441. DIAZOTROPHIC MEDIUM (RBA)

Solution A:

KH_2PO_4	0.100	g
K_2HPO_4	0.900	g
NaCl	0.100	g
$CaCl_2$ x 2 H_2O	0.100	g
$MgSO_4$ x 7 H_2O	0.100	g
Na_2MoO_4 x 2 H_2O	0.005	g
$NaVO_3$ x H_2O	0.005	g
$MnSO_4$ x H_2O	0.005	g
$FeSO_4$ x 7 H_2O	0.010	g
Yeast extract	0.050	g
Trace element sol. SL-6 (see medium 27)	3.000	ml
Distilled water	950.000	ml
Agar (if necessary)	15.000	g

Adjust pH to 7.3.

Solution B:

Na_2-succinate	1.000	g
DL-Malate	2.000	g
Na-pyruvate	1.000	g
D-Mannitol	2.000	g
D-Glucose	2.000	g
Distilled water	50.000	ml

Adjust pH to 7.3.

Sterilize solution A separately at 121°C for 15 min., cool to 50°C and then mix aseptically with filter-sterilized solution B and 5.0 ml of filter-sterilized standard vitamin solution (see medium 428).

RBA is an ammonium-free medium which has successfully been used for the isolation, growth and purity check of a broad spectrum of nitrogen fixing bacteria (Ref. 3363). For microaerophilic nitrogen-fixing bacteria use semisolid medium with 0.3% end concentration of agar and incubate the liquid cultures under 10% (v/v) air and 90% (v/v) N_2.