**Biochemical characterization of compost**

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Since microorganisms form a vital part of the food web inside the compost microcosm, microbial biomass serves as a potential measure of ecosystem functioning. Soil dehydrogenase is thought to be an indicator of overall microbial activity, because it occurs intracellular in all living microbial cells and is linked with microbial oxidoredution processes. Hence, microbial activity in terms of fluorescein diacetate [FDA] hydrolysis, dehydrogenase and alkaline phophatase is determined at monthly intervals in the crude extract of compost.

1. **Determination of Dehydrogenase enzyme activity (Casida et al., 1964).**

Biological activity/index of a soil is function of number of organisms present in soil coupled with their physiological efficiency. The rate of respiration can be used as an index of the biological activity of soil as it reflects the physiological efficiency of the organisms. Monitoring of dehydrogenases, which are respiratory enzymes and integral part of all soil organisms, give a measure of biological activity of soil at a given time.

During respiration, biological oxidation of reduced compounds occur which is catalyzed by dehydrogenases. During this process energy is evolved. The process can be represented as

RH2 + A → R + AH2 (2H+.2e-)

Where RH2 represents a reduced compounds (hydrogen donor) and A is the electron acceptor, which in aerobic organisms is oxygen. Under anaerobic condition compounds like 2,3,5 triphenyl tetrazoliumchloride (TTC) can act as an electron acceptor. In the process TTC gets reduced to a pink coloured compound triphenyl formazan (TPF) which can be quantitatively extracted by methanol and measured colorimetrically.

TTC + 2H+.2e- → TPF

The dehydrogenase can be assayed and activity can be expressed as the rate of formation of TPF from TTC. Higher the biological activity faster will be the formation of TPF.

***Material:***

* 1. Air tight screw cap test tube.
	2. Parafilm

***Reagents***

1. 2,3,5- Triphenyl tetrazolium chloride (TTC) solution (3%): Dissolve 3 g TTC in 100 mL water. Store in an amber coloured bottle.

2. Methanol (AR grade)

3. Glucose solution (1%): Dissolve 1 g glucose in 100 mL water.

4. Standard Triphenyl Formazan (TPF, 1000 ppm): Dissolve 100 mg TPF in 100 mL alcohol.

***Equipment/instruments***

* 1. Incubator
	2. Spectrophotometer
	3. Sonicator

**Procedure**

1. 1g of air-dried sample was taken in an air-tight screw capped test tube (15ml cap).
2. Add 0.2 ml of 3% TTC solution in each of the tubes to saturate the sample and add 0.5 ml of 1% glucose solution in each tube.
3. Gently tap the bottom of the tube to drive out all trapped oxygen and a water seal is formed above the soil.
4. Ensure that no air bubbles are formed, left the tubes for incubation at 37°0.5ºC for 24 hours.
5. After incubation add 10 ml of methanol, shake vigorously and allow to standing for 6 hour.
6. Withdraw clear pink colored supernatant liquid and take readings with a spectrophotometer at a wavelength of 485nm (blue filter).
7. Extrapolate Triphenyl formazan formed from the standard curve drawn in range of 10 to 90 mg TPF ml¯¹. Express results as mg TPF formed per hour per gram soil.
8. **Fluorescien Diacetate (FDA) hydrolysis activity (Green et al., 2006)**

During the past few years, interest in the size and activity of the soil microbial biomass is increasing, partly due to the importance of this information in integrated bio- ecosystem and global change provides general measures of organic matter turnover in natural habitat as about 90% of the energy in the soil environment flows through microbial decomposers

Fluorescein diacetate [3’, 6’- diacetylfluorescein diacetate (FDA)] can be used to measures microbial activity in soil. Fluorescein diacetate is hydrolyzed by a number of different enzymes, such as proteases, lipases and esterase’s. The equation of the reaction is



The product of this enzymatic reaction is fluorescein which can be visualized within cells by fluorescein microscopy. Fluorescein released by spectrophotometer.

***Equipment:***

1. Soil.
2. Conical flasks.
3. Stoppers.
4. Centrifuge.
5. Whatman filter paper.

 ***Reagents:***

1. Sodium Phosphate buffer: 60mM (pH 7.6) – 22.74 g Na3PO4.12H2Owere dissolved in approximately 800 ml distilled water and make the volume to 1L. The buffer was stored in the fridge (40 C) and pH checked on the day of use.
2. Acetone
3. 4.9 mM FDA; Dissolve 20 mg FDA substrate in 10 ml Acetone.
4. 2000µg Fluorescein ml-1 Stock solution – Fluorescein solution salt (0.2265g) was dissolved in approximately 80mL of 60mM Potassium Phosphate buffer pH 7.6 and the content made up to 100ml with buffer.
5. 20 µg Fluorescein ml-1 Standard solution – Stock solution (1ml of 2000µg Fluorescein mL-1) is added to a 100 ml volumetric flask and made up to mark with 60 mm Potassium phosphate buffer pH 7.6 .
6. 1-5 µg ml-1 standards were prepared by using Fluorescein.

***Procedure:***

1. Take 1g soil in 100ml of conical flasks/plastic bottle and add 50ml of 60mM sodium phosphate buffer.
2. Add 0.5ml 2000 µg FDA ml -1 stock solution.
3. Stoppard the flasks and then shake by hand.
4. Placed the sample in incubator at 370C for 3 hr.
5. Once removed from the incubator add 2ml of acetone immediately and swirl to mix the content and terminate FDA hydrolysis.
6. Transfer about 30 ml of soil suspension to a 50 ml centrifuge tube and centrifuge at 8000 RPM for 5 min.
7. The supernatant from every sample filtered and read at 490 nm on spectrophotometer.
8. **Determination of Alkaline Phosphatase Enzyme Activity**

The general name phosphatase has been used to describe a broad group of enzymes that catalyze the hydrolysis both esters and anhydrides of H3PO4. In this are included five major groups:

1. Phosphoric monoester hydrolases (phosphomonoesterases)
2. Phosphoric diester hydrolases (phosphodiesterases)
3. Triphosphoric monoester hydrolases
4. Enzymes acting on phosphoryl-containing anhydrites
5. Enzymes acting on P-N bonds like phosphoamidases

Due to relative importance of phosphomonoesterases in soil organic P mineralization and plant nutrition, their assay in soil assumes more importance. The enzymes are classified as acid and alkaline phosphomonoesterases, because they show optimum activities in their respective pH ranges. Alkaline phosphomonoesterase activity is derived from microorganisms only, while acid phosphomonoesterase is contributed both by plant roots and soil-inhabiting microbes.

Of the various methods available for assay of phoshomonoesterases activity in soils, the \method developed by Tabatabai and B remner (1969) is the most rapid, accurate and precise. It involves colorimetric estimation of the p-nitrophenol released by phosphomonoesterase activity, when the soil is incubated with buffer (at pH 6.5 and 11 for acid and alkaline phosphomonoesterase activities, respectively) sodium p-nitrophenyl phosphate solution and toluene. Alkaline phenol has a yellow colour, allowing it to be estimated colorimetrically. The CaCl2-NaOH treatment described for extraction of p-nitrophenol after incubation serves to stop the phosphomonoesterase activity, to develop yellow colour and to provide quantitative recovery of p-nitrophenol from soils.

***Reagents:***

1. Toluene
2. Modified universal buffer (MUB) stock solution: Dissolve 12.1 g tris (hydroxymethyl) aminomethane (THAM), 11.6 g maleic acid, 14.0 g citric acid and 6.3 g boric acid in 488 mL of 1N sodium hydroxide. Dilute the solution to 1L with distilled water. Store it in refrigerator.
3. MUB pH 6.5 and 11: Pour 200 mL of stock solution in a 500 mL beaker containing a magnetic stirrer bar, and place the beaker on the magnetic stirrer. Titrate the solution to pH 6.5 with 0.1N hydrochloric acid or to pH 11 by o.1N sodium hydroxide. Adjust the volume to 1L with water.
4. p-Nitrophenyl phosphate solution (0.025M): Dissolve 0.42 g p-nitrophenyl phosphate tetrahydrate (or 0.464 g hexahydrate salt) in about 40 mL MUB of 6.5 (for assay of acid phosphomonoesterase), and dilute the solution to 50 mL with MUB of the same pH. Store the solution under refrigeration.
5. 0.5M Calcium chloride solution: Dissolve 73.5 g CaCl2. H2O in about 700 mL of water and make up the volume to 1L.
6. 0.5M Sodium hydroxide solution: Dissolve 20 g NaOH in about 700 mL water and make up the volume to 1L.
7. Standard p-nitrophenol solution (1000 ppm): Dissolve 1.0 g p-nitrophenol in about 70 mL of water and dilute the solution to 1L. Store the solution under refrigeration.

***Apparatus:***

1. Incubator
2. Spectrophotometer.

***Procedure:***

One gram of sample is taken in a 50-ml flask, 0.2 ml of toluene is added and 4ml of MUB (pH 6.5 for assay of acid phosphatase solution or pH 11 for assay of alkaline solution), 1ml of p-nitrophenyl phosphate solution made in the same buffer, and swirl the flask for a few seconds to mix the contents then stopper the flask, and place it in an incubator at 37ºC. After 1 hour remove the stopper, add 1 ml of 0.5 M CaCl2 and 4 ml of 0.5 M NaOH. Swirl the flask for a few seconds, and filter the soil suspension through a Whatman no.2v folded filter paper. Measure the yellow color intensity of the filtrate with a Spectrophotometer at 440 nm wavelength.

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