1042. VULCANIBACILLUS MEDIUM

Sea Salts (SIGMA) 30.00 g
NaNO₃ 1.00 g
Wolfe's mineral elixier (see medium 792) 2.00 ml
VOSO₄ x 5 H₂O solution (0.01% w/v) 0.50 ml
Na-resazurin solution (0.1% w/v) 0.50 ml
FeSO₄ x 7 H₂O 0.10 g
NaHCO₃ 1.00 g
Yeast extract 0.50 g
Na-pyruvate 1.00 g
Vitamins solution (see medium 141) 20.00 ml
Distilled water 1000.00 ml

Dissolve ingredients (except ferrous sulfate, bicarbonate, yeast extract, pyruvate and vitamins), adjust pH to 7.0 and sparge medium with 100% N₂ gas for 30 – 45 min to make it anoxic. Dispense the medium under same gas atmosphere into Hungate-type tubes or serum vials and autoclave. Add ferrous sulfate, bicarbonate, yeast extract, pyruvate and vitamins from sterile anoxic stock solutions prepared under 100% N₂ gas and bicarbonate from a sterile anoxic stock solution prepared under 80% N₂ and 20% CO₂ gas atmosphere. Prepare the stock solution of ferrous sulfate by dissolving 1% (w/v) FeSO₄ x 7 H₂O in 0.1 N H₂SO₄. The stock solutions of pyruvate and vitamins should be sterilized by filtration. Adjust pH of the complete medium to 6.8-7.0.
Note: It may be necessary to add 10-20 mg sodium dithionite per liter (e.g. from 5% (w/v) solution, freshly prepared under 100% N₂ and filter-sterilized), if the medium is not completely reduced after inoculation.

For DSM 23802 replace pyruvate with 2.50 g/l D-glucose added to the autoclaved medium from an anoxic sterile stock solution.