Soil Enzyme Activity (Soil Microcosms) Part 1

Materials

Equipment
- Balance
- Scissors

Supplies
- Plastic flowerpots
- Dry, sieved soil
- Yeast extract
- Fertilizer (15-30-15)
- Markers
- Filter paper

Activities

- Set up five soil microcosms with different fertilizer treatments

Treatment	Fertilizer	Yeast Extract
Organic	0.0 g	1.6 g
Inorganic	0.2 g	0.0 g
Combined Low	0.1 g	1.6 g
Combined High	0.4 g	1.6 g
Unamended	0.0 g	0.0 g

Procedure

1. Cut out a circle of filter paper to fit the bottom of the flower pot.
2. Use a glass beaker to measure out ~300ml of soil.
3. Weigh out the appropriate amount of fertilizer and mix it into the soil.
4. Put the filter paper circle into the bottom of the flowerpot
5. Pour in the soil and fertilizer mix until it is ~1/2 inch from the top.
6. Water the soil until it is all moist.
7. Keep moist and incubate at room temperature for 2-3 weeks.

Soil Enzyme Activity (Alkaline Phosphatase Assay) Part 2

Introduction

Bacteria and fungi that break down insoluble nutrient sources in the soil produce extracellular enzymes. These are proteins that are produced inside the cell and exported out into the soil solution. The enzymes are active outside the cell where they catalyze reactions to break down the structure of the nutrient source to make it more accessible. The amount of an extracellular enzyme in the soil depends on the metabolic abilities of the soil organisms, the number of organisms present, the presence of substrate and the environment of the soil (pH, temp., ionic strength etc.). Because enzymes are costly for the cells to make, they are tightly regulated. Enzymes will only be made when they are needed.

One example of a common extracellular enzyme in soil is alkaline phosphatase. This enzyme is produced by many organisms in the soil. Its purpose is to remove the phosphate molecule from organic compounds such as phospholipids and nucleic acids. Once the phosphate is cleaved it becomes soluble and can be taken up by the cell. This is a very important activity because phosphate is often the limiting nutrient for microbial growth in soil.

In this lab you will be measuring the amount of active enzyme in soil samples by using a chromogenic substrate assay. In the presence of alkaline phosphatase, the colorless chemical para-nitrophenol phosphate is converted to para-nitrophenol, which is bright yellow. The amount of product formed can be measured using a spectrophotometer and the amount of enzyme activity can be calculated. You will also calculate the dry weight of the soil in order to standardize the results. The soils that you will be analyzing have been kept moist and incubated for ~2 weeks with the following amendments: 1.6g of yeast extract, 0.2g of inorganic fertilizer, 1.6g of yeast extract and 0.1g of inorganic fertilizer, 1.6g of yeast extract and 0.4g of inorganic fertilizer, or no addition.

Materials

Equipment

- incubator (37°C)
- clinical centrifuge
- 5 ml pipettes and pumps
- screw-top tubes (wide-mouth)
- 16 X 100 mm test tubes
- balance
- spectrophotometer (440nm)
- drying oven (100°C)
- aluminum weighing dishes

Samples

- soils from microcosms

Media and Reagents

- buffer (pH 10)
- 2 mM *p*-nitrophenol
- 0.5 M $CaCl_2$
- PNPP test solution

(para-nitrophenol phosphate in buffer)

Procedures

Phosphatase Assay

1. Weigh out two 2-gram portions of your group's soil sample and pour them into screw-cap tubes labeled "test" and "soil blank".
2. Label one other screw-cap tube as "reagent blank"
3. Pipette 5ml of 0.5 M $CaCl_2$ solution into each of the three tubes and shake well.
4. Pipette 1ml of PNPP solution into the tubes labeled "test" and "reagent blank".
5. Pipette 1ml of phosphate buffer into the "soil blank" tube to serve as a control.
6. Incubate all three tubes at 37°C for 1 hour.
7. Transfer 4ml of the liquid from each tube into labeled 16 X 100mm test tubes. (Be careful to avoid transferring sediment.)
8. Centrifuge the test tubes for 5 min. at 2500 rpm.
9. Transfer 3ml of the supernatant into clean test tubes (re-centrifuge if liquid is at all cloudy).
10. Set the wavelength on the spectrophotometer to 440nm.
11. Set the absorbance to zero with the "soil blank" tube.
12. Read and record the absorbance for the "test" and "reagent blank" tubes.
13. Set the absorbance to zero with a blank tube containing 3 ml of $CaCl_2$.
14. Read and record the absorbance of the prepared standards.
15. Plot the absorbance vs. concentration to make a standard curve.

Water Content Analysis

1. Weigh an aluminum dish and record the weight.
2. Weigh out ˜10g of your soil sample in the aluminum dish. Record the exact weight.
3. Put the samples in a 100°C oven overnight and let them cool in a desicator.
4. Weigh the dried sample and record the weight.

Lab Report

Name:_____ Date: _____

Alkaline Phosphatase Assay

Spectrophotometer Readings

	Absorbance	Net Absorbance (test – soil blank)	Concentration of *p*-Nitrophenol	Enzyme Activity
Organic fertilizer (test)				
control (reagent blank)		XXXX	XXXX	XXXX
Inorganic fertilizer				
control (reagent blank)		XXXX	XXXX	XXXX
Combined Low				
control (reagent blank)		XXXX	XXXX	XXXX
Combined High				
control (reagent blank)		XXXX	XXXX	XXXX
Unamended				
control (reagent blank)		XXXX	XXXX	XXXX

Standard Curve

Concentration	2.0 mM	1.0 mM	0.5 mM	0.25 mM	0.125 mM	0.063 mM
absorbance						

Water Content Analysis

Sample	Organic	Inorganic	Combined low	Combined high	unamended
Dish weight					
Wet weight with dish					
Wet weight of soil					
Dry weight with dish					
Dry weight of soil					
Water content					

Calculations

Water Content of Soil and Dry Weight

Water content = (wet weight of soil – dry weight of soil) / dry weight of soil

Dry weight of sample = (wet weight of sample / water content + 1)

Enzyme Activity

One unit of enzyme activity (U) is defined as the amount of enzyme that is able to convert 1 µmole of substrate to product in one minute. For soil assays, activity is reported as U per gram of dry soil

1. Calculate the amount of *p*-nitrophenol that was produced using the standard curve (remember that the total volume of liquid was 6ml even though you only measured the concentration in 3ml).

   _____ µmoles in 6 mls

2. Divide the amount of product by the number of minutes that the samples were incubated to find the value of U

   _____ µmoles / minute

3. Calculate the dry weight of the soil sample that was used in the incubation.

   _____ grams

4. Calculate the activity per gram of dry soil.

   _____ U / gram of dry soil