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ANOXIC MICROENVIRONMENTS: A SIMPLE GUIDE

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Until relatively recently, the idea of putting a museum artifact in a low oxygen or anoxic atmosphere was not taken very seriously, except in very special circumstances. Because of the difficulty of creating and maintaining such an environment, the effects of anoxia also received comparatively little study within the conservation community. With the availability of inexpensive barrier plastics and oxygen absorbers, however, this situation has changed dramatically.

Today, creating an anoxic microenvironment is remarkably simple. Because of its lack of toxicity to people and collections, anoxia has become the method of choice for treating infested objects in museums. Inexpensive storage containers that radically reduce some kinds of deterioration, such as the oxidation of metals and even the fading of many colorants, are now feasible. Oxygen levels for these purposes must be so low that a microenvironment needs to be designed and constructed carefully if it is to be at all useful. The purpose of this leaflet is to explain how this can be done.

ANOXIC MICROENVIRONMENTS

The first thing to know is that the earth's atmosphere is always about 20% oxygen, the other 80% being mostly nitrogen and a few other gases. The next thing to know is that almost all containers leak, to a greater or lesser degree. To control the leak rate, we need to consider three basic factors: the permeability of the walls and seals of the container, the buffering capacity of the system, and the degree to which the environment inside the container differs from its surroundings. (An environment in a container, or any other small area, is called a micro-environment.)

The greater the difference between the conditions inside a microenvironment and the environment outside, the harder the microenvironment is to maintain. This becomes very important when the conditions we want to achieve are radically different from ambient conditions. In an anoxic microenvironment of less than 0.5% oxygen (40 times lower than normal air) even the smallest leak becomes significant. The stability of the microenvironment will also be affected by the ability of the system to buffer or compensate for changes. This ability can be increased in two ways: by providing a scavenger that will consume whatever unwanted material may get in, or conversely by actively supplying a surplus of the desired environment under a slight positive pressure. A low oxygen atmosphere can be maintained either way: with an oxygen absorber, and/or a constant stream of a gas, such as nitrogen, that continually dilutes the air to the desired oxygen concentration.

The most important factor affecting a microenvironment, however, is the permeability of the materials from which it is made. Many of the materials normally used to make containers, aside from metal and glass, are more or less permeable to oxygen. Increasing the thickness of the walls will certainly increase efficiency, but choosing an efficient material in the first place makes better sense. Some plastics are vastly better oxygen barriers than others. Yet, even with the best barrier material, the greatest weakness in any construction is the strength of the seams where its parts are joined together. This is especially true of anoxic microenvironments, where the success or failure of a barrier bag will most often depend on the efficiency of its seams and closures. Even the smallest pinhole may be enough to render the construction useless.

MATERIALS

Barrier Films

Most anoxic microenvironments for pest eradication are made with flexible plastic films that are heat-sealed into a pillow-shaped bag. Not just any kind of plastic will do. What is commonly used is a highly efficient plastic called a barrier film. Since the physical properties of different plastics (such as transparency, flexibility, strength or permeability) vary tremendously, the packaging industry has developed a wide assortment of laminated films to meet specific requirements.

Barrier films are laminated composites that usually combine a low transmission rate plastic with a high bursting strength plastic, often with a low melting point heat-seal layer on one side. The least expensive of these laminates are aluminized and thus opaque, but many kinds of transparent barrier films are also available. The efficiency of these barrier films is rated in terms of their water vapor transmission rate (WVTR) or their gas transmission rate. These transmission rates are commonly expressed as the amount of material (grams of water, or cubic centimeters of a specific gas) that can pass through 100 square inches (or 1 square meter) of the film in 24 hours. For single plastics it is common to see this value expressed per mil (0.001 of an inch) of film thickness. For laminated composites the transmission rate applies to the thickness of the film as supplied, for instance 6 mils thick.

Low transmission rates for one substance, e.g. moisture vapor, do not necessarily correspond to low transmission rates for another, e.g. oxygen. Common polyethylene and a polyester like Mylar® are both highly efficient as water vapor barriers. Polyethylene is, however, more than 100 times more permeable to oxygen at normal temperatures. Either of these plastics might be used when a simple moisture barrier is needed (and for this purpose the polyethylene would be less expensive), but polyethylene by itself would be a poor choice for an anoxic enclosure. To be used for anoxia, a plastic film will need to have an oxygen transmission rate that is lower than the oxygen removal rate of the system. For a positive pressure system almost any plastic could be used, since the flow of gas into the container can be increased to compensate for the leak rate. In reality, this can become expensive in terms of materials and labor if the leak rate is too great.

If an oxygen scavenger is used inside a microenvironment, the transmission rate of the film will need to be lower than the rate at which the oxygen scavenger can absorb oxygen. Since the absorption rate decreases over time as the scavenger becomes exhausted, the required efficiency of the barrier film will depend on the intended service life of the microenvironment. For insect eradication treatments that typically take a week or two, barrier films with oxygen transmission rates somewhere under 100 cc/m^2 are suitable. For longer term storage, barrier films with transmission rates of under 1 cc/m^2 are be more appropriate. The most efficient laminates contain a thin layer of aluminum sandwiched between layers of nylon or polyester (e.g. MarvelSeal 360™ or BellFibre 2175™). The transmission rates of these aluminized films approach zero, and are excellent choices for general purpose barrier films.

Aluminized films are among the least expensive barrier films available, but their complete opacity can be a drawback. For transparent oxygen barrier films, those based on polyester (PET, trade name Mylar®), polyvinylidene chloride (PVDC. trade name Saran®), or oriented nylon are good choices for short term purposes such as insect eradication treatments, shipping or temporary storage containers. For long-term applications, transparent films based on fluoropolymers (CTFE, trade name Aclar®) or ethylene vinyl alcohols (EVOH) have lower transmission rates. Of these two, EVOH laminates are less expensive and can have oxygen transmission rates almost as low as the opaque aluminum-based films.

Most barrier films include a polyethylene layer on the inner surface that enables them to be heat sealed at temperatures around 117° C (350° F). Seals of at least 3/8" (1cm) wide are necessary. This is because polyethylene is a relatively poor oxygen barrier, and a thick ribbon of it is needed to match the transmission characteristics of the rest of the film. If the seal is very thin, the leak rate of the bag will be greatly increased by transmission through the polyethylene.

For these polyethylene-lined films, a variety of heat sealers are available, from hand held models costing a few hundred dollars, to much more expensive table top models. It is possible to use a tacking iron as long as it gets hot enough. Considering the importance of this aspect of fabrication, if packages are going to be made with any regularity, a more expensive model is well worth the price.

Oxygen Absorbers

The brand of oxygen absorber commonly used in anoxic microclimates is called Ageless™ (Mitsubishi Gas Chemical Company, Inc.), although other brands are becoming available. Ageless ™ is composed of finely divided ferrous oxide, a chloride salt and a humectant sealed inside a permeable paper or plastic packet much like that used for silica gel. Unlike silica gel, the packets cannot be reused once exhausted. As supplied, a number of packets are vacuum-sealed inside a transparent oxygen barrier pouch along with a single in-package oxygen monitor called an Ageless Eye™.

Ageless™ is available in several types and capacities. The type normally used for storage of collection materials and insect eradication is Ageless™ Z, which is effective at relative humidities below 85%. It can be used in conjunction with desiccants, although at very low relative humidities its effectiveness decreases. Ageless™ RP, which includes its own desiccant, will create very low relative humidity conditions, and is used principally for the preservation of metals. Ageless™ FX is used in conditions above 80% RH, and is inactive below 70% RH. Ageless™ types S and SS are fast acting varieties designed for use at cold and freezing temperatures respectively, and require relative humidities of 65% or more.

Ageless™ capacity is rated in the number of milliliters of oxygen that a single packet can absorb. For example, Ageless™ Z-1000 will absorb one liter (1000ml) of oxygen before it is exhausted. Since the atmosphere is about one-fifth oxygen, this means that one packet of Ageless™ Z-1000 can remove all the oxygen from five liters of air. It may also be helpful to remember that it takes about six packets of Ageless™Z-1000 to remove the oxygen from one cubic foot of air. Ageless™ is available in sizes as small as 20 ml capacity up to a maximum of 2000 ml. In most cases, the higher capacity packets are preferred, since fewer would be required, and the costs per ml are roughly the same.

As an Ageless™ packet reacts with oxygen some heat is generated. Just how warm the packet will get depends on the rate of the reaction, which is faster at higher oxygen concentrations and higher temperatures. In normal air, packets of Ageless™ have been observed to get as hot as 46° C (115° F). This is more likely if many packets of Ageless™ are clumped closely together. If the packets are spread out or the amount of oxygen in the air is reduced by dilution with another gas such as nitrogen, warming is less likely. Ageless™ can also absorb carbon dioxide, making dilution with CO_2 inadvisable. Additionally, some types of Ageless™ may produce a rise in RH in the microenvironment; care should be taken with humidity-sensitive objects or specimens.

As an oxygen scavenger absorbs oxygen, the volume of air inside a container will decrease. If the oxygen is not diluted with some other gas, this will mean a decrease in volume of 20%, which may have disastrous consequences if not anticipated.

The Ageless EyeÔ

As mentioned above, every pouch of Ageless™ packets includes a single in-package oxygen monitor called an Ageless Eye™. This is a small tablet containing a color indicator that changes from purple to pink when the oxygen concentration falls below 0.5%, and serves primarily to indicate that the Ageless™ packets are fresh when purchased. At concentrations above 0.5% oxygen the pill quickly changes back to purple. If the oxygen concentration is again made to fall below 0.5%, the pill will revert to pink, but much more slowly. These color changes to the eye are reversible, meaning that the indicator can be reused many times, but only up to a point. After a while the eye will become slower to respond, or may not change color at all, rendering it useless. Several factors besides the number of cycles will affect the useful life of the eye. Extended exposure to oxygen will tend to deteriorate the eye, as will storage at higher temperatures. The eye is also somewhat sensitive to light, and can easily fade until it becomes difficult to read. For these reasons, Ageless Eyes™ should be stored in an oxygen barrier bag and kept in the refrigerator or freezer until needed. During use, if the anoxic enclosure is in bright light, the eye should be protected from undue exposure.

Each Ageless Eye™ is enclosed in its own small cellophane envelope. It is not necessary to open this envelope; both sides of the envelope are perforated with tiny holes to allow air circulation.

Inert Gas

Although a perfectly serviceable anoxic microenvironment can be made using the materials already discussed, it is usually a good idea to flush out as much oxygen as possible at the beginning with some inert gas like nitrogen or argon. This not only saves on Ageless™ but results in faster anoxia and less collapse in volume. This requires a tank of compressed gas, a regulator to control the pressure, and some kind of hose and nozzle by which to introduce the gas into the bag. A suitable set-up is inexpensive, but should be handled with respect. Consult with a supplier for safety instructions. Strap the tank securely to a wall, and make sure to turn off the valve when the tank is not in use.

Most compressed gasses contain less than 5% relative humidity. In the average Ageless™ microenvironment this may not be a problem, since only a moderate amount of dry gas is introduced at the beginning and most hygroscopic objects will reach equilibrium with only a modest drop in relative humidity. Furthermore, since Ageless™ relies on an inner source of moisture for its reaction, an increase in relative humidity is more likely. In a positive pressure system however, a continual stream of dry gas could dangerously desiccate the objects in the microenvironment. In this situation, it is possible to pre-condition the gas coming from the tank by splitting the gas stream and bubbling half of the gas through water. (It should be noted that insect eradication treatments require normal to dry conditions.)

PROCEDURES

The process of constructing an effective anoxic microenvironment is fairly straightforward, but needs to be done carefully, following this step-by-step guide, if the system is to be useful. This involves making an air-tight bag out of a barrier film, calculating how much oxygen absorber will be needed, placing the absorber and the object or specimen inside the bag, and sealing it closed. It is also important to be able to determine how much oxygen is inside the bag as time goes by, and whether there are any leaks.

Making the Bag

First, estimate the size of the bag needed. Making the bag as small as practical is a good idea, since a larger bag requires more Ageless™ and longer seals are more likely to leak. Cut the plastic carefully so that both sides are the same size. When heat-sealing a bag, it is a lot easier to avoid leaky crimps and wrinkles if the sides match. Cutting twice the length of the bag from a roll and folding it over avoids an unnecessary seam.

Practice seals on scrap material before beginning the final product. To tell if the seal is acceptable, heat-seal two pieces of scrap barrier film together, let them cool for a few minutes, and then tear the seal apart. If the heat was not high enough, the pressure not great enough or the dwell time not long enough, the seal will peel apart with little or no damage to the plastic. If everything is right, the layers of the barrier laminate will separate or tear before the seal lets go. A few quick tests will make this clear.

If the barrier film is opaque, a small window should be made on the top of the bag to observe the Ageless Eye™. This is a difficult operation, and prone to causing leaks. One approach is to cut a one-inch hole in the barrier film, and a 2” piece of clear plastic from a discarded Ageless™ pouch. Using a small tacking iron, carefully heat seal the inside surfaces of both films together to make a window. It is a good idea to practice this with scrap pieces first. Always make the window before making the bag.

Plastic clips in the form of a rod and semi-circular tube are also made for sealing barrier bags without heat, and are useful when there is no source of electricity (such as in field work or when a bag will need to be repeatedly opened and closed). In this case, three sides of the bag should be heat-sealed first. The fourth side is then folded over the rod onto which the semi-circular tube is pressed, forming a tight seal.

Preparing the Object

Before the object or specimen is placed inside the barrier bag, determine whether it needs any special preparation. Will the object be harmed by the weight of the plastic or any collapse in the volume of the bag? Conversely, are there any sharp points on the object that can puncture the bag itself? It is not a problem if the object is wrapped in paper or even tucked inside a cardboard box, since oxygen will move quite freely through these materials.

Is the object sensitive to any humidity changes that may occur, or will the purpose of the microenvironment (insect treatment, corrosion prevention, etc.) require a certain relative humidity? If so, preconditioned silica gel at the rate of approximately one pound per cubic foot of volume can be used to stabilize the RH. Make sure that the Ageless™ in use will be active at the relative humidity created. When placing the object or specimen inside the bag, be careful not to stress the seams or puncture the plastic. Always test the closure of the bag to make sure that the final side can be easily sealed shut before proceeding.

Calculating the Amount of Oxygen Absorber

As stated above, it will take about six packets of Ageless™ Z-1000 (three packets of Ageless™ Z-2000, twelve packets of Ageless™ Z-500, etc.) to remove the oxygen from one cubic foot of air. This cubic foot estimate is a handy rule of thumb to use when making an enclosure. A more accurate calculation is as follows:

(VOLUME OF BAG IN CM (L X W X H) - WEIGHT OF OBJECT IN GMS) / 5
= ML OF OXYGEN IN BAG

In practice, it is common to use two or three times more Ageless™ than is actually needed. There are several reasons for this. Often, temperature and relative humidity conditions may be unpredictable and volume calculations inexact. More importantly, the integrity of the plastic and the effectiveness of the seal may be questionable. Time is usually a factor. It is important to know as soon as possible whether the oxygen absorber is working. If only the minimum adequate amount of Ageless™ is used, absorption may be so slow that it could be several days before a problem is discovered, at which point the only thing left to do is start over. If, however, several times more Ageless™ than calculated necessary is used, it should be obvious overnight if things are going well, and sufficient reserve capacity of Ageless™ available to compensate for any leaks that may need to be found and resealed.

This is also not as wasteful as it seems, since after the treatment is completed the partially used Ageless™ packets can be sealed up again in their own bag, flushed with nitrogen, and refrigerated for another use until they are exhausted. New microenvironments can use fresh Ageless™ as the primary absorber, and the second-hand packets as reserve.

Employing the Oxygen Absorber

Cut open the Ageless™ pouch and insert as many packets into the microenvironment as were determined to be necessary. There is no need to hurry, since the oxygen absorber reacts very slowly at first, especially if it has been refrigerated. Try to distribute the packets around the interior of the bag so that there is a maximum amount of Ageless™ packet surfaces exposed. Avoid piling them in one place. Also avoid letting the Ageless™ packets rest directly on the object or specimen.

Using two small pieces of adhesive tape, secure an Ageless Eye™ inside the bag where it will remain easily visible. Remember that the Eye should stay in its small cellophane envelope, and be careful not to cover the perforations in the envelope with tape. It is helpful to also place an Ageless Eye™ on the outside of the bag to compare with the one on the inside; the beginning shades of color change are subtle, and the comparison can be helpful. In fact, since the Ageless Eye™ is so prone to failure, using two or three inside the microenvironment may be even safer. As long as one turns pink, the microenvironment is a success.

Seal up any unused Ageless™ in its original pouch, leaving about a one inch opening unsealed. Through this unsealed hole, insert the tube from the nitrogen tank and fill up the bag several times with gas. After pushing out most of the excess one last time, seal up the final inch. Leave the Ageless™ pouch on the table long enough for the Eye to turn pink (this may take a day or two) and then store it in a refrigerator or freezer to keep it fresh.

Closing and Monitoring the Bag

As soon as the unused Ageless™ is sealed up, perform a similar operation on the anoxic bag. Seal up the final side except for a small hole through which the bag can be flushed with nitrogen. Fill the bag with nitrogen several times (sometimes a second hole on the other side helps circulation), and then quickly seal up the remainder. It helps to leave the package slightly puffed up. This will compensate for any shrinkage due to absorption of oxygen by the Ageless™, as well as protecting the object by making the bag more resistant to compression.

Depending on the size of the bag, the amount of Ageless™ and the degree of nitrogen flushing, the Ageless Eye™ may take up to several days to turn pink, indicating that the oxygen concentration inside the bag has dropped below 0.5%. If after a day or two the eye shows no signs of change, it may be that there is a leak, a depleted Ageless Eye™ or an insufficient quantity of Ageless™. Larger bags and colder temperatures will both result in slower response.

Once the eye has turned pink, mark the date of the change on the outside of the bag. Check the bag every day for the first few days. If there is a slow leak, the Ageless™ may be consumed before the completion of the treatment, at which point the eye will begin changing to purple.

Checking for Leaks

Leaks often occur because of wrinkles and small imperfections in the seal, or stressed and perforated areas in the plastic. If a number of anoxic bags are going to be made, a useful piece of equipment for locating leaks is a flammable gas detector, available at laboratory and safety supply vendors for less than $300. These detectors give an audible signal in the presence of flammable gases (such as butane or propane as well as alcohols, ketones and even Freon, which is non-flammable) in very low concentrations, often as low as 50ppm (0.005%).

Inject a small amount (10-50 cc) of a suitable tag gas into the bag just before it is sealed up (the gas can also be injected with a hypodermic needle after the bag is sealed and the hole covered with high efficiency pressure-sensitive tape). Allow the detector to warm up for a minute, then move it slowly around the bag with the detector head very close to potential sources of leaks. Pay particular attention to seams, folds, windows, or areas that may have been creased or punctured. Leaks at the seam may only require additional attention with the heat sealer, while small pinholes can be sealed by covering with a broad piece of high efficiency pressure-sensitive tape, such as 3M aluminized polyester tape #850. Be careful to let freshly heat-sealed areas cool before using the detector, since vapors from the melting plastic can give false readings.

Ultrasonic detectors are available for about the same cost. These units employ a small ultrasonic transmitter that is placed inside the package, and a detector that locates leaks and holes by detecting sound waves. Although these units are extremely sensitive and do not need a tag gas for their operation, a separate detector must be used for each simultaneous package and battery performance in the transmitter may limit very long-term applications.

Length of Anoxic Exposure

Once the Ageless Eye™ has turned pink, the length of time the anoxic microenvironment will be maintained is dependent on its purpose. For storage of collection materials, there is no harm in leaving the materials in the bag indefinitely, or at least as long as the Ageless™ is active. Double bagging can greatly increase the lifetime of a storage microenvironment.

Anoxia will prevent fungal growth, but will not sterilize or kill fungal spores. This can be useful where humid conditions need to be maintained, but mold growth is undesirable. Over long periods, some materials that depend on oxygen for their coloration may exhibit color shifts. While this is usually reversible, rare cases have been observed where color shifts after prolonged light exposure are unusual and permanent. For this reason, the use of anoxia as a means of reducing fading still needs further research.

For the purpose of insect eradication, a two week exposure is more than sufficient. Most insects and their eggs will be killed effectively in less than five days at concentrations of 0.5% oxygen, but a few resistant species like the cigarette beetle may require 10-12 days for 100% elimination. It is important to recognize that oxygen concentrations greater than 1% do not seem to be effective, even for longer exposures. This is because anoxia works by dehydration rather than suffocation. At very low oxygen concentrations the spiracles of the insect open so much that the body of the insect desiccates. Dry and warm conditions will accelerate this effect. If conditions are damp or cold, however, the insect may receive sufficient moisture or have a slow enough metabolism to make anoxia unsuccessful.

When a microenvironment is ready to be opened, cut it open near one of the seals, and remove and prepare the Ageless™ for refrigeration as described above. If the empty bag is stored carefully, with minimum folding and creasing, it may be reused several times.

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