How Degrading!

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Grade Level: Middle School

Discipline: Environmental Science/Biology

Goals:

This project provides students with an understanding of what the term "biodegradable" means and provides them with the awareness that man-made products just don't go away when they discard them. Students will also develop an understanding of the difference between synthetic (man-made) and natural materials. They should be able to reason that man tries to imitate nature in terms of what is produced but that imitation fails in terms of degradation.

Objectives:

- To observe the degradation or lack of degradation of certain materials by soil microorganisms.
- To conduct a controlled scientific experiment.
- To understand the meaning of degradation.
- To recognize the stability of man-made polymers and their lack of biodegradability.
- To appreciate the persistence of products in the environment.
- To develop an understanding of how slowly changes may occur to materials when they are disposed of.
- To recognize the responsibility we all have to reduce, reuse and recycle our "trash."
- To evaluate the need to produce products that are biodegradable.

Background:

We are members of a disposable society. We dump or landfill what we no longer need. Just what happens to discarded trash after we have no need for it? Contrary to what you believe, there is no "trash fairy." What happens then to that trash? The problem is, maybe nothing at all. That's a dilemma. Our trash may remain in a stable state for years, decades, or even millennia. Maybe even forever. That's frightening.

The real problem lies with those products that we label disposable. It is estimated that about 100,000,000 tons of packaging is discarded worldwide per year. This includes sheets, strapping, shrink wraps, trash bags, bottles, and beverage rings. The United States alone generates 160,000 tons per year of solid waste; 18% of this volume is plastic, 38% is paper, and 2% is glass. Most of these products are inherently stable and do not degrade.

Just recently the word biodegradable has appeared on the labels of some trash bags, diapers, and various other plastics. Even more recently manufacturers have been asked to remove the word biodegradable from their labels because these products are not truly biodegradable, rather they disintegrate into smaller units of the whole, but the original product is still there.

Making products that degrade adequately into environmentally safe byproducts should be the concern of every manufacturer in the world. We must remember that manufacturing is consumer-driven. We have to consider what kinds of things we'd be willing to give up to clean up! If we don't approach this problem very soon our children are destined to live in a world of trash piles and pollution. Recycling is a partial solution to waste reduction, but it's not a solution to the long range problem of cleaning up the environment. It is important that students be aware that the trash they create may be around for a very long time. How can they help as individuals? They can reduce, reuse, and recycle and be aware.

Many of us believe that all of our trash degrades by some natural mechanism after it has been taken to the landfill or enters the natural environment. It's true that many things that we dispose of are recycled back into the environment. Some materials deteriorate and some degrade. There are three ways materials may degrade. Photodegradation is stimulated by sunlight, usually ultraviolet radiation. Biodegradation is stimulated by the enzymatic action of microorganisms. Chemical degradation occurs when the bonds between molecules break due to inherent instability of the material. These processes aid in breaking down (degrading) materials so that they can be recycled by natural processes.

Biodegradation, the focus of this lesson, is accomplished by microorganisms. Microorganisms are capable of utilizing many types of carbon-containing molecules as substrates for growth, metabolism and cell division. The major elements required for microbial growth in oxygen-containing environments are carbon, nitrogen, hydrogen and phosphorous. Elements that are also essential but required in much smaller quantities include sodium, potassium, calcium, magnesium, sulfur, iodine, iron, boron, copper, cobalt, molybdenum and zinc. This project focuses upon the ability of soil microorganisms to use natural and man-made materials as carbon sources. Complete degradation would result in a product being degraded to carbon dioxide, water and minerals and could be accomplished by one or more of the processes of chemical, photo- or microbial degradation. Some products do not detectably degrade at all (styrofoam and many plastics, for example) or are only partially degraded (plastics, paper products). In some instances, intermediates formed during degradation may be more toxic than the starting material.

The students should be introduced to the term "polymer." There are many familiar examples of naturally-occurring polymers, including cellulose, DNA, proteins, starch. Naturally-occurring polymers are biodegradable — they are synthesized and degraded by enzymatic processes. Natural products degrade at very different rates depending upon chemical composition; chemical or structural modifications can alter the rate of degradation. Some natural products, such as lignin from trees, degrade only very slowly (consider how long it takes a fallen tree to decompose in the forest). An important reason why these naturally-occurring polymers are not incorporated into consumer products is that they do not generally possess the physical characteristics that make chemically synthesized polymers indispensable to our life-style, or the expense of using naturally-occurring materials is too high.

Many chemically-synthesized polymers are very rich in carbon, for example, polyethylene, polystyrene and poly(vinyl chloride); however, organisms do not possess enzymes that degrade these materials, and they are generally very stable to chemical or photolytic degradation. Thus, these materials persist for very long times in landfills and the open environment.

In this lesson, students will develop an awareness of the persistence of man-made polymers; those various plastic products that participate in every aspect of our lives. They include six pack beverage rings, diapers, styrofoam cups, milk and juice jugs, food wraps, detergents to list a few. The efficacy of these products is remarkable, but what of their final destination?

Vocabulary:

Degradation – a chemical reaction leading to the breaking of bonds in molecules such that new molecules may be formed as opposed to deterioration. This process uses carbon, nitrogen, moisture and microbes.

Biodegradation – a degradation process in which a living organism, like bacterium or fungus, metabolizes or breaks down a material through an enzymatic process.

Recycle – to use again; pass through a series of changes or treatments in order to regain materials for human use.

Photodegradation – A degradation process initiated by light, such as sunlight or a UV source.

Deterioration – The fragmentation of an article in which the individual fragments retain the properties of the original article and which is caused by environmental or physical forces.

Polymer – A large molecule built by the repetition of small, simple chemical units (they are the basis for all plastics).

The following are three separate procedures that can be followed in order for students to observe the process of biodegradation or the lack of it in certain materials. It would be advisable to begin these procedures at the start of the school year so that students may follow their results for as long as possible, even the entire year. It must also be noted that bacteria and or fungi may grow prolifically, and should be grown in closed containers, and should also be sterilized in an autoclave when the procedure is completed. **These microorganisms should not be handled by students. Before beginning experiments, be sure to read the article on "Safety in Microbiology Experiments" available from Sister Helen M. Burke, Ph.D., Chemistry Department, Chestnut Hill College, Philadelphia, PA 19118, (215) 248-7194.**

Experiment #1

Observing Degradation Using Soil in "Zipper" Bags

Materials:

"Zipper" plastic bags, quart size
Compost or rich garden soil (compost may be made or obtained at your local county recycling center for free)
Water
Labels
Variety of materials to be tested for biodegradability (cellulose filter paper, chewing gum and packaging, toilet and facial tissue, paper bags, newspaper, styrofoam, aluminum foil, leaves, fruit, grass clippings). **Do Not Use Animal Products. They Will Putrefy!**

Procedure:

1. Give each student a plastic bag partially filled with three cups of uniformly moistened compost soil.
2. Let each student choose one item to be placed in the bag to observe for degradation.
3. Thoroughly wet the items with water, blot excess water away from the surface, place the item inside the bag on the soil so that the item is in good contact with the soil and may be easily observed through the bag.
4. Insert a plastic straw at one edge of the bag, zip the bag closed so that the straw extends out of one side of the bag to allow some air into the bag. Do not insert the end of the straw into the soil.
5. Label each bag with student's name, date, item added, soil type, or other treatment , if applicable.

6. Place the bags in a drawer or box and leave in a warm location away from drafts and temperature fluctuations. Bags should not be left in direct sunlight.
7. Observe and record results weekly or as appropriate in a journal. Have students compare and contrast their variables, using information about the chemical and physical composition and natural or synthetic origin when making their observations. Handle the bags with care so that the material remains in good contact with the soil and also remains visible.
8. Have students make a hypothesis about how their material will look at the end of the experiment.
9. **Disposal:** Remove straws from bags, and destroy living microorganisms by heat sterilization or addition of chemical disinfectant to the bags. For chemical disinfection, prepare a solution of Lysol®, Clorox® or alcohol according to the recommendations of Pogosian in the article "Safety in Microbiology Experiments". Allow the bags to sit overnight, pour off liquid, seal bags and dispose of bags and soil in the trash.

Extensions:

- Try using different types of soil (sterile, garden, worm, mushroom, etc.)
- Try setting up other variables such as temperature, humidity, radiation.
- Try heating the soil to kill organisms before adding item.
- What conditions could you devise that would speed up degradation? (Will a gum wrapper on top of a table degrade as fast as a wrapper in water?)
- Try adding fertilizer, such as plant food in water solution, to speed the rate of degradation (see supplement for explanation).

Questions:

1. Before beginning the experiment, what did you think your variable would look like over time? Should it degrade or not?
2. What kind of changes took place in your unit?
3. Is your material biodegradable? How do you know?
4. What is your material made of?
5. What do you think will happen to your material over the next 10 years?
6. As a class make a list of products that will stay stable in the environment and not degrade for a very long time.
7. Make a list of things that you would **not** want to degrade (e.g. paints, clothing)
8. What did people do before plastics and disposables were available?
9. How could we minimize the use of disposables by reusing products and still maintain food safety, hygiene, freshness, etc.?
10. Make a list of products that will outlive their usefulness.

Experiment #2:

Observing Degradation In A Minimal Liquid Media

Materials:

Minimal liquid media (prepare or purchase)
Distilled water (if media is to be made by teacher or students, can be purchased at grocery store or pharmacy)
125-milliliter flasks, baby food jars, other clear containers (disposable plastic flasks can be purchased from suppliers such as Fisher Scientific or Carolina Biological Supply)
Soil (garden soil, sterile potting soil, compost)
Cellulose filter papers or chewing gum and wrappers, or other materials to test

Preparation of Minimal Liquid Media:
Although bacterial media and culture containers are normally sterilized before use, this experiment may be successfully conducted using unsterilized media and containers, since only minimal media is used and very few microorganisms survive under these conditions. Appropriate controls (media with no substrate) should be set up to observe whether unsterilized media supports microorganism growth. If appropriate facilities are available, the media and glassware may be sterilized before use.

To prepare 1000 milliliters of minimal media:

1. In a 2-Liter container, add 800 milliliters of distilled water
2. In the water, dissolve the following:

2 grams	K_2HPO_4 (dibasic potassium phosphate)
1 gram	NaH_2PO_4 (monobasic sodium phosphate)
2 grams	$(NH_4)_3PO_4$ (ammonium phosphate)

3. After salts are dissolved, bring the volume to 1000 milliliters with distilled water.
4. Check pH using pH paper or pH meter; pH should be between 6.5 and 7.5 and does not need further adjustment.

Procedure:

1. Add 30 milliliters minimal liquid media to each container.
2. Add 2 grams of soil to appropriate containers (should have at least one control lacking soil)
3. Wet the item to be tested with water or minimal liquid media.
4. Place the item to be tested in the container with media. You may want to use a plastic straw to support the item so that it does not rest against the sides of the container (if the item absorbs water, water will wick up the side of the container and observations will not be as clear as if water surface is undisturbed around the item being tested).
5. Close the container with a lid or a cotton plug. Do not seal tightly, as air must be able to enter the container during the experiment.
6. Incubate the containers at room temperature. Keep away from drafts and sunlight.
7. Observe and record results once a week as long as possible over the school year.
8. Dispose of containers after decontamination by heat sterilization or chemical disinfection following the instructions in Pogosian's article "Safety in Microbiology Experiments".

Experiment #3 **Observing Degradation On Agar Media In Petri Dishes**

Materials:

Minimal liquid media (see Experiment 2, prepare or purchase)
Bacteriological Agar (Difco)
Parafilm (American Can Co)
Disposable plastic petri plates
Soil (compost, garden soils)
Materials to be tested for biodegradability

Procedure:

1. Prepare minimal liquid media as in Experiment #2. Add 12 grams agar per liter.
2. Heat media in an open vessel, preferably a large beaker, at least twice the volume of the media.
3. Heat solution gently until agar dissolves. The liquid need not boil but should be watched carefully so that the agar does not burn. The solution should be yellowish but completely clear when agar is completely dissolved.
4. Allow agar to cool to about 45 to 50 degrees C, then dispense into petri plates. Pour agar media into a smaller vessel (500-mL Erlenmeyer flask or beaker) to make dispensing easier. Open each plate only long enough to dispense media, avoid unnecessary exposure of media to the open air.
5. Allow agar to solidify, about one hour. Plates should be poured at least one day in advance of the experiment.
6. Wet the material to be tested, and place the item firmly in contact with the medium using forceps. Do not touch agar with fingers (although this is not a sterile system, any extraneous contamination should be avoided). Place a small scoop of thoroughly moistened soil at the edge of the material, in contact with the agar.

7. Close the plate and seal edges with parafilm. Label plates on the bottom (lids can be switched, bottoms cannot!).
8. Observe and record results at least once weekly for as long as possible during the school year.
9. Disposal- Dispose of containers after decontamination by heat sterilization or chemical disinfection following the instructions in Pogosian's article "Safety in Microbiology Experiments".

In this series of experiments, the students will study whether familiar materials, both natural and man-made can be utilized as carbon sources by consortia of microorganisms obtained from soil samples. The students will prepare a minimal bacterial growth medium which supplies nitrogen in the form of ammonium salt, oxygen from the air, essential microelements from tap water, a buffer to maintain physiological pH, and an object that will be tested as a source of carbon. Suggestions for biodegradable materials include filter paper (pure cellulose), different types of processed papers (cellulose plus other additives), plant matter (grass clippings, leaves, fruit peels.) Nondegradable materials include synthetic polymers (polyethylene, polystyrene), or metals (aluminum foil).

A stick of gum in its wrappers is a good substrate to test, as it is a familiar object composed of parts that are very different in their compositions, and therefore their biodegradability. The outer paper wrapper is composed of cellulose, dyes, polymeric binders that hold the paper together and possibly some wax. Over time, microbial growth should develop on paper surfaces, and the paper should discolor and deteriorate. The inner aluminum foil wrapper is a paper-backed sheet of metal. Inorganic aluminum will not support microbial growth, as it does not contain carbon. Degradation of the backing may be observed. Gum sticks are usually coated on the outside with some kind of sugar and flavoring agents. Sucrose, fructose or glucose coat "sugared" gums, while sugar alcohols (sorbitol, xylitol) are used to sweeten many "sugarless" gums. These sugars are excellent carbon sources for microbial growth, and very shortly after the experiment is initiated, microbial growth will coat the gum stick surface. The gum itself, however, is a nonbiodegradable petroleum products which will not support microbial growth, and therefore, will not degrade. The stick may swell as it becomes hydrated.

In addition to gum, cellulose filter papers can be used as substrates for soil microorganisms in liquid media or on agar in petri plates.

While this experiment can be carried out with any of the media described in Experiments 1, 2, or 3, it is best suited to testing on agar plates. The substrate, or carbon source, used in this experiment is cellulose filter paper, which can be purchased from any scientific supply source. Cellulose filter paper is uniform in composition and is not treated with any preservatives that may interfere with microbial activity (many consumer products are treated with preservatives to inhibit microbial activity).

Controls are incorporated into this experiment which should provide students with the opportunity to observe and discuss the roles of the various components in the experiment: microorganisms are necessary, but they require carbon (cellulose) and nitrogen (ammonium ions in media).

Supplement to Experimental Section:

Selection and Containment of Bacterial Samples:
Teachers are strongly advised to read the article "Safety In Microbiology Experiments" before planning student experiments. Microbiological experiments can be conducted safely, however proper precautions, such as judicious choice of bacterial sources, proper containment and disposal practices must be followed.

Preparation of Media For Flask and Petri Dish Experiments:
Many schools lack equipment and facilities to prepare the media for the experiments which call for liquid and agar media. Custom-made, sterile liquid or agar media may be obtained from Carolina Biological Supply, Burlington, North Carolina. Orders for custom-preparation of the media suggested in this lesson, may be placed by contacting Ms. Juliana Hauser, Head- Microbiology Department, Carolina Biological Supply, Burlington, NC (919)-584-0381. Prices will be approximately $3.00 per 100 mL of media (Petri plates required approximately 15 milliliters each of agar media). In addition, Carolina Biological Supply offers a wealth of live and prepared specimens, hands-on kits with full instructions, equipment and supplies useful for biology, chemistry, physics, environmental science study appropriate for students from kindergarten to high school. Teachers may obtain a free copy of Carolina's catalog by calling 1-800-334-5551).

Disposal of Samples Containing Live Bacterial and Fungal Cultures:
Proper disposal of live cultures after experiments are terminated can be accomplished by pressurized heat sterilization (autoclaving) or by chemical disinfection. Instructions on how to chemically disinfect cultures using alcohol or commercial disinfectants, such as Lysol® or Clorox® are included in the article "Safety In Microbiology Experiments" by Barbara Pogosian.

Additional Suggestion for "Zipper" Bag Experiment
The Exxon Valdez oil spill off the Coast of Prince Edward Sound in 1990, while an environmental disaster of epic proportions, provided a fascinating example of the ability of endemic populations of microorganisms to cleanse the environment, provided that adequate supplies of nutrients are available. While many people spent months hosing and wiping oil from the beaches, scientists studying bioremediation as an alternative to physical cleanup found that when the beach was sprayed with fertilizer (plenty of Nitrogen and a balance of other essential nutrients), the surface of the beach was cleaned of oil (carbon!!) in a remarkably short time. This study is a dramatic example of the adaptability of endemic microbial populations to using novel carbon compounds when the environment is rich in essential nutrients. A similar situation could be stimulated in the soil bags by adding fertilizer, for example a solution of Miracle Grow® or Peter's plant food, to the soil in the bags.