

Denitrifying bacteria in the limnetic zone of Lake Tota, Colombia

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Composition of culture media for the isolation and preservation of denitrifying bacteria

Minimal medium for denitrifying bacteria (MMD). Following Atlas, 2005: Solution A (980 mL), Solution B (10 mL), and Solution C (10 mL). Solution A (980 mL): KNO_3 (5 g), Carbon source (4 g), $(\text{NH}_4)_2\text{SO}_4$ (1 g), K_2HPO_4 , 3 H_2O (0.87 g), KH_2PO_4 (0.54 g), deionized water (980 mL). Solution B (in 100 mL): $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ (2 g). Deionized water (100 mL). Solution C (In 100 mL of deionized water): $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$ (0.2 g), $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$ (0.1 g), $\text{MnSO}_4 \cdot \text{H}_2\text{O}$ (0.05 g), $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ (0.01 g), $\text{Na}_2\text{MoO}_4 \cdot 2\text{H}_2\text{O}$ (0.01 g), HCl, 0.1 N (100 ml). These three solutions were stored at 25 °C.

To prepare 1000 mL of MMD, the following volumes of solutions A to C were aseptically mixed: 980 mL cold sterile of solution A, 10 mL of cold sterile solution B, and 10 mL of cold sterile solution C.

Medium for nitrite oxidizing bacteria (NOB medium). Following Atlas, 2005 and APHA, 2012. Composition (g L^{-1}): KNO_2 (0.3 g), $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ (0.1875 g), KHCO_3 (1.5 g), K_2HPO_4 (0.5 g), KH_2PO_4 (0.5 g), NaCl (0.1875 g), $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$ (0.0125 g), $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$ (0.01 g), and deionized water (1 L). Preparation: Dissolve the ingredients in deionized water and adjust to a pH of 7.8 to 8.1 with diluted NaOH, allowing for a change in pH during sterilization.

Sodium nitrate agar. Sodium nitrate medium (Atlas, 2005). Composition (g L^{-1}): KNO_3 (2 g or 0.5 g), CaCO_3 (5 g), $\text{C}_6\text{H}_{12}\text{O}_6$ (10 g), Saline solution (50 mL), trace elements (1 mL) agar (if medium solid (18 g), and deionized water (950 mL).