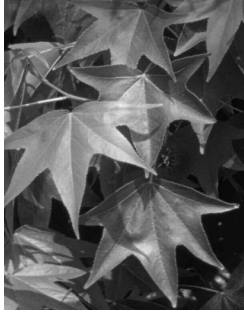


4

MONITORING THE COMPOSTING PROCESS

As decomposition proceeds, a number of changes occur in the physical, chemical, and biological characteristics of the compost mix. Monitoring these changes allows you to assess the progress of your compost, identify potential problems, and compare systems with different initial conditions or ingredients.

Simple observation of the physical changes that occur during composting is one form of monitoring. It is useful to keep a log book, not only to record data but also to note daily observations about the appearance of the compost. Does it appear soggy or dry? Is it shrinking in volume? Is there any odor? Any leachate? At what point do the various types of ingredients become unrecognizable? Have flies or other pests become a problem? If problems do develop during the course of composting, steps can be taken to correct them (Table 4–1).

Another form of monitoring is to take periodic measurements of variables such as the temperature, moisture content, pH, and biological activity. This chapter presents techniques for monitoring these physical, chemical, and biological characteristics of compost. Students can design and conduct a wide array of experiments using these monitoring techniques.

Table 4–1. Troubleshooting Compost Problems.

Symptom	Problem	Solution
Pile is wet and smells like a mixture of rancid butter, vinegar, and rotten eggs	Not enough air	Turn pile
	Or too much nitrogen	Mix in straw, sawdust, or wood chips
	Or too wet	Turn pile and add straw, sawdust, or wood chips; provide drainage
Pile does not heat up	Pile is too small	Make pile larger or provide insulation
	Or pile is too dry	Add water while turning the pile
Pile is damp and sweet smelling but will not heat up	Not enough nitrogen	Mix in grass clippings, food scraps, or other sources of nitrogen
Pile is attracting animals	Meat and other animal products have been included	Keep meat and other animal products out of the pile; enclose pile in 1/4-inch hardware cloth
	Or food scraps are not well covered	Cover all food with brown materials such as leaves, woodchips, or finished compost

TEMPERATURE

Temperature is one of the key indicators of changes occurring during thermophilic composting. If the compost does not heat up, it may be deficient in moisture or nitrogen (Table 4–1). Once the compost does heat up, temperature provides the best indicator of when mixing is desirable (see Figure 1–2, p. 4).

To take temperature readings, use a probe that reaches deep into the compost. Leave the probe in place long enough for the reading to stabilize, then move it to a new location. Take readings in several locations, including various distances from the top and sides. Compost may have hotter and colder pockets depending on spatial variability in the moisture content and chemical composition of the ingredients. Can you find temperature gradients with depth? Where do you find the hottest readings? For systems in which air enters at the bottom, the hottest location tends to be in the core, about two-thirds of the way up. You might expect it to be in the exact center, where insulation by surrounding compost is the greatest, but the core temperatures are affected by the relatively cool air entering at the bottom and warming as it rises through the compost.

Your students might decide to design compost experiments to look for variables or combinations of variables that produce the highest temperatures in the shortest amount of time, or perhaps those variables that maintain hot temperatures for the longest period. One useful way to present your data is to plot the maximum temperature and the time to reach maximum temperature for each compost system as a function of the experimental variable. For example, you could plot the maximum temperature versus the initial moisture content of the compost ingredients. A second graph could show the time to reach maximum temperature versus the initial moisture content.

MOISTURE

Composting proceeds best at moisture contents of 50–60% by weight. During composting, heating and aeration cause moisture loss. That's OK—you want finished compost to be drier than the initial ingredients. Sometimes, however, adding water may be necessary to keep the compost from drying out before decomposition is complete. If the compost appears to be dry, water or leachate can be added during turning or mixing. Below a moisture content of 35–40%, decomposition rates are greatly reduced; below 30% they virtually stop. Too much moisture, on the other hand, is one of the most common factors leading to anaerobic conditions and resulting odor problems.

When you are choosing and mixing your compost ingredients, you may wish to measure the moisture content using the procedure in Chapter 3 (p. 44). After composting is underway, you probably don't need to repeat this measurement because you can observe whether appropriate moisture levels are being maintained. For example, if your compost appears wetter than a wrung-out sponge and starts to smell bad, mix in absorbent material such as dry wood chips, cardboard pieces, or newspa-

per strips to alleviate the problem. If you are composting in a bioreactor with drainage holes, excess moisture will drain out as leachate. You may find it useful to record the amount of leachate produced by each system, for comparison with initial moisture content, temperature curves, or other variables. If you have a microscope available, try observing a sample of leachate—you will probably find that it is teeming with microbial life.

If you are blowing air through your compost system, you will need to be careful not to create conditions that are too dry for microbial growth. If the temperature drops sooner than expected and the compost feels dry to the touch, moisture may have become the limiting factor. Try mixing in some water and see if the temperature rises again.

ODOR

A well-constructed compost system should not produce offensive odors, although it will not always be odor-free. You can use your nose to detect potential problems as your composting progresses. For example, if you notice an ammonia odor, your mix is probably too rich in nitrogen (the C:N ratio is too low), and you should mix in a carbon source such as leaves or wood shavings.

If compost is too wet or compacted, it will become anaerobic and produce hydrogen sulfide, methane, and other odorous compounds that are hard to ignore. If this occurs in indoor bioreactors, you may wish to take them outside or vent them to the outside, then mix in additional absorbent material such as wood chips or pieces of paper egg cartons. Make sure that you do not pack down the mixture; you want it to remain loose and fluffy to allow air infiltration. In an outdoor compost pile, turning the pile and mixing in additional high-carbon materials such as wood chips should correct the anaerobic condition, although initially the mixing may make the odor even more pronounced.

pH

Why is compost pH worth measuring? Primarily because you can use it to follow the process of decomposition. As composting proceeds, the pH typically drops initially, then rises to 8 or 9 during the thermophilic phase, and then levels off near neutral (see Figure 1–4, p. 8).

At any point during composting, you can measure the pH of the mixture. While doing this, keep in mind that your compost is unlikely to be homogeneous. You may have found that the temperature varied from location to location within your compost, and the pH is likely to vary as well. Therefore, you should plan to take samples from a variety of spots. You can mix these together and do a combined pH test, or you can test each of the samples individually. In either case, make several replicate tests and report all of your answers. (Since pH is measured on a logarithmic scale, it does not make sense mathematically to take a simple average of your replicates. Instead, either report all of your pH values individually, or summarize them in terms of ranges rather than averages.)

pH can be measured using any of the following methods. Whichever method you choose, make sure to measure the pH as soon as possible after sampling so that continuing chemical changes will not affect your results. Also, be consistent in the method that you use when comparing different compost mixtures.

pH PAPER

The least expensive option for measuring compost pH is to use indicator paper. If the compost is moist but not muddy, you can insert a pH indicator strip into the mixture, let it sit for a few minutes to become moist, and then read the pH using color comparison. If the compost is too wet, this technique will not work because the indicator colors will be masked by the color of the mud.

SOIL TEST KIT

Test kits for analysis of soil pH can be used without modification for compost samples. Simply follow the manufacturer's instructions. These kits also rely on color comparison, but the color develops in a compost-water mixture rather than on indicator paper. Soil pH kits are available from garden stores or biological supply catalogs for $5 or more, depending on the number and accuracy range of the tests.

ELECTRONIC METER

The most accurate, but also the most expensive and time consuming method of measuring compost pH, is with a meter. First, you must calibrate the meter by using solutions of known pH. Next, mix the compost with distilled water to make a suspension. Since the amount of water affects the pH reading, it is important to be consistent in the ratio of compost to water and to start with air-dry compost. Finally, place the electrode into a compost/water solution and take a reading. For a detailed description of this method, see *Methods of Soil Analysis*.[1]

MICROORGANISMS[2]

by Elaina Olynciw

A wide range of bacteria and fungi inhabit compost, with species varying over time as changes occur in the temperature and the available food supply. You can simply observe compost microorganisms under a microscope, or culture them for more detailed observation. A third possibility is to measure their metabolic activity, which does not indicate what types or populations of microbes are present but does give an indication of their level of enzymatic activity.

OBSERVING COMPOST MICROORGANISMS

USE: To make simple observations of the microbial communities in compost. Comparisons can be made over the course of several weeks or months as the compost heats up and later returns to ambient temperature.

MATERIALS

- compound microscope
- microscope slides and cover slips
- eye dropper
- 0.85% NaCl (physiological saline)

For bacterial staining (optional)

- 1.6 g methylene blue chloride
- 100 ml 95% ethanol
- 100 ml of 0.01% aqueous solution of KOH
- distilled water
- Bunsen burner
- toothpick
- blotting paper or filter paper

PROCEDURE

1. Make a wet mount by putting a drop of water or physiological saline on a microscope slide and transferring a small amount of compost to the drop. Make sure that you do not add too much compost, or you will have insufficient light to observe the organisms.
2. Stir the compost into the water or saline (the preparation should be watery), and apply a cover slip.
3. Observe under low and high power. You might be able to see many nematodes squirming and thrashing around. Other possibilities include flatworms, rotifers (notice the rotary motion of cilia at the anterior end of the rotifer and the contracting motion of the body), mites, springtails, and fast-moving protozoa. Strands of fungal mycelia may be visible but difficult to recognize (see #5 below). Bacteria appear as very tiny round particles that seem to be vibrating in the background.
4. If you want to highlight the bacteria and observe them in greater detail, you can prepare stained slides:

a) Prepare methylene blue stain by adding 1.6 g methylene blue chloride to 100 ml 95% ethanol, then mixing 30 ml of this solution with 100 ml of 0.01% aqueous solution of KOH.
b) Using a toothpick, mix a small amount of compost with a drop of physiological saline on a slide, and spread it into a thin layer.
c) Let the mixture air-dry until a white film appears on the slide.
d) Fix the bacteria to the slide by passing the slide through a hot flame several times.
e) Stain the slide by flooding it with the methylene blue stain for one minute. Rinse the slide with distilled water and gently blot it dry using blotting or filter paper.

5. Fungi and actinomycetes may be difficult to recognize with the above technique because the entire organism (including the mycelium, reproductive bodies, and individual cells) will probably not remain together. Fungi and actinomycetes will be observed best if you can find them growing on the surface of the compost heap. The growth looks fuzzy or powdery. Lift some compost with the sample on top, and prepare a slide with a cover slip to view under the microscope. You should be able to see the fungi well under 100x and 400x. The actinomycetes can be heat-fixed and Gram-stained to view under oil immersion at 1000x (See procedure under **Culturing Bacteria**, p. 57–59).

6. To collect nematodes, rotifers, and protozoa for observation, use the **Wet Extraction** (p. 69).

CULTURING BACTERIA

USE: To culture bacteria, for colony counts or observation of specific microorganisms.

MATERIALS

Growth media
- 2 g trypticase soy agar (TSA)
- 7.5 g bacto agar
- 500 ml distilled water

For making plates
- 100 ml 0.06M $NaHPO_4/NaH_2PO_4$ buffer (approximately 4:1 dibasic:monobasic, pH 7.6)
- 5 test tubes
- 5 1-ml pipettes
- 5 0.1-ml pipettes
- 3 petri dishes
- glass spreader (glass rod bent like a hockey stick)
- autoclave
- blender
- compost sample, air-dried

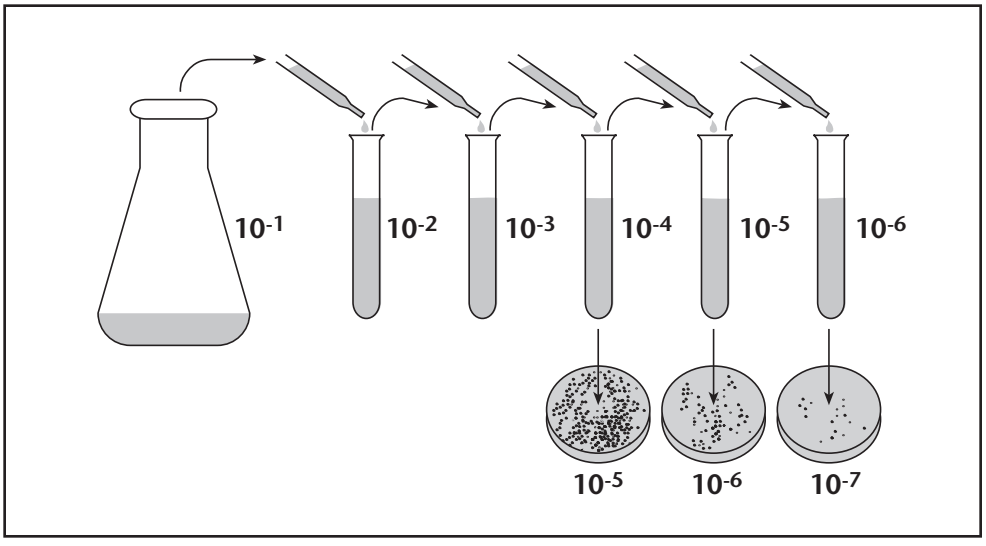
For making and observing slides
- inoculating needle
- 0.85% NaCl (physiological saline)
- Bunsen burner
- microscope and slides
- ethanol

For staining slides:
either:
- a Gram stain kit

or:
- 2 g crystal violet
- 100 ml 95% ethanol
- 80 ml 1% ammonium oxalate
- 1 g iodine crystals
- 3 g potassium iodide
- 500 ml distilled water
- amber-colored bottle
- 2.5 g safranin

PROCEDURE
1. Make the TSA media by mixing 2 g TSA, 7.5 g bacto agar, and 500 ml distilled water. Autoclave for 20 minutes, cool until comfortable to touch, then pour into sterile petri dishes and allow to solidify.
2. Autoclave 100 ml 0.06M $NaHPO_4/NaH_2PO_4$ buffer solution in an Erlenmeyer flask. Also autoclave five test tubes, each containing 9 ml of buffer solution, five 1-ml pipettes, and five 0.1-ml pipettes.

3. Autoclave the blender, or sterilize it by rinsing with ethanol. In the blender, mix 5 g compost with 45 ml sterilized buffer solution for 40 seconds at high speed.
4. Perform serial dilutions to 10^{-7}:
   a) Label the five test tubes containing sterilized buffer solution: 10^{-2}, 10^{-3}, 10^{-4}, 10^{-5}, and 10^{-6}.
   b) The mixture in the blender is a 10^{-1} dilution, since 5 g compost are mixed with 45 ml solution (which is equivalent to 45 g). Using a sterile pipette, transfer 1 ml of the compost/buffer mixture into the first test tube, labeled 10^{-2}.
   c) Mix thoroughly, then use another sterile pipette to transfer 1 ml of the solution in the 10^{-2} test tube to the one labeled 10^{-3}. The solution in each test tube will be ten times more dilute than the previous solution from which it was made.
   d) Continue with this sequence until all five test tubes have been inoculated.
   e) Label three petri dishes with the type of compost, date, and dilution: 10^{-5}, 10^{-6}, and 10^{-7}.
   f) Sterilize a glass spreading rod by holding it in the Bunsen burner flame until heated, then cool thoroughly.
   g) Using a sterile pipette, transfer 0.1 ml from the 10^{-4} test tube onto the agar in the petri dish labeled 10^{-5}. Spin the dish and spread the liquid as evenly as possible using the glass spreading rod. Follow this same procedure using 0.1 ml from the 10^{-5} test tube into the dish labeled 10^{-6}, and so forth until all dilutions have been plated.



5. Cover the petri dishes and turn them upside down so that any water that condenses will not drip into the cultures. Incubate them at 28°C if possible. (If you do not have access to an incubator, room temperature is acceptable.)

6. After four days, count the colonies and prepare microscope slides. Use an inoculating needle to add a drop of saline to a clean slide. Take a sample of a single bacterial colony and mix it into the saline. Let it air-dry until a white film appears. Heat-fix the slide by passing it through a flame a few times.
7. To highlight bacteria and actinomycetes on the slides, treat them with Gram stain (below).

Gram Staining

1. Prepare Gram stains (unless using a prepared kit):
   Crystal violet:
   Dissolve 2 g crystal violet in 20 ml 95% ethanol. Add this solution to 80 ml of a 1% ammonium oxalate solution. Let the mixture stand for 24 hours, then filter it.
   Gram iodine:
   Add 1 g iodine and 3 g potassium iodide to 300 ml distilled water. Store this solution in an amber bottle.
   Decolorizer: 95% ethanol
   Safranin:
   Add 2.5 g safranin to 10 ml 95% ethanol. Add this solution to 100 ml distilled water.
2. Flood slide with crystal violet for 20 seconds.
3. Gently rinse slide by dipping into a beaker of distilled water.
4. Flood slide with Gram iodine for 1 min. Gently rinse as above.
5. Decolorize by tilting slide and applying 95% ethanol one drop at a time, stopping as soon as no more color is washed out. Gently rinse. At this point, gram-positive bacteria will be purple colored and gram-negative bacteria will be colorless.
6. Flood with safranin for 20 seconds, then gently rinse. This step stains the gram-negative bacteria, and they become pink.
7. Air-dry or gently blot dry. Observe under oil-immersion lens.

CULTURING ACTINOMYCETES

USE: To culture actinomycetes, making it possible to count colonies or to observe individual species.

MATERIALS

Growth media

- 0.4 g typticase soy agar (TSA)
- 10.0 g bacto agar
- 500 ml distilled water
- 10 mg polymixin B in 10 ml 70% ethanol

For making plates

- 100 ml 0.06M $NaHPO_4/NaH_2PO_4$ buffer (approximately 4:1 dibasic:monobasic, pH 7.6)
- 500 ml distilled water
- 5 test tubes
- 5 1-ml pipettes
- 5 0.1-ml pipettes
- 3 petri dishes
- glass spreader (glass rod bent like a hockey stick)
- autoclave
- blender
- compost sample, air-dried

For making and observing slides

- inoculating needle
- scalpel
- 0.85% NaCl (physiological saline)
- Bunsen burner
- microscope and slides

PROCEDURE

1. Mix 0.4 g TSA, 10.0 g bacto agar, and 500 ml distilled water. Autoclave for 20 minutes, then cool to the touch. Add the polymixin B and pour into sterile petri dishes.
2. Autoclave 100 ml 0.06M $NaHPO_4/NaH_2PO_4$ buffer solution.
3. Autoclave the blender, or sterilize it by rinsing with ethanol. In the blender, mix 5 g compost with 45 ml sterilized buffer solution for 40 seconds at high speed.
4. Perform serial dilutions to 10^{-7} using Step 4 of the **Culturing Bacteria** procedure (p. 58).
5. Cover the petri dishes and turn them upside down so that any water that condenses will not drip into the cultures. Incubate them at 28°C if possible. (If you do not have access to an incubator, room temperature is acceptable.)
6. After 14 days, take counts and samples of actinomycetes colonies. Many of the colonies will look powdery white. However, some may take on a rough appearance and produce a variety of pigments. Use an inoculating needle to add a drop of saline to a clean microscope slide. Lifting with a scalpel is a good way to get an intact portion of an

actinomycete colony onto a slide. Allow to air-dry until a white film appears. Heat-fix the slide by passing it through a flame a few times.
7. Observe under a microscope. The actinomycetes probably will be too thick to observe on most of the slide, but at the edges of the colony you will be able to see the pattern that the filaments form. Gram staining can be used to highlight the actinomycetes (see procedure under **Culturing Bacteria**, p. 59).

CULTURING FUNGI

USE: To culture fungi, making it possible to count colonies or to observe individual species.

MATERIALS

Growth media

- 6.5 g potato dextrose agar (PDA)
- 5.0 g bacto agar
- 500 ml distilled water
- 15 mg rifampicin in 10 ml methanol
- 15 mg penicillin G in 10 ml 70% ethanol

For making plates

- 100 ml 0.06M NaHPO4/NaH2PO4 buffer (approximately 4:1 dibasic:monobasic, pH 7.6)
- 3 test tubes
- 3 1-ml pipettes
- 3 0.1-ml pipettes
- 3 petri dishes
- autoclave
- blender
- compost sample, air-dried

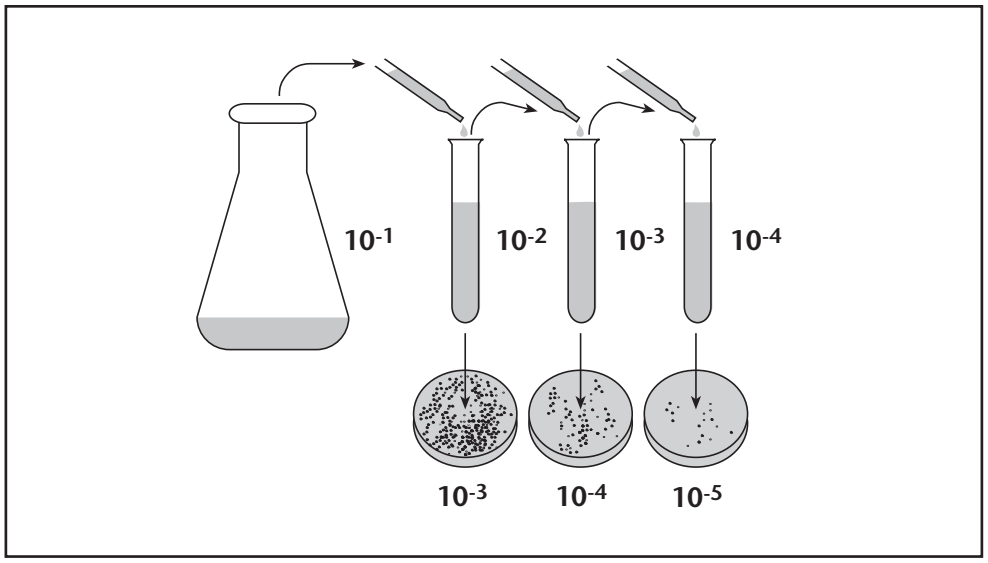
For making and observing slides

- scalpel
- microscope, slides, and cover slips

PROCEDURE

1. Mix 6.5 g PDA, 5.0 g bacto agar, and 500 ml distilled water. Autoclave for 20 minutes. Cool until comfortable to handle, then add the antibiotics rifampicin and penicillin G and pour the mixture into sterile petri dishes.
2. Autoclave 100 ml 0.06M $NaHPO_4/NaH_2PO_4$ buffer solution in an Erlenmeyer flask. Also autoclave three test tubes, each containing 9 ml of buffer solution, three 1-ml pipettes, and three 0.1-ml pipettes.
3. Autoclave the blender, or sterilize it by rinsing with ethanol. In the blender, mix 5 g compost with 45 ml sterilized buffer solution for 40 seconds at high speed.
4. Perform serial dilutions to 10^{-5}:
   a) Label the three test tubes containing buffer solution: 10^{-2}, 10^{-3}, and 10^{-4}.
   b) The mixture in the blender is a 10^{-1} dilution, since 5 g compost are mixed with 45 ml solution (which is equivalent to 45 g). Using a sterile pipette, transfer 1 ml of the compost/buffer mixture into the first test tube, labeled 10^{-2}.
   c) Mix thoroughly, then use another sterile pipette to transfer 1 ml of the solution from the 10^{-2} test tube to the one labeled 10^{-3}. The solution in each test tube will be ten times more dilute than the previous solution from which it was made.

d) Continue with this sequence until all three test tubes have been inoculated.
e) Using a sterile pipette, transfer 0.1 ml from the 10^{-2} test tube onto the agar in the petri dish labeled 10^{-3}. Spin the dish and spread the liquid as evenly as possible using the glass spreading rod. Follow this same procedure using 0.1 ml from the 10^{-3} test tube into the dish labeled 10^{-4} and so forth until all dilutions have been plated.



5. Cover the petri dishes and turn them upside down so that any water that condenses will not drip into the cultures. Incubate them at 28°C if possible. (If you do not have access to an incubator, room temperature is acceptable.)
6. After three days, take counts and samples of fungal colonies. Lift a portion of a fungal colony intact onto a clean slide (it will still be attached to the agar), add a cover slip, and observe without staining. Look at the edges of the colony where the sample will be thin enough for light to pass through.

MEASURING MICROBIAL ACTIVITY

FLUORESCEIN DIACETATE (FDA) HYDROLYSIS AS A MEASURE OF TOTAL MICROBIAL ACTIVITY IN COMPOSTS AND SOILS[3]

USE: To measure the level of metabolic activity of microbes in a compost sample.

BACKGROUND

During the course of composting, the size and activity level of microbial populations will vary. For example, as the temperature drops at the end of the thermophilic phase, activity levels may be relatively low because the populations of heat-loving microbes are diminishing and the mesophilic microorganisms are just beginning to recolonize. Another time of low activity is at the end of composting, after the curing process has left little available carbon to support microbial growth.

The FDA test measures microbial activity through color change. As microorganisms hydrolyze fluorescein diacetate to fluorescein, the solution changes from colorless to yellow. The amount of yellow pigment measured by a spectrophotometer will give an indication of how much hydrolysis has occurred, and, therefore, it will give an indication of the total metabolic activity of the microbial population.

MATERIALS

- 20 mg fluorescein diacetate (FDA)
- 10 ml acetone ACS grade
- 20 ml 0.06M sodium phosphate buffer solution (pH 7.6)
- 14 125-ml Erlenmeyer flasks
- 14 small test tubes with screw caps
- 4-cm Büchner funnel
- Whatman #1 filter paper
- 125-ml side flask for vacuum filter
- shaker (If there are enough students in a group doing this experiment, they can take turns swirling the flasks for a total of 20 minutes.)
- centrifuge
- small test tubes (to fit in centrifuge)
- spectrophotometer
- drying oven
- 10-g sample of fresh compost

METHOD

1. Place 0.5 g compost in a 105°C oven overnight for dry weight determination.
2. Make duplicate samples by placing undried compost equivalent to 0.5 g dry weight in each of two 125-ml flasks.
3. Add 20 ml of phosphate buffer solution to each flask.
4. Make FDA stock solution by mixing 20 mg FDA in 10 ml acetone. Add 0.2 ml of FDA stock solution to each flask containing compost and buffer solution.
5. Place flasks on a shaker (90 rpm) at 25°C for 20 minutes.

6. Using a Büchner funnel, filter through Whatman #1 paper with a light vacuum.
7. Transfer 2 ml of the filtrate into a small test tube and add 2 ml acetone to stop the reaction.
8. Centrifuge samples for 5 minutes at 5000 rpm to remove particulates.
9. Using a spectrophotometer, read the absorbance at 500 nm against a buffer blank.

Preparation of a standard curve

1. Make duplicate samples of a range of FDA concentrations by adding 0, 0.02, 0.04, 0.05, 0.08, and 0.1 ml FDA stock solution to 5 ml of PO_4 buffer solution in each of 10 capped test tubes.
2. Cap tightly and heat in a boiling water bath for 60 minutes to hydrolyze the FDA.
3. Cool 10 minutes and add hydrolyzed FDA to flasks containing 0.5 g compost and 15 ml phosphate buffer. (Note: You will have to set up a different standard curve for each compost you are analyzing because a certain percentage of the fluorescein will adhere to the compost and this will vary from compost to compost.)
4. Place on a shaker or swirl fairly rapidly and consistently for 20 minutes.
5. Follow Steps 6-9 in the Methods section above.
6. Record your absorbance data using the table below.

FDA solution (ml)	**FDA** (ug)	**ABSORBANCE**		
		Rep. #1	**Rep. #2**	**Mean**
0	0			
0.02	40			
0.04	80			
0.05	100			
0.08	160			
0.10	200			

ANALYSIS

Make a standard curve by graphing FDA concentrations versus optical density readings. Then, use the graph to determine the FDA concentrations for your compost samples based on their optical density readings.

By comparing samples from different stages in the composting process, what observations can you make about the relative activity of the microbial populations?

INVERTEBRATES

In outdoor compost piles, a wide range of invertebrates takes part in the decomposition of organic matter. Try monitoring invertebrate life in the pile over the course of the compost process. Do invertebrates appear while the pile is hot, or not until it cools? Do you find different types of organisms during the various stages of decomposition?

In indoor composting you may find fewer (or no) invertebrates. In vermicomposting, worms will likely be the only macro-invertebrates unless leaves or dirt were introduced into the bin for bedding. Worms can be observed by using the methods for larger invertebrates that are described in this section.

Three methods are described here for collection of organisms living in compost. For the larger compost invertebrates, such as earthworms, sowbugs, or centipedes, the **Pick and Sort** method works well. To find smaller invertebrates, you may wish to try a **Berlese Funnel**, which concentrates creatures by collecting them in a vial placed below a funnel containing compost. Tiny invertebrates that live in the films of water surrounding the organic matter or soil particles, including nematodes and flatworms, are best collected by using the **Wet Extraction**, a variation of the Berlese Funnel in which the compost is soaked in water rather than dried.

PICK AND SORT

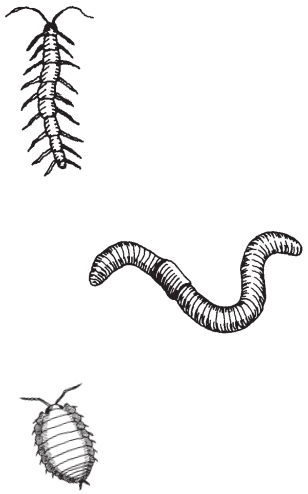
USE: To collect organisms that are easily visible to the naked eye.

MATERIALS

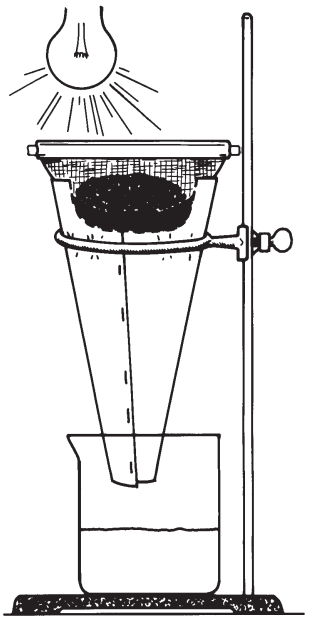
- light-colored trays or pans
- tweezers or spoons
- jars for temporary sorting and display of organisms
- flashlights (optional)
- magnifying lenses or dissecting microscopes
- petri dish or watch glass for use with microscope
- fresh compost sample, preferably from an outdoor compost pile

PROCEDURE

1. Take samples of compost from various locations in the heap. Are there some organisms that you find near the surface and others only at greater depths? Spread each compost sample in a large tray or pan, or on a large piece of paper, preferably light in color for maximum contrast. When sorting through the compost, students should use soft tweezers, plastic spoons, or other instruments that will not hurt the organisms.
2. Flashlights and magnifying lenses can be used to enhance the observation. The larger organisms, such as worms, centipedes, millipedes, and sowbugs, can easily be seen with the naked eye, but they can be observed more closely under the microscope. Place samples of the compost in petri dishes or watch glasses and observe them under a dissecting microscope.
3. In a classroom setting, you can use an overhead projector to show the outline of the organisms. It can be fun for the students to see the organisms projected to large sizes, crawling around larger than life on the screen. Caution: If you are using live organisms, it is best to have someone standing next to the projector to catch the organisms as they crawl away from the projection platform.



BERLESE FUNNEL



USE: To concentrate into a vial small organisms not easily collected through picking and sorting.

MATERIALS

- ring stand and ring
- funnel lined with small piece of window screen, or kitchen sieve enclosed in paper funnel
- beaker or jar
- light source (25 watt)
- fresh compost sample, preferably from an outdoor compost pile
- magnifying glass or dissecting microscope
- petri dish or watch glass
- optional: 100 ml of 90% ethanol/10% glycerol solution (if you wish to preserve invertebrates)

PROCEDURE

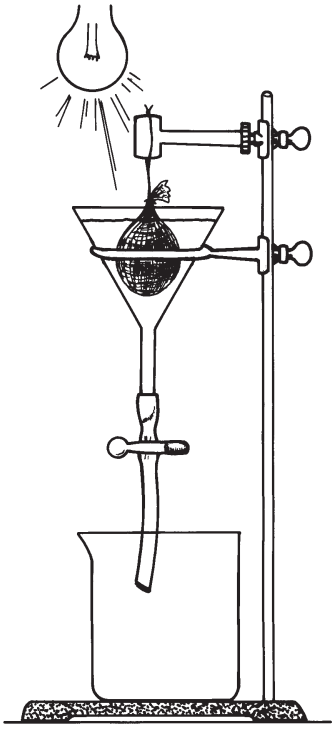
1. Assemble a funnel and sieve, either by lining the bottom of a funnel with a small piece of window screen, or by making a funnel to fit over a kitchen sieve.
2. Into a beaker or jar, add 100 ml of water if you wish to collect live organisms or a mixture of 90% ethanol and 10% glycerol if you wish to preserve them. Place the beaker just below the funnel to collect the specimens.
3. Position a light source (25 watt) 2–5 cm above the funnel, or place the collecting apparatus in a sunny location. The light, heat, and drying will gradually drive the compost organisms downward through the funnel and into the collecting jar. If you use too strong a light source, the organisms will dry up and die before making it through the compost and into the funnel.
4. Place compost in the sieve or funnel, making a layer several centimeters deep. Leave for several hours or overnight.
5. Place your collected organisms in a petri dish or watch glass, and observe them under a dissecting microscope or with a magnifying glass.

WET EXTRACTION

USE: To collect nematodes, rotifers, enchytraeids, and other small organisms that live in aqueous films surrounding compost particles.

MATERIALS

- ring stand and attachments
- funnel
- rubber tubing to fit small end of funnel
- pinch clamp
- beaker or jar
- 20-cm^2 square of cheesecloth
- 25-cm length of string
- fresh compost sample
- light source (25 watt)
- light microscope
- microscope slides and cover slips



PROCEDURE

1. Assemble the apparatus as shown in illustration, with the funnel suspended above the beaker and the rubber tubing leading from the bottom of the funnel into the beaker. Close the tubing with a clamp.
2. Make a bag of compost by placing a sample on the cheesecloth, gathering the edges, and tying them tightly together at the top. The amount of compost you use will depend on the size of your funnel (the finished bag should be small enough to fit within the funnel with space for water to flow around the edges).
3. Suspend the bag of compost, locating it so that it hangs inside the funnel with clearance around its edges.
4. Fill the funnel with water, making sure that the compost bag is submerged but not sitting on the funnel walls.
5. Place the light above the funnel and turn it on.
6. After 24 hours, open the clamp and allow the water to drain into the beaker.
7. Observe drops of water from the collected sample under the microscope. What types of organism can you identify? You may wish to count organisms from a specified amount of compost, comparing quantities and types of organisms found to those in other types of compost or soil.

[1] Page, A. L., R. H. Miller, and D. R. Keeney, eds. 1982. *Methods of Soil Analysis*, Part 2, 2nd ed. American Society of Agronomy, Inc., Soil Science Society of America, Inc., Madison, WI. pp. 208–209.

[2] The activities in the Microorganisms section were written by Elaina Olynciw, biology teacher at A. Philip Randolph High School in New York City. The descriptions assume a working knowledge of microbiological techniques. For those who have not previously worked with microorganisms, the following laboratory manual provides a good overview of techniques: Selley, H. W., Jr., P. J. Vandemark, and J. J. Lee. 1991. *Microbes in Action*, 4th ed. W. H. Freeman & Co., NY.

[3] Adapted from Schnurer, R. and T. Rosswall. 1982. Fluorescein diacetate hydrolysis as a measure of total microbial activity in soil and litter. *Applied and Environmental Microbiology* 43:1256–1261, and
Craft, C. M. and E. B. Nelsen. 1996. Microbial properties of composts that suppress damping-off and root rot of creeping bentgrass caused by *Pythium graminicola*. *Applied and Environmental Microbiology* 62:1550–1557.