
<td>5.6</td>
<td>5.4</td>
<td>5.4</td>
<td>5.4</td>
</tr>
<tr>
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<td>5.3</td>
<td>5.3</td>
<td>5.2</td>
<td>5.6</td>
<td>5.4</td>
</tr>
<tr>
<td>FSFg</td>
<td>5.3</td>
<td>5.3</td>
<td>5.4</td>
<td>5.6</td>
<td>5.4</td>
</tr>
<tr>
<td>BSRT</td>
<td>5.2</td>
<td>5.2</td>
<td>5.6</td>
<td>5.8</td>
<td>5.4</td>
</tr>
<tr>
<td>BSFg</td>
<td>5.2</td>
<td>5.2</td>
<td>5.6</td>
<td>5.8</td>
<td>5.4</td>
</tr>
</tbody>
</table>

a = unsterilized treatment
b = sterilized treatment
* = evidence of contamination
For meaning of treatment symbols see text.
In summary, it can be concluded that refrigeration was superior to room temperature in all cases because it resulted in low counts of fungi. Taken in isolation, refrigeration alone was not the best method of storing fresh capsicums. Storage was, however, improved when combined with the addition of salt. One can therefore make a general statement that effective preservation would depend on the storage temperature and the concentration of salt in the food preserved.

Acknowledgement

The authors wish to thank the Director, Commonwealth Mycological Institute, Kew, Surrey, for identifying most of the cultures.

References


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Changes in properties of finished Indian leather in store due to fungal infestation. O.P. Sharma and K.D. Sharma

CHANGES IN PROPERTIES OF FINISHED INDIAN LEATHER IN STORE DUE TO FUNGAL INFESTATION

O.P. Sharma¹ and K.D. Sharma¹

Summary

Finished Indian leathers viz: vegetable sole, kattai and chrome upper (all Cow) were studied using Indian standardized methods, for changes in physical and chemical properties due to fungal attack during storage for 60, 120, and 180 days at 75% and 90% relative humidities and 28°C±1°C. Tensile strength exhibited only small changes whereas chemical matters showed significant reduction at both humidities after 180 days. The greatest reduction occurred in oils and fats and in water solubles, whereas nitrogen (protein) was reduced only slightly.

Introduction

It is well established that vegetable and chrome tanned finished leathers are attacked by many fungi during usage and storage under environmental conditions suitable for mould growth (Sharma and Sharma, 1977; 1978; 1979). The fungi grow and infest the corium causing undesirable changes in the physical and chemical properties rendering the leather unfit for commercial purposes (Sharma, 1979). It is therefore desirable to evaluate the extent of damage which is brought about as a result of infestation by fungi, and their biochemical activities during storage.

A number of workers have discussed the significance of various parameters in biodeterioration of leathers. However, their studies have mostly been conducted under conditions optimal for growth of fungi (95–100% R.H. and 28°C–30°C). There is little information on the quantitative and qualitative changes in properties due to fungal growth in more normal conditions of humidity. In the present investigations the changes in physical and chemical properties of chrome and vegetable tanned leathers during storage for various periods at various levels of relative humidity, and a constant optimum temperature (28±1°C) have been studied.

Materials and Methods

Cow hide is considered to be typical of mammalian hides and skins, therefore cow leathers, viz: vegetable sole, kattai (vegetable tanned), and chrome upper, were selected so as to avoid possible experimental variations due to varying original sources. The leather pieces, (165 x 65 mm) were stored in the laboratory at 70% and 95% relative humidities controlled by saturated solutions (C.M.I., 1968) in large size desiccators. Prior to storage the pieces were exposed to open atmosphere to get them charged with fungal spores present in the environment.

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Observations were made after 60, 120, and 180 days to study the mould development and changes in physical and chemical properties following standard (Indian Standards Association) methods (ISI, 1970; I.S. 5914 and I.S. 582). The results were compared with those of sterilized control sets kept at each relative humidity under aseptic conditions.

**Sterilization of controls** Simultaneously with exposure to atmosphere of the experimental test pieces, leather pieces for control were sterilized in a desiccator 200 mm in diameter containing 100 ml of methanol, the pieces being kept on a wire gauze about 50 mm above the surface of the alcohol for 24 hours at room temperature. During this period the methyl alcohol vapours were able to sterilize the leather pieces completely. The pieces were then transferred aseptically to another desiccator which could be evacuated through a stopcock to remove the alcohol. They were then again transferred into sterilized desiccators maintained at 75% and 90% to serve as controls.

**Isolation of fungi** Fungi were isolated following the usual methods as described by the Commonwealth Mycological Institute (CMI 1968) as followed also by Smith (1969) and Orlita (1975). Two methods were used:

1. **Direct agar inoculation method**; the samples were observed under a binocular microscope for fungal growth and spores were picked up by inoculating needle from distinct colonies and coloured spots which had developed on the leather surface and directly inoculated on to agar plates under aseptic conditions. The plates were incubated at 28±1°C for up to 7 days.

2. **Serial dilution method** The fungi from deteriorated surfaces were removed by swabbing the sample with sterilized moist cotton which was shaken in 10 ml of sterile distilled water. Serial dilutions (1/10, 1/100, 1/1000, and 1/10,000) were made from this and 1 ml of each was aseptically transferred to petri dishes to each of which 20 ml of sterilized medium was added. These dishes were incubated for 7 days at 28±1°C.

The media used were potato dextrose agar and Czapek-Dox agar. Fungal isolates were purified by the spore suspension streak method and pure cultures were maintained on agar slants. Fungi were identified following standard books and were finally confirmed by courtesy of the Director of the Commonwealth Mycological Institute, Kew, England.

**Results**

The results of observations are given in Tables 1, 2, and 3 for the three types of leather.

**Mould growth** Profuse fungal growth was observed on the grain and flesh sides of the leather at both 75% and 90% relative humidity. Since the control sets were maintained in sterilized conditions, no fungal growth was recorded. In all, 33 species were isolated (Sharma, 1979; Sharma and Sharma, 1980b).

**Odour** The samples developed a change in odour during storage. They were unpleasantly smelling and gave off a mouldy to musty odour. It was very sharp in vegetable sole and kattai leathers.

**Discolouration** Discolouration and formation of coloured unbleachable spots were observed on the grain and flesh sides of the leathers. This was very significant after only 60 days at 90% R.H. Various fungi each caused distinctive coloured spots, viz: blackish brown stain caused by Aspergillus niger and Drechslera papendorfii on vegetable sole and kattai; red spots caused by Penicillium purpurogenum on vegetable sole and chrome upper at 75% and 95% relative humidity after each storage period. A change from the original colour into faint or dark shades was observed in all samples. No discolouration was observed in the control sets.

**Crackiness of the grain** The grain surface of all experimental samples exhibited crackiness on single and double folding after 60 days at each humidity. The grain was considerable weakened at 90% R.H. at the end of the storage period.

**Stiffness** A gradual increase in stiffness was noted when leathers were folded. All showed stiffness at the higher relative humidity which increased with extended storage. The samples were very stiff after 180 days.

**Tensile strength** Tensile strength showed a slight but definite decreasing trend, but the loss in strength at 75% R.H. was only slight. At 90% R.H. in 180 days, kattai leather lost 13.6% of its strength and chrome upper lost 1.27%, but at 75% R.H. the same leathers lost only 9.06% and 0.72% of their strength respectively. The tensile strength of vegetable tanned sole leather was not tested as it was too strong for the tensile tester available.

**Moisture content** The moisture content of both control and test specimens increasing during storage, the increase being greater at 90% R.H. than at 75% R.H. Some parallel samples stored at 65% R.H. did not change in moisture content.

**Oils and fats** Solvent extraction showed a reduction in total oil and fat content of all samples, greater at 90% R.H. than at 75% R.H. Some parallel samples stored at 65% R.H. did not change in moisture content.

**Water solubles** Water soluble components decreased at both relative humidities, the greater reduction being at 90% R.H.

**Chromium** The chromium content of chrome tanned upper leather, expressed as chromium oxide (Cr₂O₃) decreased at both relative humidities, the greater reduction being at 90% R.H.
Changes in properties of finished Indian leather in store due to fungal infestation. O.P. Sharma and K.D. Sharma

Table 1

Changes in properties of Vegetable Tanned Sole leather during storage at 75% and 90% relative humidity and 20°C±1°C

<table>
<thead>
<tr>
<th>Properties</th>
<th>75% relative humidity</th>
<th>90% relative humidity</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control 180</td>
<td>Control 180</td>
</tr>
<tr>
<td>Odour</td>
<td>Control 60 120 180</td>
<td>Control 60 120 180</td>
</tr>
<tr>
<td>Colour</td>
<td>Control 60 120 180</td>
<td>Control 60 120 180</td>
</tr>
<tr>
<td>Crackiness of grain</td>
<td>Control 60 120 180</td>
<td>Control 60 120 180</td>
</tr>
<tr>
<td>Stiffness</td>
<td>Control 60 120 180</td>
<td>Control 60 120 180</td>
</tr>
<tr>
<td>Moisture%</td>
<td>Control 60 120 180</td>
<td>Control 60 120 180</td>
</tr>
<tr>
<td>Percent change</td>
<td>Control 60 120 180</td>
<td>Control 60 120 180</td>
</tr>
<tr>
<td>Oils &amp; fats %</td>
<td>Control 60 120 180</td>
<td>Control 60 120 180</td>
</tr>
<tr>
<td>Percent change</td>
<td>Control 60 120 180</td>
<td>Control 60 120 180</td>
</tr>
<tr>
<td>Water solubles %</td>
<td>Control 60 120 180</td>
<td>Control 60 120 180</td>
</tr>
<tr>
<td>Percent change</td>
<td>Control 60 120 180</td>
<td>Control 60 120 180</td>
</tr>
<tr>
<td>Nitrogen %</td>
<td>Control 60 120 180</td>
<td>Control 60 120 180</td>
</tr>
<tr>
<td>Percent change</td>
<td>Control 60 120 180</td>
<td>Control 60 120 180</td>
</tr>
</tbody>
</table>

Table 2

Changes in properties of Kattai leather during storage at 75% and 90% relative humidity and 28°C±1°C

<table>
<thead>
<tr>
<th>Properties</th>
<th>75% relative humidity</th>
<th>90% relative humidity</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control 180</td>
<td>Control 180</td>
</tr>
<tr>
<td>Odour</td>
<td>Control 60 120 180</td>
<td>Control 60 120 180</td>
</tr>
<tr>
<td>Colour</td>
<td>Control 60 120 180</td>
<td>Control 60 120 180</td>
</tr>
<tr>
<td>Crackiness of grain</td>
<td>Control 60 120 180</td>
<td>Control 60 120 180</td>
</tr>
<tr>
<td>Stiffness</td>
<td>Control 60 120 180</td>
<td>Control 60 120 180</td>
</tr>
<tr>
<td>Tensile strength Kg/cm²</td>
<td>Control 60 120 180</td>
<td>Control 60 120 180</td>
</tr>
<tr>
<td>Percent change</td>
<td>Control 60 120 180</td>
<td>Control 60 120 180</td>
</tr>
<tr>
<td>Moisture %</td>
<td>Control 60 120 180</td>
<td>Control 60 120 180</td>
</tr>
<tr>
<td>Percent change</td>
<td>Control 60 120 180</td>
<td>Control 60 120 180</td>
</tr>
<tr>
<td>Oils &amp; fats %</td>
<td>Control 60 120 180</td>
<td>Control 60 120 180</td>
</tr>
<tr>
<td>Percent change</td>
<td>Control 60 120 180</td>
<td>Control 60 120 180</td>
</tr>
<tr>
<td>Water solubles %</td>
<td>Control 60 120 180</td>
<td>Control 60 120 180</td>
</tr>
<tr>
<td>Percent change</td>
<td>Control 60 120 180</td>
<td>Control 60 120 180</td>
</tr>
<tr>
<td>Nitrogen %</td>
<td>Control 60 120 180</td>
<td>Control 60 120 180</td>
</tr>
<tr>
<td>Percent change</td>
<td>Control 60 120 180</td>
<td>Control 60 120 180</td>
</tr>
</tbody>
</table>
Table 3

Changes in the properties of Chrome Upper leather during storage at 75% and 90% relative humidity and 28°C ± 1°C

<table>
<thead>
<tr>
<th>Properties</th>
<th>Control 60</th>
<th>120</th>
<th>180</th>
<th>Control 180</th>
<th>75% relative humidity</th>
<th>90% relative humidity</th>
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<td>120</td>
<td>180</td>
<td>Control 180</td>
<td>75% relative humidity</td>
</tr>
<tr>
<td>Colour</td>
<td>OC</td>
<td>SC</td>
<td>SC</td>
<td>SC</td>
<td>OC</td>
<td>SC</td>
</tr>
<tr>
<td>Crackiness of grain</td>
<td>CX</td>
<td>C1</td>
<td>C1</td>
<td>C2</td>
<td>CX</td>
<td>C1</td>
</tr>
<tr>
<td>Stiffness</td>
<td>SO</td>
<td>S1</td>
<td>S1</td>
<td>S2</td>
<td>SO</td>
<td>S1</td>
</tr>
<tr>
<td>Tensile strength</td>
<td>234.02</td>
<td>234.0</td>
<td>233.0</td>
<td>230.0</td>
<td>234.0</td>
<td>234.0</td>
</tr>
<tr>
<td>Moisture %</td>
<td>9.86</td>
<td>13.50</td>
<td>15.20</td>
<td>16.60</td>
<td>16.60</td>
<td>11.08</td>
</tr>
<tr>
<td>Oils &amp; fats %</td>
<td>3.0</td>
<td>2.80</td>
<td>2.45</td>
<td>1.80</td>
<td>3.0</td>
<td>2.99</td>
</tr>
<tr>
<td>Water solubles %</td>
<td>2.82</td>
<td>2.55</td>
<td>2.20</td>
<td>1.70</td>
<td>2.80</td>
<td>2.78</td>
</tr>
<tr>
<td>Chromium %</td>
<td>2.50</td>
<td>2.35</td>
<td>2.00</td>
<td>1.85</td>
<td>2.50</td>
<td>2.51</td>
</tr>
<tr>
<td>Nitrogen %</td>
<td>11.43</td>
<td>11.42</td>
<td>11.39</td>
<td>11.36</td>
<td>11.42</td>
<td>11.40</td>
</tr>
</tbody>
</table>

Percent change:

- Odour: - OO = Original odour
- Colour: - OC = Original colour (Vegetable tanned sole - brown; Kattai - black; Chrome Upper - red) SC = Coloured spots
- Crackiness of grain: - CX = No crackiness; C1 = Less crackiness; C2 = More crackiness
- Stiffness: - SO = No change; S1 = Less stiffness; S2 = High stiffness
- Control = Original control
- Control 180 = Sterile control kept 180 days

Tensile strength was not measured for Vegetable Tanned sole leather because its strength exceeded the capacity of the tensile tester available.

Percent change is the value applicable to the samples stored under the experimental conditions expressed as a percentage of the sterile control kept 180 days, except moisture, which is the percentage of the original control.

Chromium was measured only in the Chrome Tanned Upper leather.

Total Proteins (Hide substance) can be calculated from the nitrogen figures by multiplying by 5.62.
Total nitrogen and protein. Protein is calculable from total nitrogen by multiplying by 6.25. Total nitrogen, and therefore protein, including that incorporated during finishing, decreased slightly at both relative humidities, the greater loss being at 90% R.H.

Discussion

It is evident from the results that relative humidity is the factor which chiefly affects the stored samples when the temperature is suitable. Under high levels of relative humidity in the surrounding atmosphere, the moisture content of the leathers was raised considerably which caused rapid mould development. Greater moisture uptake occurred in vegetable tanned samples than in chrome tanned. This difference in water absorbing capacity of different types of leather is due to the compactness of leather fibres and the relative amounts of fat and water solubles present. The more compact leathers with high fats and low water soluble content absorb the least amount of moisture while those of loose structure with high water solubles and low fat gain the greatest amounts, (Nandi et al, 1968, and Sarkar, 1974).

All three types of leather showed luxuriant mould growth at 75% and 90% relative humidity and gave off an unpleasant odour within 60 days. The marked discoloration and formation of coloured stains which developed during storage was due to secretion of various water soluble pigments by the fungi. Sen (1963) observed the appearance of red pigmented patches on chrome tanned leather caused by the growth of Penicillium rubrum. Krishnamurthi et al. (1968) reported black spots on vegetable tanned belting leather by Aspergillus niger, a pink stain on vegetable sole due to Penicillium purpureogenum, and violet spots in E.I. kips due to Aspergillus nidulans. Similar observations were also recorded by Orlita (1966) and Sharma and Sharma (1977, 1978).

The loss of oil and fat during storage was correlated with the high leather moisture contents (16%-30%) favouring the growth of fungi which have been shown to utilize the fat liquors for their growth and establishment (Sharma and Sharma, 1980). The reduction in total water solubles during storage was presumably due to their utilization as carbon sources by the fungi during their active growth.

The undesirable increase in crackiness of the grain, increase in stiffness, and loss of tensile strength, may be due to removal of the water solubles and the oils and fats which impart flexibility and softness to the finished leather. Similar views have been expressed by other workers. Kanagy et al. (1946) studied the effect of microorganisms on vegetable tanned strap leathers exposed to hot humid conditions and stated that fat, waxes, greases, and organic oils incorporated into leather are considerably utilized by the growth of fungi. Banghoorn (1950) and Hyde et al. (1951) also suggested that fungi derive their nutrients chiefly from water solubles, oils and fats, and other constituents from leather surfaces. Pelczar and Reid (1965) and Sharma and Sharma (1980a) opined that the destruction of these substances affects leather products by increasing stiffness, weakening grain, and a tendency to crack and lose tensile strength.

Chrome tanned leather is usually assumed to be resistant to fungal growth due to the presence of chromic oxide (Colin-Russ, 1940; Gray, 1959). In the present investigation chromium content was found to be reduced in deteriorated leathers. Either this decrease is due to the utilization of chromium by fungi during their growth, or to the secretion of organic acids which cause reduction of chromic oxide due to chemical action. However, laboratory studies with basic chromium sulphate have shown that fungi could grow with up to 2.5% concentration of chromium incorporated in the medium (Sharma, 1979). There are several examples in the literature which testify to the adaptive capacity of microorganisms when they are placed in a medium containing some toxic substances injurious to growth. Walker and Smith (1952) reported utilization and detoxification of cyclohexamidine by Myrothecium verrucaria. Lukens (1971) pointed out that fungi can prevent the toxic nature of a chemical in liquid phase.

In the present studies slight reductions in nitrogen and protein contents were recorded but earlier workers have not shown changes in these constituents. This difference in findings is probably due to the fact that earlier workers have confined their studies to tanned collagen which is the constituent protein of leather, whereas in the present investigation, the substrate is finished leather which is enriched in finishing with a number of proteinaceous substances, e.g. casein, gelatin, albumins, pigments and other protein binders to make the leather soft and the surface smooth. Therefore, in finished leather, measurable quantities of extra proteins are present in the finished surface in addition to collagen and hence quantitative reductions were obtained. Whether or not the hide substance (collagen) is attacked by moulds during long term storage is beyond the scope of the present study.

Orlita (1978) stated that degradation of finished leathers by fungi begins by the hydrolysis of substances introduced into leather during its manufacture and the collagen is attacked only in the last phase. Generally it can be said that the biodegradation of tanned collagen is very slow and takes place only after other more accessible nutrients contained in leathers are utilized (Orlita, pers. comm.)
Acknowledgements

We are grateful to Dr. A. Orlita, Shoe and Leather Research Institute, Gottwaldov, Czechoslovakia, for valuable suggestions; to C.S.I.R. New Delhi, India for financial assistance and to the Principal, Agra College, Agra, India, for facilities.

References

Barghoorn, E.S. (1970)
Histological study of the action of fungi on leather JALCA 45 (10): 688–700

C.M.I. (1968)
Plant Pathologists Pocket Book
Commonwealth Mycological Institute, Kew, England

Colin-Russ, A. (1940)
A contribution to the study and control of mould growth in leather and other materials

Gray, W.D. (1959)

A study of mould growth in pyrogallol tanned leather and the associate changes of chemical composition and physical properties.
Journ. Society of Leather Trade Chemists 35: 82–103

I.S.I. (1970)
Indian Standard Methods of Physical Testing (IS: 5914) and Chemical Testing (IS 582) of Leather Indian Standards Institute, New Delhi

Effects of mildew on vegetable tanned strap leather JALCA 61(5): 198–213

Krishnamurthi, V.S., Sen, S.M. and Bhaskaran, R. (1968)
A note on permanent stains on leather caused by fungi
Leather Science 15: 88–91

Lukens, R.J. (1971)
Action of fungus on fungicides; chemistry of fungicidal action
In: Molecular Biology, Biochemistry and Biophysics Chapman and Hall, London

Effect of delay in cure on the rate of vegetable tanning and on the properties of sole leather made from buffalo hide
Proc. Seminar on Biological Aspects of Leather Manufacture, C.L.R.I., Madras, India

Orlita, A. (1968)
Biodeterioration in leather industry

Orlita, A. (1975)
The occurrence of moulds on shoe making materials
Kozarstvi 25(9): 267–269

Pelczar, M.J. and Reid, R.D. (1965)
Deterioration of Materials by Organisms

Theory and Practice of Leather Manufacture
Sharif Publishing Co. Madras, India

Sen, S.N. (1963)
Pigment formation by Penicillium rubrum Stoll.
Nature (Lond) 199: 71–72

Sharma K.D. and Sharma, O.P. (1978)
A new variety of Aspergillus sydowii
Current Science 47(7): 239

Sharma, O.P. and Sharma, K.D. (1977)
Drechslera papendorfii — a new record from finished leathers
Leather Science 24(7): 245

Sharma, O.P. and Sharma, K.D. (1979)
Succession of mycoflora on finished leathers during storage
Defence Science Journal 29: 77–78

Sharma, O.P. and Sharma, K.D. (1980a)
Utilization of fatliquors by fungi
Defence Science Journal 30(2): 93–94

Sharma, O.P. and Sharma K.D. (1980b)
Application of fungicides in control of fungal deterioration of finished leathers in India
International Biodeterioration Bulletin (In Press)

Sharma, O.P. (1979)
Studies on mycobial deterioration of finished leather
PhD Thesis, Agra University, Agra, India

Smith, G. (1969)
An Introduction to Industrial Mycology
Edward Arnold Ltd. London

Walker, A.T. and Smith, F.G. (1952)
Effect of actidione on growth and respiration of Myrothecium verrucaria
A BACTERIAL CONTRIBUTION TO WOOD NITROGEN

B. King¹, W.J. Henderson¹ and M.E. Murphy¹

Summary

The contribution of bacterial nitrogen to the total nitrogen content of wood during decomposition was estimated by impregnating wood blocks with bacterial suspensions at known concentrations and subsequently analyzing the treated wood for total nitrogen content. The results show that a bacterial presence in wood, in numbers commonly found in soil or in decayed wood, may contribute significantly to total nitrogen estimates of wood during decomposition.

Introduction

The importance of nitrogen to wood utilising insects and fungi is well established. However since Savory (1954) noted that soft rot fungi have a requirement for nitrogen in excess of that found in wood before they become actively cellulolytic, the importance of nitrogen to this particular microbial grouping has been especially recognised. The recent failure of tropical hardwoods and treated hardwoods in soil in tropical environments due to this form of decay has again pointed to the nutrient needs of fungi as an important consideration in understanding decomposition processes and in evaluating control measures. While it is known that the nutrient requirements of wood destroying insects may be satisfied in a number of ways including utilisation of microorganisms and microbial metabolites in complex symbiotic relationships, little information is available on nutrient balances in the complex of microbial interactions which take place in decomposing wood in soil.

Recent studies in a number of environments (Henningsson & Nilsson 1976, King, Oxley and Long 1976, Oxley, King and Long 1976, Waite and King 1979), and using different soil types, have shown consistently that the nitrogen proportion of decaying wood increases and that this increase is considerably greater than that which would be accounted for by carbohydrate loss alone during decomposition. King et al. have also shown that there is constant correlation between these increases and the extent of soft rot which takes place, and have suggested (King and Waite, 1979; Waite and King, 1980) that biotic transport is the means by which wood nitrogen is increased. This hypothesis is supported by the findings of King (1980) and King, Smith and Bruce (1980) who showed that nitrogen movement to wood is a preliminary to failure of preservative treated material.

Alternatively Levy (1962) suggested that wood in soil may act as a sink and as such absorb nutrients. Baines and Levy (1979) have shown in laboratory studies that if small stakes have one end enclosed in sealed containers of water and the other end exposed to the atmosphere, dependent upon the relative humidity surrounding the latter, some liquid throughput may take place. These authors suggested that such liquid movement might contribute to nutrient uptake in wood in service confirming the observation of Friis Hansen (1976) that wood immersed in water throughout the year was subject to soft rot at evaporative surfaces. Henningsson (1976) observed that micro-organisms of

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soil salts might be responsible for nitrogen increases observed in decayed transmission poles. However, the studies of King, Oxley and Long (1976) and Waite and King (1979) have shown that dissolved nitrogen did not contribute to nitrogen increases observed by them in wood buried in a normal soil despite liquid uptake. At this laboratory we have quantified nitrogen increases in wood after exposure to micro-organisms in soil and in pure culture and have determined the proportions of such increases which might be allocated to such factors as soil salts and microbial presence. We have also developed quantitative and semi-quantitative techniques for isolation and enumeration of organisms colonising wood and have isolated large numbers of bacteria and actinomycetes from decayed wood in soil. As an adjunct to this work it was decided to evaluate the influences of bacterial numbers on the total nitrogen content of wood. This paper summarises the results of this work.

Materials and methods

Four bacterial species Bacillus polymyxa (Prazmowski) Maco. B. subtilis (Elhernberg) Cohn, Paracoccus denitrificans (Beijerinck), and Micrococcus luteus (Schroeter) Cohn, commonly found in soil and decomposing plant litter, were used for these studies. Bacilli were grown in 50 ml of Oxoid nutrient broth in shake flasks at concentrations of 1.0%, 0.5%, 0.3%, 0.2% and 0.1% (w/v) for 3 and 5 days. Glucose at the same concentrations was added to the flasks of B. polymyxa as this bacterium grows poorly without glucose addition in nutrient media. Coci were grown in static culture in a similar medium to B. subtilis. At the end of the growth periods cells were harvested, by centrifugation at 6000 r.p.m., washed three times in Ringers physiological saline solutions and made up to volumes of 20 ml. 10 ml allquots of these suspensions were used to determine population density, biomass and bacterial nitrogen contents. Population densities were evaluated microscopically using a bacterial counting chamber; biomass was determined on a dry weight basis and bacterial nitrogen content by a micro-kjeldahl technique (Humphries 1956). The remaining 10 ml of each solution was used to impregnate approximately one-centimetre cubes of the sapwood of Lime (Tilia vulgaris Hayne), Beech (Fagus sylvatica L.), and Pine (Pinus sylvestris L.). Pre-weighed dry blocks of each wood were placed in the centre well of a vacuum desiccator and were covered by the bacterial suspension. A vacuum was pulled for five minutes by which time the blocks usually became water-logged and sank. They were then removed, excess liquid allowed to drain off and the blocks were weighed to calculate liquid uptake. The total nitrogen content of blocks was then determined using the Kjeldahl technique. Results

Mean values for biomass, total cell count, bacterial nitrogen content and total nitrogen content of each wood species after impregnation with each organism under all growth conditions are presented in Figure 1. Both biomass production and population levels were greater when organisms were grown at higher nutrient concentrations than when they were grown at lower levels. Mean biomass per ten ml of impregnation fluid rose from 10 mg to 38 mg over the range of concentrations used and mean values for cell counts rose from less than 1 x 10^6 per ml to 7 x 10^8 cells per ml. The nitrogen content of cells also increased with increasing nutrient values of basal media, mean values rising from 3% to over 6% calculated on a basis of bacterial dry weight.

These mean values conceal the extent of variation in population sizes and bacterial nitrogen contents which were observed depending upon organism type, nutrient concentration, age of culture and amount of liquid uptake. Similar variations must also occur in soil. It can be seen from the individual data for B. polymyxa and P. denitrificans (Fig. 2) that whereas suspensions of 1.0 x 10^8 cells/ml could be prepared from cultures of B. polymyxa after incubation for 3 days, suspensions of only 3 x 10^6 cells per ml could be prepared from cultures of P. denitrificans when both strains were grown on 1% nutrient broth. Furthermore, the nitrogen content of B. polymyxa ranged from 3% to 15% over the concentrations of broth used as support media, and that of P. denitrificans ranged from 3% to over 10% under the same conditions of growth. Further studies showed that if broths were incubated for periods up to five days, the nitrogen contents of washed cell suspensions could be as low as 1.5% even when grown on 1% nutrient broth presumably as a result of autolysis and leaching of nitrogen-rich cellular components during stationary and decline phases of growth and the removal of these during subsequent washing procedures. The mean nitrogen contents of lime, beech and pine after impregnation with bacterial suspensions are shown in Figure 3. These results show that lime and pine nitrogen values increased by broadly similar extents when impregnated with bacterial suspension grown in the broth range 0.1% to 0.5%, whereas when bacteria were grown in 1% broth, lime increased less than pine. Beech blocks increased less in nitrogen content on impregnation than either lime or pine. Bacteria grown in 1% broth were shown to increase the mean nitrogen content of lime from 0.08% to 0.12% (an increase of 50%), of pine from 0.05% to 0.11% (an increase of 120%); and of beech from 0.06% to 0.09% (an increase of 50%) with lesser values for organisms grown at lower concentrations.

The lower nitrogen increases of beech are probably accounted for by the lesser liquid uptake by this wood species at all bacterial concentrations after impregnation (Fig. 4). A similar effect may have resulted in the depressed mean nitrogen increases in lime when impregnated with bacteria grown on 1.0% broth. Figure 4 shows that with increasing concentrations of bacteria

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**Figure 1** Mean nitrogen content (% dry weight) cell concentrations (cells/ml) and biomass of bacteria when grown at a range of nutrient concentrations.

**Figure 2** Nitrogen content (% dry weight) and cell concentration (cells/ml) of *Bacillus polymyxa* and *Paracoccus denitrificans* when grown at a range of nutrient concentrations. The nitrogen content of pine after impregnation with both organisms is included in the figure.

**Figure 3** Mean total nitrogen contents of Beech, Lime and Pine after impregnation with bacterial suspensions grown at different nutrient concentrations.

The extent of liquid uptake decreased, presumably as a consequence of blockage of liquid penetration pathways by the heavy bacterial suspensions forced in under pressure. Isolation experiments on impregnated blocks using the technique of Carey (1979) showed that bacteria were concentrated in the outer 2 mm of blocks and that there was little bacterial penetration beyond this point. On this basis, the anatomy, density and porosity of the wood species tested was probably a significant determinant of the degree of nitrogen increase due to bacterial impregnation.

As with bacterial nitrogen contents and population levels, the mean figures for wood nitrogen increases (irrespective of species) do not reflect the variations which were produced by individual organisms. These variations are illustrated in Figure 2 from which it can be seen that *Bacillus polymyxa* increased the nitrogen content of pine from 0.05% to 0.15% when grown on the 1% broth for 3 days (an increase of 200%) whereas *P. denitrificans* in the same time period increased the nitrogen content of pine from 0.05% to 0.10% (an increase of 100%). When *B. polymyxa* and *P. denitrificans* were grown at lower nutrient concentrations the lower population sizes and bacterial nitrogen contents produced proportionately lower increases in total nitrogen content of wood.
The bacteria used in this study were impregnated into the wood and obviously this situation would not occur in nature. However, many soil bacteria are motile and it has been shown that motile bacteria may respond chemotactically by directed movement to even micro-molar concentrations of nutrients (Lynch and Poole, 1979). Wood in service in soil may have considerable concentrations of soluble nutrients (consisting of soluble amino acids and carbo-hydrates) deposited at soil exposure faces. Our work to date on nitrogen accumulation in wood in soil and on quantitative bacterial isolation shows a considerable stimulus by soluble nutrients in wood for biomass transfer from soil, and it is suggested that these nutrients act as chemotactic stimuli. In this way nitrogen, in the form of motile or chemotropic micro-organisms (derived initially from the soil), will be stimulated to enter wood thus enhancing its basic nitrogen content.

The bacilli used in this study measured approximately 3 μm x 1 μm and the cocci 1 μm x 1 μm. However, many rod-shaped bacteria in soil measure up to 7 μm x 2 μm (Bergey, 1973). Traditionally, the zymogenous population is considered to consist of these rods and the smaller mycelial bacteria and it is this population which is likely to immobilise nutrients and invade organic material impregnated in soil contact (Hawker and Linton, 1979). Many of these have high specific growth rates which not only confer competitive advantage but also maintain high inoculum potentials. The presence in wood of larger organisms such as these, at the same nitrogen values and population levels as the bacteria used in the experiments described, would have increased the total nitrogen contents to even a greater using nitrogen content of biomass, cell count, volume of liquid uptake, cell size of individual organisms and block mass showed good agreement with values determined by analysis for the larger bacilli grown at higher nutrient concentrations. Poor correlation existed when this value was calculated for cocci. This is probably explained by different absorption and adsorption characteristics of bacilli and cocci by the various wood species used in the experiments and the lack of total penetration by the bacteria when impregnated into the wood.

Apart from the influence of nutrient medium concentration and generic differences, variations in the amount of nitrogen content increase as a result of bacterial impregnation are also explicable at a secondary level by such features as specific growth rates of individual organisms, particularly in the presence of limited nutrient concentrations, and viability duration during stationary phase of growth. The mean generation time of many bacteria is very rapid and may be as low as 20 minutes for Escherichia coli when grown under optimal conditions (Stanier, Douderoff and Adelberg 1971). Bacteria with high specific growth rates maximise biomass production more quickly than the slower growing organisms. However, on depletion of nutrients and after stationary phase, rapidly growing organisms enter decline phase of growth much more quickly, autolysis then takes place, resulting in biomass

![Figure 4](image-url) Liquid uptake by Beech, Lime and Pine after impregnation with bacterial suspensions grown at different nutrient concentrations.
A bacterial contribution to wood nitrogen. B. King, W.J. Henderson and M.E. Murphy

The studies described in this paper were undertaken to explore the general hypothesis that bacteria, in numbers found in practice in soil or wood, had a significant influence on the total nitrogen content of wood and the results clearly show that this influence may be considerable. As part of this influence they may also contribute, during autolysis, significant quantities of growth factors in which wood may be deficient. Biotic determination of nutrient availability such as this is a recognised feature in soil fertility and organic matter decomposition in soil, and its role in the decay of wood, though little studied, may determine the performance of both preserved and non-preserved wood in service. Much work is required in this area and the concept should also be considered in the development of rapid monoculture and mixed culture techniques for evaluation of antimicrobial preservatives.

Using the data for total nitrogen content of pine after impregnation with \textit{B. polymyxa} and \textit{P. denitrificans} presented in Figure 2 it is possible to show the influence of bacterial presence on the total nitrogen proportion of wood during various stages of loss of weight by decomposition. For the purposes of this transformation the bacterial populations were assumed to be $1.6 \times 10^9$ and $3 \times 10^8$ for \textit{B. polymyxa} and \textit{P. denitrificans} respectively at all decomposition levels and the weight of the wood was corrected in 10% weight loss intervals over the range of 10 - 80%. The nitrogen content of the wood was presumed for the purpose of the transformation to remain unchanged. The transformed data are shown in Figure 5 and it is clear that if the bacterial population of wood is similar to that in soil, the total nitrogen content of decayed wood is increased to a considerable extent by the presence of bacterial cells alone.

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Soft rot in poles treated in the years 1940–1954
In: Soft Rot in Utility Poles Salt-treated in the Years 1940–1954
Swedish Wood Preservation Institute report No. 117 E
(Published 1976): 3.1–3.25

Hawker, L.E. and Linton, A.H. (1979)
Microorganisms, function, form and environment
(2nd. Edition)
Edward Arnold

Henningsen, B. (1976)
Cu- and As- resistance of wood-attacking fungi in relation to the nitrogen content of the substrate.
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Soft rot in poles treated in the years 1941–1946
In: Soft Rot in Utility Poles Salt-treated in the Years 1940–1954
Swedish Wood Preservation Institute report No. 117 E
(Published 1976): 1.1–1.25

Humphries, E.C. (1956)
Mineral components and ash analysis
In: Modern Methods of Plant Analysis. Paech, K and Tracey, M.V. (Editors)
Springer Verlag, Berlin

King, B. (1980)
The durability of wood and wood products
Proceedings of the Symposium on Biodegradation of Polymeric Materials, Strathclyde University, April 1979
(In press)

Some biological effects of redistribution of soluble nutrients during drying of wood.
Material und Organismen 11(Suppl): 264–276

King, B., Smith, G.M. and Bruce, A. (1980)
Soluble nutrient influences on toxicity and permanence of CCA preservatives in wood.
International Research Group on Wood Preservation Document No. IRG WP 3144
11th. meeting, Raleigh, N. Carolina, May 1980

King, B. and Waite, J. (1979)
Translocation of nitrogen to wood by fungi

Lynch, J.M. and Poole, N.J. (1977)
Microbial Ecology: a Conceptual Approach
Blackwell, London. 266pp

Some effects on decay of wood caused by redistribution of nutrients during drying.
Record of the 1976 Annual Convention of the British Wood Preservation Association: 87–96

Savory, J.G. (1954)
Damage to wood caused by microorganisms
Journ. of Applied Bacteriology 17: 213–219

General Microbiology (3rd. edition)

Waite, J. and King, B. (1979)
Total nitrogen balances of wood in soil.
Material und Organismen 14(1) 27–41

Waite, J. and King, B. (1980)
Quantification of microbial invasion of wood.
Pitman, London.
BOOK REVIEW


This book is not, and does not claim to be, a textbook on the subject: rather it is one-third a literature review and two-thirds a report of detailed studies on the biological treatment of mixed industrial wastewaters at four sites in USA, carried out for the Environmental Protection Agency (EPA). Thus the title is misleading and should have been changed to reflect the actual content.

The mixed waste waters dealt with at the four sites are limited to those from petrol refining, petro-chemicals and other synthetic organic chemicals, paper mills, textiles and pharmaceuticals, while the literature review covers a wider range. Some eleven "innovative applications" of biodegradation techniques are given in the review but are not described in sufficient detail for full understanding without reference to the original papers. The techniques range from biological seeding to fluidized-bed reactors, and comparisons of performance are made where this is possible. The processes used, and described in much detail, at the four sites are limited to facultative lagoons, high-rate activated sludge, deep shaft and the UNOX oxygen facility. Not only are the characteristics of the wastes, the design, operation and performance of the processes presented, but also estimates of costs are given, together with the legal requirements where these are known.

There follow, in a very useful separate section, results of GC-MS analysis of influents, effluents, sludges and sediments taken from three of the sites. About 50 compounds spread over some nine groups were identified and conclusions are made from the concentrations found at each stage as to the biodegradability of these rather recalcitrant substances.

The book concludes with a section on what amounts to guidelines for making engineering and economic comparisons between biodegradation techniques, bibliography and notes on sampling and analysis.

There is no doubt that this book contains a wealth of material and that the investigations described were carried out painstakingly, but because of the method of presentation the reader might well be put off. The printing (photo-offset of the typed original) is unattractive and the publishers admit that some parts "may be less legible than desired". The original reports to EPA may well have been suitable for their intended purpose but drastic editing, to avoid repetition, and a gathering together of principles are necessary when the material is presented to a wider audience. The lack of an index and the reliance on a table of contents do not provide easy access to the information, as the publishers claim. Even when the information is found it is too often in an undigested or incomplete form, particularly in tables in the literature review section. For example, flows and loadings are sometimes given in non-specific terms (i.e. m³/d and kg/d) without the size of the treatment installation, instead of in terms which gives readers the full picture (i.e. m³/m³ d and kg/m³ d or kg/kg d). Some sets of results are presented unnecessarily in both graphical and tabular form, while some figures give quite irrelevant information such as the structure of a hut, or the single change in components making up an influent. A source of confusion, at least to the non-American reader, relates to abbreviations such as "IGPD", "S⁰" and "P⁰". It is also disturbing to find a switch within a paragraph from "kg/1000 m³" to "mg/l d" describing a given loading. Finally, there are more than the usual number of "typographical" errors, some of which are obvious but many of which remain unresolved; there is even a wrong "pagination" — pages 215 and 217 should be interchanged.

In summary, there is much useful information in this book for those seeking to treat industrial waste waters by biological methods, especially the more recent modifications, but the reader will have to work hard to find it.

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