Main sol. 28 (from medium 28)

Solution A	460.00	ml

1. Aliquot Solution A into 100 mL screw-cap bottles, filled with 46 mL each. Bubble with N_2/ CO_2 and autoclave at 121°C for 15 min (as decribed below).

2. Prepare the following solutions (resazurin, bicarbonate and Pfennig's heterotrophic salts) and sterilize as given below.

Resazurin solution	450.00	ml
Bicarbonate solution	50.00	ml
Pfennig's heterotrophic salts solution	26.00	ml

3. Add bicarbonate solution and Pfennig's heterotrophic salts to the resazurin (complete volumina, i.e. 50 mL bicarbonate solution and 26 mL Pfennig's heterotrophic salts solution). Bubble with CO_2 in an ice bath under sterile conditions.

4. Fill 50 ml of this mixture to each bottle of solution A (46 mL + 50 mL).

5. Before use, add 4 ml sulfide solution (1.5%) and 0.1 ml Vitamin B_{12} solution to each 100 mL bottle.

Sulfide solution, 1.5%	40.00	ml
Vitamin B_{12} solution	1.00	ml

6. Adjust the pH with filter-sterilised 1M Na_2CO_3 to 7.1-7.3.

7. If needed, aliquot into sterile, N_2 gassed screw-cap tubes under N_2 gas.

8. During the first 24 h, the iron of the medium precipitates in the form of black flocks. No other sediment should arise in the otherwise clear medium.

9. Feed the actively growing culture periodically with neutralized 3% solution of sodium sulfide (use 1 -3 mL/100 mL depending on strain and cultivation stage) to replenish sulfide and with other supplement solutions (see Ref. 3365).

Neutralized sulfide solution 3% (w/v)	10.00	ml