UREASE PROTOCOL
Steve Allison
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based on Sinsabaugh (1998, unpub) and Kandeler and Gerber 1988

Purpose: measure the activity of urease (amidohydrolase, EC 3.5.1.5) in environmental samples. Urease is the common name for enzymes that decompose urea into carbon dioxide and ammonium. The enzyme is considered important in soil nitrogen cycling, particularly in agricultural systems.

1. Prepare reagents:

40 mM urea
- 0.240 g urea
- 100 ml sodium acetate buffer, pH 5

0.3 M NaOH
- 12 g NaOH
- 1000 ml DI water

Na salicylate solution
- 17 g sodium salicylate
- 120 mg sodium nitroprusside
- 100 ml DI water

Na salicylate/NaOH solution (make fresh daily)
- 1 part 0.3 M NaOH
- 1 part Na salicylate solution
- 1 part DI water

Na dichloroisocyanide solution (make fresh daily)
- 0.1 g sodium dichloroisocyanide
- 100 ml DI water

2. Sample preparation (refer to pNP protocol): Blend 2 g wet soil with 60 ml, 50 mM pH 5 acetate buffer on whip setting for 1 min and pour suspension into a screw-cap Nalgene bottle.

3. While stirring vigorously, pipet 750 µl suspension into 2 ml centrifuge tubes. Use 3-4 replicates and 2 replicate suspension controls (controls get acetate buffer in place of substrate). Also use 4 substrate controls/11 samples: replace soil suspension with acetate buffer.

4. Add 750 µL of urea solution to each of the tubes (except suspension controls, which get acetate buffer). The final substrate concentration is 20 mM urea.

NOTE: pH optima for ureases is reportedly in 7-8 range. Depending on experimental design, you may want to select another buffer. Apparent Km is a function of particle dispersion and mixing. This substrate concentration (20 mM) may not be high enough to reach substrate saturation in all systems.

5. Standards: Alpkem standard (1000 ppm NH_4-N) is 71.43 mM. Dilute this 100X in acetate buffer to get a standard of 714 nmol/ml. 2X serial dilutions of this solution yield standards of 357, 179, 89.3, 44.6, and 22.3 nmol/ml. Contstruct a standard curve using this concentration range.

6. Incubate the tubes at room temperature for 2-18 h while shaking.

7. Centrifuge tubes at 10,000 rpm for 1 min.

NOTE: The next part is from Kandeler and Gerber 1988 (in Alef and Nannipieri, Methods in Applied Soil Microbiology and Biochemistry, p. 318). It omits the addition of an acid KCl solution to terminate the reaction and dilute the sample.

8. Ammonium determination: Pipet 0.5 ml supernatant or standard into 10 ml culture tubes, add 2.5 ml NaOH/Na salicylate solution and 1 ml Na isocyanide solution. Also prepare a blank with acetate buffer instead of supernatant. Let stand at room temperature for 30 min.

9. Read optical density of each sample/standard on spectrophotometer at 690 nm, using the blank to zero the spectrophotometer.

10. Calculate activity as nmole of ammonium released per hour per gram of soil.

Final OD = (mean OD of assay wells) - (mean OD of sample control wells) - (mean OD of substrate control wells)

Activity (µmole NH_4 h^{-1} g^{-1}) =

final OD/[(EC/µmole/ml)/(1.5 ml/assay) (incubation time, h) (g dry soil used to make sample suspension/60 ml sample suspension) (0.75 ml sample suspension)]