DENITRIFIER ENZYME ACTIVITY

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Materials

225 mL jelly jars, with lids fitted with Hungate septa
Glucose
KNO_3
Acetylene
10 mL disposable plastic syringes
N_2
Shaker

Preparation

1. 1 m*M* Glucose - 1 m*M* KNO_3 solution: Dissolve 0.180 g glucose and 0.101 g KNO_3 to 1,000 mL in a volumetric flask.
2. Purify the acetylene by passing the gas through a solution of $CuCl_2$ in concentrated HCl and then water.

Procedure

1. Place 50 g soil and 30 mL glucose-KNO_3 solution in jelly jar, and seal with a fresh lid. Shake vigorously.
2. Include 2 control jars, with 30 mL glucose-KNO_3 solution but no soil.
3. Alternately evacuate and flush with N_2 four times. Equilibrate with atmospheric pressure after the last N_2 flush using a glass syringe.
4. Remove 20 mL of the headspace and replace with 20 mL purified acetylene.
5. Shake the flask vigorously, then incubate on a rotary shaker (about 100 rpm).
6. Draw at least 4 syringe samples during an incubation period not to exceed 2 h. For each injection, add 3 mL of room air, pump the syringe 3 times with headspace, then draw the 3 mL sample.

References

1. Tiedje, J.M. 1982. Denitrification. pp 1011-1026. *IN*: Page, A.L., R.H. Miller, and D.R. Keeney (eds). Methods of soil analysis. Part 2. Second edition. American Society of Agronomy, Madison, WI, USA.
2. Tiedje, J.M. 1994. Denitrifiers. pp 245-267. *IN*: Weaver, R.W., J.S. Angle, and P.S. Bottomley (eds). Methods of soil analysis. Part 2. American Society of Agronomy, Madison, WI, USA.