Do All Plastics Safely Break Down in the Environment?

Space available for landfills is rapidly decreasing. Within the next five years, many of the landfills in the United States will close because they're full. Current research is investigating the benefits of making plastic from renewable resources that are more environmentally friendly and don't rely on fossil fuels to synthesize. This new technology will hopefully reduce the amount of solid waste in landfills.

Under normal composting conditions, both abiotic and biotic factors play a part in the degradation of materials. In this lab, you will determine how biodegradable various plastics are using microbial degradation and whether they leave harmful byproducts in the soil, which could potentially harm the environment.

Materials

- PLA plastic
- Cast-Film plastic
- BioBag plastic film
- Polystyrene sample
- Polyethylene sample
- Plastic eating utensil
- Bioreactor (3)
- 300 g of soil
- Digital Balance
- Digital Camera
- Soil pH Test Kit
- Micrometer
- Microscope
- 100 mL graduated cylinder
- Plastic scoop
- tweezers
- newspaper
- nonlatex gloves
- petri dishes (4)
- lettuce seeds
- filter paper

Safety

- Goggles must be worn at all times
- Do not touch face, including eyes and mouth during the lab
- Wash hands thoroughly after completing the lab

Procedure

1. Spread the newspaper across your working space.
2. Obtain three bioreactors. Fill each of them with 250 grams of soil.
3. Use the biopolymer you made in the *Comparing Plastics Lab*. Choose one of the synthetic polymers from the materials list. Your third sample should be either the BioBag or the plastic eating utensil. You need to have a minimum of 5 pieces of each type of plastic.
4. Make a data table using the template below:

	Date:		Date:		Date:	
Type of Plastic	Mass of all pieces	Average Mass	Mass of all pieces	Average Mass	Mass of all pieces	Average Mass

Continue table for two additional data columns. →

5. Measure the initial mass of all the plastic pieces to be composted. Determine the average mass for one piece of plastic by dividing the total mass by the number of pieces.
6. Determine the average thickness of each individual sample of plastic using a micrometer. Take thickness readings from 3 different areas of each piece of plastic. Record your data for each area, then determine the average by adding up all the measurements and dividing by 3. Determine the overall average thickness by adding up the average thickness for each sample and dividing by the total number of pieces of plastic. Record your data using the template below:

Type of plastic	3 spot thickness measurements (mm)	Average thickness for each piece (mm)	Average thickness for type of plastic (mm)
Example 1	1. 0.1, 0.15, 0.12	1. 0.123	*Add up all the values in column three and divide by 5*
	2. o.15, 0.13, 0.17	2. 0.45	
	3.	3.	
	4.	4.	
	5.	5.	

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7. Determine the soil pH by following the directions given in the soil pH test kit. Make a data table following the template below:

	Date:	Date:	Date:	Date:	Date:
Type of plastic	Initial Soil pH	Soil pH	Soil pH	Soil pH	Soil pH

8. Arrange each piece of the same type of plastic on the surface of the soil in one of the bioreactors. Evenly cover the plastic with 50 g of soil.
9. Add 100 mL of distilled water to the surface of the soil. Close the lid of the bioreactor and seal it shut with tape.
10. Label the outside of the bioreactor with the *type of plastic*, *the date*, and *your group names*.
11. Repeat steps 4-6 for the other two types of plastic.
12. Place all bioreactors in the area designated by your teacher. These will be checked once a week for 4 weeks.
13. Clean up your area when finished.

Weekly Measurements

14. Spread newspaper across your working space.
15. Carefully remove the tape from one of the bioreactors. Use a scoop to carefully remove the top layer of soil.
16. Carefully wash off the soil from each piece of plastic. Allow the samples to air dry on a piece of paper towel.
17. Repeat steps 4 and 5 to determine the average mass and thickness for the plastic. Record the data in the data tables made previously.
18. Determine the soil pH by using the soil directly beneath where the samples were resting. Follow the directions given in the pH test kit.
19. Once the measurements have been taken and recorded, place the pieces of plastic back into the bioreactor and cover completely with soil.
20. Add 20 mL of distilled water to the surface of the soil.
21. Seal the bioreactor with tape.
22. Repeat steps 14-19 for the other two bioreactors.
23. Clean up your area when finished.

Bioassay

1. Take the four petri dishes and label them control, Plastic #1 (name), Plastic #2 (name), etc.
2. In the control dish, place 2 grams of regular potting soil. Cover the soil with a filter paper circle.
3. Dampen the filter paper with 2 mL of distilled water. Arrange 5 lettuce seeds on the surface of the filter paper. Place the top on the petri dish and secure it with tape.
4. For petri dish 2, place 2 grams of soil from one of the bioreactors. Cover the soil with filter paper.
5. Repeat step 3. Make sure the petri dish is labeled with the type of plastic that was in the bioreactor.
6. Repeat steps 4-5 with the remaining petri dishes.
7. Place all petri dishes in a gallon Zip-lock bag. Place in your lab drawer for 5 days.
8. At the end of 5 days: Measure the root lengths in cm for each lettuce seed and the % sprouted for each petri dish. Record the root lengths as an average. Use the template below to record your data:

Type of Plastic	% Seeds Sprouted	Average Root Length (cm)

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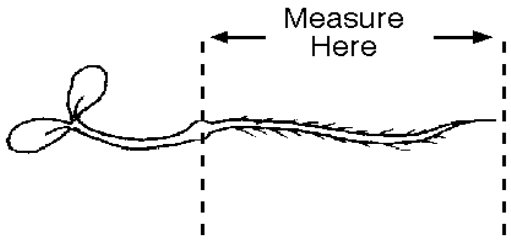
Measuring Lettuce Seeds:



9. Note any variations in appearance in the lettuce seeds that might be useful in your final analysis.

Final Analysis

24. Plot the rate of degradation by making a graph of the number of days in compost vs. the % mass loss for each type of plastic. To determine % mass loss, divide each weekly average mass by the initial mass taken before composting.
25. You should have a total of four plots for each type of plastic. Make sure you have a key to identify the different plots.
26. Using the data collected weekly for each type of plastic, make a conclusion that addresses the biodegradability of the plastic, overall suitability for compost, ability to reduce solid waste in landfills, and the toxic effects on the environment using soil pH and the results of the bioassay.

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Teacher's Notes

- Digital balance should measure to the 0.01 g.
- Microscopes can be substituted with hand-held microscopes. These can be purchased from Radio Shack for $10.00 each and can magnify up to 100X.
- One bioreactor can be made using two 2-liter soda bottles. These can be prepared prior to conducting the lab, or you can have students make them. Caution: sharp knives will have to be used to cut through the plastic. Exercise extreme caution. Use the procedure below to make the bioreactor:

Bioreactor Construction

Materials (for each bioreactor)

- 2-liter soda bottle (2)
- Utility knife
- Screening material square (5 cm^2)
- Rubber band
- Dissecting needle or small nail
- Candle

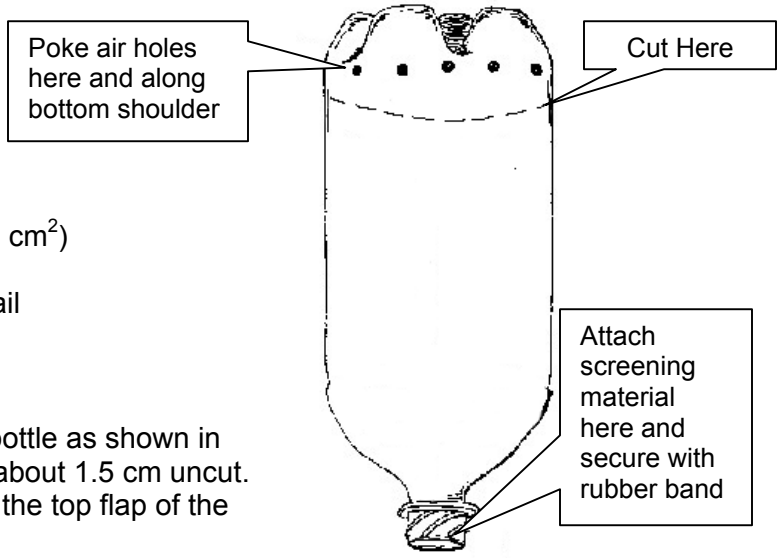

Figure 1

Set-up

1. Cut along the bottom of the bottle as shown in figure 1. Leave a section of about 1.5 cm uncut. This will serve as a hinge for the top flap of the bioreactor.
2. Secure the screening material to the mouth of the bottle using the rubber band.
3. Use the candle to heat up the end of a small nail. Poke the hot nail through the top of the bioreactor and along the lower third. These holes will help air to circulate through the reactor, which is vital for composting to take place.
4. Preparing the base: Cut a 2-liter bottle in half. Use the bottom half as the base to rest the bioreactor (you can save the top to use as a funnel). If you don't want to use another half of a 2-liter bottle as the base, substitute a 600 mL beaker. The bioreactor needs to sit off the bottom of the container as much as possible.

An alternative to having each group compost three types of plastic would be to assign each group a different pair of plastics to test, preferably one synthetic and one biopolymer. Each group should use a minimum of 5 pieces of the plastic in their bioreactor. You could also use the plastic utensil as an unknown. Have students try to match it with one of the other plastics (it is made of the same material as the BioBag).

Lettuce Seed Bioassay

- The lettuce seeds have to be soaked in a 10% bleach solution (10 mL of bleach to 90 mL distilled water) for 20 minutes prior to use. This is done to prevent mold growth which would negatively affect the results of the bioassay.

References

Green Plastics; Stevens, E. S.; Princeton University Press: Princeton, 2002.

Tuominen, Jukka; Kylma, Janne; Kapanen, Anu; et. al. *Biomacromolecules*. **2002**, *3*, pp 445-455.

Bioassays: Testing Environmental Samples With Lettuce Seeds. *http://ei.cornell.edu/toxicology/bioassays/lettuce/EnvSample.asp.* (accessed 8/13/03).

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