

# **Biology Curators Group Newsletter**

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### BIODETERIORATION

## A. DEFINITIONS AND EXAMPLES

Biodegradation can be defined as "the harnessing of the decay capabilities of living organisms (mainly micro-organisms, but also includes fungi and algae), to transform a waste material into a more acceptable form or to use the waste as a growth medium to produce a useful end product". It is a term also used to describe chemical formulae that appear to carry out some of the above mentioned capabilities.

Some examples of biodegradation are:

- a) fungal protein for animal feed from food industry wastes.
- b) low volume sanitary landfill by composting town waste.
- c) protein from natural gas. Methanol is produced from natural gas. Bacteria (Methylophilus methyotropus) feed on this and produce a substance containing nearly 80% crude protein.

Biodeterioration is defined as "any undesirable change brought about by the activities of living organisms on material of economic or other, importance"

It is a process of considerable concern to the museum biologist, leading to the breakdown of museum specimens and other material used in the field of museology.

The breakdown of substances by biological action can take many forms. For example, tannic acid in oak wood used in cupboards may produce sufficient acid to cause decalcification of mollusc shells in a dry collection. Sulphur bacteria have long been suspected of having something to do with the formation of oil, this breakdown occuring when the bacteria play a part in releasing the oil from oil shale deposits to build underground oil deposits. This is happening gradually in the oil shale area of the Lowlands of Scotland at the present time and can be considered a useful biodeterioration process. Concrete thiobacillus will corrode and break down concrete, especially in new buildings when water is being passed into the atmosphere from the drying concrete. This has caused pyritisation problems in the new Palaeontology Wing of the B. M. (N.H.). Another form of this bacteria causes oxidation of iron sulphide in mines to form acid waters which will severely damage pumping machinery, and it is also responsible for the breakdown of fire hoses due to the presence of sulphur in the hose lining (used in vulcanisation). None of these bacteria can function without water so dry climate is the remedy.

Bacteria can infect fluid preserved specimens, especially if part of the preserving formula contains glycerin (Bacillus subtilis), and it can destroy the specimen. Moulds grow readily on the surface of the preserving fluid formaldehyde in tanks or large containers not properly sealed. Colonies of fruit flies and house flies can then breed in the area of mould. Constant vigilance is necessary to keep biodeterioration at bay.

### B. HAZARDS AND PRECAUTIONS

In preserving specimens, and preventing biodeterioration, the museum biologist will encounter

a variety of hazards. Some of these are:

- 1. <u>Preserving fluids</u> formaldelyde, alcohol, mercuric chloride, acetic acid, nitric acid, osmium tetroxide.
- 2. Live or freshly dead specimens. Quite small animals can inflict damage, which must never remain untreated. Poisons from mosquito, bee and wasp stings may cause serious effects to some people. Exudates from certain animals, and hair, fur and feathers can cause allergic reactions and even amphylatic shock. Parasites, both ectoparasites (fleas, lice and ticks) and endoparasites (nematodes etc) will certainly be present on and in freshly killed specimens. All material from the sea is potentially dangerous because of the marine bacteria present. Stale seawater is highly 'septic', and echinoderms and crustacea are particularly dangerous. Freshwater snails and bivalves, and terrestrial molluscs are also dangerous and should be handled with care. Insect larvae are also a source of allergic reaction due to the hairs, many of which when broken release poison from associated glands. The hairs also float in the air and can cause inflammation of the eyes and skin. Infection by bacteria and viruses is also a considerable hazard. Many animal and plant tissues are reservoirs of viruses and/ or bacteria, and although human skin is normally an efficient barrier, lesions on its surface will allow entry to the body, as will certain other areas including the conjunctiva of the eye, the respiratory and urinary tracts and the gut. Wear rubber gloves when handling any preserved or freshly dead material on all occasions.
- 3. <u>Preserved material</u>. Take care in handling poisonous snakes even after years in alcohol as time could merely concentrate the toxin and a scratch could give a less but still dangerous effect of a snake bite. Handling large numbers of frogs and other amphibians preserved in alcohol can sometimes give a allergic reaction in certain people wear gloves and take sensible precautions.

Material preserved in formaldehyde solutions are usually safe to handle but improperly preserved material (failure to inject a body cavity for example) could remain a dangerous source of sepsis and possible allergy – again take reasonable safety precautions. (The presence of a germicide does not necessarily mean safety from infection).

Handling numbers of skins that may have been treated with arsenical soap will give violent allergic reactions - rash and swollen eyes a little while after handling skins may be the symptoms.

Precautions against biological hazards are based on the following principles:

1. Protection: use the appropriate protective clothing, appliances and prophylatic measures.

2. Restriction: limit the number of people in contact with the biological materials.

3. Education: ensure that all personnel are aware of any hazards etc and any procedures to be adopted.

4. Substitution: do not use dangerous substances when non dangerous material may be available.

### C. THE CAUSES OF DETERIORATION

- 1. Humidity:
- a) excessive wetness rotting, damp heat, mildewing, bacteria and fungi (moulds), pyritisation.
- b) excessive dryness desiccation and splitting of skin and other preparations, damage to herbaria, insect and other dry collections, evaporation in spirit collections.
- 2. Contaminated air:

sulphur dioxide (bleaching), hydrogen sulphide (tarnishing), soot and dust (staining), grease from oil vapour from central heating, apparatus etc.

3. Neglect:

pest infestation can only occur in neglected collections. Pests include moth, silver fish, book lice, flies, various beetles (particularly the dermestes), rats and mice, squirrels (problem in rural museums and those set in parkland or garden areas).

It is important to point out that neglect, although seemingly an act of incompetence and lack of interest could just as easily be a series of unfortunate happenings. A slight change of undetected humidity or dryness could cause untold damage.

## D. THE PREVENTION OF DAMAGE TO DRY BIOLOGICAL MATERIAL

Many reagents act in several different ways:

for example:

Thymol is a useful bactericide, fungicide and fumigant, and much underrated.

Camphor is a good bactericide, fungicide and fumigant. It is also underrated mainly because the synthetic reagent does not have the effect of the natural chemical. This is true of the majority of chemicals mentioned.

p. Dichlobenzene is also a good bactericide, fungicide and insecticide. The colour loss noted is perhaps due to an impure chemical.

In the following notes many chemicals will be mentioned and repeated for different uses.

## 1. Bacteria and fungi infestations

These usually occur in conditions of high temperature and humidity, and although individual specimens can be dried out (ovens or desiccants such as silica gel, salts of cobalt or phosphorus pentoxide) climate control is the only real answer.

The lower permissible limit of relative humidity is set by hygroscopic materials which are the most sensitive to over drying conditions because they contain moisture. Skin and leathers may suffer from a low RH and a safety limit should be set at 50% RH.

A high level of relative humidity results in the development and growth of moulds and fungion any material that might provide nutriment. 'Moulds' and 'fungi' are words used indiscriminately to describe growths of minute fungi of which there are many species. Tiny threads called hyphae form a mycelium "mat" which throws up fruiting bodies called spores. Fungithrive on conditions of damp, warmth and darkness but growth may be prevented by keeping the RH below 68%, although in actual practice it might be safer to go to 65% RH.

The limits of atmospheric relative humidity are thus defined as lying between 50 and 65% RH, and temperatures of 60 to  $75^{\circ}$ F (16 to  $25^{\circ}$ C).

#### Mould and bacteria treatments:

Thymol. 1 oz. of crystals in a tin suspended over a 40w electric bulb is sufficient to sterilise 16 cubic feet of cupboard space – keep doors shut for 24 hours after light has been switched off. B.M. use a thymol chamber to treat oil paintings, but it can be used to fumigate skin collections. Thymol does not remove the mould or bacteria colony, but simply kills it, and adequate cleaning is necessary after treatment. Thymol papers can be prepared from 10% thymol in alcohol. White blotting or filter paper are soaked in this solution and allowed to evaporate (usually overnight). Alternatively crystals of thymol can be ironed between sheets of filter paper. Used between mildewed sheets of paper (herbaria) and in books etc.

Chlorocresol. An efficient bactericide and fungicide. Crystals kept in container in case or can be used as with thymol. It is also used in the field for collecting, a tin with crystals covered with tissue paper. Specimens are treated as a Riker mount, and have been kept for up to 6 months without any change - a useful new method.

Camphor. A much underrated reagent although the rate of evaporation is a disadvantage.

Naphthalene flake. One of the oldest and safest fumigants used scattered in containers, cases, etc. Can be used as thymol is used.

P. Dichlobenzene. Often causes changes in colours of specimens but still widely used nevertheless.

Pentachlorophenol - Mystox - used usually 5% in a selected solvent.

## Formaldehyde and Potassium permanganate

and

Formaldehyde and Chloride of Lime (Calcium hypochlorite) "Bleaching powder". Both used in the same way. A small pile of chemical is placed in a tin and the formaldehyde (40%) added. Take care as the rapid oxidation causing the release of formic acid gas very occasionally flares (hence the use of tin). After 24 hours or so thoroughly ventilate the area and to finally remove traces of the gaseous formaldehyde sprinkle the floor with ammonium hydroxide which converts the residual formaldehyde to hexamethylenetetramine which is odourless and harmless. No lasting protection but very effective.

### Specific problems:

a) Bone collections - look for mould or bacteria staining. Keep bones in plastic bags or cover skeletons etc not in display cabinets. Fat often forms a corrosive area which can break down.

- b) Skin collections should be kept at 60 to 65°F (16 to 18°C) and very slightly humid. Dressed skins should be refrigerated at 4°C at low humidity.
- c) Egg collections: Change cotton wool every 2 years (non absorbent is best). Never use coloured wool. Check for fungi and mould infestation with ultra violet light a colour in degrees of orange usually denotes contamination.

### 2. Insect infestations

These can only occur due to neglect. The most widely used insecticides are:

Paradichlorbenzene – used as a dry crystal, spread in cupboards and through skin collections. It induces foxing in skins and it seems reasonable to confine this reagent to plant material although that too has colour problems. Still widely used. Some toxic effects (do not spend too much time in heavy atmosphere of this reagent!)

Flake naphthalene - one of the oldest of reagents used - more effective if powdered - needs replacement at frequent intervals as it volatilises rapidly.

<u>Camphor</u> - still widely used in entomological collections. Its insecticidal action is probably suspect but it probably acts as a deterrent.

<u>DDT</u> - the most effective contact insecticide which is not now used for obvious reasons. Even if the reagent is taken out of the collections the DDT will remain.

Vapona strip (dichlovos) - used at Tring, B.M. (N.H.), for bird skin collections. There is some evidence of cumulative poisoning and precautions should be taken to avoid inhalation of fumes.

Carbon disulphide - a dangerous toxin still in use, particularly in the U.S.A. 24 hour treatment usually sufficient.

Xylene as a killer of all insects in spray form.

Ethylene dichloride/Carbon tetrachloride. A mixutre of 3 parts Ethylene dichloride to 1 part Carbon tetrachloride is used in the National Museum of Canada – a spray toxic to most insects.

Mercuric chloride Possibly the most efficient agent against pest infestation of any sort is mercuric chloride used in alcoholic or ageous solution. It is diluted until when brushed onto black paper it does not leave a white deposit - there is so much variation between batches of mercuric salt that this crude method is still the best. Probably around 0.5%.

Formaldehyde Fumigation (as previously described)

Mystox LPLX A chemical based on pentachlorophenol, used 5% in white spirit. Try to make sure that no liquid condenses onto specimens, as it can cause colour change.

Mystox LSE Use as 5% in distilled water it is an inhibitor used in a similar manner to Eulan.

## 3. Preserving and protecting skins

Alum dressing This is the oldest of skin preparation methods, "tawing" the skin with an excess of alum after preliminary cleaning. Action is reversable, water must therefore not be allowed to come into contact with tawed skins. Tawing is still used for the preparation of wool skins, tawed leather is used for gloves, and to a limited extent in bookbinding.

Oil dressing used in chamois leather making. An oxidised oil is used, often cod oil or Lankrolene. Skin may be fixed in formaldehyde after cleaning which is treated with dilutions of the oil well rubbed in. The skill is in removal of excess oil with a detergent, which gives an excellent result.

Tanning Vegetable tanning - infusion of oak bark. In ancient Egypt acacia pods were used rather than oak bark. Mineral tanned skin - although alum tawing is a form of mineral tanning the term is usually reserved for the use of salts of chrome.

Deterioration of skins is due to:

moisture: at worst converted into a black syrup-fungus and moulds-mystox compounds can be used to combat this.

insect attack: pyrethrum and lethane in an odourless distillate (obtainable from Shell-Mex).

An additional problem is dealing with taxidermy specimens of mammals and birds. To keep the hair and skin in reasonable condition after many years exposure in galleries the following formula was used at Baroda Museum.

Lanolin	100 gms
Naphthalene flake	50 gms
Camphor oil	25 ccs
Pyrethrum 2%	10 ccs
(extract)	
Phenol	10 ccs
Hexane	1000 ccs

Dissolve lanoline in the hexane (say 600 ccs) and the rest of the hexane is used to dissolve the naphthalene flake. Two solutions mixed and the pyrethrum added. Stir well and add camphor oil. Continue stirring and then finally add the phenol.

Brush on to specimens. It can be sprayed but care needs to be taken in re-arranging fur or plumage. Dry mounted specimens keep supple and soft and do not crack. Useful for mounted heads etc.

# Lankrolene treatment for skins

Lankrolene is an oil readily emusifiable in water. Skins prepared for tanning are placed in 5% formaldehyde for at least two weeks. They are then thoroughly washed to remove this reagent and then the flesh surface is rubbed with a solution of lankrolene in water. The skin needs to be well impregnated and the process may take several hours. Excess oil is then removed and the skill is in removing by using a detergent sufficient of the oil to leave the skin soft and dry. Lankrolene is available from Lankro Chemicals, Eccles,

#### Lancashire.

### Eulan treatment for skins

Eulan, Edolan in the U.S.A., is an aromatic sulphonamide derivative, the formula of which is not divulged as it is used in the cloth and carpet industry. Used as a dilute 1% aqueous solution. Skins may be dipped or material may be sprayed and allowed to dry. Eulan is persistant and will remain in spite of any other treatment.

Fresh skins need not be tanned. After skin has been removed wash all blood and grease from the skin and soak in a solution of Eulan in water at 100F. The quantity of water should be adequate to well cover the skin and the quantity of Eulan calculated at 1.5 to 2% of the skin weight. Submerge the skin for 10 to 15 minutes – measure out an equal quantity of acetic acid equal to 1% skin weight and soak the skin for the same time in the acid solution at 100F (38C). This is to lower the pH and to allow the skin to fix the Eulan. (Not necessary in dried or otherwise treated skins). Rinse in cool water, degrease etc. and dry skin using usual techniques or dress as required. Eulan is not affected by any reagent after fixation and does not wash out or deteriorate. Reason it is not necessary to fix prepared skins is that most tanning and preserving agents will have a sufficiently low pH to fix the Eulan.

Important Note - this reagent does NOT kill any invading pest, but merely discourages them. It can be used on dry insect collections, trophy heads and on material on open display without hinderance. It would appear to have no affect on colour.

Safety precautions are necessary when using Eulan, which is harmful if swallowed. It may cause eye and skin irritation in strong solutions, so do not get on skin or clothing. Use face mask and gloves when using the concentrate.

## 4. Conservation of bone

Bones are easily warped by exposure to heat and damp, and they are decomposed by the prolonged action of water due to hydrolysis of the ossein, the inorganic framework is easily disintegrated by acid contact.

If bones are in good condition they are easy to conserve. If fragmentary waterlogged or in a fossil state they have to be cleaned, strengthened and stabilised. Restoration fully may be impossible.

General: wash in soap and water using a detergent - surface only using brush (not cotton or a sponge).

Bones may be strengthened by the use of polyvinyl acetate although it has been largely superseded by the use of Bedacryl (a polymethacrylate emulsion) diluted from a concentrate to use as required.

Horns and antlers require similar treatment as for bone. An acrylic polymer in toluene can be used to protect the often loose, scaly surface.

### 5. Treatment of Stains

(a) Oil, fat and grease stains. Pyridine in its purest form is a valuable solvent for old, partially oxidised oil, and for asphaltic stains, much more effective than

benzene.

- (b) Wax and candle grease stains. Some grease and wax can be removed with a fine scalpel or razor blade. Soak area in petrol and brush away the stain. Xylene almost as good.
- (c) Fly stains. Hydrogen peroxide at 20 vols. Stipple any spots with a mixture of equal parts Hydrogen peroxide (20 vols) and 80% alcohol.
- (d) Tea and Coffee stains. Damp the areas, stipple 2% aqueous potassium perborate and expose to sunlight for an hour or so (also use electronic flash).
- (e) Ink stains. A number of methods are available, necessary due to the differing formulae.

  5% oxalic or citric acid removes most iron ink stains.

As a last resort paint over stain with 0.5% potassium permanganate, after 5

minutes cover with 2% oxalic acid. Wash well.

(f) Oil paint. Use paint stipper "Nitromors Green Label" - (ventiliation essential) any residue remove with pyridene.

(g) Stains caused by algae - lichens etc. Use a little dilute (2%) Ammonia or treat with 5% formaldehyde.

### E. DAMAGE TO FLUID PRESERVED SPECIMENS

Loss of preserving fluid can result in the drying up of specimens. Recovery of alcohol preserved specimens is possible by immersion in dilute solutions (aqueous) of sodium orthophosphate (1 to 3%). Evaporated containers should never be topped up directly. Test the remaining alcohol if possible, sometimes the remaining solution becomes stronger on evaporation while at other times it avaporates almost to water. Topping up without testing could result in complete disassociation of the cells making up the tissue of the specimen and a sludge in the bottom will be all that remains. This sludge will curiously enough almost always contain perfect separate cells.

Formaldehyde preserved specimens require similar treatment, although there is less chance of complete breakdown unless completely dried out. Dried out formaldehyde specimens are very prone to mould which is very difficult to remove. Modern preservatives contain humectants (which means that even if evaporation takes place no specimen will ever completely dry up). Propylene glycol is a humectant and fungicide and is used in post fixation preservation.

Reconstitution methods – use 1 to 5% aqueous (sodium orthophosphate ) for most specimens, exceptions being archaeological material including human remains. May go up to a maximum of 15% aqueous for reconstitution, and heat slightly to increase the action. Very good method for reconstituting herbaria for close microscopic examination.

Procedure for the transference of material from alcohol to post fixation reagents.

Wash samples in de-ionised water to remove excess alcohol and then transfer for several days to the following:

A. 40% formaldehyde 10 ml
Propylene glycol 5 ml
Phenoxetol 1 ml
Water 84 ml

(this reagent may be used for several transfers)

Wash again in de-ionised water and then transfer finally to the following:

B. Propylene glycol 10 ml
Phenoxetol 1 ml
Water 89 ml

The possibility of transferring several specimens from alcohol to solution A at the same time may be considered providing that they are properly labelled and easily identifiable.

Propylene glycol is a humectant and no sample immersed in solutions containing this reagent will ever completely dry up even when all solution is evaporated off. Propylene glycol as an additive to preserving fluids has a softening effect, relaxing stiff tissues - 2 to 5% added to alcohol reduces the rigidity of arthropodlimbs. It is a powerful inhibitor of moulds and appears to assist the penetration of formaldehyde. It lowers the freezing point of preserving fluids and has solvent properties so that phenoxetols may easily be dissolved. It also breaks down to pyruvic acid and acetic acid and is therefore relatively harmless to man - preferred to the use of glycerine which can encourage moulds and bacteria.

### Treatment of discoloured alcohol

Mix in activated charcoal and allow to stand for 24 hours. Filter through thick Whatman filter paper (glycerin grade).

## Raising the percentage alcohol

Dehydrate copper sulphate by heating in metal container or hot air oven until colourless. Place cooled reagent in bottom of a bottle (Winchester Quart) to a depth of 3". Pour in the alcohol and allow to stand for at least a week. If during this time the reagent turns blue pour off the alcohol into another bottle, remove the copper sulphate and re-dry in an oven etc. Pour treated alcohol into another bottle with the 3" dehydrated copper sulphate. Check the alcohol percentage with an alcoholometer.

### F. PYRITE DISEASE

The problems of pyrite disease are well known, and most treatments are only partially successful. Much depends on obtaining a suitable museum climate to house the material below 60°F and above 80% RH are optimum for pyrite growth. Most treatments are a neutralisation of the breakdown by exposure to ammonia followed by impregnation or coating. New ideas may be to freeze dry in a suitable climate produced by a dehumidifier using lithium chloride. The major problem is how to deal with the specimen when at constant weight. Some form of coating while still under vacuum might work satisfactorily.

A good deal of research has been carried out on this and other problems of biodeterioration at the Biodeterioration Unit at the University of Aston, Birmingham. A publication (SB16), 'Biodeterioration of Books and Museum Specimens' should be available in the near future. A catalogue of potentially biodeteriogenic fungi is available at a cost of £2.50.

### G. CONCLUSION

It would appear that the work against biodeterioration is not taken seriously enough. Bearing

in mind that the curators of today are in charge of material that will be required for study well into the next century and beyond. Too often I have been told "We are too busy to take time to look seriously at the collections". When that occurs a serious mixup of priorities has occurred. The care and upkeep of a biological collection is the most important part of a museum curators job. However, this task could be made far easier with the installation of proper climate controls in all museums – this could effectively mean the end of biodeterioration.

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## Some recent references to Biodeterioration and Biodegredation.

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- 6. Deterioration by Insects, Rodents, Birds and Animals. Sessions 2 and 8 of the 3rd International Biodegredation Symposium 1976. £4.00 ISBN 085334 704 2
- 7. Fungicide Toxicity and Metabolism. Session 20 of the 3rd International Biodegredation Symposium 1976. £3.00 ISBN 085334-713-1
- 8. General biodeterioration and deterioration of organic wastes. Sessions 11, 12, and 16 of the 3rd International Biodegredation Symposium 1976. £4.00 ISBN 085334 7077
- 9. Materials deterioration and mechanisms of deterioration. Sessions 3, 9, 10, and 24 of the 3rd International Biodegrations Symposium 1976. £12.00 ISBN 085334 7050
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