## 332. METHANOGENIUM BOURGENSE MEDIUM

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|---|---|---|
| $NH_4Cl$ | 1.0 | g |
| $K_2HPO_4$ x 3 $H_2O$ | 0.4 | g |
| $MgCl_2$ x 6 $H_2O$ | 0.1 | g |
| Na-formate | 5.0 | g |
| Na-acetate | 1.0 | g |
| Trypticase peptone (BD BBL) | 1.0 | g |
| Yeast extract (OXOID) | 1.0 | g |
| Na-resazurin solution (0.1% w/v) | 0.5 | ml |
| L-Cysteine-HCl x $H_2O$ | 0.5 | g |
| $Na_2CO_3$ | 1.5 | g |
| $Na_2S$ x 9 $H_2O$ | 0.2 | g |
| Distilled water | 1000.0 | ml |

Dissolve ingredients except cysteine, carbonate and sulfide. Sparge medium with 80% $H_2$ and 20% $CO_2$ gas mixture for 30 – 45 min to make it anoxic. Add and dissolve cysteine, then dispense medium under same gas atmosphere into anoxic Hungate-type tubes or serum vials and autoclave. Add carbonate from a sterile anoxic stock solution prepared under 80% $N_2$ and 20% $CO_2$ gas mixture and sulfide from a sterile anoxic stock solution prepared under 100% $N_2$ gas. Prior to use check pH of complete medium and adjust to 6.8 - 7.0, if necessary.
*Note: After growth has started and the culture is becoming turbid add sterile 80% $H_2$ and 20% $CO_2$ gas mixture to 0.5 - 1 bar overpressure.*