BIODETERIORATION RESEARCH 1

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PLENUM PRESS • NEW YORK AND LONDON

Library of Congress Cataloging in Publication Data

Pan American Biodeterioration Society. Meeting (1st: 1986: Washington, D.C.)
Biodeterioration research 1.

"Proceedings of the First Annual Meeting of the Pan American Biodeterioration Society, held July 17-19, 1986, in Washington, D.C."—T.p. verso.
Includes bibliographies and indexes.
1. Materials—Biodeterioration—Congresses. 2. Materials—Microbiology—Congresses. 3. Mycotoxins—Congresses. I. Llewellyn, Gerald C. II. O'Rear, Charles E. III. Title. [DNLM: 1. Biodegradation—congresses. W3 PA187 1st 1986b / WA 671 P187 1986b]
TA418.74.P36 1986 620.1'1223 87-25494
ISBN 0-306-42764-8

Proceedings of the First Annual Meeting of the Pan American Biodeterioration Society, held July 17-19, 1986, in Washington, D.C.

A Division of Plenum Publishing Corporation
233 Spring Street, New York, N.Y. 10013

Printed in the United States of America

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PRELIMINARY SCANNING ELECTRON MICROSCOPY STUDY OF MICROBIOLOGICALLY INDUCED DETERIORATION OF HIGH ALKALI LOW-LIME GLASS

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INTRODUCTION

The existence of microbiologically caused deterioration of historical glass has not been experimentally verified to data. Further, it is a difficult hypothesis to prove from static examination of historical glass in the field or laboratory. Isolation and identification of microbes, from glass surfaces provide circumstantial evidence for an association, but do not prove a causal relationship.

A wide variety of forms of deterioration can be observed on historical glasses, depending on the chemical composition of the glasses and environmental exposure conditions. Conditions include burial in the earth, submersion in water, atmospheric exposure to weathering elements, and storage in damp indoor rooms. The most common visual effects of deterioration include fragile or brittle weathering crusts, iridescence, surface scum, pitting, mechanical erosion, dulling, and crizzling. Ordinarily, the mechanisms proposed to account for these phenomena have been based on purely chemical or physical factors, while microbiological factors have either been rejected summarily or been attributed little importance.

Only a few studies have investigated the possibilities of microbial attack (Prod'Homme, 1965; Kerner-Gang, 1968; Collongues et al., 1976; Perez y Jorba, 1981; Perez y Jorba et al., 1978; 1980). The corrosion type associated with high-potassium glass, which begins with circular pits that eventually form craters, has been attributed to microbial attack and/or atmospheric attack (Perez y Jorba, 1981).

The purpose of this study was to ascertain whether the potential for microbiological attack indeed exists, and if so, whether such attack produces a characteristic appearance.

MATERIALS AND METHODS

This study made use of test glasses melted for The Corning Museum of Glass by the late Dr. A.A. Erickson of Corning Glass works. The glasses are a highly degraded potassium glass and a degradable high soda glass. These were originally prepared as part of a set of glassed with a range of durabilities replicating actual Medieval and Renaissance glasses.

The organisms selected for the initial tests were ones isolated from a previous study on calcite and dolomite stone (Koestler et al., 1985), and have not been associated with glass deterioration before. Isolates of a Tricothecium spp. fungus, a chlorophyte of the chlorococcales family, and a cyanobacterium were collected in that study. Isolates were kept in culture in Sabaroud's medium for the fungus, and soil extract for the alga and bacterium.

For the experiment, composite samples of each microorganism were washed three times with tap water, spun at 1000 rpm for 5 minutes each time, vertexed for several seconds, and then 1-2 ml containing approximately 10^5-10^6 cells was used for the algae and bacteria. An equivalent optical density was used for the fungus inoculate on the horizontal surfaces of glass 'squares,' approximately 2.5 x 2.5 x 0.6 cm. The glass was placed on Whatman #2 filter paper, which was moistened with tap water, in glass Petri dishes and placed in temperature- and light-controlled biological chambers. Dishes were checked periodically to ensure maintenance of moist conditions. Wet and dry glass controls were prepared in the same manner, but not inoculated. After six months the experiment was terminated and samples were prepared for light microscopy (LM), scanning electron microscopy (SEM), and energy dispersive X-ray spectrometry (EDS).

Samples for SEM observations were air dried and mounted on 2.54 cm aluminum pin-type stubs which had been previously coated with carbon paint. Mounted samples were coated with approximately 10 nm of spectroscopically pure carbon in an Edwards 505 vacuum evaporator and examined with an AMRay 1600T SEM operated at 15 kV. Energy dispersive X-ray spectrometry was performed with a Kevex 0700 system; spectra were collected for 200 seconds at 15 kV and analyzed with Kevex's standardless routine with ZAF corrections by MAGIC V.

RESULTS

Chemical Characterization

The chemical characterization of both glass types, as determined by EDS, are presented in Table 1, as oxide weight percentages. It

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Table 1. Energy Disper

Oxide
SiO_2
K_2O
Na_2O
Al_2O_3
CaO
MgO
MnO

a = Means.
b = Standard deviation
ND = Not detected (dete

Visual Observations

Figure 1 depicts a
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AW Glass (sodium-rich)

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should be noted that the chemical composition of both glass were similar except for a high potassium content in the AV glass and high sodium content in the AW glass.

Table 1. Energy Dispersive X-ray Spectrometric Analysis of Test Glass

Oxide	AV Glass		AW Glass	
	X^a	sd^b	X^a	sd^b
SiO_2	80.4 ±	0.4	74.9 ±	0.3
K_2O	16.5 ±	0.6	2.5 ±	0.7
Na_2O	ND		20.7 ±	0.9
Al_2O_3	0.1 ±	0.07	1.3 ±	0.1
CaO	1.5 ±	0.1	1.3 ±	0.1
MgO	1.0 ±	0.09	0.07 ±	0.03
MnO	0.1 ±	0.13	0.2 ±	0.1

[a] = Means.

[b] = Standard deviation where population parameter is taken to be "N".

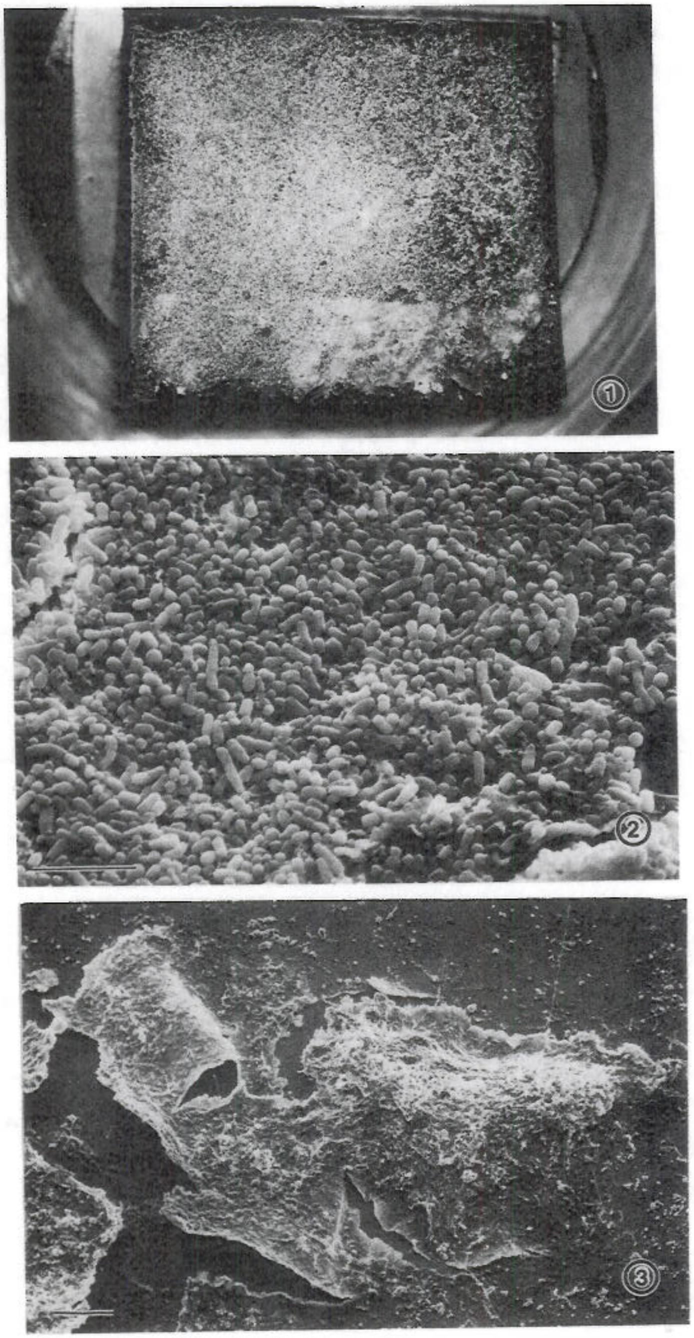
ND = Not detected (detection limits are approximately 0.1%).

Visual Observations

Figure 1 depicts a low magnification (2.5 X) light micrograph of the surface of one of the tests glasses. The extent of coverage by the microorganisms can be readily discerned. Microorganism growth, while not quantified, was extensive on the surface of all test samples, generally covering 80-90% of the surface.

AW Glass (sodium-rich)

Large numbers of microorganisms were supported on this glass as depicted in Figure 2. Extensive cracking and/or crizzling of the glass surface was noted where dense mats of the microorganisms were present (Figure 3). Algal growth on AW glass resulted in surface cracking in a jagged meandering fashion (Figure 4). In addition, the presence of microorganisms seems to be associated with increased subsurface breakup and/or etching (Figure 5). The spalling surface of the AW water control is seen in Figure 6. The thickness of the spalling layers averaged about 3 μm. The glass surface underneath the



Figures 1-3. Microorganism-Incubated Na-Rich Glass. Figure 1 is a light micrograph approximately 2.5 cm on a side. Figures 2 and 3 are scanning electron micrographs. Figure 2 (marker--10 μm) illustrates the extensive microbial coverage. Figure 3 (marker--100 μm) shows crizzling and/or cracking.

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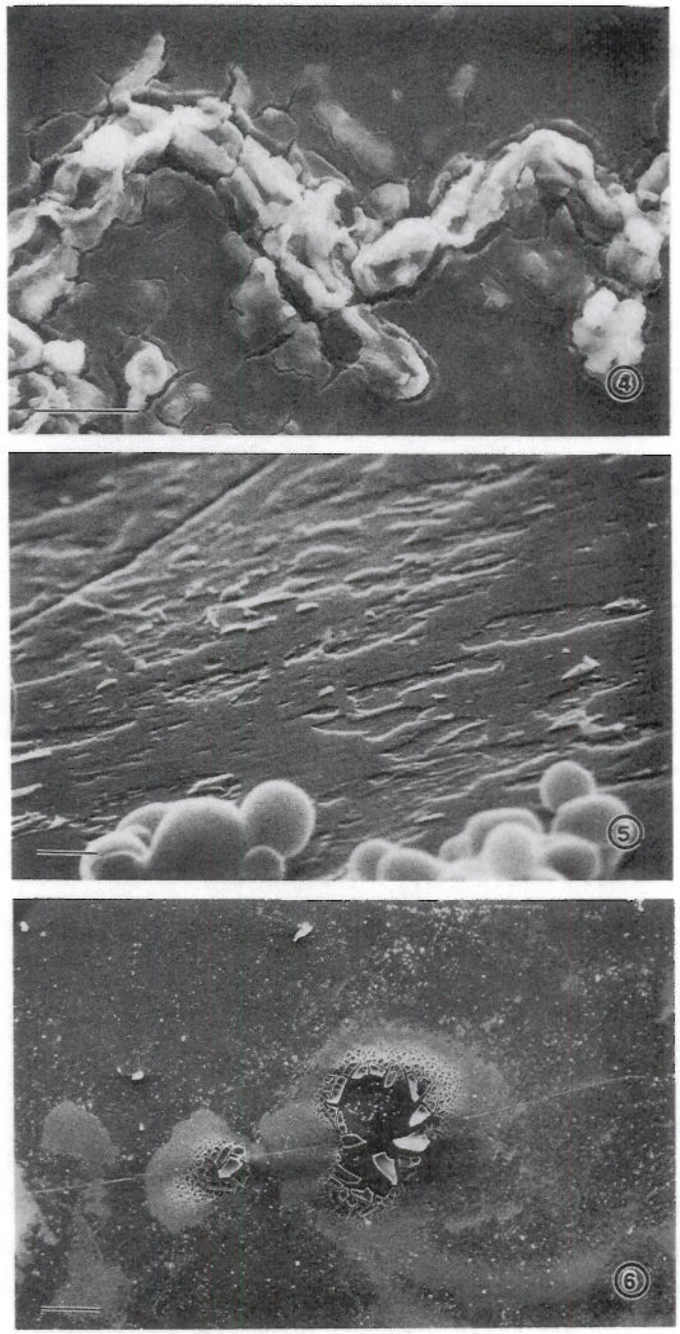

Figures 4-6. Scanning Electron Micrographs of Na-Rich Glass. Figures 4 and 5 (markers--10 μm and 1 μm, respectively) show microorganisms-etched surface under a spalling layer. Figure 6 is the water control illustrating typical water attack effects. Marker--100 μm.

uplifted spalling layers revealed initial stages of water attack (Figure 7). See Figure 5 for a comparison of microorganism attack. Energy dispersive X-ray Spectrometric analysis of AW wet controls showed significant surface depletion of sodium from hydration factors alone; this phenomenon was not observed in the dry controls.

<u>AV Glass (potassium-rich)</u>

This glass also supported large numbers of microorganisms and showed surface irregularities in both the control and experimental groups. In microorganism-exposed surfaces extensive films similar to those on the AW glass were noted (Figure 8). Fungal growth on the potassium-rich glass surface was associated with smooth round holes and deep pits (Figures 9 and 10). This was not seen in either the AV control, or the AW glass. The AV dry control (Figures 11 an 12) showed subsurface breakup and crizzling. The AV wet controls showed only minor surface effects (Figures 13 and 14).

Energy dispersive X-ray spectrometric analysis inside pits and holes revealed the presence of high levels of phosphorus. In this experiment the presence of phosphorous is associated with the microorganisms. It is believed that the pits and holes were either newly created by the fungi or pre-existing holes that had been enlarged and smoothed by them. Smaller, jagged holes were evident in both the wet and dry controls (Figure 14). Energy Dispersive X-ray spectrometry analysis also revealed that potassium leached out of the water controls and experimentals.

DISCUSSION

<u>Moisture Attack</u>

The non-specialist presumes that glass does not corrode. Such is not the case. For example, when glass reacts with an aqueous solution both chemical and structural changes occur at the surface. Clark et al. (1979) noted that as the corrosion cause the pH of the surrounding solution to change. Several investigators (Wang and Tooly, 1958; Bacon and Calcomuggio, 1967), suggests that these glass-water reactions occur in two stages.

In the first stage (in glasses containing sodium such as the AW glass and hydrogen ions from the aqueous solution. The remaining constituents of the glass are not altered. Coupled with the stage one reaction, an increase in surface area occurs (Walker, 1977). During the second stage of the attack, the remaining structure of the leached surface layers of the affected glass breaks down and begins to dissolve.

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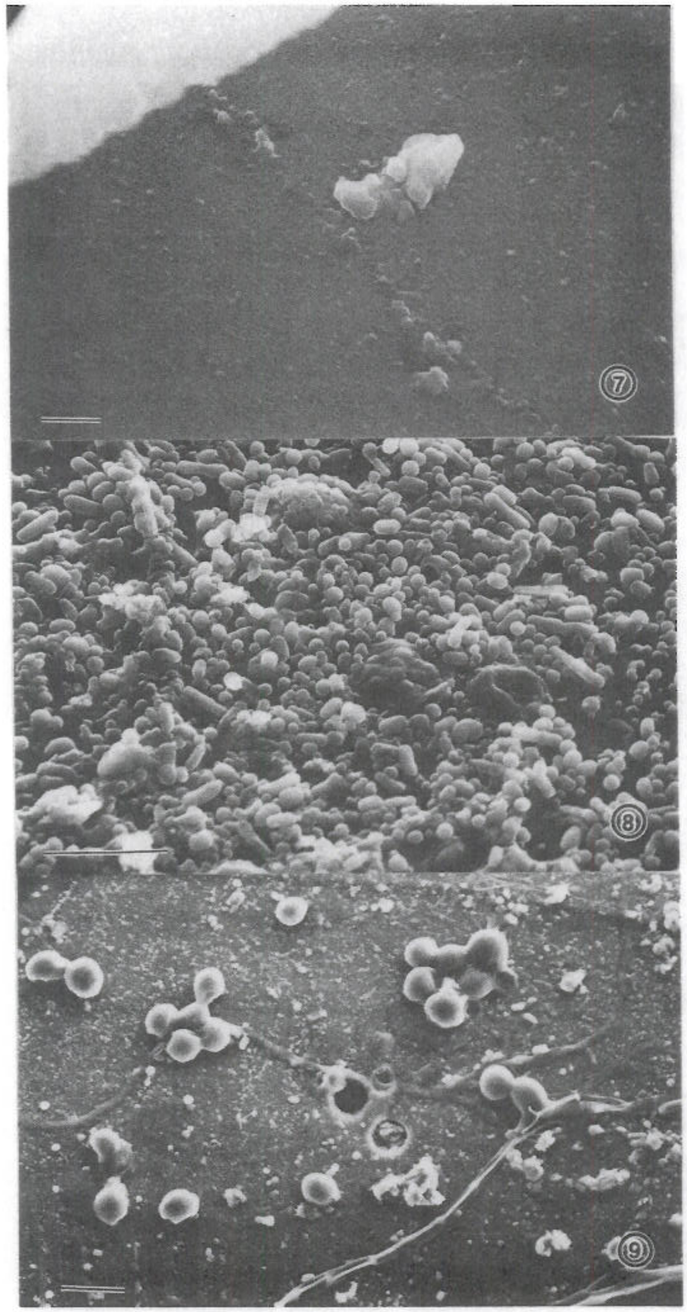
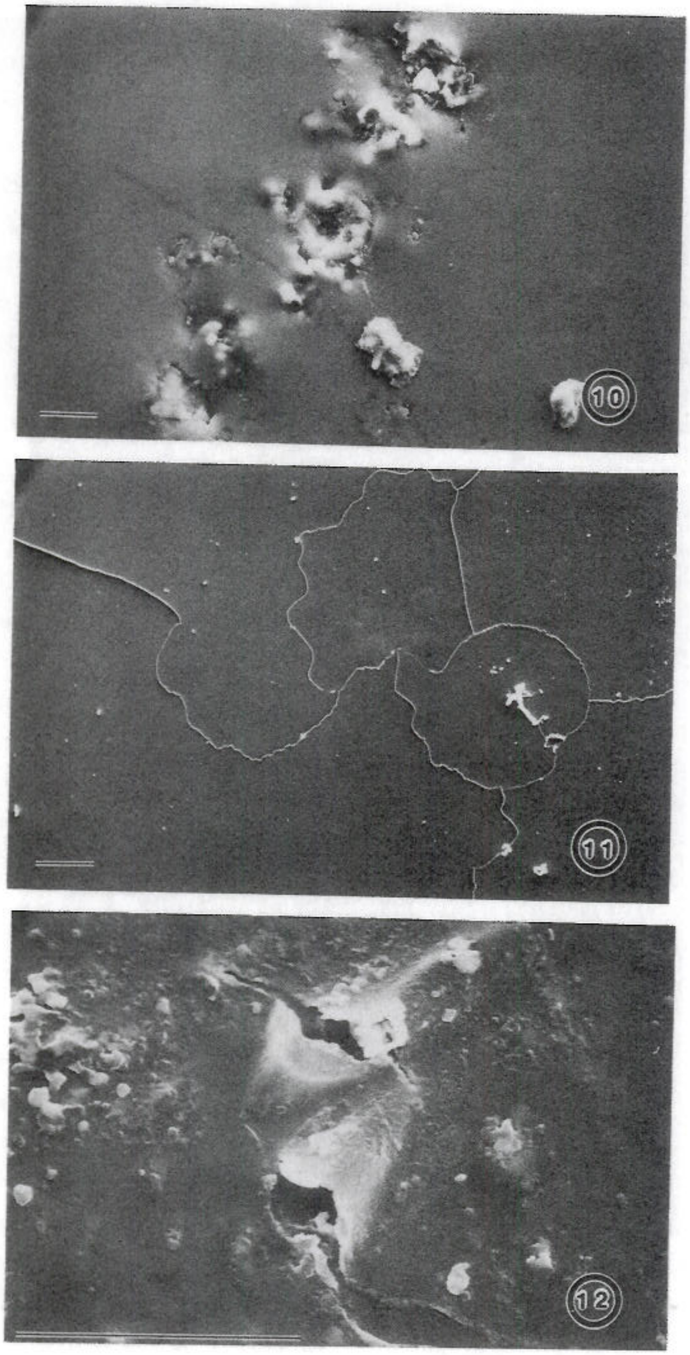


Figures 7-9. Scanning Electron Micrographs. Figure 7 is of Na-Rich Glass--surface underneath a spalling layer showing initial water attack. Marker--1 μm. Figures 8 and 9 are of K-rich glass showing (Figure 8, marker--10 μm) extensive microorganism growth and (Figure 9, marker--μm) circular pits, apparently created or enlarged by the fungus.



Figures 10-12. Scanning Electron Micrographs of K-Rich Glass. Figure 10 (marker--10 μm), glass after surface cleaning, shows numerous pits. The K-rich dry control in Figures 11 and 12 shows crizzling and subsurface breakup, respectively (markers--100 and 10 μm).

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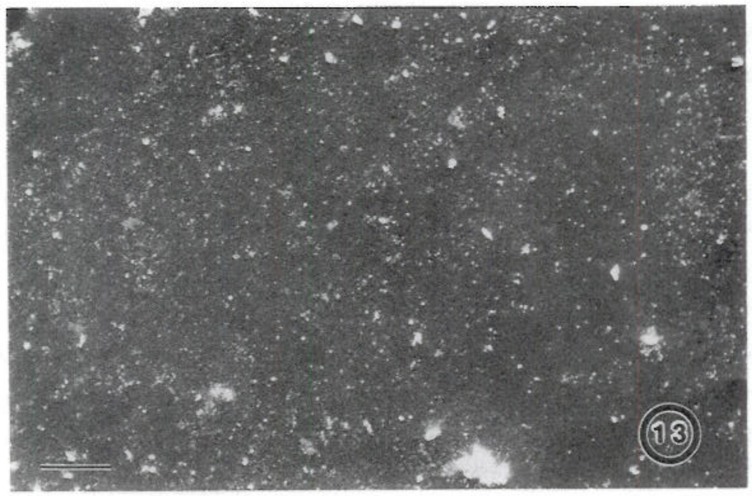

Figure 13-14. Scanning Electron Micrographs of K-Rich Glass Wet Control Show, Respectively, Minor Effects and Very Small Irregularly-Shaped Holes (markers--100 and 10 μm).

The glasses tested in the present study are thought to be representative of the stage-two-type attack as EDS and SEM analysis of samples revealed destruction and dissolution of leached surface layers in both glasses (cf. Figure 6).

It can be noted, from the present study, that the AW glass deteriorated rather vigorously in an aqueous solution as opposed to dry conditions, with a spalled surface layer approximately three µm thick appearing on the wet control sample. In contrast, the high potassium AV glass wet control shows only minimal surface marring with minor surface eruptions and no apparent weakening or surface spalling. The AV dry control shows the breakup and apparent crizzling of the glass surface.

Exposure to Microbiological Attack

Exposure of the two glass-types to microorganisms resulted in their growth over most of the glass surface with no distinguishable difference in mass organism present on either glass type. The effects of the presence of the microorganisms differed in the two glasses. The high-potassium (AV) glass has been noted in the present study to have deep circular pit formations. This type of pit formation has been described by Perez y Jorba et al. (1980) in high-potassium glass from Evron and Bourges medieval windows. The study reported herein provides some experimental evidence to support statements by Perez y Jorba that fungi are responsible for these types of pits. There was no noticeable effect of moisture in the microorganisms treated samples, other than a depletion of the alkali as seen by EDS analysis.

One observable effect of the microorganisms present on the surface of high-sodium (AW) glass was to etch the surface and subsurface layers (cf. Figure 5). This was considerably different from water attack alone. In water attack alone, the spalled surface was approximately three µm thick, and fractured in a mud-crack pattern (Figure 6). The surface beneath this layer appeared to be relatively unmarred and resemble the original sample surface (Figure 7). By contrast, the spalled layer of the microorganism incubated samples were considerably thinner (approx. 0.5 µm thick) and lifted up in blister-like fashion (Figure 3). The surface underneath this layer appeared to be biologically etched (cf. Figure 5). The thinner spalling layers with biological growth may actually represent a reduction of leaching of alkali as compared to the water controls, perhaps by reducing the ion-proton exchange. (See Newton, 1985, for an extensive review of leaching problems). An additional note is that thin "tree-ring-like" layers, corresponding to the sizes and thickness found herein for AV glass, have been reported by Perez y Jorba et al. (1980) in glass from Evron. Weathering layers in ancient glasses have been discussed by Brill and Hood (1961).

In addition to the etching phenomenon of microorganisms noted above, there is the potential for mechanical disruption of the surface layers by the microbial film created by the organisms. The biofilm adheres strongly as evidenced by attempts to scrape it off with orange wood sticks, which were completely unsuccessful. It was possible, however, to remove the biofilm by ultrasonication for 30 seconds in H_2O_2, but an unknown alteration in the surface may have occurred. When the biofilm dries it could pull the hydration-weakened or microbe acid-attack weakened layers away from the subsurface (cf. Figure 3). This effect was more pronounced than in the high potash glass.

SUMMARY

Mixed cultures of microorganisms--a fungus, a cyanobacterium, and an alga--were incubated on Corning high-alkali low-lime glass (similar in composition to selected types of unstable historical glass) for six months. Wet and dry controls were utilized for testing in conjunction with mixed cultures. Evidence for microbiologically caused deterioration of the glasses was gathered with scanning electron microscopy. Visual and SEM observations revealed that both glass types supported large populations of microorganisms. The surface of Na-rich glass was affected by moisture alone, as noted in the wet control, while the K-rich glass showed spalling and fissures in the "dry" control not seen in the wet control. Potassium-rich glass was found to have deep circular pits associated with fungal hyphae. Na-rich glass did not have pitting, instead spalling of layers was evident, with etching of the glass subsurface by the microorganisms. Other surface irregularities were noted for both glass types in both controls and experimentals.

This set of experiments points out three distinct possibilities associated with the growth of microorganism on the surface of glass: (1) Direct attack of the surface and/or subsurface by the microorganisms which can be differentiated from other observable effects; (2) mechanical disruption by microbial adhesion phenomena; and (3) constant wetting of the surface by water held in place by the microbial biofilm.

REFERENCES

Bacon, F.R., and G.L. Calcomuggio. (1967). Effect of heat treatment in moist and dry atmospheres on chemical durability of soda-lime glass bottles. Am. Ceram. Soc. Bull. 46(9), 850-855.

Brill, R.H. and H.P. Hood. (1961). A new method for dating ancient glass. Nature 189(4758), 12-14.

Clark, D.E., C.G. Pantano, and L.L. Hench. (1979). Corrosion of Glass. Magazines for Industry Inc., pp.22-37.

Collongues, R., M. Perez y Jorba, G. Tilloca, and J.P. Dallas. (1976). Nouveaux aspects du phenomenon de corrosion des vitraux anciens des eglises francaises. Verres Refract. 30(1), 43-55.

Kerner-Gang, W. (1968). Zur Frage der Enstehung von Schimmelpilzspuren aufoptischen Glasern. Material und Organismen, 3, 1-17.

Koestler, R.J., A.E. Charola, M. Wypyski, and J.J. Lee. (1985). Microbiologically induced deterioration of dolomitic and calcitic stone as viewed by scanning electron microscopy. In: Proceed. 5th Int. Cong. Deter. Conserv. Stone. pp. 617-626. Lausanne, Switzerland.

Newton, R.G. (1985). The durability of glass. Glass Tech. 16(1), 21-38.

Perez y Jorba, M. (1981). Deterioration of stained glass by atmospheric corrosion and by microorganisms. pp. 96-98. In: Conservation and Restoration of Stained Glass. Int. Cong.

Perez y Jorba, M., J.P. Dallas, R. Collongues, C. Bahezre, and J.C. Martin. (1978). Etude de l'alteration des vitraux anciens par microscopie electronique a balayage et microsonde. Silicates Industriels 1978-4-5, 88-99.

Perez y Jorba, M., J.P. Dallas, R. Collongues, C. Bahezre, and J.C. Martin. (1980). Deterioration of stained glass by atmospheric corrosion and micro-organisms. J. Material Sci., 15, 1640-1647.

Prod'Homme, M. (1965). Actions des microorganisms sur les surfaces vitreuses. Proc. VII Int. Cong. Glass, Brussels, Belgium. Gordon & Breach, New York, Paper no. 17.

Walker, M.M. (1977). An investigation into the bonding mechanism of bioglass, Ph.D. Dissertation, Univ. of FL.

Wang, F.F., and F.V. Tooley. (1958). Detection of reaction products between water and soda-lime-silica glass. J. Amer. Ceramic Soc., 41(11), 467-469.

Acknowledgements

The authors thank
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Acknowledgements

The authors thank Christopher Blair for microscopical and photographical assistance, Mark Wypyski for initial culturing of the microorganism, John Lee for use of his culturing facilities, Victoria Riba Koestler for editing, and Marie Koestler for word processing. Cindy Goss assisted with some clerical aspects of the paper.