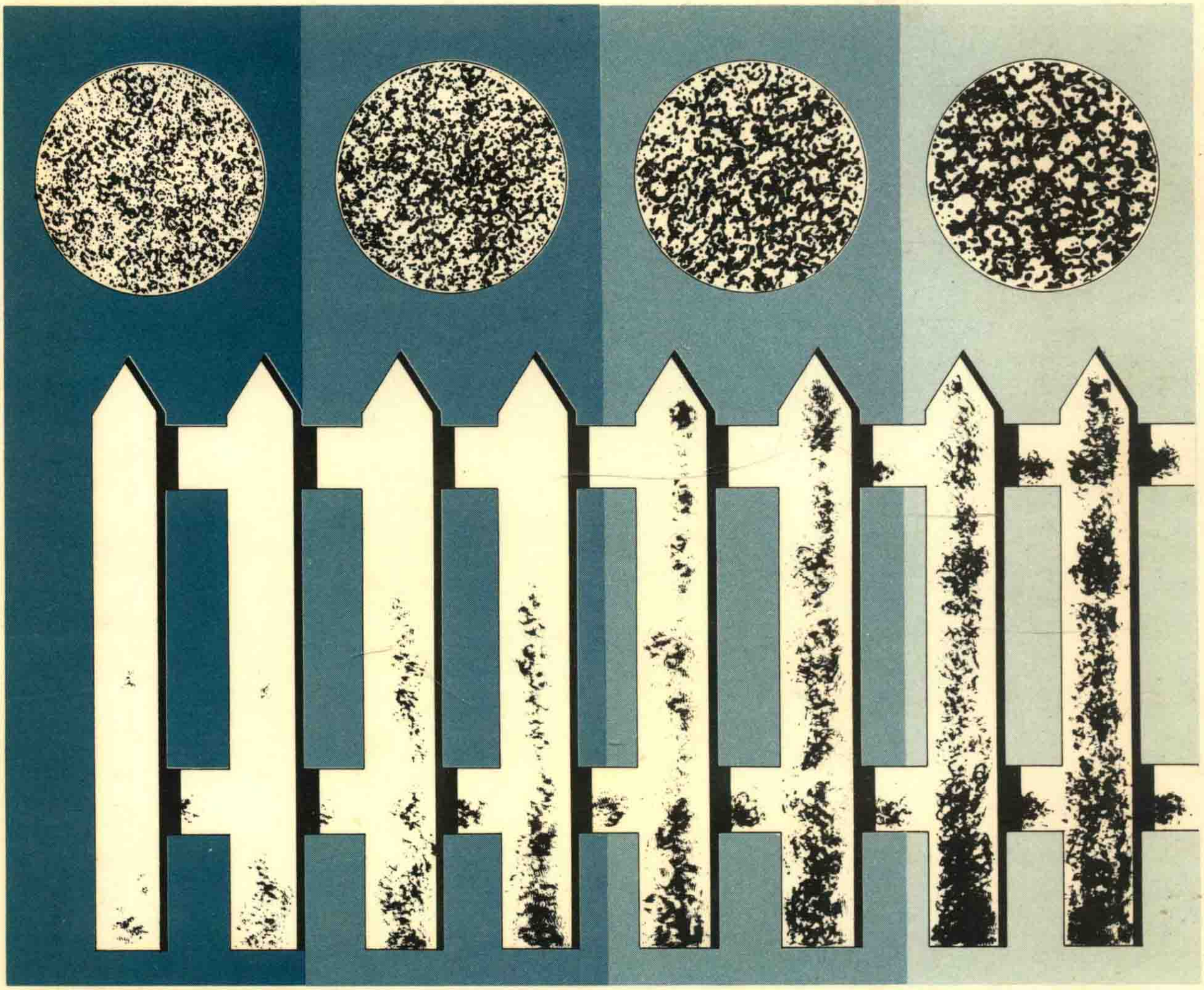
C·A·B INTERNATIONAL MYCOLOGICAL INSTITUTE

THE BIODETERIORATION SOCIETY

Biodeterioration 6

PROCEEDINGS OF THE SIXTH INTERNATIONAL BIODETERIORATION SYMPOSIUM



Biodeterioration 6

Papers Presented at

The 6th International Biodeterioration Symposium
Washington, DC, August, 1984

Sponsored by

The George Washington University
Virginia Commonwealth University

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Published by
C·A·B International,
Farnham House,
Farnham Royal,
Slough SL2 3BN,
United Kingdom.

Tel: Farnham Common (02814) 2281
Telex: 265871 (MONREF G) for BOX CAU001
Telegrams: Comag Slough
Telecom Gold/International Dialcom: 84: CAU001

ISBN 085198 555 6

Printed in Great Britain by: The Cambrian News Ltd., Aberystwyth.

Foreword

This issue of the Proceedings of the Sixth International Biodeterioration Symposium held in Washington in August 1984, forms a very worthy record. It represents the culmination of the untiring endeavours of Dr Charles O'Rear and Dr Gerald Llewellyn, together with their able band of Committee Members.

The topics of the Symposium represent the current concerns of biodeterioration and as such encompasses a wide range of interests from rodent and bird control to the preservation of museum materials or the assessment of new cutting oil biocides.

Perhaps it is useful to consider the meaning of biodeterioration. Hueck at the OECD Working Party in 1962 stated that:—
"Biodeterioration is the study of deterioration of materials of economic importance by organisms". Later, structures and processes were also linked to materials in this definition which has become widely accepted.

Within their definition the word "deterioration" requires some further comment. Words such as "decay", "spoilage" and "damage" could also be included, and for some, biodeterioration is probably used in the sense that there should be actual physical breakdown, or "decay", of a material. But, of course, the attack on materials by organisms is much wider than this and it seems to include all forms of damage, or loss of economic value caused by organisms interacting with materials, as biodeterioration. Hueck in his classic paper listed a large number of ways in which organisms could damage or deteriorate materials, not all of which involve the physical breakdown of the material, to cause economic loss.

Perhaps at this stage it is worthwhile to discuss the origins of the terms "biodeterioration" and "biodegradation". Tom Oxley and myself published a paper on this topic in the *International Biodeterioration Bulletin.* Undoubtedly, the two terms have been used interchangeably; however, hopefully, they now have distinct and different meanings, although they are closely linked. The word "deteriorate" comes directly from the Latin and means (as in the Latin) "to make worse" and for us to make something worse by biological agents may imply fungi rotting timber, bacteria blocking a pipeline or barnacles growing on the side of a ship thereby reducing its streamlining. In only one of these instances does the organism cause damage by breaking down a material, but in each example things are "made worse" by biological activity. Thus biodeterioration covers a wide range of biological activities involved in man's materials and constructions where the effect is to "make things worse" and thus adversely or negatively affect man's economy.

The word "degrade" also comes directly from Latin and means "to step down" and obviously when applied to things rather than people means "to break down". I hope it thus becomes clear straightawaythat biodeterioration and biodegradation are not interchangeable, biodeterioration having a wider meaning in terms of the unpleasant things that organisms can do to materials. However, biodegradation could properly mean those activities of organisms that result in the breakdown of a material either to man's detriment or good. Today it is generally considered that it is in the latter sense that "biodegrade" has a special meaning and has come to be applied to those activities where materials or chemicals are broken down to man's benefit, and where new substances of value may be synthesized, and where the activity can be thought of as the opposite of a biodeteriorative activity.

Biodeterioration as a technical subject can only justify its demand for study if it can be shown that the concept helps us to control the problem thus designated. I believe that the commitment to this concept does not justify it. However, the relationships to certain other areas need to be mentioned. Pathology relates to the attack by organisms on other growing organisms, e.g. standing crops, whilst biodeterioration is concerned with crops once they have been harvested. Clearly there is overlap between pathology and biodeterioration, and this is perfectly proper. Secondly, there is a concern with public health aspects that need consideration. Public health is concerned when organisms that can cause disease are passed on to human beings either by touch, inhalation or ingestion. Some organisms are merely passively carried to the human host and cause disease only when they are multiplying on or in the host. Others though, interact first with a material, multiplying on it and subsequently causing disease by the inhalation or ingestion of spores or toxic compounds. The latter are the proper concern of those involved in the study of biodeterioration.

There are several reasons why there is more attention being given to the control of biodeterioration today, First, in real terms the value of materials is increasing and manufacturers are continually improving their designs and using less materials to achieve constant performance. Any attack by organisms is thus more important than before and, as a result, there is a continual and increasing demand for a wider range of susceptible materials to be protected against attack—clearly this could mean expanding markets for biocides. In line with this trend is the demand for increasing standards of quality control in products and the heightened awareness of deviation and increased sensitivity towards organism contamination. Again safety standards have pointed to the dangers of uncontrolled organism involvement with Man and his materials, for instance, the possible dangers from saprophytic fungal spores, mycotoxins and household mite infestations. Running counter to these trends is the heightened awareness of the improper and misinformed use of biocides generally—"throw another litre in if in doubt" will no longer satisfy. This interesting balance of trends has led to a more professional outlook on these matters.

Clearly the Symposium demonstrated that there are still many problems in biodeterioration which need solving and many new problems that arise. However it is obvious that biodegradation, the harnessing of activities that are familiar to all of us, is the beginning to play a more important role, particularly in the context of biotechnology.

Although developmental work has often a long way to go before many of these processes are truly economic, I believe that events are making it likely that more will be economic in the future. I hope sincerely that our Society will play an important part in those developments. I should like to record our thanks and indebtedness of Charles O'Rear and to Gerry Llewellyn and their splendid team. I would also like to thank most sincerely the Virginia Commonwealth University, and the George Washington University for its hospitality and excellent organization; and finally to Henry Solomon, Dean of the Graduate School of Arts and Sciences at George Washington for his warm welcome on behalf of the University.

H. O. W. Eggins BSc, PhD, FIBiol
Chairman, Standing Committee for International Biodeterioration Symposia.

Preface

The present volume contains the majority of the papers given at the 6th International Biodeterioration Symposium held at the George Washington University, Washington, USA, in August, 1984. The program was organised by members of the USA Program Planning Committee. They invited leading scientists in specific topic areas and accepted contributed papers from individuals and laboratories actively involved in these areas of research. The Society thus ensured that the program reflected current developments, informed reviews and critical assessment of the present state of knowledge.

All the presentations and papers underwent both scientific and technical review. A few of them do not appear in this volume because they were considered unsuitable for publication. The order and organization of the contributions varies somewhat from that of the actual Symposium and some sessions have been combined where the topics have a natural affinity to each other.

The result of the efforts of the USA Program Planning Committee, Symposia Organisers, SCIBS, Session Chairmen and authors in this publication which is considered to be a significant contribution to the general area of biodeterioration. We believe it will be of value to a broad range of scientists.

The Editors.

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Acknowledgements

The 6th International Biodeterioration Symposium was organised by the USA Program and Planning Committee on behalf of the Standing Committee for International Biodeterioration Symposia (SCIBS) of the Biodeterioration Society. The sponsors were the George Washington University, Department of Forensic Sciences and the Virginia Commonwealth University, Department of Biology. The former provided the venue and played host to the Symposium.

The Biodeterioration Society wishes to record its grateful thanks both to the George Washington University and the Virginia Commonwealth University and in particular to the Co-Chairmen Drs. C. E. O'Rear and G. C. Llewellyn.

The facilities provided prior to and during the Symposium and the editing of these Proceedings by the Ministry of Defence UK and the Virginia Department of Health were much appreciated.

Thanks are also due to the USA Program and Planning Committee, SCIBS, the Symposia Organisers and Session Leaders for their efforts in making the Symposium a great success. The contributions of Mr. K. Cooper, Mrs. C. Clark, Dr N Lappas, Dr W Rowe and Dr H Solomon are gratefully acknowledged. Acknowledgements are also due to Mrs. H. Minter who prepared the Index.

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Session 1 - Biodegradation of Effluents

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Municipal and industrial wastes contain both organic and inorganic complexes which should be biodegraded so that neither the environment nor man be adversely affected. These wastes, included in effluents from the industrial or the municipal treatment system require that a healthy biological ecosystem be available to receive them to accomplish biodegradation or biomodification of the wastes. Biota, to function in this process, must have physical and chemical environmental conditions that permit them to alter the complex wastes with which the receiving system is confronted. These were the topics for discussion in Session 1.

The approaches to studying the problems of biodegradation of wastes in effluents have run the gamut from the study of the degradation of a single chemical compound by a species of bacteria in a plasmid-mediated system, to mixtures of 13 synthetic organic compounds treated with the mixture of organisms present in an activated sludge system, and to mixtures of domestic wastes with sewage sludge applied to open land areas. At various levels between these approaches, discussions have centered on, and found considerable room for agreement in, certain factors affecting biodegradation of waste effluents.

The processes of biodegradation of effluent wastes may require variously; 1, digestion of complex molecules; 2, combination of simpler molecules; 3, dissociation of metallic element complexes; 4, incorporation of metallic elements into biota; 5, rearrangement or restructuring molecules within the waste; or 6, some combination of any or all of these steps.

It is generally conceived that microbial populations are most likely to carry out these processes, at least in the initial phases, and that in order to do so, the waste compounds must be accessible to the organisms. A recurring topic in the discussions of this session was the effect of sorption of chemicals to particulates in the environment. Such sorption affects the availability of the waste to the metabolic processes of the organisms.

A second recurrent theme in these discussions was the effect of acclimatization of microbial populations to waste materials. If biotic populations have been exposed to a waste compound for some period of time, then it is most common that the biota will be able at least to tolerate the compound, and given sufficient time for acclimatization, either the original or the modified (selected) population will be able to metabolize the compound. Periods of acclimatization appear particularly needed when new mixtures of waste compounds are released to effluents. In these cases lowered waste concentrations combined with the allowance of acclimatization time have proven helpful.

The discussion of factors influencing biodegradation included the effects of pH and of waste concentrations. One report dealt with the bioconcentration of heavy metals in waste effluents and the effect of effluent pH on bioconcentration, mobilization and cycling of these wastes. The potential toxicity of some elements to the metabolic processes required for biodeterioration of complex molecules points up the importance of efficient cycling of these chemicals to allow biodegradation of effluent wastes to occur.

Discussion of bacterial biodegradation of phenanthrene provided the first report of plasmid-mediated degradation associated with estuarine organisms, and perhaps points the way for future studies involving the aquatic environment and effluent wastes.

In this and other discussions the necessity for consideration of microbial populations present in all toxicity testing is a unifying thread. Certainly if microbial populations are the primary mechanisms for biodegradation of waste effluents (toxicants), which they seem to be, these organisms and their activities will dramatically affect the results of toxicity testing using other organisms. Such potential effects are largely ignored by most investigators.

The Role of Plasmids in Degradation of Phenanthrene by Bacteria Isolated from the Estuarine Environment

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Summary

A differential phenanthrene enrichment agar plating technique was used to isolate phenanthrene-degrading bacteria from water and sediment samples collected from Chesapeake Bay. One of the isolates, a yellow-pigmented, slime-producing, Gram-negative rod, identified as a *Flavobacterium* sp. has been found to carry a single plasmid of about 34 x 10^6 mw. Results of hydrocarbon adherence tests showed that the organism adhered only minimally to n-octane and n-hexadecane, but emulsified cyclohexylbenzene and 1, 2, 3, 4 - tetrahydronaphthalene (tetralin). Curing of the plasmid with 3 μg/ml novobiocin resulted in loss of phenanthrene clearing ability, as well as loss of aromatic hydrocarbon emulsification. Upon transformation of the cured derivative with the 34 Mdal plasmid DNA, the phenanthrene clarification phenotype was reintroduced. However, the transformed strain did not reveal the 34 x 10^6 mw plasmid by agarose gel electrophoresis, an observation suggesting integration of the plasmid into the chromosome. The degradative ability of these strains was confirmed by C^{14} - phenanthrene uptake experiments. These results provide the first instance of plasmid-mediated phenanthrene degradation associated with estuarine bacteria.

I. Introduction

Polycyclic aromatic hydrocarbons (PAHs) are widely distributed in the environment, partly as a result of high temperature pyrolytic processes (Blumer 1976). They are important contaminants because they persist in natural systems (Platt & Mackie 1980). Using a replica plate method, it has been observed that hydrocarbon-polluted sediments in central and northern Chesapeake Bay are enriched in phenanthrene-degrading microorganisms (Cooney & Shiaris 1982; West *et al.* 1984). Phenanthrene is not acutely toxic, carcinogenic, or mutagenic; however, it has been used as a model substrate in studies of environmental degradation of polycyclic aromatic hydrocarbons, since its structure is found in the nucleus of carcinogenic PAHs, such as benzo(a) anthracene and 3-methylcholanthrene (Cerniglia & Yang 1984). Hydrocarbon degrading bacteria play an important role in the clean-up of oil pollution (Walker & Colwell 1977; Atlas 1981) and in the cycling of carbon in the environment (Gibson 1977). Plasmid mediation of key steps in the degradation of hydrocarbon molecules is now well established (Chakrabarty 1980; Yen & Gunsalus 1982). In general, degradation requires an interaction between chromosomal and plasmid genes (Fennewald *et al.* 1978; Zuniga *et al.* 1981).