

5-Day Biochemical Oxygen Demand Test (BOD5)

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Preparation of Reagents

1. *Phosphate Buffer*—Dissolve 8.5 g KH_2PO_4 , 21.75 g K_2HPO_4 , 33.4 g $\text{Na}_2\text{HPO}_4 \cdot 7\text{H}_2\text{O}$, and 1.7 g NH_4Cl in 500 mL distilled water. Stirring and/or heating may be required. Dilute to 1 L.
2. *Magnesium sulfate solution*—Dissolve 4.5 g $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ in distilled water, dilute to 200 mL.
3. *Calcium chloride solution*—Dissolve 5.5 g CaCl_2 in distilled water, dilute to 200 mL.
4. *Ferric chloride solution*—Dissolve 0.05 g $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$ in distilled water, dilute to 200 mL.
5. *Sodium sulfite solution*—Dissolve 1.575 g Na_2SO_3 in 1000 mL distilled water.
**Not stable so prepare daily.
6. *Glucose-glutamic acid solution*—Add 150 mg of pre-dried glucose and 150 mg of pre-dried glutamic acid to distilled water, dilute to 1 L.
**Prepare immediately before use, unless maintained in sterile condition.
**Store mixtures at 4 degrees C or lower.
7. *Ammonium chloride solution*—Dissolve 1.15 g NH_4Cl in 500 mL distilled water and adjust pH to 7.2 using NaOH solution. Dilute to 1 L.
8. *Allylthiourea (ATU) solution*—Dissolve 2.0 g $\text{C}_4\text{H}_8\text{N}_2\text{S}$ in 500 mL distilled water, dilute to 1 L.
**Store at 4 degrees C.
**Not stable for more than 2 weeks.
9. *Seed suspension*—Dissolve the contents of 5 capsules of PolySeed in 2500 mL

Preparation of Samples

1. Transfer 25, 50, and 100 mL of sample water from each site to 300 mL glass incubation bottles.