Main sol. 667

Solution A	973.00	ml
Solution B	30.00	ml
Solution C	1.00	ml
Solution D	5.00	ml
Solution E	1.00	ml

Solution A is sparged with 80% N_2 and 20% CO_2 gas mixture to reach a pH below 6 (at least 30 min), then distributed under same gas atmosphere into anoxic Hungate-type tubes or serum vials and autoclaved. Solution B is autoclaved separately under 80% N_2 and 20% CO_2 gas atmosphere. Solutions C is prepared under 100% N_2 gas atmosphere and sterilized by filtration. Solution D is autoclaved under 100% N_2 gas. Solution E is prepared under 80% N_2 and 20% CO_2 gas atmosphere and sterilized by filtration. To complete the medium appropriate amounts of solutions B to E are added to the sterile solution A in the sequence as indicated. Final pH of the medium should be at 7.0 - 7.2.