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...
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INTERNATIONAL BIODETERIORATION BULLETIN

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INTERNATIONAL
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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 x 29.7 cm; or 8.27 in. x 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 x 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 Levinson (1952).

and in the bibliography:

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BIODETERIORATION SOCIETY NEWSLETTER

Special Meeting on Decay of Wood in Boats

A special meeting, convened at the request of a member of the Society, Mr. C. J. Chapman, a consultant and surveyor of small boats, was held at the Royal Commonwealth Society on Wednesday, 19th February. Sixteen persons were present, mainly members of the Society but including, also, members of the boating press, a Naval Architect and a representative of the wood preserving industry.

Mr. Chapman addressed the meeting and most of those present participated in the discussion. It was concluded that many of the problems raised were the result of misunderstanding by practical, experienced men of the literature produced by mycologists and other scientists. It is expected that the opportunity will be taken when some of the well known publications on biodeterioration of wood are re-issued, to present them in such a way that absolute clarity is preserved. This will ensure that intelligent laymen will find no conflict between their experience and the principles and practice laid down by experts.

Several of those present expressed the view that the Society had done a useful job in organising this meeting.

Forthcoming Meetings - 1975

16th April 1975
"Mechanisms of biocidal and bio-static activity."
University College of Wales, Aberystwyth. (Jointly with Industrial Group of The Biochemical Society).

11th-12th July, 1975
Summer Scientific Meeting University of Reading.
11th—Symposium on "Biodeterioration problems in agriculture."

12th—Presentation of short (15 minute) original papers on any aspect of biodeterioration.

Offers of all papers should be made to the Hon. Programme Secretary as soon as possible.

17th-23rd August, 1975
3rd International Biodegradation Symposium incorporating the Society A.G.M. University of Rhode Island, U.S.A.

28th November 1975
"Cargo biodeterioration problems" University of Cambridge.

Offers of papers should be made to the Hon. Programme Secretary by 1st August, 1975.

Hon. Programme Secretary:
Dr. R. H. Tilbury,
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BIODETERIORATION INFORMATION CENTRE

REVISION OF THE TECHNICAL ENQUIRY AND PHOTOCOPYING SERVICES

1) Technical Enquiry Service Reorganisation

Since the Centre has become self supporting it has been necessary to review the economic efficiency of the enquiry service. As a result, from April 1st 1975, all enquiries involving a literature search will be charged at a rate of £8.00, inclusive of up to 50 pages of photocopying (anything over 50 pages will be charged at the rate of 5p per page). If the search time required exceeds two hours, subsequent time will be charged at £5.00 per hour. British enquirers will be advised by telephone if it is found that more than two hours or extra photocopying is needed. Overseas enquirers will be sent the results of up to two hours work and up to 50 pages of photocopying, if appropriate, and told if further work is likely to yield further results. Where enquiries can be answered without undertaking a literature search no charge will be made. The rates above apply to both subscribers and non-subscribers to the Centre's publications.

Sustaining Associates

The special enquiry facilities offered to Sustaining Associates remain unchanged. (i.e. free enquiry time but a charge on any photocopying over 50 pages at 5p per page).

Personal callers at the Centre

The Centre welcomes visitors to the Centre; individuals may search the document collection, free of charge (but a charge of 5p per page will be made for any photocopies taken).

2) Revision of Photocopying Rates

From April 1st 1975, photocopying charges will be 5p per page (minimum 50p), although there will be higher rates for copies of items held by the Centre as microfiche or microfilm.
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<td>7-9 April 1975</td>
<td>International Symposium on Food from Waste</td>
<td>Weybridge, Surrey, England</td>
<td>The Symposium Committee, c/o The Secretary, National College of Food Technology, St. George's Avenue, Weybridge, Surrey.</td>
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<td>2-4 June 1975</td>
<td>2nd International Hydromicrobiology Symposium</td>
<td>Smolenice, Czechoslovakia</td>
<td>Dr. I. Daubner, Limnobiology Institute Slovakian Academy of Sciences, ul. Obrancov mieru 1/4, 865 34 Bratislava, Czechoslovakia</td>
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<tr>
<td>20-26 July 1975</td>
<td>6th International Continuous Culture Symposium</td>
<td>Oxford, England</td>
<td>Mr. A. Fleming, Secretary, Microbiology Group, Society of Chemical Industry, Biochemistry Dep., Imperial College, London SW7 England. (Tel: 01-589 5111 ex. 1104)</td>
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<tr>
<td>29 September-10 October 1975 Repeated on 24-26 November 1975</td>
<td>The Control of Insects and Rot in Buildings (3 day course)</td>
<td>Princess Risborough, England</td>
<td>Mrs. Susan Hobbs, Building Research Establishment, Princess Risborough Laboratory, Princess Risborough, Aylesbury, Bucks. HP17 9PX</td>
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<td>14-17 October 1975</td>
<td>International Rubber Conference 1975</td>
<td>Tokyo, Japan</td>
<td>Secretariat, IRC '75—Tokyo, Society of Rubber Industry, 5-26 Motoakasaka, 1-chome, Minato-ku, Tokyo 107, Japan</td>
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<td>20-25 October 1975</td>
<td>International Rubber Conference 1975</td>
<td>Kuala Lumpur, Malaysia</td>
<td>Chairman, Executive Committee IRC '75—Kuala Lumpur, Rubber Research Institute of Malaysia, P.O. Box 150, Kuala Lumpur, 01-02, Peninsular Malaysia.</td>
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<tr>
<td>14-19 June 1976</td>
<td>4th International Congress on Marine Corrosion and Fouling</td>
<td>Juan-les-Pins, Antibes, France</td>
<td>Secretariat, Centre de Recherches et d'Etudes Oceanographiques 73/77, rue de Septes, 92100 Boulogne-sur-Seine</td>
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ON THE EFFECT OF SYNTHETIC POLYMERS ON ENZYMES BIOSYNTHESIS. I. POLYFORMALDEHYDE

V. N. Kestel'man\(^1\), R. D. Koshkina\(^1\), G. L. Vinogradova\(^1\), M. A. Ostrovskaya\(^1\)

Summary. The presence of paraformaldehyde in the culture medium was found to alter the fermentative activity of *Aspergillus oryzae* and *Bacillus mesentericus*; that of *A. oryzae* was enhanced while that of *B. mesentericus* was suppressed.

**Introduction**

The industrial microbiological production of enzymes, vitamins, antibiotics etc. is a process of great importance, and the problem of increasing the fermentative activities of microorganisms is, therefore, of great interest.

It was previously shown (Kestel'man and Vilnina, 1971; Trifonova *et al.*, 1970) that the presence of polymeric samples in media for the cultivation of microorganisms leads to a change in the process of fermentative synthesis, involving either the enhancement or the suppression of the fermentative activities in comparison with a control.

In this work we investigated the influence of the presence of polyformaldehyde samples on the biosynthesis of enzymes by the fungus *Aspergillus oryzae* and the bacterium *Bacillus mesentericus*.

**Materials and Methods**

*B. mesentericus* was cultivated by deep culture in a medium consisting of 4% malt sprout water with 1% soluble starch and *A. oryzae* in a medium consisting of 4% rye flour, trace elements (0.5%KH\(_2\)PO\(_4\); 0.001%MgSO\(_4\); 0.001%FeSO\(_4\); 0.001%ZnSO\(_4\)) and tap water.

The polyformaldehyde samples were placed in the experimental Erlenmeyer flasks together with 50ml of the respective nutrient medium. Control flasks contained medium only. The flasks and their contents were then sterilised in an autoclave for 30 min. at a pressure of 0.1 MPa (1 atm.).

The respective flasks were then inoculated with 1ml of water suspension of *B. mesentericus* and incubated at 30°C for 24-30 hours rocked at 180 r.p.m. and with 5% of water suspension of *A. oryzae* spores and incubated at 26°-30° for 37-39 hours.

Each polyformaldehyde sample had a surface area of 20cm\(^2\). Surface area of the samples was increased by increasing the number of samples per flask.

Milk-clotting activity (CA) was determined according to the method used in cheese production (Inihov, 1962).

Proteolytic activity was defined by the Leiljan-Folgard method in Babakina's (1962) modification and Anson's (1938) method. Amylolytic activity was determined by the technique of Klimovsky and Rodzevich (1952).

**Results and Discussion**

The results show that the presence of polyformaldehyde in the nutrient medium for the cultivation of *A. oryzae* is accompanied by the increasing of the synthetic ability of this microorganism. Moreover the proteolytic activity (MC) increased by 220-260% and the amylolytic activity (AC) by 140-160% (Table 1).

---

\(^1\)Technological Institute of the Food Industry, Moscow, U.S.S.R.

(Copy originally received September 1973; in final form August 1974)
TABLE 1. Fermentative activities of *Aspergillus oryzae*.

<table>
<thead>
<tr>
<th>Object</th>
<th>Proteolytic activity</th>
<th>Amylolytic activity</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Alkaline protease</td>
<td>Acid protease</td>
</tr>
<tr>
<td></td>
<td>I H II III</td>
<td>I I II III</td>
</tr>
<tr>
<td>Polyformaldehyde</td>
<td>0.740 0.830 0.900</td>
<td>350 330 1.00 0.89 0.92</td>
</tr>
<tr>
<td>control</td>
<td>0.308 0.308 0.400</td>
<td>138 138 0.68 0.55 0.57</td>
</tr>
</tbody>
</table>

The numerator expresses the activity in unit/ml the denominator expresses the activity in per cent.

I, II, III—successive cycles of cultivation.

The kinetics of the process of proteolytic activity accumulation in the cultural liquid of *A. oryzae* testifies to the higher speed and intensity of the process occurring when polyformaldehyde has been added (Figure 1).

![Figure 1](image1.png)

Time in hours

**Figure 1.** The dynamics of protease accumulation by *Aspergillus oryzae*.

OM = experiment
K = control
AC = proteolytic activity

The maximum proteolytic activity of the cultural liquid in the experimental flasks coincides with that in the controls. It corresponds to 37-39 hours of mould growth.

Figure 2 expresses the kinetics of the accumulation of *α*-amylase in the cultural liquid in the presence of polyformaldehyde. From the figures it can be seen that the process is slower in the experimental flasks. Possibly it is connected with the adaptation of the strain to the changed conditions. After 21 hours of mould growth a sharp increase of amylolytic activity in the experimental flasks is observed and the process becomes stable after 37-39 hours of growth.

![Figure 2](image2.png)

**Figure 2.** The dynamics of *α*-amylase accumulation by *Aspergillus oryzae*.

OM = experiment
K = control
AC = amylolytic activity

In order to determine whether the synthetic ability of the mould depends on the surface area of the polymer samples in the medium, a further series of experiments was carried out in which the surface area of the samples was changed from 20 cm² to 160 cm² in 20 cm² stages.

The results represented in figure 3 show that the increase of the surface area of the samples to 120 cm² provokes a simultaneous increase in the proteolytic and amylolytic activities. Further increase of the surface area is accompanied by a decrease in the quantity of the exoenzymes synthesised by the mould.

![Figure 3](image3.png)

**Figure 3.** Dependence of enzymatic activity in *Aspergillus oryzae* cultures on the surface area of samples.

AC = amylolytic activity
MC = proteolytic activity
When *B. mesentericus* was cultivated with polyformaldehyde the intensive bactericidal action of this plastic was discovered (Table 2).

### TABLE 2. Fermentative activities of *Bacillus mesentericus*.

<table>
<thead>
<tr>
<th>Object</th>
<th>Milk clotting activity</th>
<th>Proteolytic activity</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>I</td>
<td>II</td>
</tr>
<tr>
<td>Polyformaldehyde</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Control</td>
<td>300</td>
<td>3428</td>
</tr>
</tbody>
</table>

*The numerator expresses the activity in unit/ml; the denominator expresses the activity in per cent.*

I, II, III—successive cycles of cultivation.

Growth of *B. mesentericus* is not inhibited with separate sterilisation as it is with joint sterilisation and fermentative activity is equal to that in the controls.

### References


Adding the cultural liquid to petri-dishes containing agar medium, the intensive suppression of bacterial growth was observed.

Polyformaldehyde is liable to depolymerise at a temperature of 130°. To discover the substances which may migrate out of the polymer into a liquid medium during high temperature sterilisation, experiments were carried out in distilled water and individual compounds were identified by gas liquid chromatography. The results showed the presence of formic and acetic acids as depolymerisation products of polyformaldehyde under these conditions.

To exclude the influence of these substances on the cultivation of the microorganisms, the polymer samples were sterilised separately from the nutrient media and then added to the flasks before inoculation. The experimental results are shown in Table 3.

In the case of *A. oryzae* the fermentative activity is lower with separate sterilisation than with joint sterilisation but it is still higher than in the controls by 1.7 times with acid protease and 1.8 times with alkal protease. The amylolytic activity remains at the same level as in the controls.

### TABLE 3. The effects of joint and separate sterilisation of medium and polymer on enzymatic activity of *A. oryzae* and *B. mesentericus*.

<table>
<thead>
<tr>
<th>Experimental conditions</th>
<th>Aspergillus oryzae</th>
<th>Bacillus mesentericus</th>
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<tbody>
<tr>
<td></td>
<td>Proteolytic activity</td>
<td>Amylolytic activity</td>
</tr>
<tr>
<td></td>
<td>alkali unit/ml</td>
<td>acid %</td>
</tr>
<tr>
<td>Joint sterilisation of medium and polymer</td>
<td>4.15</td>
<td>300</td>
</tr>
<tr>
<td>Separate sterilisation of medium and polymer</td>
<td>2.40</td>
<td>180</td>
</tr>
<tr>
<td>Addition of formic acid to the medium</td>
<td>2.26</td>
<td>170</td>
</tr>
<tr>
<td>Control</td>
<td>1.32</td>
<td>100</td>
</tr>
</tbody>
</table>
ARTIFACTS IN THE REGULATORY ANALYSIS OF MARKET SAMPLES OF FOODGRAINS USING URIC ACID CONTENT AS A PARAMETER FOR QUALITY

S. P. Pillai1, M. V. Sharangapani1, S. K. Majumder1 and B. L. Amla1

Summary. Analysis of market samples for kernel damage, insect count, frass, uric acid and market quality indicated anomalies in the correlation characteristics between 'true' uric acid content and organoleptic quality (F.A.Q.). Controlled laboratory samples and market samples were compared for kernel damage, insect count, 'true', 'total' and 'apparent' uric acid, and frass contents. Data indicated a close relationship of 'Benedict and Franke's Reaction Complex' and 'apparent' uric acid with organoleptic quality, while 'true' uric acid did not. Hence further investigation is needed to establish 'Benedict and Franke's Reaction Complex' as the index of grain quality for regulatory purposes.

Introduction

In regulatory assessment for quality of foodgrains, several parameters have been suggested as indices of biodegradation. Quality considerations for foodgrains like cereals and pulses are based on their nutritional characteristics. No convenient criterion has yet been established to estimate the biological or nutritive quality of grain samples which could be adopted for regulatory purposes. Since the nutritional or biological tests are time-consuming, there is a need for evolving rapid and indirect indices for the quality of the foodgrain.

Earlier work relates to the measurement of free fat acidity as a parameter for quality of stored grain. Fifield and Bailey (1929), Zeleny and Coleman (1938), Hunter et al. (1951) and Baker et al. (1957) showed that the level of fat acidity varied with commodity and conditions of storage.


Since stored grains are susceptible to insect infestation, Subramanyan et al. (1955) were the first to investigate the uric acid content as an index of insect activity in cereals and cereal products. Venkat Rao et al. (1959) improved on the method of estimation of uric acid by differentiation between uricase active and uricase inactive uric-acid like substances. Although several workers such as Howe and Oxley (1944) considered several physical, chemical and biological characteristics, Nicholson et al. (1953) recommended the use of visual examination for the direct assessment of insect damage. Linko and Sogin (1960) reported on the relationship of germination percentage and storage deterioration to glutamic acid decarboxylase activity in wheat. Harris and Knudsen as early as 1948 recommended the use of insect fragment count as a parameter for insect infestation in milled materials.

Apart from the insect activity, mould growth in stored grain also brings about changes in grain

1Central Food Technological Research Institute, Mysore-13, India.

(Copy received September 1974)
Artifacts in the regulatory analysis of market samples of foodgrains using uric acid content as a parameter for quality. S. P. Pillai, M. V. Sharangapani, S. K. Majumder and B. L. Amla.

quality. While Hunter et al. (1951) reported on the increase of free fat acidity on rice with the increase of moisture, Hummel et al. (1954), Domenig et al. (1955a, 1955b), and Baker et al. (1959) established a high positive correlation between mould damage and free fat acidity.

Deterioration is a complex phenomenon and consists of physical scouring of material, changes in fatty acids, uric acid, fragment count and other criteria, but these criteria do not show a close relationship with the quality status of the stored cereals. Parvathappa et al. (1970) were the first to recognise the significant relationship between fungi and "uric acid like substances" which reacted with Benedict and Franke's reagent. Working on physical and biochemical changes in grain sorghum they obtained a high degree of correlation of moisture with fungi and of fungi with "apparent" uric acid.

During the course of a survey of market samples parameters such as moisture, kernel damage, insect count, frass, germination percentage, weight/volume ratio, free fat acidity, and uric acid ('total', 'apparent' and 'true') were employed for obtaining reliable information on the quality of samples. In this survey of market samples, several anomalies appeared in the analysis and in the subsequent interpretation of the data. Although relationship between moisture and fat acidity indicated a positive correlation, parameters like kernel damage, insect count, frass, and uric acid content did not establish the expected trend in correlation characteristics with any of the factors in these market samples. Therefore attempts have been made to analyse and isolate the factors which are responsible for the anomalies in the analytical data, which have been obtained in the market samples as compared to the expected results under controlled conditions. In the present paper the relevant data on the above parameters in market samples as compared to the expected results under controlled conditions. Therefore, in addition to the "total" uric acid.

Materials and Methods

i) Sampling: Samples of wheat, rice, greengram, fieldbeans and cowpea were drawn from different market centres. The samples were analysed for moisture content, free fat acidity, uric acid ('total', 'apparent' and 'true'), germination percentage, weight/volume ratio, kernel damage, insect count and frass, by the methods described below.

ii) Analytical Methods


Kernel damage—Number of insect-damaged kernels per 100 kernels, the value expressed as percent.

A kernel was considered as insect damaged if insect devouring, emergence or entry holes are present on visual examination by the method of Nicholson et al. (1953).

Insect count—The samples were sieved and only emerged adults were counted; both dead and live adults were included in the count. The results are expressed as the total count/100 g.

Frass—100 g of the sample was sieved through standard sieve No. 8, 10 or 12 as the size of the grain warranted and the weight in g of the sievings expressed as percent.

Germination percentage—Method of Linko and Sogn (1960).

Wt/Vol. ratio—100/cc of the sample was measured in a measuring cylinder. The weight of the material expressed in g/100 cc.


'Total' Uric acid—A protein-free water extract of finely powdered grain material when treated with Benedict and Franke's reagent in presence of sodium cyanide develops a blue colour which is measured colourimetrically. The fraction positive to this test is 'total' uric acid.

'Apparent' Uric acid—Uric-acid-like substances giving a positive colour reaction to Benedict and Franke's reagent in presence of sodium cyanide after the removal of "true" uric acid by the action of uricase enzyme in a protein-free water extract of finely powdered grain material.

'True' Uric acid—The end product of purine metabolism in insects. The fraction removed by the action of uricase enzyme in the protein-free water extract of finely powdered grain material. Estimated experimentally by subtracting 'Apparent' uric acid from 'Total' uric acid.

iii) Simulated studies: In the market samples some of the components such as the frass and excreta of insects may not be included due to loss of frass material from the damaged kernels. Under the conditions of incubation in the laboratory these materials, however, could be included in the composite samples prior to subjecting them for analysis, so as to study the distribution pattern of 'total', 'apparent' and 'true' uric acid in frass-free fraction of grain infested under controlled conditions. Therefore, in addition to the 206 market samples on which the above parameters were employed.
Artifacts in the regulatory analysis of market samples of foodgrains using uric acid content as a parameter for quality. S. P. Pillai, M. V. Sharangapani, S. K. Majumder and B. L. Amla.

samples of grains viz. wheat, greengram and rice were incubated under controlled conditions to assess the changes in the parameters of biodeterioration.

1 kg. each of the 3 commodities (insect and damage free and fresh) were incubated in glass bottles covered with cloth at 25 ± 2°C at RH 75% for 90 days with 100 insects respectively in each sample. In rice and wheat Sitophilus oryzae adults and in greengram Callosobruchus chinensis adults were released. After 90 days the material was sieved through a standard No. 12 sieve to separate the frass from the kernels. The frass material and the frass-free fraction were analysed to estimate the amount of uric acid present.

Physical and organoleptic characteristics such as odour, colour, lustre and feel, and presence of re-fractions (broken or imperfect grains) or foreign matter are employed by purchasers of raw grains. Market quality for on the spot inspection mostly depends on these factors. Recent approaches in advanced countries employing X-ray radiography and ninhydrin tests with special equipment are increasingly finding application. For the purpose of quick assessment of the physical and organoleptic qualities of the grain samples they were classified as 'good', 'fair' and 'bad' following the normal quality parameters employed under marketing regulations. The fair average quality (F.A.Q.) as employed under the Indian Food Laws (1954) was followed for grading and classifying the samples.

TABLE 1.
F.A.Q. grading vs. distribution of kernel damage, insect count, frass and uric acids of market samples of grains. (Mean values)

<table>
<thead>
<tr>
<th>Number of samples</th>
<th>Kernel damage %</th>
<th>Insect count no./100g</th>
<th>Frass mg/100g</th>
<th>Uric acid mg/100g</th>
<th>F.A.Q. classification</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Total mg/100g</td>
<td>Apparent mg/100g</td>
<td>True mg/100g</td>
</tr>
<tr>
<td>Wheat (Triticum vulgare)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>14</td>
<td>6.0</td>
<td>66.1</td>
<td>440</td>
<td>5.3</td>
<td>4.5</td>
</tr>
<tr>
<td>21</td>
<td>10.4</td>
<td>38.8</td>
<td>220</td>
<td>7.0</td>
<td>6.2</td>
</tr>
<tr>
<td>46</td>
<td>57.3</td>
<td>150.2</td>
<td>2250</td>
<td>17.6</td>
<td>16.0</td>
</tr>
<tr>
<td>Rice (Oryza sativa)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>.</td>
<td>12.7</td>
<td>140</td>
<td>5.6</td>
<td>3.0</td>
</tr>
<tr>
<td>10</td>
<td>.</td>
<td>8.1</td>
<td>130</td>
<td>7.3</td>
<td>3.8</td>
</tr>
<tr>
<td>2</td>
<td>.</td>
<td>5.0</td>
<td>250</td>
<td>17.8</td>
<td>10.0</td>
</tr>
<tr>
<td>Greengram (Phaseolus aureus Roxb.)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>25</td>
<td>1.5</td>
<td>64</td>
<td>20</td>
<td>11.9</td>
<td>10.5</td>
</tr>
<tr>
<td>11</td>
<td>9.7</td>
<td>62.4</td>
<td>100</td>
<td>19.8</td>
<td>18.2</td>
</tr>
<tr>
<td>10</td>
<td>26.6</td>
<td>312.1</td>
<td>1650</td>
<td>39.4</td>
<td>33.6</td>
</tr>
<tr>
<td>Cowpea (Vigna catjang Burm.)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>27</td>
<td>3.1</td>
<td>2.2</td>
<td>0</td>
<td>13.1</td>
<td>11.5</td>
</tr>
<tr>
<td>9</td>
<td>6.5</td>
<td>9.1</td>
<td>60</td>
<td>17.9</td>
<td>15.7</td>
</tr>
<tr>
<td>8</td>
<td>50.7</td>
<td>256.0</td>
<td>1430</td>
<td>33.0</td>
<td>25.7</td>
</tr>
<tr>
<td>Fieldbeans (Dolichos lablab L.)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>6.3</td>
<td>3.3</td>
<td>0</td>
<td>13.3</td>
<td>12.3</td>
</tr>
<tr>
<td>6</td>
<td>18.8</td>
<td>101.8</td>
<td>330</td>
<td>27.7</td>
<td>17.7</td>
</tr>
<tr>
<td>3</td>
<td>46.7</td>
<td>117.7</td>
<td>0</td>
<td>28.6</td>
<td>25.0</td>
</tr>
</tbody>
</table>

Results and Discussion

In spite of closely following the F.A.Q. standards for categorisation of the samples into 'good', 'fair' and 'bad' qualities, striking anomalies could be noted in per cent kernel damage, insect count per 100 g and mg frass content per 100 g when compared with these quality criteria. Even with a higher insect count than 'fair' quality a sample could be classified as 'good'. This might be due to the fact that a pre-cleaning and conditioning operation such as sieving could remove the frass, remove the damaged kernels and also to some extent reduce the insect count. A similar anomaly also could be noted when 'true' uric acid was compared with quality classifications according to F.A.Q. This also may be because the 'true' uric acid is mostly bound in the frass and removed during sieving and pre-cleaning operations along with the frass. Therefore the results in Table 1 clearly indicate that the classification with respect to 'good', 'fair' and 'bad' did not fall within any well defined class intervals with regard to the criteria such as kernel damage, insect count, frass and 'true' uric acid content. However the mean values of the above parameters did show significant differences with respect to the three quality groups. The main anomaly seems to be in the data obtained on 'total', 'apparent' and 'true' uric acid contents when these are compared with quantitative figures for kernel damage and insect count. In contrast to the laboratory studies carried out and reported earlier by Venkat Rao et al. (1959) the 'true' uric acid content did not reflect the kernel damage and
Artifacts in the regulatory analysis of market samples of foodgrains using uric acid content as a parameter for quality. S. P. Pillai, M. V. Sharangapani, S. K. Majumder and B. L. Amla.

Insect count quantitatively in this market survey, only a gross difference between the three qualities could be noted. The data obtained (Table 2) on the infestation of soft wheat and rice by *Sitophilus oryzae* and greengram by *Callosobruchus chinensis* have shown that a significant amount of frass is produced during incubation under controlled conditions. Even within 90 days of incubation the frass produced by the infestation of *Callosobruchus chinensis* on greengram was 5300 mg/100g and *Sitophilus oryzae* on wheat was 1500 mg/100g. In contrast to this, in the commercial samples of wheat, rice, and greengram, in proportion to the insect count quantitatively in this market survey, only a gross difference between the three qualities could be noted. The data obtained (Table 2) on the infestation of *Callosobruchus chinensis* on greengram was 5300 mg/100g and *Sitophilus oryzae* on wheat was 1500 mg/100g. In contrast to this, in the commercial samples of wheat, rice, and greengram, in proportion to the high kernel damage observed the frass content should have been several times higher than what is estimated. In commercial samples of wheat up to 100% kernel damage is noted and mean kernel damage in samples in category 'bad' is 57.3%. Under this high degree of kernel damage there is always some dilution factor with the frass as the kernel material collapses and breaks into smaller fragments which are hard to separate from the frass.

The published data of Parvathappa *et al.* (1970) Subramanyan *et al.* (1955), Venkat Rao *et al.* (1957, 1958) and Sen (1968) have conclusively established that with the progress of infestation, kernel damage, insect count and uric acid content become progressively higher under controlled conditions. The uric acid content shows a direct correlation with insect population, while 'apparent' uric acid has an indirect and partial correlation with the total biodeterioration caused by moisture and mould. The 'total' uric acid in the product varies with the insect population, mould count and moisture damage.

There is no doubt that stored product insects are uricotellic. They produce uric acid in crystalline form in the malpighian tubules and excrete this in the kernels during their growth and development. The excretory crystalline uric acid mingles with the frass as it is free flowing in character. It is quite evident that in commercial samples of cereals and pulses the uric acid estimated is derived mostly from grains depleted of the frass content. Simulated studies in which analysis of 'total', 'apparent' and 'true' uric acid content were analysed in greengram, rice and wheat subjected to a regulated degree of infestation for a period of 90 days have indicated that 'apparent' uric acid adheres to the kernel material while the 'true' uric acid, being crystalline and free-flowing, is recovered in the frass fraction. It appears that there is a progressive depletion of 'true' uric acid due to mechanical scouring, ingestion and excretion of the insect; 'apparent' uric acid consists of metabolites, produced in the micro-climate within the kernel outside the insect body, which give a positive reaction to Benedict and Franke's reagent and are products of interaction between insect, mould, and grain of high moisture percentage. The studies (Table 3) have confirmed that about 73% of the 'true' uric acid content is recovered from the free flowing frass in the case of greengram and rice. The corresponding figure is 47% for wheat. Of 'apparent' uric acid 82%, 70% and 51% respectively for greengram, rice and wheat remain on the frass-free kernel fraction. Although no information is available on this "Benedict and

### Table 2. Laboratory simulated studies.

<table>
<thead>
<tr>
<th>Commodity</th>
<th>Infestation</th>
<th>Number of days of incubation</th>
<th>Kernel damage %</th>
<th>Frass mg/100g</th>
</tr>
</thead>
<tbody>
<tr>
<td>Greengram</td>
<td><em>C. chinensis</em></td>
<td>90</td>
<td>40</td>
<td>5300</td>
</tr>
<tr>
<td>Rice</td>
<td><em>S. oryzae</em></td>
<td>90</td>
<td>..</td>
<td>2500</td>
</tr>
<tr>
<td>Wheat</td>
<td><em>S. oryzae</em></td>
<td>90</td>
<td>18</td>
<td>1500</td>
</tr>
</tbody>
</table>

The published data of Parvathappa *et al.* (1970) Subramanyan *et al.* (1955), Venkat Rao *et al.* (1957, 1958) and Sen (1968) have conclusively established that with the progress of infestation, kernel damage, insect count and uric acid content become progressively higher under controlled conditions. The uric acid content shows a direct correlation with insect population, while 'apparent' uric acid has an indirect and partial correlation with the total biodeterioration caused by moisture and mould. The 'total' uric acid in the product varies with the insect population, mould count and moisture damage.

### Table 3.

Distribution of 'Total', 'Apparent' and 'True' uric acid in frass and frass-free fraction of grain infested under controlled laboratory conditions. (Mean of 3 replications). The percentage figures indicate the proportion of the 'Total', 'Apparent' or 'True' uric acid in the original sample which is found in the 'Frass Free' or 'Frass' fraction.

<table>
<thead>
<tr>
<th>Grain</th>
<th>Total uric acid</th>
<th>Apparent uric acid</th>
<th>True uric acid</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Frass fraction</td>
<td>frass-free fraction</td>
<td>frass fraction</td>
</tr>
<tr>
<td></td>
<td>g/100 g</td>
<td>mg/100 g</td>
<td>mg/100 g</td>
</tr>
<tr>
<td></td>
<td>Greengram-infestation, <em>C. chinensis</em></td>
<td></td>
<td></td>
</tr>
<tr>
<td>94.7</td>
<td>5.3</td>
<td>68.0</td>
<td>60.0</td>
</tr>
<tr>
<td>97.5</td>
<td>2.5</td>
<td>10.6</td>
<td>7.3</td>
</tr>
<tr>
<td>Wheat-infestation, <em>S. oryzae</em></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>98.5</td>
<td>1.5</td>
<td>26.6</td>
<td>12.0</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Grain</th>
<th>Total uric acid</th>
<th>Apparent uric acid</th>
<th>True uric acid</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Frass fraction</td>
<td>frass-free fraction</td>
<td>frass fraction</td>
</tr>
<tr>
<td></td>
<td>g/100 g</td>
<td>mg/100 g</td>
<td>mg/100 g</td>
</tr>
<tr>
<td></td>
<td>Greengram-infestation, <em>C. chinensis</em></td>
<td></td>
<td></td>
</tr>
<tr>
<td>94.7</td>
<td>5.3</td>
<td>68.0</td>
<td>60.0</td>
</tr>
<tr>
<td>97.5</td>
<td>2.5</td>
<td>10.6</td>
<td>7.3</td>
</tr>
<tr>
<td>Wheat-infestation, <em>S. oryzae</em></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>98.5</td>
<td>1.5</td>
<td>26.6</td>
<td>12.0</td>
</tr>
</tbody>
</table>
Artifacts in the regulatory analysis of market samples of foodgrains using uric acid content as a parameter for quality. S. P. Pillai, M. V. Sharanapani, S. K. Majumder and B. L. Amla.

Franke's reaction complex" regarding its nature, experimental evidence obtained in the present study indicate that most of the 'apparent uric acid' remains adhering to the damaged kernel.

The artifact in the regulatory analysis of grain, using uric acid content as a parameter of quality, stems from the loss by handling or processing of frass produced by insect activity on the samples. Though the trend of the results may indicate to some extent the differences in the samples with respect to deterioration, they may not give quantitative evidence with respect to the status of damage. The results of the present report have suggested the need for detailed and critical investigation on the acceptability of 'total' uric acid content as a reliable index of biodeterioration in market samples as 'true' uric acid is not retained by the damaged kernels. The 'total' uric acid includes the major fraction of 'apparent' uric acid and a small fraction of 'true' uric acid, elaborated by insects, mould, enzymes and moisture in situ within the kernels.

References


Microbial corrosion of electrical equipment: analysis of causes and consequences: a review.

R. Wasserbauer and R. Blahnik

Summary. The microclimate, in which the electrical equipment is situated, as well as the cryptoclimate proper to the electrical equipment, are characterized by specific physical and chemical effects that can influence the growth of moulds and bacteria both positively and negatively. The task of the microbiologist, who together with the designer designs the electrical equipment for regions with extreme climatic conditions, is to forecast the possibility of the occurrence of such effects and to suggest suitable measures to minimize those effects which stimulate microbial deterioration. This task is complicated by interactions between the single effects of microclimate or cryptoclimate.


Détérioration microbienne de l'appareillage électrique: étude des causes et conséquences; compte rendu. Le microclimat dans lequel se trouve l'appareillage électrique ainsi que le cryptoclimat propre à l'appareillage électrique se caractérisent par des actions physiques et chimiques spécifiques qui peuvent influencer la croissance des mousses et des bactéries d'une manière positive ou négative. Le travail du microbiologiste, qui en même temps que le dessinateur, conçoit l'appareillage électrique pour des régions à climats exceptionnels, est de prédire la survenance possible de telles actions et de suggérer des mesures appropriées en vue de minimiser les actions favorisant la détérioration microbienne. Cette tâche n'est pas facilitée par les interactions entre les effets particuliers du microclimat ou du cryptoclimat.

The ever increasing complexity of electrical equipment is accompanied by ever increasing demands for the application of various plastic insulating materials which may be attacked by moulds and, in some special cases, by bacteria in humid environments. The task of the microbiologist, who, in cooperation with the designer, must solve the problem of the suitability of the electrotechnical product for humid areas, does not consist only in determining the resistance of single insulators used in the product but also in anticipating and limiting single phenomena, correlations and regularities which make themselves felt in the environment effects on the technical product and which represent a risk of its damage and/or of the deterioration of its function by microorganisms.

It is evident that the character of the environmental effects on the technical equipment is that of the complex, temporarily variable effect of a number of factors, which involve climate, physical elements, biological and chemical agents and, particularly, factors conditional on the equipment's operation, technology and design. These factors condition the complex of partial deterioration processes. The mentioned simple scheme, qualifying the factor as the cause of the passing degradation process and the degradation process as the consequence of the factor effect, does not give the true picture of the existing multiplicity of the technical product deterioration in natural conditions. In fact, a chain of causes and consequences is influential, it being possible that a certain degradation process will generate a factor which will cause the further degradation process. Besides, a certain factor or a certain degradation process can condition directly or influence positively or negatively another factor or degradation process.

We meet the opinion, in many cases, that the microbial corrosion of a number of insulators, especially those based on natural materials, is regular under favourable atmospheric conditions (Haldenwanger, 1970; Ritter, 1965; Rosato and Schwarz, 1968). However, the below mentioned analysis of effects, which encounter in the function of electrotechnical products located in the humid environment, suggests that only very favourable climatic and operating conditions allow such an intensive attack by microorganisms on products that would cause significant deterioration of their function.

Certain possible interactions and correlations of environment factors and deterioration processes, which may play a part in the attack by microorganisms on electrical equipment, are stated in Table 1.

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1Electrotechnical Testing Institute, Prague, Czechoslovakia.
2G. V. Akimov State Research Institute of Material Protection, Prague, Czechoslovakia.

(Copy received September 1974)
TABLE 1. Mutual effects of factors influencing the growth of biodeteriogens in the electrical equipment and their consequences.

<table>
<thead>
<tr>
<th>Factors resulting from the electrotechnical function assembly technology, design and function</th>
<th>Material properties</th>
<th>Outside generated factors</th>
</tr>
</thead>
<tbody>
<tr>
<td>Temperature and humidity</td>
<td>Resistance changes</td>
<td>Thermooxidative material ageing, photooxidative material ageing, change in water vapour pressure, moisture content increase, etc.</td>
</tr>
<tr>
<td>Electric field</td>
<td>Effect upon surface charge density, change in resistivity and dielectric constant, etc.</td>
<td>Biomicrobic agents</td>
</tr>
<tr>
<td>Rel. humidity</td>
<td>Effect upon surface tension, change in electrical conductivity, etc.</td>
<td>Biomicrobic agents</td>
</tr>
<tr>
<td>Vapour pressure</td>
<td>Effect upon solvent capacity, change in sorption properties, etc.</td>
<td>Biomicrobic agents</td>
</tr>
<tr>
<td>Contact</td>
<td>Effect upon surface tension, change in electrical conductivity, etc.</td>
<td>Biomicrobic agents</td>
</tr>
</tbody>
</table>

Consequences:
The natural selection of biodeteriogens according to the character of the surface and inner insulation resistances, of flashover voltage, the change of surface and inner insulation resistances of insulators, the different degrees of attacks and of growing intensity, mutual relations of biodeteriogens on insulators, biodeteriogenesis, etc.

R. Wasserfarb and R. Blanka.
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The degree of destruction of the electrical equipment as a result of such attacks is influenced by the mutual interactions of biodeteriogens on insulators (Eggin, 1968; Hollingsworth, Eggin, and Allsopp, 1973; Walsh 1968). The practical consequence of these interactions may be a change in the electroinsulating properties of the insulators, especially of the surface and internal insulation resistance and of the surface flash-over voltage. The degradation of the electroinsulating properties tends to be more intensive in combined attacks than during the growing of biodeteriogens in monoculture (Ganz and Wälchli, 1955; Pitis, 1965; Teitel, Berk, and Kravitz, 1955; Wasser­ bauer, 1967, 1968, 1969, 1971; Blahnik and Wasserbau­ er, 1972, 1973).

The affinity of particular biodeteriogens for certain insulators also plays an important part in the micro­ bial attack of insulators, so that certain biodeteriogens may only normally be expected on certain insulators (Agarwal and Amar, 1971; Lazar and Ionita, 1971). The practical consequence of these effects appears in the insulant etching, colouring by metabolic products, lustre loss, pitting, transparency loss of transparent materials. Another consequence is the humidity increase near the mould mycelium, which, together with metabolic products of moulds, may result in the contact corrosion of metallic parts near to attacked insulators (Flerow, Maslenikova and Surovtsova, 1963; Ganz and Wälchli, 1955).

Another range of interactions comes into consideration in relations between microorganisms and equipment proper. It is necessary to hold the soil, where the decisive majority of biodeteriogens develop, as the main source of the contamination of electrical equipment by micro­organisms. The micro­organisms are transported to the equipment mostly by air, less often by insects, higher animals as well as by man (transfer vectors) (IEC TC 50 Wg 5 (Sec) 22; Hueck, 1965). As it is impossible, in principle, to influence or anticipate the presence of single biodeteriogens without intricate air-conditioning equipment and without a thorough knowledge of the place of exposure of the electrical equipment, the electrical equipment may become infected during its production (Planina, 1960),—certain selected species which have often been isolated from electrical equipment are used for tests (Courtois, 1959, 1960; Dayal, Agarwal and Nigam, 1973; Nigam, Agarwal and Dayal, 1973, Jones, 1968).

It is natural that specific conditions of relative humidity and temperature are necessary for microbial germination and microbial attack on the insulators. Cardinal values of relative humidity in relation to substrate and temperature are known for a number of biodeteriogens isolated from electrical equipment (Ayerst, 1965, 1966, 1968; Blahnik and Záňová, 1963; Genovová, 1971; Ritter, 1965). Therefore, it is not too difficult to determine the probability of germina­ tion and growth of particular biodeteriogens, if meteorological data of the place where the electrical equipment is to be exposed are known. Relations between atmospheric conditions and the degree of attack have been subject to intensive studies lately and a considerable range of knowledge has been obtained, which makes it possible to define the so-called “dangerous periods” within the local climate, i.e. the periods favourable for the growth of biodeteriogens on the product (Rychtera and Niederführövá, 1965a, 1965b). A method of predicting the microbial corrosion of electroinsulating materials on the basis of hourly or average monthly meteorological data has been developed, taking into account that the process slows down or even stops as a result of the periodic occurrence of subcritical levels in one or more of the environmental agents (Machová, 1973; Rychtera, Genovová and Machová, 1974).

While it is possible to determine the local climate parameters on the basis of detailed meteorological data with relative precision, this method is not successful in the microclimate, in which the electrical equipment is situated, and/or in its kryptoclimate. A number of influences, whose mutual relationships have not yet been studied in detail, occur in the electrical equipment microclimate and specific degradation processes with interesting relations appear. These processes include, particularly, the formation of exhalations from plastics (Knotkova, 1971; Cawthorne et al., 1966). Volatile substances from the plastics, within the enclosed spaces in electrical equipment, reach critical values, although they occur in only unmeasurable, biologically quite ineffective values outside. Formic, acetic, butyric acids, ammonia, formaldehyde, hydrogen sulphide, some volatile amines and amides can be expected in the electrical equipment kryptoclimate. The origin of these low­molecular products is a consequence of photo- and thermo-oxidation degradation and hydrolytic processes. The catalytic effect of metal ions on rubber, polyvinyl chloride and cellulose degradation and the diffusion of monomer, lower­molecular products of the polycondensation of catalysts and softening agents to the plastic material surface may also be involved. The growth of biodeteriogens is not dependent solely on the substrate character and on relative humidity but also on the character of these exhalations, especially those in materials with cellulose-based fillers (Puckman, 1960; Suolahti 1951), which may inhibit or stimulate the growth of biodeteriogens.

Certain biodeteriogens can either inhibit or stimulate corrosion processes of metals in the electrical equipment kryptoclimate. For example, it has been confirmed experimentally that the presence of moulds on phenoplasts filled with wood flour or on cellulose­ based plastics reduced considerably the corrosion of some metals present in the kryptoclimatic spaces. Further, it has been ascertained that the growth of moulds on phenoplasts can also regulate the quantity of ammonia to a certain extent. On the other hand, a reverse effect has been observed in some rubbers, where the growth of certain moulds considerably
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Microbial corrosion of electrical equipment: analysis of causes and consequences: a review.

reduced aggressiveness of the kryptoclimate to metals. A considerable percentage of formates and acetates has been found in corrosion products (Wasserbauer and Knšťková, 1967).

The growth of biodeteriogens on combinations of plastic materials is a very interesting phenomenon from the microbial corrosion point of view. An intensive development of biodeteriogens has been noticed at the point of contact of different plastic insulators much sooner than on other insulant surfaces. The precise mechanism of this phenomenon is not yet known. It is probable that besides the increase of moisture content at points of contact there is a preferential source of trace substances from the materials which are more accessible on the edges than on the smooth surfaces of the insulators. In some cases, it is possible even to talk about foci, from which the mould mycelium extends on to further insulators. Such places, determined by the methods in Czechoslovak Standard CSN 03 8181, comprise e.g. the contact of PVC insulations with some pressing materials, the contact of painted glass fabric with some sorts of hardened paper or the contact of some phenol-, urea- or melamine-formaldehyde pressing materials to one another. The task of the microbiologist consists in determining and eliminating these combinations that may affect the operational reliability of the electrical equipment.

Further influences which make themselves felt in krypto- or microclimatic conditions include operational effects, particularly rise in air temperature and both spontaneous and forced flowing. These influences affect the growth of biodeteriogens negatively (Niederfůhróvá, 1961; Pitis, Lacatusu and Polcovník, 1970; Ayerst, 1966). In some cases, also the electrical field may have an effect, especially a high-frequency alternating-current field or a direct-current field of a higher intensity (Abramova, Jarovskaya and Karakash 1969; Achasova and Vladimírský, 1969; Kryzhunovský, Berenňíkovská and Rodinová, 1972; Lazarenko, Kpepis and Pimenov, 1968; Pareilleus and Sicard, 1970).

It is natural that operational effects (particularly temperature rise and radiation) considerably affect the susceptibility of plastics to moulds, as thermo-oxidation and photo-oxidation processes connected with the origin of low-molecular substances and oligomers stimulate or inhibit the biodeterioration processes under certain conditions. These influences are significant as regards the equipment with an interrupted function, when the function delay is long enough as to allow the microbial attack on insulators (Wasserbauer, 1973).

The accelerated thermo-oxidation ageing of materials exposed at their thermal class limit has resulted in an average two- to three-times increase in the rate of surface colonisation by moulds in all cellulose-filled phenolformaldehyde pressing materials. On the contrary, a considerably growing inhibition has been found in glass and cotton fabrics painted with alkyd varnish. The measurement of the permeability and the water vapour sorption in material has shown that the reduction of the resistance of the cellulose-filled phenolformaldehyde pressing material is caused by an increase of the water vapour sorption in the material. Not only the increased sorption, but also the growth inhibition have been noticed in painted fabrics. On the basis of conductivity measurements oxidation products of ageing are expected with the inhibitory effects on mould growth. The increase in resistance in softened polyvinyl chloride and triacetate and acetybutyrate of cellulose has been attributed to the volatilisation of the softener, while in the case of some electroninstallation varnishes the increase in resistance is caused by final setting (cross linking). Somewhat different results have been obtained by the accelerated photo-oxidative ageing of materials. As for softened polyvinyl chloride, polypropylene, acetybutyrate and triacetate of cellulose as well as cellulose-filled phenolformaldehyde pressing materials, considerable growth inhibition followed by mycelium autolysis have been noticed. The growth stimulation has been noticed in polyamide, rubbers based on natural rubber and nitro-cellulose lacquers and oil varnishes (Wasserbauer, 1973, 1974).

Antimicrobial substances that increase the resistance of electrical equipment to microorganisms are applied in insulators in a number of cases. Considerable attention has been paid to the problem of antimicrobial protection. Problems of the corrosiveness of antimicrobial substances for metals as well as problems of changes of the electro-insulating parameters of insulators after the addition of antimicrobial substances have been studied. It was also found that the antimicrobial protections lost their effectiveness during the operation of the electrical equipment, especially as a result of temperature rise, radiation and leaching due to relative humidity variations. The stability problems of mercuric compounds in particular were studied in detail and the deactivation mechanism was determined. Also the successive adaptation of biodeteriogens to certain antimicrobial compounds was studied (Iacob and Lacatusu, 1971; Genovová, 1973; Jakubovská, Kusewicz and Scopa, 1972; Muskalová, Litvinenková and Tyltin, 1973; Nelson et al., 1973; Sanjay et al., 1973; Tonomura et al., 1968; Bennet, Adamson and Feisal, 1959; Taylor, 1965; Ramp and Grier, 1961; Pitis, Focsamaeanu and Antoniu, 1963; O'Neill, 1963). The application of antimicrobial protective substances in insulators is no simple matter and it requires the knowledge of facts for the selection of a suitable antimicrobial compound.

It is possible to determine, on the basis of the above properties of the environment and of electrical equipment, whether the biodeteriogens will be capable of conforming to some unfavourable effects of microphoneclimates or not. It is natural that the entire complex of influences seldom makes itself felt in one particular piece of electrical equipment and, therefore,
it is necessary to regard this survey as a summary of different possibilities and variants which must be taken into consideration by the microbiologist for the preparation of electrical equipment for use in humid regions, especially the tropics.

References


R. Wasserbauer and R. Blahnik.
Microbial corrosion of electrical equipment: analysis of causes and consequences: a review.


Summary. A synopsis of the investigations carried out into the problems of the microbiological deterioration of plastics, the first of this kind in Romania, is presented. Different plastic types were investigated: phenol-furfural and phenol-formaldehyde resins, plasticised and unplasticised PVC and polyethylene of high and low density. The results concerning the resistance of these materials to the attack of fungi, obtained with different methods proposed by the International Organisation for Standardisation are discussed, as well as the investigations on the main enzymic activities of the fungi attacking these substrates, which were carried out to elucidate the attack mechanism.

In conclusion, the possibilities of preventing and controlling this deterioration are discussed.

Materials and Methods

Investigations were performed on 21 types of experimental plastics, namely: 6 kinds of resins, 13 of polyvinyl chloride (PVC) and 2 of polyethylene, whose composition is given in Table 1.

Results

1. Research on the resistance of indigenous experimental plastics to fungus attack

From Table 3 it can be seen that the least resistant type of plastic was composition 2 (phenol-furfural resin), which supported maximum attack by all the inoculated fungal species. Also strongly attacked, though not equally by all the species, was composition 1 (phenol-formaldehyde resin). The most resistant kind was the composition 8 (PVC plasticised with dibutylphthalate) which was not attacked by any of the inoculated fungal species. Good resistance was shown by the unplasticized PVC types stabilized with lead stearate (compositions 18 and 19), and high density polyethylene (composition 21).

2Institute of Biological Sciences, Department of Microbiology, Spl. Independentei 296, Bucharest VII, Romania. (Copy received April 1973)
The study of microbiological corrosion of plastics in Romania. V. Lazar.

### TABLE 1. Materials investigated

<table>
<thead>
<tr>
<th>Resins</th>
<th>PVC</th>
<th>Unplasticised PVC</th>
<th>Polyethylene</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phenol-furfural resin</td>
<td>Phenol-formaldehyde resin</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1. Press powder, wood flour, alkaline phenol-furfural resin (unwashed)</td>
<td>7. PVC-S</td>
<td>15. PVC-S</td>
<td>20. of low pressure</td>
</tr>
<tr>
<td>2. Press powder, wood flour, neutral phenol-furfural resin (washed with distilled water)</td>
<td>8. DBP</td>
<td>16. PVC-E</td>
<td>21. of high pressure</td>
</tr>
<tr>
<td>3. Press powder, graphite, phenol-furfural resin</td>
<td>9. TCP</td>
<td>CaSt</td>
<td></td>
</tr>
<tr>
<td>4. Textile bakelite, phenol-furfural resin</td>
<td>10. DBP</td>
<td>CaSt</td>
<td></td>
</tr>
<tr>
<td>5. Press powder, wood flour, phenol-formaldehyde resin</td>
<td>11. DOS</td>
<td>CaSt</td>
<td></td>
</tr>
<tr>
<td>6. Textile bakelite, phenol-formaldehyde resin</td>
<td>12. DOS</td>
<td>CaSt</td>
<td></td>
</tr>
<tr>
<td></td>
<td>13. DOS</td>
<td>CaSt</td>
<td></td>
</tr>
<tr>
<td></td>
<td>14. Saprarex IX</td>
<td>CaSt</td>
<td></td>
</tr>
</tbody>
</table>

E = stabiliser in emulsion
S = stabiliser in suspension
p = parts

DBP = dibutylphthalate
DOS = dioctylsebacate
CaSt = Calcium stearate
TCP = tricresylphosphate
Saprarex IX = alkylphthalate (import)
 PbSt = lead stearate

### TABLE 2. Methods used.

I. Testing of the resistance of materials to the attack of microorganisms

1. Adapted method after method A from First draft ISO Czech proposal (International Organisation for Standardisation, a)
2. Methods A and B from Second draft ISO Czech proposal (International Organisation for Standardisation, b)
3. Project de norme Francais ISO NF X 41-504 (Norme Francais).
4. Agar plate method (EMPA)

II. Investigation of the action mechanism of fungi upon materials

1. Cellulolytic activity was estimated by using as substratum either cellulose or carboxymethylcellulose in citrate buffer medium pH = 4.75. The released reducing substances were determined by the anthrone method (Dische, 1955).
2. Lipasic activity was determined titrimetrically using polyoxyethylene sorbitan monolaurate (Tween 20) in buffer solution pH = 7.2 as substratum (Bier, 1955).
3. Pyrophosphatase activity was estimated using as substratum sodium pyrophosphate in buffer system pH = 7.2 (Greehouse and Wessel, 1954).
4. Proteolytic activity was determined according to Anson's (1938) method using haemoglobin as substratum. Proteolytic enzymes of the pepsin type (pH = 2) and of the trypsin type (pH = 7) were studied.
5. Catalasic activity was determined according to the Jolles-Josephson method, H2O2 in phosphate buffer pH = 6.8 being employed as substrate (Colowick and Kaplan, 1955).

The variants were the following: different species of fungi, different ages of the investigated species, different methods of cultivation and various contact intervals between enzyme and substrate.
The study of microbiological corrosion of plastics in Romania. V. Lazar.

TABLE 3. The resistance of plastics to fungi.

<table>
<thead>
<tr>
<th>Inoculated fungus species**</th>
<th>Resins</th>
<th>Plasticised</th>
<th>Unplasticised</th>
<th>Polyethylene</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Phenol-furfural resin</td>
<td>Phenol-formaldehyde resin</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td>Aspergillus amstelodami</td>
<td>1.2</td>
<td>4</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>Neurospora sitophila</td>
<td>1.8</td>
<td>4</td>
<td>2</td>
<td>1.8</td>
</tr>
<tr>
<td>Stachybotrys atra</td>
<td>1.6</td>
<td>4</td>
<td>0.8</td>
<td>1.8</td>
</tr>
<tr>
<td>Chaetomium globosum</td>
<td>1.6</td>
<td>4</td>
<td>1</td>
<td>0.4</td>
</tr>
<tr>
<td>Memnoniella echinata</td>
<td>1</td>
<td>4</td>
<td>1</td>
<td>0.4</td>
</tr>
<tr>
<td>Penicillium luteum</td>
<td>3.6</td>
<td>4</td>
<td>0.8</td>
<td>0.2</td>
</tr>
<tr>
<td>Trichoderma sp.</td>
<td>1.6</td>
<td>4</td>
<td>0.6</td>
<td>3.4</td>
</tr>
<tr>
<td>Sterigmatocystis nigra</td>
<td>2.6</td>
<td>4</td>
<td>2.2</td>
<td>0.8</td>
</tr>
<tr>
<td>Myrothecium verrucaria</td>
<td>2</td>
<td>4</td>
<td>0.4</td>
<td>0.4</td>
</tr>
<tr>
<td>Aspergillus flavus Acrostalagnus koningi</td>
<td>1.2</td>
<td>4</td>
<td>0.2</td>
<td>2.6</td>
</tr>
<tr>
<td>Pseudallescheria variotii</td>
<td>1.4</td>
<td>4</td>
<td>0.8</td>
<td>1</td>
</tr>
</tbody>
</table>

* = the plastic compositions are numbered as in Table 1.
** = the inoculation was with each species separated

Visual evaluation of the fungal growth (Norme Francais PN X 41-504)

0 = no fungal growth visible with the naked eye or with the magnifying glass.
1 = no fungal growth visible with the naked eye, but with the magnifying glass an invasion of the extremities of the sample by a sterile and less abundant mycelium can be seen.
2 = a growth visible with the naked eye, as an important invasion of the extremities of the sample or moderate invasion of the surface.
3 = the invasion of at least half of the sample by a fructified mycelium.
4 = the total invasion of the sample by the fructified mycelium.

TABLE 4. Resistance of various PVC compositions to fungi.

<table>
<thead>
<tr>
<th>Method</th>
<th>Plasticised</th>
<th>Plasticised</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Unplasticised</td>
<td>Plasticised</td>
</tr>
<tr>
<td></td>
<td>15</td>
<td>17</td>
</tr>
<tr>
<td>I B method (Czech) Czech strains</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>French strains</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>II Agar plate method (EMPA) Czech strains</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>French strains</td>
<td>0</td>
<td>2</td>
</tr>
</tbody>
</table>

* Mixed inoculum (spore mixture) according to methods
** The PVC compositions are numbered as in Table 1.

Visual evaluation of fungal growth (The EMPA agar plate method)

0 = no fungal growth—good.
1 = a very slight growth, hardly visible with the naked eye, more with the microscope—satisfactory.
2 = slight growth, less than 25% of the surface is covered—unsatisfactory.
3 = medium growth, 25-50% of the surface is covered—unsatisfactory.
4 = strong growth, 50% and more of the surface is covered—unsatisfactory, strong growth.
5 = very strong growth, the samples completely covered—very strong growth, unsatisfactory.
From the results it can be seen that the fungi which developed on most materials, in a decreasing order of their incidence, were: *Neurospora sitophila*, *Aspergillus amstelodami*, *Chaetomium globosum*, *Penicillium luteum*, *Aspergillus niger* and *Trichoderma sp*. The most powerful attacks were induced by the species *Neurospora sitophila* and *Aspergillus niger*, while the feeblest attacks were by the species *Stachybotrys atra*, *Mennonietta echinata* and *Aspergillus flavus*.

It would appear that *Aspergillus niger*, *Stachybotrys atra*, *Mennonietta echinata* and *Aspergillus flavus* generally attack the resins, while *Neurospora sitophila* attacks PVC and polyethylene.

In Table 4 the results concerning the resistance of some PVC types to the attack of a mixture of fungal species are presented. It was found that the strongest growths were obtained by the B method in which compositions 12 and 13 were noted with growth rating 5. These compositions, plasticised with a mixture of DBP and DOS in various proportions, presented optimum growth by both methods. It was generally ascertained that the most sensitive to attack were compositions of plasticised PVC. The most resistant was the variant plasticised with Sapraflex IX (alkylic phthalate plasticiser with fungicidal action) and the polymer, composition 15 from unplasticised PVC, presented no growth by any of the methods used.

From the inoculated fungus mixture only *Aspergillus niger* developed, while on a few samples, by the agar plate method, *Chaetomium globosum* also developed.

Observations at the end of the test period (after sample washing) showed that transparent (plasticised) samples became opaque with their smooth surface being modified into a rough one, while for other compositions a mosaic appearance was noted. Comparison with the respective controls showed that these modifications were produced under the action of high moisture levels during the testing period. The strong black-brown colouring in several attacked samples was due to fungal pigments.

### 2. Mechanism of fungal action upon plastics

The results of investigations, performed in collaboration with the Biochemistry Department of the "Dr. I. Cantacuzino" Institute, on 5 categories of...
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enzymic activity in 6 fungus species which attack plastics, are presented in Table 5. It was ascertained that these activities vary according to the fungal species, the age of species (growth stage), culture medium and the presence of inhibitors (with fungistatic or fungicidal action). Table 5 shows the growth stages in which the species studied attained the maximum of the various enzymic activities. From the results it was perceived that the species studied may be divided, according to their enzymic activities, into 3 categories: with high, medium and low activity, the highest enzymic activity being recorded in *Neurospora sitophila*, *Chaetomium globosum* and *Aspergillus niger*. In regard to the maximum enzymic activity depending on the species age, it was observed that cellulolytic and proteolytic activity is generally more specific to the mycelial stage, or mycelium with few conidia, while lipase and pyrophosphatase activity are specific to the mycelium with numerous conidia and to the aged culture stage. For catalase activity the highest values were generally those of cultures in the mycelial stage with numerous conidia, except for the species *Aspergillus niger* and *A. amstelodami* which also showed activity in the mycelium with few conidia, and for the former species, in that of mycelium alone. In the aged culture stage certain activities are reduced or absent.

Table 5 shows the growth stages in which the species studied attained the maximum of the various enzymic activities. From the results it was perceived that the species studied may be divided, according to their enzymic activities, into 3 categories: with high, medium and low activity, the highest enzymic activity being recorded in *Neurospora sitophila*, *Chaetomium globosum* and *Aspergillus niger*. In regard to the maximum enzymic activity depending on the species age, it was observed that cellulolytic and proteolytic activity is generally more specific to the mycelial stage, or mycelium with few conidia, while lipase and pyrophosphatase activity are specific to the mycelium with numerous conidia and to the aged culture stage. For catalase activity the highest values were generally those of cultures in the mycelial stage with numerous conidia, except for the species *Aspergillus niger* and *A. amstelodami* which also showed activity in the mycelium with few conidia, and for the former species, in that of mycelium alone. In the aged culture stage certain activities are reduced or absent.

Table 5. Action mechanism of fungi upon plastics

<table>
<thead>
<tr>
<th>Fungal species</th>
<th>Aspergillus amstelodami</th>
<th>Aspergillus niger</th>
<th>Neurospora sitophila</th>
<th>Chaetomium globosum</th>
<th>Penicillium cyclopium</th>
<th>Paeilomyces variotii</th>
</tr>
</thead>
<tbody>
<tr>
<td>Culture medium</td>
<td>Growth phases</td>
<td>Growth phases</td>
<td>Growth phases</td>
<td>Growth phases</td>
<td>Growth phases</td>
<td>Growth phases</td>
</tr>
<tr>
<td>1</td>
<td>2</td>
<td>1</td>
<td>2</td>
<td>1</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>Cellulolytic activity</td>
<td>++</td>
<td>MC</td>
<td>++++ + + M</td>
<td>+++ + M</td>
<td>+++ + + M</td>
<td>M + + MC</td>
</tr>
<tr>
<td>Proteolytic activity</td>
<td>+</td>
<td>MCv</td>
<td>+++ + + M</td>
<td>+++ + + M</td>
<td>+++ + + M</td>
<td>M + + MC</td>
</tr>
<tr>
<td>Lipase activity</td>
<td>+++</td>
<td>MC</td>
<td>+++ + + Mcv</td>
<td>+++ + + Mcv</td>
<td>+++ + + Mcv</td>
<td>M + + MC</td>
</tr>
<tr>
<td>Pyrophosphatase activity</td>
<td>+++</td>
<td>MCv</td>
<td>+++ + + M</td>
<td>+++ + + M</td>
<td>+++ + + M</td>
<td>M + + MC</td>
</tr>
<tr>
<td>Catalase activity</td>
<td>+++</td>
<td>Me,Mc</td>
<td>+++ + + M</td>
<td>+++ + + M</td>
<td>+++ + + M</td>
<td>M + + MC</td>
</tr>
</tbody>
</table>

1 = malt-agar medium
2 = PVC with 35% DOS
++ = low activity
+++ = medium activity
++++ = high activity

M = mycelium without conidia
Me = mycelium with few conidia
MC = mycelium with numerous conidia
MCv = old culture

Discussions and Conclusions

The results obtained in testing the resistance of various plastics to fungal attack agree with those of other authors (Berk et al., 1957; Blahnik and Zanova, 1963; Bomar, 1956, 1957, 1959; Dolezel, 1955, 1967; Genin, 1968; Greathouse and Wessel, 1954; Klausmeier, 1972; Merz and Dolezel, 1967). Considering that the resistance of a mixture is determined by all its components and even by its pH, the behaviour of the various types of plastic is to be expected. The sensitivity of resins with acid or neutral pH, and of those with wood flour as filling material, can thus be explained. In the case of PVC the data confirmed those of Dolezel (1955) and Bomar (1956, 1957, 1959), the plasticised types being the most sensitive to microbiological attack, except those plasticised with DBP and Saprafort IX; the microbistatic activity of the phthalic acid derivatives used as plasticisers being well known (Bomar, 1956). Subsequently, Merz and Dolezel (1967) reaffirm that the resistance of PVC is influenced by the composition of plasticisers and of additives, as well as by the polymer purity; while Dolezel (1967) in his classification presents plasticised PVC as the most sensitive from the microbiological point of view. Also of interest are Klausmeier's (1970)
very recent findings from the results obtained by several laboratories, which show that the sole resistant composition was the one plasticised with diisooctyl phthalate and that the loss in weight of samples (by which the degree of deterioration was assessed) was due to the loss in plasticiser. These data are confirmed by Merz and Dolezel (1967), who state that the phthalates are the most resistant plasticisers.

From the results it was also ascertained that DBP in mixture with other plasticisers no longer retains its fungitoxic properties. This fact was also established by Klausmeier (1972) who showed that PVC plasticised with diisooctylsebacate and diisooctylphthalate mixtures were not resistant.

The low resistance of compositions stabilised with calcium stearate when compared to those stabilised with lead stearate agreed with Boman's (1956, 1959) data and that of Merz and Dolezel (1967), concerning the inhibitory action of lead stearate to fungi.

In regard to the development of the fungal species used as inocula, we attempted for the first time to assess some specialisation of the species against the various groups of plastics. In the case of mixed inocula their behaviour in the mixture could be observed and if we take into account the complex enzymatic apparatus of *Aspergillus niger*, the predominance of this species in the mixtures employed is accountable.

Regarding the number of fungal species used as inocula, similar results for both groups of strains showed that the Czech assortment was sufficiently complete. Dolezel likewise showed that an increase in the number of fungi does not affect the degree of their growth on plastics, and that the best growths were obtained by the B method. Similarly the results presented by Klausmeier (1972) concerning the use of various inocula with individual species or in mixture support the finding that a smaller number of species is sufficient as inoculum. A fact, shown by this author, is also interesting; that though the various inocula may be classified according to the degree of deterioration they induce, these differences almost disappear if the "plastic-fungus" interaction is examined when the weight losses become no longer significant. This would support the results of investigations concerning physical modifications of resins submitted to biological tests, which established that modifications occurring in the behaviour of materials are induced, particularly by the conditions necessary for the development of micro-organisms: high moisture and temperature and the action of fungi for the duration of the biological test is only limited to growth at the surface or in the superficial layers of the material (Savulescu et al., 1960).

This research concerning the attack mechanism of fungi, attempted to explain the mode of action of these micro-organisms on plastics, and to offer the possibility of assessing the resistance of the various kinds of materials in accordance with their components, which can represent substrates for the different fungal enzymes. The results showed a direct connection between the biological attack and the enzymic activities of fungi. Thus, the high enzymic activities of *Aspergillus niger*, *Neurospora sitophila* and *Chaetomium globosum* explain their high attacking power, determined in the resistance tests (Savulescu et al., 1960).

The connection between culture medium and enzymic activity determined also by Jermyn (1952, 1953) and Shipe (1951), accounts for the high sensitivity of materials whose constituents represent food sources for micro-organisms: resins, plasticised PVC.

Age relationship between enzymic activity of a fungus and its growth stage, shown by Heppeh (1955), Shiu and Sinden (1951) and Vasiu (1963) suggested that the highest activities are in the mycelium with few conidia, and in the mycelium with numerous conidia, which justifies determining the age of the cultures used as inoculum in resistance tests.

The action exerted by the various fungal enzymes makes possible the development of these micro-organisms on and within the various materials, by macromolecular hydrolysis, and favouring at the same time the attack of other factors, with subsequent destructive action. In support of our results, the research recently performed by Kestelman et al. (1972) shows that within the framework of enzyme synthesis processes, fungi played a preponderant role in the corrosion of polymeric materials. By their electron microscopic structural investigations of materials submitted to fungal action, they showed the modification of structure both in the superficial layers, as well as in depth. This opens new perspectives to investigations concerning the "plastic-fungus" interaction.

The Romanian research performed within the framework of the study of microbiological corrosion of plastics, offers the possibility of comparing the results of recent foreign investigations, which confirm the data presented, and developed the study of the fungal enzymic activity, in the problem of elucidating the attack mechanisms on plastics.

References


1 Researches performed by the Chemical Research Centre of RSR Academy.
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Norme Francais NF X 41-504


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Summary. The genus Curvularia Boedijn is important both in biodeterioration and in plant pathology. Its taxonomy, general distribution, cultural characteristics, morphology, physiological attributes and sensitivity to biocides are described.

Taxonomy

When the genus Curvularia was first described (Boedijn, 1933) it was defined so that it included those darkly pigmented microfungi that produced spores with three or four transverse septa, with the middle cells enlarged and often asymmetrical so that the spores appeared curved or bent. Boedijn described five new species, C. intermedia, C. brachyspora, C. pallescens, C. fallax and C. affinis and also included thirteen which had previously been ascribed to the genera Acrothecium, Brachycladium, Brachysporium, Helminthosporium, Napicludium and Spondylocladium.

When Ellis (1966) reviewed the genus, he included twenty nine species, one variety, C. lunata v. aeria (Batista, Lima and Vasconcelos) Ellis and one forma specialis, C. trifolii f. sp. gladioli Parmelee and Luttrell. In a later summary of the genus (Ellis, 1971) he included two further species, C. ovidea (Hirole and Watanobe) Muntanola (1957) and C. robusta Kilpatrick and Luttrell (1967). Other new species described include C. eutinula Reddi and Bilgrami (1968) C. desmodii Bharadwaj (1969), C. richardiae Alcorn (1971) and C. tritici Kumar and Nema (1969).

Spore morphology is the main feature in the Curvularia taxonomy. In Ellis's key, the primary characteristic was the prominence of the hilum, the texture (rough/smooth) of the wall (Figure 1) and the number of septa. Other characteristics used to distinguish between species included size and shape of spores, relative positions of septa, and production and appearance of stromata. However, after prolonged culture on artificial media, certain of these features, which are distinct in freshly isolated strains, become less clear or even lost. Species with rough-walled conidia produce conidia with smooth walls (e.g. C. verruculosa), 4-septate species produce a greater proportion of 3-septate conidia (e.g. C. senegalensis) and stromata cease to be formed. There are other less precise signs of degeneration in which the degree of asymmetry or curvature decreases and the pigmentation is less intense and becomes more uniform. With

Figure 1. Conidia of C. verruculosa showing typically roughened surface (s.e.m.) × 1300.
prolonged culture, the ability to sporulate is often reduced or lost altogether, as is common in some other microfungi.

With the description of *C. catenulata* and *C. tritici*, a further characteristic, spore proliferation, was introduced into the taxonomy of *Curvularia*. In *C. catenulata*, the distal cell becomes sporogenous and spore chains develop acropetally and in *C. tritici*, the proximal and distal cells give rise to secondary conidiophores (Figure 2). The formation of conidial chains has been observed in other species, but this is rare.

![Figure 2. Sporulation of *C. tritici* showing development of secondary conidiophores × 1300.](image)

Perfect stages have been recorded for four species, all belonging to the genus *Cochliobolus*, having ostiolate perithecia and filiform or flagelliform ascospores; viz. *C. lunatus* (Nelson and Haasis, 1964), *C. intermedius* (Nelson, 1960), *C. geniculatus* (Nelson, 1964) and *C. cymbopogon* (Hall and Sivanesan, 1972).

**General Distribution**

*Curvularia* is an inhabitant mainly of tropical and subtropical areas, with only a few species, including *C. lunata*, *C. pallescens* and *C. inaequalis* occurring regularly in cool, temperate regions (Ellis, 1966). In nature *Curvularia* is most often present on plant material, either as a pathogen on live tissue or as a saprophyte on moribund or decaying herbage. Probably its greatest economic importance is as the causative agent of diseases of graminaceous crops, including barley, maize, oats, rice, rye, sorghum, sugar cane and wheat, and turf; other crops and decorative plants are also attacked (Ellis, 1966; Anon., 1968). Barron (1968) regarded *Curvularia* as an infrequent inhabitant of soil. At Innisfail, North Queensland (hot-wet tropical), however, it was present in 80% of lawn soil samples at 5 cm depth, although in nearby jungle it was present in only 20% of samples. (Upsher 1971a). In arable soils in Egypt, it showed a seasonal incidence, being absent during the hottest months and most plentiful in the autumn (Moubasher & El Dohlob, 1970). Pugh (1966) found that *Curvularia* accounted for six per cent of the total isolates from Madras soils and that *C. lunata* was the major species. Several species were isolated from soils in the Sonoran desert (Ranzoni, 1968) and from various soils in Iraq (Al-Doori, Tolba & Al-Ani, 1959) but not from semidesert soils of Nevada (Durrell & Shields, 1960).

*Curvularia* is a major component of the tropical air spora (Meredith, 1962; Sreeramula & Seshavataram, 1962; Dransfield, 1966; Turner, 1966; Upsher & Griffiths, 1973). When a seasonal incidence has been observed, the greatest abundance has been shortly after the start of the rainy season (Dransfield, 1966; Upsher & Griffiths, 1973). Dransfield proposed that this was associated with new growth of grass. There is a rapid increase in airborne *Curvularia* spores after dawn culminating in a maximum at about noon (Sreeramula & Seshavataram, 1962; Meredith, 1962; Upsher & Griffiths, 1973). Meredith (1963) observed two methods by which spores of *C. geniculata* and *C. lunata* are violently discharged into the air in a dry or drying atmosphere. The method accounting for the discharge of most spores was associated with the rapid appearance of gas bubbles in the conidial cells often following some movement of the spore, pivoted at the point of attachment. Some spores were also seen to become detached by twisting of the conidiophores.

*C. lunata* was the main species in Nigeria where it exceeded a quarter of the fungal air spora (Dransfield, 1966). The same species predominated at Hong Kong (Turner, 1966) and at Innisfail, *C. senegalensis* was the major species (Upsher & Griffiths, 1973). As an agent of biodeterioration the greatest significance of *Curvularia* has been its ability to colonize and break down cellulosic materials. It has been frequently reported as colonizing cotton fabrics (White, Darby, Stechert and Sanderson, 1947; Siu, 1951; Ellis, 1966; Upsher, 1971b). At Innisfail, we have found it present on almost every cotton fabric which bore any fungal growth; *C. senegalensis*, *C. ergrostidis*, *C. pallescens*, *C. lunata* and *C. affinis* have been isolated. On cotton fabrics at the sunny, cleared exposure site, *Curvularia* grew and formed spores within seven days but on similar fabrics in the

25

jungle, it grew but did not sporulate. Despite its ability to attack cellulose, *Curvularia* has not been found on timbers at Innisfail.

*Curvularia* shares a noteworthy ability with certain other dematiaceous hyphomycetes, including *Aureobasidium* and *Cladosporium*, to colonize surfaces fully exposed to the tropical sun. In this situation, at Innisfail, it has been isolated from a variety of plastics, rubbers, paints, and adhesives, and was the second most frequent fungus, after *Rhinocladiella elatior* on plasticized poly(vinyl chloride); it was also present, with the blue-green alga *Seytonema stuposum*, on polyolefin and natural fibre ropes and was also frequently isolated from polyurethane coated nylon fabrics.

**Cultural Characteristics**

The walls of conidia, conidiophores and mycelium are generally darkly pigmented, and the colonies are thus some shade of brown or grey to black. The amount of aerial mycelium varies with the strain of the culture and the nature of medium. At its most sparse, there may be only a few conidiophores rising from the surface of the medium whereas the most vigorous colonies produce a deep lanose growth. For standard observations, cultures are generally grown on potato dextrose agar; to produce stromata, they are grown on corn meal agar scattered with unpolished rice grains. Colony growth is often rapid, spreading to about 50 mm diameter in 7 days, though some types remain small—not exceeding about 10 mm.

Conidia are broadly fusiform or elliptical and in most species this outline is bent, hooked or sigmoid because the enlarged middle cells are asymmetrical. Typically there are three or four transverse septa though more or less are not infrequent. The terminal cells are often much paler than the others. This and other features of *Curvularia* conidia are shown in figures 3-5. Conidia are produced through pores at the tip of the conidiophore, which itself elongates. On mature conidiophores, conidia may be in a dense cluster near the tip or in groups or verticils along their length. The conidiophore is septate, usually unbranched and in the fertile area is scarred and knobby.

*Figure 4. C. eragrostidis* with symmetrical outline and with the middle septum heavily pigmented and in median position × 1300.

Stromata (Figure 6) are formed by most species particularly on hard substrata such as cereal grains. They can grow to about 10 mm in length, being simple or branched and may bear conidiophores or contain perithecia. Chlamydospores are also produced by most species, often in aggregates, forming microsclerotial structures. Detailed accounts of the morphologies of the species are given by Ellis, (1966), and other authors quoted above.
Physiological Attributes

The ability to degrade cellulose is widespread among members of the genus Curvularia. Siu (1951) reported several species as being strongly or moderately cellulolytic, and in tests with cotton yarn, we found cultures of C. clavata, C. eragrostidis, C. affinis and C. inaequalis were strongly cellulolytic (Upsher, 1973). Curvularia is associated with the breakdown of cellulolic materials including cotton fabrics and natural fibre ropes exposed at Innisfail.

C. eragrostidis and C. senegalensis are the species we have most frequently isolated from plasticized pvc specimens exposed at Innisfail and laboratory tests have shown that some strains of these and other species are able to grow weakly on certain of the ester-type plasticizers including di(2-methyl-hexyl) phthalate, tri-toly phosphate and di-(2-ethyl hexyl) adipate, and on some polymeric plasticizers based on poly (propylene adipate) and poly (butylene 1,3-adipate). However, the potassium salts of phthalic and adipic acids did not support growth (Upsher, unpublished results).

In studies on the metabolism of racemic steroids, Lin and Smith (1970) showed C. lunata could be induced to produce an enzyme which caused hydroxylation at three sites in the natural and unnatural enantiomers of 19-norsteroids. Kosmol, Hill, Kerb and Kieslich (1970) found that the same species could also saponify the 21-trimethyl acetate of corticosterones.

The ability of Curvularia spp. to assimilate sugars and related compounds has been the subject of several investigations which have shown that all of the sugars tested support growth of most species—as summarised in Table 1.

The ability to reduce nitrate and to utilize it and ammonia as sole sources of nitrogen is apparently widespread in the genus as in most of the hyphomycetes (Bais, Singh and Singh, 1970). A variety of amino-acids including alanine, asparagine, aspartic acid, cystine, glutamic acid, glycine, leucine, methionine, ornithine, proline, tyrosine and valine have all been found to be utilized as nitrogen sources, (Bais, Singh & Singh, 1970, Panwar, 1970) but DL-histidine was not utilized (Panwar, 1970).

Although Agnihotri and Bhide (1963) suggested that the presence of certain vitamins in the culture medium had no effect on C. Lunata, Shukla and Bais (1971) found that most B group vitamins and especially thiamine and biotin improved growth and sporulation.

In buffered mineral salts solutions, with glucose as the substrate C. lunata was shown to grow between pH 2.5 and 10.0 with an optimum at pH 3.5 (Upsher, 1971a).
Sensitivity to Biocides

In comparative studies on the toxicity of benomyl (methyl 1-(butyl carbamoyl)-2-benzimidazolcarbamate), it was found that growth of *C. geniculata*, together with that of some other porospore-forming hyphomycetes, was not significantly inhibited (growth exceeded 50% of the controls) at a concentration of 1 ppm, whereas other microfungi tested (with the exception of the Phycomycetes) were inhibited at 1 ppm (Edgington, Khew & Barron, 1971). However, we have shown complete inhibition of *C. eragrostidis* and *C. senegalensis* at 1.5 ppm and *C. lunata* at 0.5 ppm. The same species were more tolerant of Thia-bendazole, (2-(4 thiazolyl)-benzimidazole); concentrations of 10 ppm being required to completely inhibit *C. eragrostidis* and *C. senegalensis* and 30 ppm to inhibit *C. lunata*.

In tests with di-2-octanone thiocarboxyhydrate, we found that a concentration of 100 ppm in the aqueous medium prevented growth of *C. lunata* but caused only slight inhibition of *C. eragrostidis* and *C. senegalensis*.

*C. lunata* was found by us to be more tolerant of bis (tributyltin) oxide and pentachlorophenyllaurate than *C. eragrostidis* and *C. senegalensis* but all were inhibited by the same level of dissolved copper as shown in Table 2.

### TABLE 1. Growth of species of curvularia on sugars and derived compounds

<table>
<thead>
<tr>
<th>Pentoses</th>
<th>Hexoses</th>
<th>Hexahydric Alcohols</th>
<th>Disaccharides</th>
<th>Tri-</th>
<th>Poly-</th>
<th>Authority</th>
</tr>
</thead>
<tbody>
<tr>
<td>Arabinose</td>
<td>Ribose</td>
<td>Xylose</td>
<td>Fructose</td>
<td>Glucose</td>
<td>Mannose</td>
<td>Sorbitol</td>
</tr>
<tr>
<td><em>C. affinis</em></td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td><em>C. clavata</em></td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td><em>C. eragrostidis</em></td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td><em>C. fallax</em></td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td><em>C. inaequalis</em></td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td><em>C. lunata</em></td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
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<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td><em>C. oryzae</em></td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td><em>C. pallescens</em></td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
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<td></td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td><em>C. prasodii</em></td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td><em>C. senegalensis</em></td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td><em>C. stidiquis</em></td>
<td>+ w</td>
<td>w</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td><em>C. trifolii</em></td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

*Results not previously published
+ = Good growth; w = weak growth; - = growth not more than controls.

### TABLE 2. Lowest levels which caused complete inhibition (ppm)

<table>
<thead>
<tr>
<th>Authority</th>
<th>Bis (tributyltin) oxide</th>
<th>Pentachlorophenyllaurate</th>
<th>Cu</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>C. geniculata</em></td>
<td>1.0</td>
<td>100</td>
<td>64</td>
</tr>
<tr>
<td><em>C. eragrostidis</em></td>
<td>0.1</td>
<td>30</td>
<td>64</td>
</tr>
<tr>
<td><em>C. senegalensis</em></td>
<td>0.1</td>
<td>30</td>
<td>64</td>
</tr>
</tbody>
</table>

*Acknowledgements*

The author thanks Dr. P. G. Valder of the University of Sydney, Dr. Dorothea Frey of the Royal
North Shore Hospital, Sydney and Dr. E. N. Fitzpatrick of the Western Australian Department of Agriculture, Perth, who so kindly provided cultures: Mrs. Veronica M. Silva (M.R.L.) for the scanning electron micrographs, Mr. P. Jackson (M.R.L.) for the other micrographs and he and Mr. T. Keen (Melbourne University late of M.R.L.) for technical assistance.

References


Summary. Laboratory experiments were conducted on the basis of the OECD Activated Sludge System (Confirmatory Test Procedure) to investigate the self-infection processes by air-borne bacteria. It could be shown that after a very short running-in period a certain "flow-balance" in the continuous culture fermenter is obtained. The main interest lay in determining the free living bacteria which were predominant during this period. More than half consisted of Pseudomonas spp. (Ps. putida, Pseudomonas sp. (non-fluorescent), Ps. stutzeri, Ps. aeruginosa); the majority of the remainder (28%) being Bacillus spp. (B. pumilus, B. cereus, B. megaterium, B. sphaericus). In these experiments the results were very reproducible.

Introduction

At CIBA-GEIGY the OECD Confirmatory Test Procedure (OECD 1971) is routinely used for testing the biodegradability of detergents in a modified Hsumann Apparatus. This procedure now prescribes that the system has not to be inoculated by bacteria from an outside treatment plant, the activated sludge being allowed to form its own flora by self-infection of air-borne bacteria directly in the apparatus when fed continuously by synthetic sewage. This OECD Activated Sludge System was used to study just this phenomenon of aerial infection in the primary running-in period; and accordingly the development of these bacteria within the first 72 hours in this system was investigated, but without adding any anionic detergent. This is because the interest was not in the acclimatisation process itself but in the self infection process of the bacteria for the given OECD nutrient solution. Primarily, the qualitative aspects of the bacteria involved were investigated but quantitative aspects of the process were also studied.

Materials and Methods

1. The OECD Confirmatory Test Procedure as described in the literature (OECD 1971), is derived from the German legislation on detergents (Federal Council of the German Federal Republic, 1962). As synthetic sewage, a nutrient solution containing 160 mg peptone (Merck Nr. 7213), 110 mg meat extract (Liebig), 30 mg urea, 7 mg NaCl, 2H2O, and 2 mg MgSO4, 7H2O per litre tap water is employed at a pH of about 7.2. For this particular investigation no methylene blue active substance (MBAS) was added. This synthetic sewage is then pumped by a dosing device B from the storage vessel A into the aeration tank C (1.8 /) in the OECD-Program (OECD Procedure) as described in the literature (OECD 1971) to obtain certain equilibria of fermentation continua of the culture. Estimating the principal processes in determining viable numbers of the bacteria growing freely that predominated during this period, the test procedure as described in the literature (OECD 1971) was followed. More than half consisted of Pseudomonas spp. (Ps. putida, Pseudomonas sp. (non-fluorescent), Ps. stutzeri, Ps. aeruginosa); the majority of the remainder (28%) being Bacillus spp. (B. pumilus, B. cereus, B. megaterium, B. sphaericus). In these experiments the results were very reproducible.

H. R. Hitz1, G. Pichtla1, Judith E. P. Young2 and J. M. Shewan2

1 CIBA-Geigy Ltd., Dyes and Chemicals Division, CH-4002 Basle, Switzerland.
2 Ministry of Agriculture, Fisheries and Food, Torry Research Station, Aberdeen, Scotland, AB9 8DG.
Primary developments of air-borne bacteria in the OECD activated sludge system. H. R. Hitz, G. Plichta, Judith E. P. Young and M. Shewan.

2. Bacterial samples were taken from the system every three hours. About 10 ml of the nutrient solution were filtered aseptically through a paper filter to separate free bacteria from sludge. For the qualitative and quantitative investigations, aliquots of the filtrate were poured into petri dishes either with Brain Heart Infusion Agar (BBL) or CM3-Agar (Oxoid), and incubated at 22°C for 72 hours. For the microscopical examination, the pure cultures were grown in Brain Heart Infusion (BBL) at 22°C overnight or on CM1-Broth (Oxoid) at 25°C for 48 hours. The nutrient solution was renewed every 12 hours.

3. Bacterial Identification. The 25 bacteria isolated during the two experiments were identified according to a variety of procedures, the most important of which are described by Shewan et al. (1960); Cowan and Steel (1965); Stanier, Palleroni and Doudoroff (1966) and Houston (1969).

Results

1. Development of bacteria in the continuous activated sludge system. The quantitative test was done in two steps. In the first run, the periods 1-12 hours, 24-36 hours and 38-60 hours were investigated; while in the second the periods 12-24 hours, 36-48 hours and 60-72 hours were studied under the same conditions.

Every three hours, samples were taken from a. the aeration chamber; b. the effluent; c. the storage vessel. The contents of the storage vessel together with the stock nutrient solution were previously sterilised to avoid additional bacterial infection. However, the tap water was not sterile, so a source of infection occurred through this route.

The progressive accumulation of airborne bacteria can be seen from Figs 2 and 3. In the first run (Fig. 2) the exponential growth in the aeration chamber starts from the very beginning reaching a maximum after about 36 hours. At this point a count of 10^8 bacteria per ml was found, which remains constant for the 72 hours of the test. The effluent shows the same shape of curve but in the storage tank growth is slower but reaches its maximum also after about 36 hours.

The second test (Fig. 3) shows the same situation as in the first run.

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Primary developments of air-borne bacteria in the OECD activated sludge system. H. R. Hitz, G. Plichta, Judith E. P. Young and J. M. Shewan.

In the aeration chamber and settling vessel the air-borne bacteria start suddenly growing, after the system is set up; the nutrient solution becomes turbid within the first 24-30 hours forming tiny sludge-flocs within 48 hours and sludge frazzles after 56 hours. After 72 hours the content of sludge reaches about 0.7-1 g/l of dry-weight. In the storage vessel the infection occurs primarily from the air, but also from the tap water used which influences the infection of the aeration chamber.

If in the two experiments, the growth kinetics of the bacteria are considered, the specific growth rate and the doubling time can be calculated from the following formula.

Specific Growth Rate ($\mu$):
$$\mu = 2.303 \frac{\log N - \log N_0}{t - t_0}$$

Doubling Time ($g$):
$$g = \frac{\ln2}{\mu} = \frac{0.693}{\mu}$$

whereas $N = \text{amount of cells at time } t$
$N_0 = \text{amount of cells at time } t_0$

These rates over the first 24 hours were then calculated in the aeration chambers. In the first run the specific growth rate was 0.61 (+ the dilution rate of $D = 0.2$) = 0.81 and the doubling time 1.13 hours. The second run showed an equal specific growth rate of 0.60 ($D = 0.2$) = 0.80 and the doubling time of 1.15 hours.

In the following stationary phase (36-72 hours) the specific growth rate and doubling time were still 0.2/h and 3.5 hours.

2. Biodegradation of the nutrient solution

In addition, we also checked the efficiency of our OECD Activated Sludge System by means of TOC-analysis. The results are expressed in the following equation:

$$\text{TOC}_{\text{EFFLUENT}} = \text{TOC}_{\text{INFLUENT}} \times \%\text{Biological \ elimination}$$

In Fig. 4 it is shown that after 3 days a 70% effect, and practically also the maximum level of total oxidisable carbon (TOC) degradation, is reached. However, the TOC-degradation curve follows the shape of bacterial growth of the aeration chamber and the effluent in Figs. 2 and 3. With other nutrient solutions a higher efficiency degree can be obtained.

3. Bacterial identifications

The identifications of the twenty five bacterial isolates are given in Table I. This shows that 13 were identified as Pseudomonas spp. (9 Pseudomonas putida, 1 Ps. aeruginosa, 1 Ps. stutzeri and 2 as non-fluorescent pseudomonads (Group II Shewan et al. 1960), 7 as Bacillus spp. (4 B. pumilus, 1 B. cereus, 1 B. sphaericus)

**TABLE I. List of the isolated bacteria (Summary)**

<table>
<thead>
<tr>
<th>Isolate No.</th>
<th>Bacterium</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Moraxella sp.</td>
</tr>
<tr>
<td>2 L</td>
<td>Pseudomonas putida</td>
</tr>
<tr>
<td>2 S</td>
<td>&quot;Mycobacterium rhodochrous&quot;</td>
</tr>
<tr>
<td>7</td>
<td>Pseudomonas putida</td>
</tr>
<tr>
<td>8</td>
<td>Pseudomonas putida</td>
</tr>
<tr>
<td>9</td>
<td>Pseudomonas putida</td>
</tr>
<tr>
<td>10</td>
<td>Bacillus megaterium</td>
</tr>
<tr>
<td>11</td>
<td>Pseudomonas sp. (non-fluorescent)</td>
</tr>
<tr>
<td>12</td>
<td>Pseudomonas putida</td>
</tr>
<tr>
<td>13</td>
<td>Bacillus pumilus</td>
</tr>
<tr>
<td>14</td>
<td>Bacillus pumilus</td>
</tr>
<tr>
<td>16</td>
<td>Bacillus cereus</td>
</tr>
<tr>
<td>17</td>
<td>Bacillus sphaericus</td>
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<td>Bacillus pumilus</td>
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<tr>
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<td>Bacillus pumilus</td>
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<td>Pseudomonas stutzeri</td>
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<tr>
<td>21</td>
<td>Pseudomonas putida</td>
</tr>
<tr>
<td>22</td>
<td>Pseudomonas aeruginosa</td>
</tr>
<tr>
<td>23</td>
<td>Pseudomonas putida</td>
</tr>
<tr>
<td>24</td>
<td>Pseudomonas sp. (non-fluorescent)</td>
</tr>
<tr>
<td>25</td>
<td>Pseudomonas putida</td>
</tr>
<tr>
<td>26</td>
<td>Staphylococcus epidermidis</td>
</tr>
<tr>
<td>27</td>
<td>Flavobacterium sp.</td>
</tr>
<tr>
<td>28</td>
<td>&quot;Mycobacterium rhodochrous&quot;</td>
</tr>
<tr>
<td>29</td>
<td>Pseudomonas putida</td>
</tr>
</tbody>
</table>

![Figure 4. Efficiency Degree of the OECD-Activated Sludge-System by TOC-Analysis.](image-url)

and 1 B. megaterium). Two of the remainder were identified as "Mycobacterium rhodoehrous," on as Staphylococcus epidermidis, one Moraxella sp. and one Flavobacterium sp.

Discussion

The OECD Confirmatory Test consists of a continuous activated sludge procedure infected naturally by airborne bacteria. It was of interest therefore to study this primary infection of the proper OECD Nutrient Solution without adding any anionic detergent, ignoring at the same time any acclimatization process of the bacteria. It was found that as an open continuous culture fermenter the system tends towards a "flow-balance" within the first 72 hours.

However, it is influenced by a lot of physico-chemical and biological factors of which temperature, pH, nutrients, microbial antagonism, symbiosis, and metabolism, are probably the most important (Daubner, 1972). Although some authors deny the reproducibility of similar sludge compositions in consecutive experiments we were able to demonstrate a good correlation between our tests which must be due to the self-infection of the system by airborne bacteria and secondly to the use of the same nutrient solution.

From an examination of the bacteria isolated and identified more than half belonged to the genus Pseudomonas, with Ps. putida, Pseudomonas sp. (non-fluorescent) and strains of Ps. stutzeri and Ps. aeruginosa predominating. In genus Bacillus the species B. pumilus dominated, in addition to B. cereus, B. megaterium and B. sphaericus. Five other isolates were grouped to Flavobacterium, "Mycobacterium rhodochrous" complex, Moraxella and Staphylococcus.

Prakasam and Dondero (1967, 1970) who studied the Aerobic Heterotrophic Bacterial Populations of Sewage and Activated Sludge cited Allen (1944) as identifying Flavobacterium, Achromobacter and Chromobacter in laboratory activated sludge.

Van Gils (1964) reported that Achromobacter, Alcaligenes and Flavobacterium were predominant in both natural and laboratory activated sludges with Pseudomonas and coryneform organisms occurring in relatively low numbers. Dias and Bhat (1964) reported the presence of Zoogloes and Comamonas in activated sludge and stated that coliforms formed only a small portion of the total population, indicating that these organisms played a minor role in sewage purification. Pike and Carrington (1972) summarised the principal bacterial genera occurring in activated sludge which with the exception of the Moraxella spp. parallel their findings in the first running-in period studied here.

Comparing the carbon sources in the nutrient solution with the biochemical reactions of the isolated bacteria it is suggested that the bacilli must be the primary carbon hydrolysers apart from Ps. aeruginosa.

However, in these experiments it was not intended to study all the very complicated relationships of bacterial succession in an activated sludge system.

In further studies similar investigations will be undertaken in the biodegradation of different substances such as MBAS products in order to investigate additionally the acclimatization process of the bacteria to the products over the whole test period.

References


Primary developments of air-borne bacteria in the OECD activated sludge system. H. R. Hitz, G. Plichta, Judith E. P. Young and J. M. Shewan.


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