



Main sol. 386a

Solution A	967.00	ml
Solution B	30.00	ml
Solution C	1.00	ml
Solution D	10.00	ml
Sulfur (powdered)	10.00	g

Solution A is sparged with 80% N₂ and 20% CO₂ gas mixture for 30 – 45 min to make it anoxic, then distributed under the same gas atmosphere into anoxic Hungate-type tubes or serum vials and autoclaved. Solution B is autoclaved separately under 80% N₂ and 20% CO₂ gas mixture. Solutions C and D are prepared under a 100% N₂ gas atmosphere and sterilized by filtration. To complete the medium solutions B to D are added to the sterile solution A in the sequence as indicated. Before inoculation, appropriate amounts of sterile sulfur are added to the vials with the medium. Sulfur is sterilized by steaming for 3 hours on each of 3 successive days (see medium 35) and added aseptically to the sterile medium while retaining anoxic conditions. The final pH of the medium should be at 7.0.