Practice Guideline: Plant Bioassays to Evaluate Biochar Compost

A Wide Variety of Biochar Composts to Compare

We can make a wide variety of compost preparations and fertilizer mixes with biochar and other organic materials including manure, food waste, crop waste, and organic fertilizers such as seed meal, rock dust, liquid fish and kelp meal. We can compost the ingredients in anaerobic compost piles with balanced C:N ratio that promotes thermophilic (heat-loving) organisms, or we can add ingredients to worm bins, or mesophilic (warm) piles that don't reach thermophilic temperatures. We can also use biochar in anaerobic ferments such as bokashi, or we can simply culture a pile of biochar by adding some nutrients and moisture and allowing native microorganisms to begin consuming nutrients and depositing metabolic products on the surfaces of biochar. These products form organic coatings on the biochar that help determine its benefits to soil fertility (Hagemann et al 2017).

Compost Needs to Mature

All of the above processes take time. During the periods of active metabolism, microbial respiration rates are high and a great variety of unstable or phytotoxic organic compounds are present. These compounds can include ammonia, volatile organic acids, bacterial enzymes, salts and other chemicals that could be toxic to plants, especially seedlings (Woods End Research Laboratory, 2005). This is known as phytotoxicity. Mature compost has broken down these phytotoxic compounds and transformed them into humus, the stable byproduct of microbial metabolism that is beneficial to plant growth. Anaerobic ferments also need to mature before adding to soil because they can acidify soil to the point where it inhibits plant growth.

Compost Can Have Both Nutrients and Toxins

Compost can be tested for nutrient content and availability using soil testing methods. Compost maturity is usually gauged by measuring respiration rate. These tests are done in a lab, yet they will not indicate the presence of phytotoxic compounds. One of the best ways to determine if phytotoxic compounds are present is to perform a plant bioassay.

This Biochar Practice Guideline was created in 2018 by South Umpqua Rural Community Partnership.
Updates available at UBETBiochar.blogspot.com and WilsonBiochar.com.

Plant Bioassay: the Basics

The plant bioassay we use is a two week germination and growth test using cucumber seeds. The basic procedure is to mix the target material with a plain, peat-based potting soil at the desired rate and plant the same number of seeds in each pot. Trays of pots are placed in a controlled environment and grown for two weeks. At the end of the growth period, we count the number of germinations (a strong indicator of phytotoxicity) and the number of secondary leaves. The number of secondary leaves may indicate different levels of growth promoting hormones, nutrients or other constituents in the target amendment. Then we cut, dry and weigh the biomass of each treatment. The biomass weight will tell us something about nutrients available for growth.

The plant bioassay is primarily used for determining whether a compost material is mature enough to use, but it is also useful for comparing the effects of different amendments on plant growth. A complete protocol is outlined below, followed by an Illustrated Guide with additional information.

Plant Bioassay Protocol for Testing Biochar Compost Materials

Materials needed:

- Plain peat-based potting soil with no added nutrients, such as Sunshine Mix #4 with perlite
- A number of 4 inch round plastic pots
- A package of 16 ounce clear plastic drink cups to use as humidity domes
- Greenhouse trays
- Label, tape and indelible markers
- 1000 milliliter beaker
- 100 milliliter beaker
- Clean bins for mixing soil. We use bus trays from a restaurant supply store.
- Mesh bags. We used a nylon net bath scrubber – unfurl it and it is one long mesh tube that can be cut into sections to use as bags to contain the plants for drying.
- Food dehydrator
- Scale accurate to .01 grams
- Controlled growth environment such as greenhouse or growth chamber

Procedure:

1. Determine number of treatments, plus a control using plain potting soil, and label all pots with treatment code and replication number. Use at least 3 replications. Five is better.
2. Determine treatment rate on volume-to-volume basis (usually 20% treatment to 80% soil).
3. Fill one pot with dry soil and tap the pot gently against the table surface to pack lightly. Weigh volume of soil that fills one pot = **a.**
4. Fill control pots with soil
    a. Multiply weight **a** times number of replicates (usually 5).
    b. Weigh that amount of soil and place in bin – this will be soil for control pots.
    c. Measure a volume of water equal to half the volume of the 5 control pots and mix with soil to moisten. Let stand for 10 minutes, then fill pots.
5. Mix treatment and fill pots with soil-treatment mix (see example, page 6).
    a. Multiply weight **a** x 80% x # replicates.
    b. Weigh that amount of dry soil and place in bin.
    c. Multiply volume of one pot by 20% for treatment volume. For example, if pot volume is 300 ml, then 20% of that volume is 60 ml.
    d. Multiply treatment volume by # replicates.

    e. Measure the total volume of treatment material into a beaker. Tap to pack lightly. Place total volume of treatment material in bin with dry soil.
    f. Mix soil and treatment thoroughly.
    g. Measure a volume of water equal to half the volume of the 5 treatment pots and mix with soil to moisten. Let stand for 10 minutes, then fill pots.
6. Mix additional treatments using same method as 5.
7. Plant seeds.
    a. Place 10 cucumber seeds from the same packet evenly spaced on the surface of each pot. Keep seeds away from sides of pot.
    b. Place about ¼ cup of moist, un-amended soil on top of the seeds and press to spread the moist soil evenly across the top of the pot, covering all the seeds with at least 1/8" of soil.
    c. Add about a tablespoon of additional water to each pot to make sure that soil is completely saturated.
8. Prepare plants for growth chamber or greenhouse.
    a. Place a humidity dome (plastic drinking cup) on each pot and secure with tape if needed.
    b. Place pots on trays, randomizing pots within each tray or block.
9. Growth Phase
    a. Determine lighting schedule and set up timers if using artificial light.
    b. If growing in greenhouse, supply heat mats, if needed.
    c. If growing in growth chamber, adjust exhaust fan temperature control as needed.
    d. Grow plants for 14 days. Do not open humidity domes or add any more water.
10. Evaluate results.
    a. Count number of germinations in each pot and record.
    b. Count number of well-formed secondary leaves in each pot and record.
    c. Count number of incipient secondary leaf buds in each pot and record.
    d. Take photographs of trays to record information such as leaf color.
    e. Snip off above ground vegetation at soil level for all of the pots in each treatment (including control) and place in one labeled mesh bag for each treatment.
    f. Place mesh bags of vegetation in drying oven or food dehydrator and dry for 24 hours at 105 degrees F.
    g. Remove dried vegetation from each bag and weigh biomass for that treatment.
    h. Record total biomass for each treatment.

References

Hagemann, N., Joseph, S., Schmidt, H. P., Kammann, C. I., Harter, J., Borch, T., ... & McKenna, A. (2017). Organic coating on biochar explains its nutrient retention and stimulation of soil fertility. Nature communications, 8(1), 1089.

Bonhotal J, Harrison EZ. (2004). Testing Composts. Cornell Waste Management Institute. Updated 2015. 6 p.

Woods End Research Laboratory (2005). Interpreting Waste & Compost Tests. Journal of the Woods End Research Laboratory, Vol 2. No 1.

An Illustrated Guide to Plant Bioassay Procedures

	1. Prepare soil for all treatments 2. Plant seeds 3. Top up moisture levels 4. Cover with humidity dome 5. Place on trays in growth chamber or greenhouse
	6. After two weeks, remove plants from growth environment 7. Count germinations in each pot and record
	8. Count number of fully developed secondary leaves and record 9. Count number of incipient secondary buds and record
	10. Cut all plants for each treatment at soil level and combine

	11. Place plants from each treatment in a separate mesh bag and label, then place on drying tray
	12. Dry plants for 24 hours at 105 degrees F in food dehydrator
	13. Weigh each treatment using a scale that is accurate to .01 grams

Germination and Growth Test Report 12-17-16

Treatments:

DM – Don Morrison's composted biochar (biochar chunks picked out of compost pile)
JL – John Livingston's vermicompost (with biochar added to vermicompost bedding)
C – Control (Sunshine #4 potting mix)

Methods:

- 5 replicates of each treatment.
- Base is peat-perlite soil-less medium manufactured by Sunshine.
- Pots are 4" round pots with individual humidity domes.
- Control pots have 330g air-dry potting soil
- Treatment pots have 275g air-dry potting soil mixed with 60ml of amendment
- Each treatment mixed with soil and wet to squeeze test – 600 ml water
- Ten cucumber seeds planted in each pot with two tablespoons of plain soil on top.
- Humidity domes attached
- Treatments randomized on one greenhouse tray and placed in growth chamber for 2 weeks.
- At 2 weeks, remove from growth environment
- Count germination numbers
- Count secondary leaves (for reporting we combined fully developed leaves and leaf buds).
- Cut plants at root level and place in drying oven. After drying, biomass of each treatment is weighed with scale (MyWeigh Balance 601)

Results:

Treatment	Germination (%)	Secondary Leaves & Buds (count)	Biomass (grams)
DM	100	67	2.04
JL	84	57	1.87
C	98	41	1.30

Figure 1. Treatments from left to right: DM, JL, C.