202. DESULFONEMA MAGNUM MEDIUM

Solution A:

- NaCl 25.00 g
- MgCl₂ x 6 H₂O 5.60 g
- MgSO₄ x 7 H₂O 6.80 g
- CaCl₂ x 2 H₂O 1.40 g
- KCl 0.72 g
- KH₂PO₄ 0.14 g
- NH₄Cl 0.25 g
- Trace element solution SL-10 (see medium 320) 1.00 ml
- Selenite-tungstate solution (see medium 385) 1.00 ml
- Na-resazurin solution (0.1% w/v) 0.50 ml
- Distilled water 1000.00 ml

Solution B:

- Na-benzoate 0.60 g
- Distilled water 10.00 ml

Solution C:

- Vitamin solution (see medium 141) 10.00 ml

Solution D:

- KAl(SO₄)₂ x 12 H₂O 0.48 g
- Distilled water 10.00 ml

Solution E:

- Na₂CO₃ 1.00 g
- Distilled water 20.00 ml

Solution F:

- Na₂S x 9 H₂O 0.30 g
- Distilled water 10.00 ml

Solution A is sparged with 80% N₂ and 20% CO₂ gas mixture for 30 – 45 min to reach a pH of around 6, then distributed under the same gas atmosphere into anoxic Hungate-type tubes and autoclaved. Solutions B, D, and F are autoclaved separately under 100% N₂ gas. Solution E is autoclaved under 80% N₂ and 20% CO₂ gas mixture. Solution C is prepared under 100% N₂ gas and sterilized by filtration.

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To complete the medium appropriate amounts of solutions B to F are added to the sterile solution A in the sequence as indicated. Final pH of the medium should be 7.0. After completion the medium should equilibrate overnight and a white precipitate should be apparent.

*Note:* Addition of 10 - 20 mg sodium dithionite per liter (e.g. from 5% (w/v) solution freshly prepared under N₂ and filter-sterilized) just before inoculation may stimulate growth at the beginning. For transfers use 5 - 10% inoculum.