

## Nutrient Media

When unsealing an ampoule with a culture obtained from the All-Russian Collection of Microorganisms (VKM) and for initial reinoculations, it is recommended to use the medium specified for the strain in the Catalogue.

General comments to the descriptions of the media

1. In all cases when the composition of a medium is given as the description of particular solutions, this implies that the given solutions should be sterilized separately and mixed upon sterilization.
2. In all cases, besides when specified otherwise, a given mode of sterilization assumes autoclaving. If the sterilization conditions of a medium are not specified, they can be set arbitrarily within the limits accepted in the general microbiological practice.
3. The purity of the reagents is not specified in the descriptions. It is assumed that the respective reagents will be used, specifically, peptone, triptone, yeast extract, etc. marked 'Bacto' or 'For microbiological work'. For some media, descriptions for preparing the components are given instead of using commercial concentrates.
4. The pH values given indicate the magnitudes, which a medium shall have prior to inoculation. In the cases when some solutions are described and errors are possible, it is specified: pH of the medium. An insufficiently alkaline medium shall be alkalinized, usually with a sterile solution of NaOH or NaHCO<sub>3</sub>; an insufficiently acidic one should be acidified, usually with a sterile solution of HCl.
5. Some media differ in a few components or their concentrations. When a medium similar to the already mentioned one is given, the reader is referred to the latter. The references can (i) specify a compound added and its concentration; (ii) specify the set of trace elements or vitamins; (iii) specify the differences in the concentrations of particular compounds. In the latter case, the zero concentration of a substrate implies that it is not to be added.

### **B 1. ALLOMONAS ENTERICA MEDIUM**

Peptone 10.0 g

NaCl 20.0 g

Beef extract 5.0 g\*

Distilled water 1000.0 ml

pH 7.0

Sterilize at 121 C for 15 min.

\*Beef extract may be replaced with 3.0 g yeast extract.

### **2. YEAST WATER**

Pressed yeast 200.0 g

Tap water 1000.0 ml

*Preparation of yeast water:* suspend 200.0 g of pressed yeast in 1000.0 ml of tap water. Twice filter hot through a paper filter or centrifuge.

Sterilize at 121 C for 15 min.

### **Ac, B 3. POTATO AGAR**

Potato 200.0 g

Agar 20.0 g

Tap water 1000.0 ml

Boil 200g scrubbed and sliced potatoes in 1000.0 ml water for 1 hour, filter cold through a cotton-gauze filter, fill up distilled water to 1000.0 ml, add agar. Do not use new potatoes.

pH 7.0

Sterilize at 121 C for 15 min.

### **4. WORT AGAR**

Wort extract (malt extract) 20.0 g

Agar 20.0 g  
Distilled water 1000.0 ml  
Sterilize at 121°C for 15 min.

**Ac, B 5. PEPTONE MEAT AGAR**

Peptone 10.0 g  
NaCl 5.0 g  
Beef extract 3.0 g  
Agar 20.0 g  
Distilled water 1000.0 ml  
pH 7.0  
Sterilize at 121 C for 15 min.

**6. PEPTONE MEAT BROTH**

Peptone 10.0 g  
NaCl 5.0 g  
Beef extract 3.0 g  
Distilled water 1000.0 ml  
pH 7.2-7.4  
Sterilize at 121 C for 15 min.

**F 7. OATMEAL AGAR (OA)**

Oatmeal 30.0 g  
Agar 15.0 g  
Tap water 1000.0 ml  
Cook 20 g of oatmeal in 1000.0 ml tap water for 20 min, filter through 2 layers of gauze, dilute to 1000.0 ml and add 15.0 g agar.  
Sterilize at 121 C for 15 min.

**Ac 8. PEPTONE MAIZE AGAR**

Peptone 5.0 g  
Maize extract 5.0 g  
Starch (soluble) 10.0 g  
NaCl 5.0 g  
CaCl<sub>2</sub> 0.5 g  
Agar 20.0 g  
Tap water 1000.0 ml  
pH 7.2-7.4  
Sterilize at 121 C for 15 min.

**B, F, Y 9. MALT AGAR 7 BALLING (MA7B)**

Malt extract Balling 7 degrees 1000.0 ml  
Agar 20.0 g  
Adjust pH to 7.0  
Sterilize at 111 C for 30 min.

**F 10. MALT AGAR 2 BALLING (MA2B)**

Malt extract Balling 2 degrees 1000.0 ml  
Agar 20.0 g  
Sterilize at 111 C for 30 min.

**F 11. MALT AGAR 3.5 BALLING (MA3.5B)**

Malt extract Balling 3.5 degrees 1000.0 ml  
Agar 20.0 g

Sterilize at 111 C for 30 min.

### **F 12. CZAPEK MEDIUM (CZ)**

NaNO<sub>3</sub> 3.0 g

K<sub>2</sub>HPO<sub>4</sub> 1.0 g

KCl 0.5 g

MgSO<sub>4</sub> x 7H<sub>2</sub>O 0.5 g

FeSO<sub>4</sub> x 7H<sub>2</sub>O 0.01 g

Sucrose 30.0 g

Agar 20.0 g

Distilled water 1000.0 ml

pH 6.0

Sterilize at 121 C for 30 min.

### **Ac, F 13. POTATO-DEXTROSE AGAR (PDA)**

Grated potato 200.0 g

Dextrose 20.0 g

Agar 20.0 g

Tap water 1000.0 ml

Boil 200g scrubbed and sliced potatoes in 1000.0 ml water for 1 hour, filter cold through a cotton-gauze filter, add water to the initial volume, add glucose and agar. Do not use new potatoes.

pH 6.5-7.0

Sterilize at 121 C 15 min.

### **F 14. POTATO-CARROT AGAR (PCA)**

Grated potato 20.0 g

Grated carrot 20.0 g

Agar 20.0 g

Tap water 1000.0 ml

Boil potato and carrot in 1000.0 ml of water for 1 h, filter cold through a cotton-gauze filter, add water to the initial volume and add agar. Do not use new potatoes.

pH 7.0-7.1

Sterilize at 121 C for 15 min.

### **15. LB MEDIUM**

Tryptone 10.0 g

Yeast extract 5.0 g

NaCl 10.0 g

Tap water 1000.0 ml

pH 7.5

Sterilize at 121 C for 15 min.

### **16. YT MEDIUM**

Tryptone 8.0 g

Yeast extract 5.0 g

NaCl 5.0 g

Tap water 1000.0 ml

pH 7.0

Sterilize at 121 C for 15 min.

### **B, Y 17. GLUCOSE PEPTONE AGAR (GPA)**

Glucose 40.0 g

Peptone 10.0 g

Yeast extract 5.0 g  
Agar 20.0 g  
Distilled water 1000.0 ml  
pH 7.0  
Sterilize at 111 C for 30 min.

**Y 18. GLUCOSE PEPTONE AGAR WITH 5% NaCl**

Glucose 40.0 g  
Peptone 10.0 g  
Yeast extract 5.0 g  
NaCl 50.0 g  
Agar 20.0 g  
Distilled water 1000.0 ml  
Sterilize at 111 C for 30 min.

**19. MALT AGAR 7 BALLING WITH 5% NaCl (MA7B+5% NaCl)**

Malt extract Balling 7 degrees 1000.0 ml  
NaCl 50.0 g  
Agar 20.0 g  
Sterilize at 111 C for 30 min.

**B 20. LIESKE MEDIUM**

Mg-acetate 0.1 g  
Agar 15.0 g  
Distilled water 1000.0 ml

**21. MANNITOL AGAR WITH YEAST WATER**

Mannitol 10.0 g  
Agar 15.0 g  
10% yeast water 100.0 ml  
Tap water 900.0 ml

*Preparation of yeast water:* suspend 200.0 g of pressed yeast in 1000.0 ml of tap water. Twice filter hot through a paper filter or centrifuge.

Sterilize at 121 C for 15 min.

**F 22. MALT AGAR 3.5 BALLING WITH 60% SUCROSE (MA60S)**

Malt extract Balling 3.5 degrees 1000.0 ml  
Sucrose 600.0 g  
Agar 20.0 g  
Sterilize at 121 C for 15 min.

**23. MALT AGAR 3.5 BALLING WITH 40% SUCROSE (MA40S)**

Malt extract Balling 3.5 degrees 1000.0 ml  
Sucrose 400.0 g  
Agar 20.0 g  
Sterilize at 121 C for 15 min.

**24. MALT AGAR 3.5 BALLING WITH 20% SUCROSE (MA20S)**

Malt extract Balling 3.5 degrees 1000.0 ml  
Sucrose 200.0 g  
Agar 20.0 g  
Sterilize at 121 C for 15 min.

**F 25. MALT AGAR 3.5 BALLING WITH FILTER PAPER**

Malt extract Balling 3.5 degrees 1000.0 ml  
Agar 20.0 g  
Sterilize at 121 C for 15 min.  
Sterilize filter paper strips with dry heat and soak it with sterile medium.

#### **F 26. MANURE AGAR**

Horse manure 100-125 g  
Agar 25.0 g  
Distilled water 1000.0 ml  
pH 6.5-7.0  
Boil manure in 1000.0 ml of water for 10 min, then keep for 16-20 hours, filter through 1-2 layers of filter paper, adjust to the initial volume, add agar.  
Sterilize at 121 C for 15 min.

#### **27. PEPTONE LACTOSE AGAR**

Peptone 10.0 g  
Lactose 10.0 g  
Agar 15.0 g  
Tap water 1000.0 ml  
Sterilize at 121 C for 15 min.

#### **28. TRYPTOSE AGAR**

Tryptose 20.0 g  
Dextrose 1.0 g  
NaCl 5.0 g  
Agar 15.0 g  
Thiamine-HCl 0.005 g  
Distilled water 1000.0 ml  
Sterilize at 121 C for 15 min.

#### **29. PEA AGAR**

Yellow peas 100.0 g  
K<sub>2</sub>HPO<sub>4</sub> 0.5 g  
Sucrose 10.0 g  
Agar 20.0 g  
Tap water 1000.0 ml  
Boil peas in 1000.0 ml of water, filter through gauze, add water to the initial volume; add phosphate, sucrose and agar.  
Sterilize at 121 C for 15 min.

#### **30. AZOTOBACTER MEDIUM 1**

Glucose 5.0 g  
Mannitol 5.0 g  
CaCl<sub>2</sub> x 2 H<sub>2</sub>O 0.1 g  
MgSO<sub>4</sub> x 7 H<sub>2</sub>O 0.1 g  
Na<sub>2</sub>MoO<sub>4</sub> x 2 H<sub>2</sub>O 5.0 mg  
K<sub>2</sub>HPO<sub>4</sub> 0.9 g  
KH<sub>2</sub>PO<sub>4</sub> 0.1 g  
FeSO<sub>4</sub> x 7 H<sub>2</sub>O 0.01 g  
CaCO<sub>3</sub> 5.0 g  
Agar 15.0 g  
Distilled water 950.0 ml  
pH 7.3  
Sterilize at 121 C for 15 min.

Sterilize glucose and mannitol separately (in 50.0 ml H<sub>2</sub>O) and add to the medium after autoclaving.

### **31. CABBAGE AGAR**

Cabbage 50.0 g

Glucose 20.0 g

Peptone 10.0 g

Agar 20.0 g

Tap water 1000.0 ml

Boil 50.0 g of cabbage in 1000.0 ml of water, filter cabbage and adjust the volume of broth to the initial value.

pH 7.0

Sterilize at 111 C for 30 min.

### **32. CURD DECOCTION**

Pour 9000.0 ml of distilled water into 3.0 kg of curd and add 150.0 of dry *Aspergillus terricola* mycelium, shake, pour chloroform, seal with a stopper. Decoct at 37 C for 10 days, adjust pH to 7.0 with 1 N NaOH. The decoction shall contain 400 mg% amine nitrogen and 300 mg% tryptophan.

### **B 33. PEPTONE MEAT AGAR WITH 0.2% UREA**

Peptone 10.0 g

NaCl 5.0 g

Beef extract 3.0 g

Agar 20.0 g

Distilled water 1000.0 ml

Do not adjust pH; pH raises to about 8 due to heat degraded urea.

Sterilize at 121 C for 15 min.

10 ml filter-sterilized 20% urea solution is added aseptically post autoclaving to 1000 ml cooled, molten, agar. The medium is then immediately dispensed aseptically.

### **34. SOIL EXTRACT**

Dry garden soil, rich in organic material, in the air by spreading in a thin layer, comminuting and stirring. Then sieve through a rough sieve, and mix 400 g of soil with 960 ml of tap water. Autoclave at 121 C for 1 hour at the end of the day but leave the autoclave open overnight. Filter the cooled extract through filter paper, autoclave 300 ml of filtrate at 121 C for 20 min and allow to stay for 2 weeks or longer to settle the sediment. Decant the clear supernatant liquid and use to prepare the medium.

### **35. NITROBACTER MEDIUM 1**

Solution 1 (see below) 0.5 ml

Solution 2 (see below) 0.5 ml

Solution 3 (see below) 1.0 ml

Solution 4 (see below) 0.5 ml

Solution 5 (see below) 0.5 ml

Solution 6 (see below) 0.1 ml

Distilled water to 1000.0 ml

*Solution 1:*

CaCl<sub>2</sub> 2.0 g

Distilled water 100.0 ml

*Solution 2:*

MgSO<sub>4</sub> x 7 H<sub>2</sub>O 20.0 g

Distilled water 100.0 ml

*Solution 3:*

Chelated iron 0.1 g

Distilled water 100.0 ml

*Solution 4:*

Na<sub>2</sub>MoO<sub>4</sub> 0.1 g

MnCl<sub>2</sub> x 4 H<sub>2</sub>O 0.2 g  
CoCl<sub>2</sub> x 6 H<sub>2</sub>O 0.002 g  
ZnSO<sub>4</sub> x 7 H<sub>2</sub>O 0.1 g  
CuSO<sub>4</sub> x 5 H<sub>2</sub>O 0.02 g  
Distilled water 1000.0 ml

*Solution 5:*

NaNO<sub>3</sub> 41.4 g  
Distilled water 100.0 ml

*Solution 6:*

K<sub>2</sub>HPO<sub>4</sub> 1.74 g  
Distilled water 100.0 ml

Sterilize solutions separately at 121 C for 15 min and mix aseptically.

**Ac 36. PROPIONIBACTERIUM MEDIUM**

Yeast extract 10.0 g  
KH<sub>2</sub>PO<sub>4</sub> 1.0 g  
Na<sub>2</sub>HPO<sub>4</sub> x 2 H<sub>2</sub>O 3.0 g  
Na-lactate (70%) 40.0 ml  
Distilled water 1000.0 ml  
Dissolve all ingredients and add lactate.  
pH 7.0  
Sterilize at 121 C for 15 min.

**F 37. KNOP MEDIUM WITH FILTER PAPER**

Ca(NO<sub>3</sub>)<sub>2</sub> 1.0 g  
KH<sub>2</sub>PO<sub>4</sub> 0.25 g  
MgSO<sub>4</sub> 0.25 g  
FeCl<sub>3</sub> Traces  
Agar 20.0 g  
Distilled water 1000.0 ml  
pH 6.5-7.0  
Sterilize at 121 C for 15 min.  
Sterilize filter paper strips by dry heat and soak with sterile medium.

**F 38. MALT AGAR 7 BALLING WITH 12% NaCl (MA12NaCl)**

Malt extract Balling 7 degrees 1000.0 ml  
NaCl 120.0 g  
Agar 20.0 g  
Sterilize at 121 C for 15 min.

**F 39. MALT 7 BALLING AGAR WITH 1% NaCl**

Malt extract Balling 7 degrees 1000.0 ml  
NaCl 10.0 g  
Agar 20.0 g  
Sterilize at 121 C for 15 min.

**B 40. AZOTOBACTER MEDIUM 2**

KH<sub>2</sub>PO<sub>4</sub> 0.2 g  
K<sub>2</sub>HPO<sub>4</sub> 0.8 g  
MgSO<sub>4</sub> x 7 H<sub>2</sub>O 0.2 g  
CaSO<sub>4</sub> x 2 H<sub>2</sub>O 0.1 g  
FeCl<sub>3</sub> Traces  
Na<sub>2</sub>MoO<sub>4</sub> Traces  
Yeast extract 0.5 g

Sucrose 20.0 g  
Agar 15.0-20.0 g  
Distilled water 1000.0 ml  
pH 7.2  
Sterilize at 111 C for 30 min.

#### **41. FLAVOBACTERIUM MEDIUM**

Na-caseinate 2.0 g  
Yeast extract 0.5 g  
Peptone 1.0 g  
K<sub>2</sub>HPO<sub>4</sub> 0.5 g  
Agar 12.0 g  
Distilled water 1000.0 ml  
pH 7.4  
Sterilize at 121 C for 15 min.

#### **42. PSEUDOMONAS SACCHAROPHILA MEDIUM**

*Solution 1:*

KH<sub>2</sub>PO<sub>4</sub> 4.4 g  
Na<sub>2</sub>HPO<sub>4</sub> 4.8 g  
NH<sub>4</sub>Cl 1.0 g  
MgSO<sub>4</sub> x 7 H<sub>2</sub>O 0.5 g  
Agar (if needed) 20.0 g  
Distilled water 985.0 ml

*Solution 2:*

Ferric ammonium citrate 50.0 mg  
CaCl<sub>2</sub> 5.0 mg  
Distilled water 5.0 ml

*Solution 3:*

Sucrose 1.0 g  
Distilled water 10.0 ml

Sterilize solutions 1 and 2 separately at 121 C for 15 min, solution 3 at 111 C for 30 min and mix aseptically.

#### **B 43. SEA WATER MEDIUM WITH YEAST EXTRACT**

Sea salt 37.9 g  
Yeast extract 3.0 g  
Peptone 10.0 g  
Agar 20.0 g  
Distilled water to 1000.0 ml  
pH 7.2-7.4  
Sterilize at 121 C for 15 min.

#### **44. HALOBACTERIUM MEDIUM 1**

*Solution 1:*

NaCl 250.0 g  
MgSO<sub>4</sub> 10.0 g  
KCl 5.0 g  
CaCl<sub>2</sub> x 6 H<sub>2</sub>O 0.2 g  
Tap water 800.0 ml

*Solution 2:*

Yeast extract 10.0 g  
Tryptone 2.5 g



Agar 20.0 g  
Tap water 200.0 ml  
Sterilize solutions separately at 121 C for 15 min and mix aseptically.

#### **45. STARVED AGAR**

Agar 20.0 g  
Distilled water 1000.0 ml  
Sterilize at 121 C for 15 min.

#### **46. SP MEDIUM FOR STIGMATELLA AURANTIACA**

Raffinose 1.0 g  
Sucrose 1.0 g  
Galactose 1.0 g  
Starch (soluble) 5.0 g  
Casitone 2.5 g  
MgSO<sub>4</sub> x 7 H<sub>2</sub>O 0.5 g  
K<sub>2</sub>HPO<sub>4</sub> 0.25 g  
Agar 15.0 g  
Distilled water 1000.0 ml  
Sterilize at 111 C for 30 min.

#### **47. CM + YE MEDIUM**

Casamino acids 7.5 g  
Yeast extract 10.0 g  
MgSO<sub>4</sub> x 7 H<sub>2</sub>O 20.0 g  
Na-citrate 3.0 g  
KCl 2.0 g  
NaCl 200.0 g  
Agar 15.0 g  
FeSO<sub>4</sub> x 7H<sub>2</sub>O in 0.01N HCl (see below) 1.0 ml  
Distilled water 1000.0 ml  
*Solution of FeSO<sub>4</sub> x 7 H<sub>2</sub>O:*  
HCl (0.01 N) 100.0 ml  
FeSO<sub>4</sub> x 7 H<sub>2</sub>O 4.98 g  
pH 7.4 (adjust with 1N NaOH)  
Sterilize solutions separately at 121 C for 15 min and mix aseptically.

#### **48. CASEIN MEDIUM**

NaCl 250.0 g  
Casein hydrolysate 5.0 g  
Yeast extract 5.0 g  
MgCl<sub>2</sub> x 6 H<sub>2</sub>O 20.0 g  
KCl 2.0 g  
CaCl<sub>2</sub> x 2 H<sub>2</sub>O 0.2 g  
Agar 20.0 g  
Distilled water 1000.0 ml  
pH 7.4 (adjust with 1N NaOH)  
Sterilize at 121 C for 15 min.

#### **49. HALOBACTERIUM MEDIUM 2**

*Solution 1:*  
NaCl 120.0 g  
MgCl<sub>2</sub> x 6 H<sub>2</sub>O 50.0 g  
K<sub>2</sub>SO<sub>4</sub> 5.0 g

CaCl<sub>2</sub> x 6 H<sub>2</sub>O 0.2 g  
Distilled water 500.0 ml

*Solution 2:*

Tryptone 5.0 g  
Yeast extract 5.0 g  
Agar 20.0 g  
Distilled water 500.0 ml  
pH 6.8

Sterilize solutions separately at 121 C for 15 min and mix aseptically.

**B 50. YEAST GLUCOSE AGAR**

Yeast extract 5.0 g  
Peptone 5.0 g  
Glucose 10.0 g  
Agar 20.0 g  
Distilled water 1000.0 ml  
pH 7.2  
Sterilize at 111 C for 30 min.

**51. CASITONE AGAR**

Casitone 3.0 g  
CaCl<sub>2</sub> x 2 H<sub>2</sub>O 1.36 g  
Agar 15.0 g  
Distilled water 1000.0 ml  
pH 7.2  
Sterilize at 121 C for 15 min.

**52. CASITONE YEAST MEDIUM**

Casitone 3.0 g  
CaCl<sub>2</sub> x 2 H<sub>2</sub>O 1.36 g  
Yeast extract 1.0 g  
Agar 15.0 g  
Distilled water 1000.0 ml  
pH 7.2  
Sterilize at 121 C for 15 min.

**53. THERMUS THERMOPHILUS MEDIUM**

Yeast extract 4.0 g  
Polypeptone 8.0 g  
NaCl 2.0 g  
Agar 20.0 g  
Distilled water 1000.0 ml  
pH 7.0  
Sterilize at 121 C for 15 min.

**54. DESULFOVIBRIO MEDIUM WITH 1% NaCl**

K<sub>2</sub>HPO<sub>4</sub> 0.01 g  
NaCl 10.0 g  
MgSO<sub>4</sub> 0.2 g  
Na-lactate (40%) 4.0 ml  
Solution of More salt (see below) 1.0 ml  
Yeast extract 1.0 g  
Ascorbic acid 0.1 g  
Agar 6.0 g

Distilled water 1000.0 ml

*Solution of More salt:*

Fe(NH<sub>4</sub>)<sub>2</sub>(SO<sub>4</sub>)<sub>2</sub> x 6 H<sub>2</sub>O 1.0 g

Distilled water 5.0 ml

Sterilize solutions separately at 121 C for 15 min and mix aseptically.

### **B 55. CAULOBACTER MEDIUM**

Peptone 2.0 g

Yeast extract 1.0 g

MgSO<sub>4</sub> x 7 H<sub>2</sub>O 0.2 g

Agar 10.0 g

Tap water 1000.0 ml

Sterilize at 121 C for 15 min.

### **B 56. CAULOBACTER MEDIUM WITH GLUCOSE**

Peptone 2.0 g

Yeast extract 1.0 g

Glucose 2.0 g

Riboflavin 1.0 mg

MgSO<sub>4</sub> x 7 H<sub>2</sub>O 0.2 g

Agar 10.0 g

Tap water 1000.0 ml

pH 7.0

Sterilize at 111 C for 30 min.

### **B 57. PEPTONE MEAT AGAR WITH 1% UREA**

Peptone 10.0 g

Beef extract 3.0 g

NaCl 5.0 g

Urea 10.0 g

Agar 20.0 g

Distilled water 1000.0 ml

Do not adjust pH; pH raises to about 8 due to heat degraded urea.

Sterilize at 121 C for 15 min.

### **58. HALOBACTERIUM MEDIUM 3**

*Solution 1:*

Casamino acids 7.5 g

Yeast extract 10.0 g

Na-citrate 3.0 g

KCl 2.0 g

MgSO<sub>4</sub> x 7 H<sub>2</sub>O 20.0 g

FeCl<sub>3</sub> traces

NaCl 250.0 g

Distilled water 750.0 ml

*Solution 2:*

Agar 25.0 g

Distilled water 250.0 ml

pH 7.4

Sterilize solutions separately at 121 C for 15 min. and mix aseptically.

### **Ac 59.**

Peptone 2.5 g

Meat extract 2.5 g

NaCl 2.5 g  
Yeast extract 0.1 g  
Glucose 2.5 g  
Sucrose 5.0 g  
Casein acidic hydrolysate 0.1 g  
Agar 15.0 g  
Distilled water 1000.0 ml  
pH 7,2  
Sterilize at 111 C for 30 min.

#### **60. ALFALFA AGAR**

K<sub>2</sub>HPO<sub>4</sub> 0.5 g  
MgSO<sub>4</sub> x 7 H<sub>2</sub>O 0.2 g  
KH<sub>2</sub>PO<sub>4</sub> 0.5 g  
NaCl 0.2 g  
CaSO<sub>4</sub> 0.1 g  
(NH<sub>4</sub>)<sub>2</sub>MoO<sub>4</sub> Traces  
Mannitol 20.0 g  
Alfalfa meal 10.0 ml  
Agar 20.0 g  
Distilled water 1000.0 ml  
pH 7.0-7.2.  
Sterilize at 111 C for 20 min.

#### **61. MEDIUM FOR NITROGEN-FIXING SPIRILLUM**

K<sub>2</sub>HPO<sub>4</sub> 0.1 g  
KH<sub>2</sub>PO<sub>4</sub> 0.4 g  
MgSO<sub>4</sub> x 7 H<sub>2</sub>O 0.2 g  
NaCl 0.1 g  
CaCl<sub>2</sub> 0.02 g  
FeCl<sub>3</sub> 0.01 g  
Na<sub>2</sub>MoO<sub>4</sub> x 2 H<sub>2</sub>O 0.002 g  
Na-malate 5.0 g  
Yeast extract 50.0 mg  
Distilled water 1000.0 ml  
pH 7.2-7.4  
Sterilize at 121 C for 15 min.

#### **62. MANURE TINCTURE**

Cow manure (fresh) 1.0 kg  
Distilled water 3000.0 ml  
Boil, squeeze through gauze into a bottle and dilute to 3000.0 ml.

#### **63. MEDIUM WITH CURD DECOCTION**

Curd decoction 61.0 ml  
Manure tincture 184.0 ml  
Na-acetate 1.0 g  
Agar 20.0 g  
Tap water 735.0 ml  
pH 7.8

*Preparation of curd decoction:* pour 9000.0 ml of distilled water into 3.0 kg of curd and add 150.0 of dry *Aspergillus terricola* mycelium, shake, pour chloroform, seal with a stopper. Decoct at 37 C for 10 days, adjust pH to 7.0 with 1 N NaOH. The decoction shall contain 400 mg% amine nitrogen and 300 mg% tryptophan.

*Preparation of manure tincture:* mix cow manure (fresh) 1.0 kg and distilled water 3000.0 ml. Boil, squeeze through gauze into a bottle and dilute to 3000.0 ml. Sterilize at 121 C for 15 min.

#### **Ac 64. PEPTONE YEAST AGAR**

Peptone 5.0 g  
Yeast extract 3.0 g  
KH<sub>2</sub>PO<sub>4</sub> 0.2 g  
Glucose 5.0 g  
Agar 20.0 g  
Tap water 1000.0 ml  
pH 7.0-7.2  
Sterilize at 111 C for 30 min.

#### **65. OATMEAL AGAR FOR BACTERIA**

Oatmeal 2.0 g  
Peptone 0.5 g  
NaCl 1.0 g  
Galactose 0.5 g  
Agar 12.0 g  
Distilled water 1000.0 ml  
pH 7.5  
Sterilize at 111 C for 30 min.

#### **Ac 66. MEDIUM WITH SOIL EXTRACT**

Peptone 5.0 g  
Beef extract 3.0 g  
Agar 15.0 g  
Soil extract 250.0 ml  
Tap water 750.0 ml  
pH 7.0  
Sterilize at 121 C for 15 min.

*Preparation of soil extract:* dry garden soil, rich in organic material, in the air by spreading in a thin layer, comminuting and stirring. Then sieve through a rough sieve, and mix 400 g of soil with 960 ml of tap water. Autoclave at 121 C for 1 hour at the end of the day but leave the autoclave open overnight. Filter the cooled extract through filter paper, autoclave 300 ml of filtrate at 121 C for 20 min and allow to stay for 2 weeks or longer to settle the sediment. Decant the clear supernatant liquid and use to prepare the medium.

#### **67. PEPTONE MEAT AGAR WITH TRACE ELEMENTS**

Peptone 10.0 g  
NaCl 5.0 g  
Beef extract 3.0 g  
Yeast autolysate 2.0 g  
Trace element solution (see below) 1.0 ml  
Distilled water 1000.0 ml  
Agar 20.0 g  
*Trace element solution:*  
H<sub>3</sub>BO<sub>3</sub> 5.0 g  
(NH<sub>4</sub>)<sub>2</sub>MoO<sub>4</sub> 5.0 g  
KI 0.5 g  
NaBr 0.5 g  
ZnSO<sub>4</sub> 0.2 g  
Al<sub>2</sub>(SO<sub>4</sub>)<sub>3</sub> 0.3 g

Distilled water 1000.0 ml  
pH 7.2-7.4  
Sterilize at 121 C for 15 min.

#### **Ac, B 68. PEPTONE MEAT AGAR WITH 3% SEA SALT**

Peptone 10.0 g  
NaCl 5.0 g  
Sea salt 30.0 g  
Beef extract 3.0 g  
Agar 20.0 g  
Distilled water 1000.0 ml  
pH 7.2-7.4  
Sterilize at 121 C for 15 min.

#### **69. DAVIS SUPPLEMENTED MINIMAL MEDIUM**

*Solution 1:*

Yeast extract 2.0 g  
Casein hydrolysate 2.0 g  
K<sub>2</sub>HPO<sub>4</sub> 7.0 g  
KH<sub>2</sub>PO<sub>4</sub> 3.0 g  
Na-citrate x 3 H<sub>2</sub>O 0.5 g  
MgSO<sub>4</sub> x 7 H<sub>2</sub>O 0.1 g  
(NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub> 1.0 g  
Agar 15.0 g  
Distilled water 980.0 ml

*Solution 2:*

Glucose 2.0 g  
Distilled water 20.0 ml  
pH 7.0  
Sterilize at 121 C for 15 min.

#### **70. MEDIUM VY/2 FOR STIGMATELLA AURANTIACA**

Baker's yeast 5.0 g  
CaCl<sub>2</sub> x 2 H<sub>2</sub>O 1.36 g  
Vitamin B<sub>12</sub> (cyanocobalamin) 0.5 mg  
Agar 15.0 g  
Distilled water 1000.0 ml  
pH 7.2 (adjust with KOH before adding agar)  
Sterilize at 111 C for 20 min. Vitamin B<sub>12</sub> sterilize separately with filtration.

#### **71. NITROSOCOCCUS MEDIUM 1**

(NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub> 1.32 g  
MgSO<sub>4</sub> x 7 H<sub>2</sub>O 380.0 mg  
CaCl<sub>2</sub> x 2 H<sub>2</sub>O 20.0 mg  
Chelated iron (13% iron) 1.0 mg  
Na<sub>2</sub>MoO<sub>4</sub> x 2 H<sub>2</sub>O 100.0 µg  
MnCl<sub>2</sub> x 4 H<sub>2</sub>O 200.0 µg  
CoCl<sub>2</sub> x 6 H<sub>2</sub>O 2.0 µg  
ZnSO<sub>4</sub> x 7 H<sub>2</sub>O 100.0 µg  
K<sub>2</sub>HPO<sub>4</sub> 8.7 mg  
Phenol red (0.04%) 3.25 ml  
Sea water 1000.0 ml  
pH 7.5-7.8 (adjust with 1 N HCl)  
Sterilize at 121 C for 15 min.

**B 72. BEAN AGAR**

Beans (peas or pulse) 100.0 g

K<sub>2</sub>HPO<sub>4</sub> 0.5 g

Sucrose 10.0 g

Agar 20.0 g

Tap water 1000.0 ml

Boil beans in 1000.0 ml of water, filter through gauze, add water to the initial volume; add phosphate, sucrose and agar

pH 7.2-7.4

Sterilize at 111 C for 30 min.

**73. GYT-AGAR**

Glucose 10.0 g

Yeast extract 1.0 g

Tryptose 2.0 g

FeSO<sub>4</sub> x 7 H<sub>2</sub>O 1.0 mg

Agar 20.0 g

Distilled water 1000.0 ml

pH 7.2

Sterilize at 111 C for 30 min.

**74. HOTTINGER BROTH**

Boil meat (1-2 cm pieces) (without fat or tendons) in 2000.0 ml of water, then mince. Adjust pH of the decoction to 8.0, mix with minced meat and cool down to 40 C. Then add 1.0 g of dry pancreatin, mix and again alkalinize to pH 7.8-8.0. Pour the mixture into a bottle with the rubber stopper (1/3 of the bottle to remain free), add chloroform (20 ml), mix and open the bottle for 1 min to remove the excess chloroform vapors. 2 hour after pancreatin was added, adjust pH to 7.4-7.6 and leave the mixture for 2 weeks at 18-20 C. The first 4 days adjust pH of the medium; shake and mix 3 times a day, then stir once a day. Two days before the end of the procedure stop mixing to allow the decoction to settle. The liquid shall be of straw color, the reaction with tryptophan with bromine water shall be positive; in the decoction hydrolysate the total nitrogen shall be no less than 1100 mg%. Filter the decoction through the linen, pour into flasks and sterilize in autoclave at 121 C for 30 min. Filter prior to use.

**B 75. MODIFICATION OF TWEEN-80 MEDIUM FOR MILK-ACID BACTERIA**

Yeast extract 5.0 g

Glucose 2.5 g

Beef extract 1.2 g

Tween-80 1.0 ml

K<sub>2</sub>HPO<sub>4</sub> 2.0 g

Na-acetate 5.0 g

NH<sub>4</sub>-citrate 2.0 g

MgSO<sub>4</sub> x 7 H<sub>2</sub>O 0.2 g

MnSO<sub>4</sub> x 4 H<sub>2</sub>O 0.05 g

Agar 5.0 g

Distilled water 1000.0 ml

pH 6.0-6.5

Sterilize at 111 C for 30 min.

**76. POTATO-PEPTONE MEDIUM**

Potato decoction 200.0 ml

Yeast extract 1.0 g

Peptone 5.0 g

Agar 30.0 g

Distilled water 800.0 ml

pH 7.0

Boil 200 g scrubbed and sliced potatoes in 1000.0 ml water for 1 hour, filter cold through a cotton-gauze filter, add water to the initial volume. Do not use new potatoes.

Sterilize at 121 C for 15 min.

#### **77. PEPTONE MEAT AGAR WITH VITAMINS**

Peptone 10.0 g

NaCl 5.0 g

Beef extract 3.0 g

Yeast extract 1.0 g

Glucose 1.0 g

B<sub>12</sub> 2.0 mg

B<sub>1</sub> 2.0 mg

Agar 20.0 g

Distilled water 1000.0 ml

pH 7.0

Sterilize at 111 C for 30 min.

#### **78. PEPTONE MEAT AGAR WITH 2% SOLUBLE STARCH**

Peptone 10.0 g

Beef extract 3.0 g

NaCl 5.0 g

Soluble starch 20.0 g

Agar 20.0 g

Distilled water 1000.0 ml

pH 7.0

Sterilize at 121 C for 15 min.

#### **B 79. PEPTONE MEAT AGAR WITH 1% SOLUBLE STARCH**

Peptone 10.0 g

Beef extract 3.0 g

NaCl 5.0 g

Soluble starch 10.0 g

Agar 20.0 g

Distilled water 1000.0 ml

pH 7.2

Sterilize at 121 C for 15 min.

#### **80. PEPTONE MEAT AGAR WITH 6% NaCl**

Peptone 10.0 g

Beef extract 3.0 g

NaCl 60.0 g

Agar 20.0 g

Distilled water 1000.0 ml

pH 7.0

Sterilize at 121 C for 15 min.

#### **Ac 81. PEPTONE MEAT AGAR WITH 1.8% SEA SALT**

Peptone 10.0 g

NaCl 5.0 g

Beef extract 3.0 g

Sea salt 18.0 g

Agar 20.0 g



Distilled water 1000.0 ml  
pH 7.0  
Sterilize at 121 C for 15 min.

### **82. POTATO AGAR WITH 2% GLUCOSE see MEDIUM 13**

Potato 200.0 g  
Glucose 20.0 g  
Agar 20.0 g  
Tap water 1000.0 ml  
pH 7.0

Boil 200 g scrubbed and sliced potatoes in 1000.0 ml water for 1 hour, filter cold through a cotton-gauze filter, add water to the initial volume, add glucose and agar. Do not use new potatoes.  
Sterilize at 111 C for 30 min.

### **83. LOPATINA MEDIUM**

Glucose 10.0 g  
KH<sub>2</sub>PO<sub>4</sub> 0.2 g  
MgSO<sub>4</sub> x 7 H<sub>2</sub>O 0.2 g  
Thyrosin 1.0 g  
NaCl 0.2 g  
CaSO<sub>4</sub> 0.1 g  
Agar 20.0 g  
Distilled water 1000.0 ml  
pH 7.0  
Sterilize at 111 C for 30 min

### **84. MEDIUM WITH CASEIN HYDROLYSATE**

Casein hydrolysate 10.0 g  
Glucose 5.0 g  
p-Aminobenzoic acid 5.0 µg  
Agar 20.0 g  
Distilled water 1000.0 ml  
pH 7.0  
Sterilize at 111 C for 30 min.

### **85. MEDIUM WITH HOTTINGER BROTH**

Peptone 10.0 g  
Yeast autolysate 10.0 g  
Hottinger broth 10.0 ml  
Phosphate solution (see below) 25 ml  
Salt solution (see below) 25 ml  
Glucose 5.0 g  
Distilled water 940.0 ml  
*Phosphate solution:*  
KH<sub>2</sub>PO<sub>4</sub> 100.0 mg  
K<sub>2</sub>HPO<sub>4</sub> 100.0 mg  
Distilled water 25.0 ml  
*Salt solution:*  
MgSO<sub>4</sub> x 7 H<sub>2</sub>O 40.0 mg  
NaCl 2.0 mg  
FeSO<sub>4</sub> 2.0 mg  
Distilled water 25.0 ml  
pH 7.0  
Sterilize phosphate and salt solutions at 121 C for 15 min, base medium at 111 C for 30 min.

### **86. NITROSOLOBUS MEDIUM 1**

CaCl<sub>2</sub> x 2 H<sub>2</sub>O 0.02 g  
MgSO<sub>4</sub> x 7 H<sub>2</sub>O 0.2 g  
Chelated iron 1.0 mg  
Na<sub>2</sub>MoO<sub>4</sub> x 2 H<sub>2</sub>O 0.1 mg  
MnCl<sub>2</sub> x 4 H<sub>2</sub>O 0.2 mg  
CoCl<sub>2</sub> x 6 H<sub>2</sub>O 2.0 µg  
CuSO<sub>4</sub> x 5 H<sub>2</sub>O 0.02 mg  
ZnSO<sub>4</sub> x 7 H<sub>2</sub>O 0.1 mg  
(NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub> 1.65 g  
K<sub>2</sub>HPO<sub>4</sub> 0.087 g  
Phenol red 5.0 mg  
Distilled water 1000.0 ml  
pH 7.5 (adjust with 0.1 M NaHCO<sub>3</sub>)  
Sterilize at 121 C for 15 min.

### **87. NITROSOCOCCUS MEDIUM 2**

NH<sub>4</sub>Cl 0.5 g  
KH<sub>2</sub>PO<sub>4</sub> 0.05 g  
CaCO<sub>3</sub> 5.0 g  
Chelated iron 1.0 mg  
Phenol red (0.04%) 3.25 ml  
Sea water 1000.0 ml  
pH 7.5-7.8 (adjust with 1 N HCl)  
Sterilize at 121 C for 15 min.

### **B 88. SPIRILLUM MEDIUM**

Peptone 10.0 g  
Succinic acid 1.0 g  
(NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub> 1.0 g  
MgSO<sub>4</sub> x 7 H<sub>2</sub>O 1.0 g  
FeCl<sub>3</sub> x 6 H<sub>2</sub>O 2.0 mg  
MnSO<sub>4</sub> x H<sub>2</sub>O 2.0 mg  
Distilled water 1000.0 ml  
pH 6.8  
Sterilize at 121 C for 15 min.

### **89. MILK MEDIUM FOR HALOPHILS**

*Solution 1:*

Milk 500.0 ml

*Solution 2:*

MgSO<sub>4</sub> x 7 H<sub>2</sub>O 10.0 g

KNO<sub>3</sub> 2.0 g

NaCl 200.0 g

Distilled water 100.0 ml

*Solution 3:*

Peptone 5.0 g

Glycerol 10.0 g

Agar 25.0 g

Distilled water 400.0 ml

pH 8.4

Sterilize at 121 C for 20 min.

Sequence of mixing: add warm skim milk to a hot mixture of solutions 1 and 2.

## **B 90. DESULFOVIBRIO GIGAS MEDIUM**

### *Solution 1:*

KH<sub>2</sub>PO<sub>4</sub> 1.0 g  
NH<sub>4</sub>Cl 0.5 g  
MgSO<sub>4</sub> x 7 H<sub>2</sub>O 0.4 g  
Na<sub>2</sub>SO<sub>4</sub> 2.0 g  
CaCl<sub>2</sub> x 2 H<sub>2</sub>O 0.1 g  
Trace element solution SL-6: (see below) 1.0 ml  
2 M H<sub>2</sub>SO<sub>4</sub> 1.0 ml  
Na L-lactate 2.0 g  
Distilled water 950.0 ml

### *Solution 2:*

NaHCO<sub>3</sub> 2.0 g  
Distilled water 40.0 ml

### *Solution 3:*

Na<sub>2</sub>S x 9 H<sub>2</sub>O 300.0 mg  
Distilled water 6.0 ml

### *Vitamine solution:*

Biotin 2.5 mg  
Nicotinic acid 25.0 mg  
Thiamine-HCl 12.5 mg  
p-Aminobenzoic acid 12.5 mg  
Calcium pantothenate 6.5 mg  
Pyridoxine-HCl 62.5 mg  
Distilled water 1000.0 ml

### *Trace element solution SL-6:*

ZnSO<sub>4</sub> x 7 H<sub>2</sub>O 0.1 g  
MnCl<sub>2</sub> x 4 H<sub>2</sub>O 0.03 g  
H<sub>3</sub>BO<sub>3</sub> 0.3 g  
CoCl<sub>2</sub> x 6 H<sub>2</sub>O 0.2 g  
CuCl<sub>2</sub> x 2 H<sub>2</sub>O 0.01 g  
NiCl<sub>2</sub> x 6 H<sub>2</sub>O 0.02 g  
Na<sub>2</sub>MoO<sub>4</sub> 0.03 g  
Distilled water 1000.0 ml

pH 7.2

Sterilize solutions 1 and 3 separately under N<sub>2</sub> at 121 C for 15 min, add 5 ml of the filter sterilized vitamine solution to 1000.0 ml of sterile solution 1. Solution 2 (sterilized at 121 C for 15 min) is not to be kept for long.

## **91. THERMODESULFOBACTERIUM MEDIUM**

### *Solution 1:*

Na<sub>2</sub>SO<sub>4</sub> 3.0 g  
NH<sub>4</sub>Cl 1.0 g  
MgCl<sub>2</sub> x 6 H<sub>2</sub>O 0.2 g  
KH<sub>2</sub>PO<sub>4</sub> 0.3 g  
Na<sub>2</sub>HPO<sub>4</sub> x 12 H<sub>2</sub>O 2.0 g  
FeSO<sub>4</sub> x 7 H<sub>2</sub>O 1.5 mg  
Resazurin 1.0 mg  
Distilled water 930.0 ml

### *Solution 2:*

Trace element solution (see below) 10.0 ml

### *Solution 3:*

Yeast extract 1.0 g

Distilled water 25.0 ml

*Solution 4:*

Na-lactate 4.0 g

Distilled water 25.0 ml

*Solution 5:*

Na<sub>2</sub>S x 9 H<sub>2</sub>O 0.5 g

Distilled water 6.0 ml

*Solution 6:*

Vitamin solution (see below) 5.0 ml

*Trace element solution:*

Nitrilotriacetic acid 12.8 g

FeCl<sub>3</sub> x 4 H<sub>2</sub>O 0.2 g

MnCl<sub>2</sub> x 4 H<sub>2</sub>O 0.1 g

CoCl<sub>2</sub> x 6 H<sub>2</sub>O 0.17 g

CaCl<sub>2</sub> x 2 H<sub>2</sub>O 0.1 g

ZnCl<sub>2</sub> 0.1 g

CuCl<sub>2</sub> 0.02 g

Na<sub>2</sub>MoO<sub>4</sub> x 2 H<sub>2</sub>O 0.01 g

NiCl<sub>2</sub> x 6 H<sub>2</sub>O 0.026 g

NaCl 1.0 g

Na<sub>2</sub>SeO<sub>4</sub> x 5 H<sub>2</sub>O 0.02 g

Distilled water 1000.0 ml

*Vitamin solution:*

Biotin 2.0 mg

Folic acid 2.0 mg

Pyridoxine-HCl 10.0 mg

Thiamine-HCl 5.0 mg

Riboflavin 5.0 mg

Nicotinic acid 5.0 mg

Ca DL-pantothenate 5.0 mg

Vitamin B<sub>12</sub> 0.1 mg

p-Aminobenzoic acid 5.0 mg

Lipoic acid 5.0 mg

Distilled water 1000.0 ml

pH 6.8-7.0

Solutions 1, 3, 4 and 5 sterilize under N<sub>2</sub> at 121 C for 15 min.

Solution 6 is filter sterilized. Before use, neutralize solution 5 by dropwise of 1 N HCl.

## **92. DESULFOVIBRIO MEDIUM WITH LACTATE**

*Solution 1:*

K<sub>2</sub>HPO<sub>4</sub> 0.5 g

NH<sub>4</sub>Cl 1.0 g

CaCl<sub>2</sub> x 6 H<sub>2</sub>O 0.1 g

MgSO<sub>4</sub> x 7 H<sub>2</sub>O 2.0 g

Na<sub>2</sub>SO<sub>4</sub> 1.0 g

Na-lactate 5.0 g

Yeast extract 1.0 g

Resazurin 0.001 g

Cysteine 0.5 g

Distilled water 950.0 ml

*Solution 2:*

NaHCO<sub>3</sub> 4.0 g

Distilled water 40.0 ml

*Solution 3:*

Na<sub>2</sub>S x 9 H<sub>2</sub>O 300.0 mg

Distilled water 6.0 ml

*Solution 4:*

FeSO<sub>4</sub> x 7 H<sub>2</sub>O 0.4 g

Distilled water 10.0 ml

pH 6.8

Sterilize all solutions separately at 121 C for 15 min. Solution 1 bring to boil while simultaneously bubbling a mixture of oxygen-free gas composed of 97% N<sub>2</sub> and 3% H<sub>2</sub> through the mixture and sterilize in atmosphere of this gas mixture. Solution 3 sterilize in atmosphere of N<sub>2</sub>.

### **93. AZOSPIRILLUM BRASILIENSE MEDIUM 1**

Ca-malate 10.0 g

K<sub>2</sub>HPO<sub>4</sub> 1.0 g

MgSO<sub>4</sub> x 7 H<sub>2</sub>O 0.5 g

CaCl<sub>2</sub> x 2 H<sub>2</sub>O 0.02 g

Distilled water 1000.0 ml

pH 6.5

Sterilize at 121 C for 15 min.

### **B 94. MEDIUM FOR CARBON MONOOXIDE OXIDIZERS**

Na<sub>2</sub>HPO<sub>4</sub> x 12 H<sub>2</sub>O 4.5 g

KH<sub>2</sub>PO<sub>4</sub> 0.75 g

NH<sub>4</sub>Cl 1.5 g

MgSO<sub>4</sub> x 7 H<sub>2</sub>O 0.2 g

CaCl<sub>2</sub> x 2 H<sub>2</sub>O 0.03 g

Fe(NH<sub>4</sub>)-citrate 0.018 g

Agar (if necessary) 1.2 g

Trace element solution SL-6 (see below) 1.0 ml

Distilled water 1000.0 ml

*Trace element solution SL-6:*

ZnSO<sub>4</sub> x 7 H<sub>2</sub>O 0.1 g

MnCl<sub>2</sub> x 4 H<sub>2</sub>O 0.03 g

H<sub>3</sub>BO<sub>3</sub> 0.3 g

CoCl<sub>2</sub> x 6 H<sub>2</sub>O 0.2 g

CuCl<sub>2</sub> x 2 H<sub>2</sub>O 0.01 g

NiCl<sub>2</sub> x 6 H<sub>2</sub>O 0.02 g

Na<sub>2</sub>MoO<sub>4</sub> 0.03 g

Distilled water 1000.0 ml

pH 7.0

Sterilize at 121 C for 15 min.

For chemoautotrophic growth incubate under gas atmosphere of a) 20-80% CO<sub>2</sub> + 10% O<sub>2</sub> + 0-70% N<sub>2</sub> or b) 70% H<sub>2</sub> + 20% O<sub>2</sub> + 10% CO<sub>2</sub> adding 2.5 g NaHCO<sub>3</sub> per liter of medium. For chemoorganotrophic growth add 3.0 g sodium acetate and incubate under air.

### **95. DESULFOTOMACULUM ACETOXIDANS MEDIUM**

*Solution 1:*

NaCl 1.17 g

MgCl<sub>2</sub> x 6 H<sub>2</sub>O 0.4 g

KCl 0.3 g

CaCl<sub>2</sub> x 2 H<sub>2</sub>O 0.15 g

NH<sub>4</sub>Cl 0.27 g

KH<sub>2</sub>PO<sub>4</sub> 0.2 g

Na<sub>2</sub>SO<sub>4</sub> 2.84 g

Na-acetate 1.4 g

Na-butyrate 1.4 g  
Yeast extract 1.0 g  
Vitamin solution (see below) 1.0 ml  
Trace element solution 1.0 ml (see below)  
Distilled water 940.0 ml

*Solution 2:*

$\text{Na}_2\text{S} \times 9 \text{H}_2\text{O}$  0.36 g  
Distilled water 10.0 ml  
Sodium bicarbonate for alkalization:  
 $\text{NaHCO}_3$  4.5 g  
Distilled water 50.0 g

*Vitamin solution:*

p-Aminobenzoic acid 4.0 mg  
D(+)-Biotin 1.0 mg  
Thiamine-HCl 10.0 mg  
Distilled water 100.0 ml

*Trace element solution:*

$\text{FeCl}_3 \times 4 \text{H}_2\text{O}$  1.5 g  
 $\text{ZnCl}_2$  68.0 mg  
 $\text{MnCl}_2 \times 4 \text{H}_2\text{O}$  100.0 ml  
 $\text{H}_3\text{BO}_3$  62.0 mg  
 $\text{CoCl}_2 \times 6 \text{H}_2\text{O}$  120.0 ml  
 $\text{Na}_2\text{MoO}_4 \times 2 \text{H}_2\text{O}$  24.0 mg  
HCl (0.05 M) 1000.0 ml  
pH 7.0-7.2

Solution 1 is boiled before sterilization for a few minutes being flushed with gas mixture of 80%  $\text{N}_2$  and 20%  $\text{CO}_2$  with sodium bicarbonate added until an equilibrium pH of 6.9 - 7.1. Solution 1 is sterilized under this gas mixture at 121 C for 15 min. Solution 2 is sterilized under 100%  $\text{N}_2$  at 121 C for 15 min. Vitamin solution is filter sterilized.

## **96. DESULFONEMA LIMICOLA MEDIUM**

*Solution 1:*

$\text{Na}_2\text{SO}_4$  3.0 g  
NaCl 13.0 g  
 $\text{MgCl}_2 \times 6 \text{H}_2\text{O}$  2.2 g  
 $\text{CaCl}_2 \times 2 \text{H}_2\text{O}$  0.15 g  
KCl 0.5 g  
 $\text{KH}_2\text{PO}_4$  0.2 g  
 $\text{NH}_4\text{Cl}$  0.3 g  
Distilled water 850.0 ml

*Solution 2:*

Trace element solution SL-10 (see below) 1.0 ml

*Solution 3:*

$\text{NaHCO}_3$  5.0 g  
Distilled water 100.0 ml

*Solution 4:*

Na-acetate  $\times 3 \text{H}_2\text{O}$  2.5 g  
Distilled water 10.0 ml

*Solution 5:*

Disodium succinate 0.1 g  
Distilled water 1.0 ml

*Solution 6:*

Vitamin solution (see below) 5.0 ml

*Solution 7:*

AlCl<sub>3</sub> x 6 H<sub>2</sub>O 245.0 mg

Distilled water 5.0 ml

*Solution 8:*

Na<sub>2</sub>CO<sub>3</sub> 170.0 mg

Distilled water 1.6 ml

*Solution 9:*

Rumen fluid, clarified 20.0 ml

*Solution 10:*

Na<sub>2</sub>S x 9 H<sub>2</sub>O 0.4 mg

Distilled water 10.0 ml

*Trace element solution SL-10:*

HCl (25%; 7.7 M) 10.0 ml

FeCl<sub>3</sub> x 4 H<sub>2</sub>O 1.5 g

ZnCl<sub>2</sub> 70.0 mg

MnCl<sub>2</sub> x 4 H<sub>2</sub>O 100.0 mg

H<sub>3</sub>BO<sub>3</sub> 6.0 mg

CoCl<sub>2</sub> x 6 H<sub>2</sub>O 190.0 mg

CuCl<sub>2</sub> x 2 H<sub>2</sub>O 2.0 mg

NiCl<sub>2</sub> x 6 H<sub>2</sub>O 24.0 mg

Na<sub>2</sub>MoO<sub>4</sub> x 2 H<sub>2</sub>O 36.0 mg

Distilled water 990.0 ml

*Vitamin solution:*

Biotin 2.0 mg

Folic acid 2.0 mg

Pyridoxine-HCl 10.0 mg

Thiamine-HCl 5.0 mg

Riboflavin 5.0 mg

Nicotinic acid 5.0 mg

Ca DL-pantothenate 5.0 mg

Vitamin B<sub>12</sub> 0.1 mg

p-Aminobenzoic acid 5.0 mg

Lipoic acid 5.0 mg

Distilled water 1000.0 ml

pH 7.6

*The trace element solution preparation:* FeCl<sub>3</sub> x 4 H<sub>2</sub>O is dissolved firstly in HCl, and then is mixed with water and other salts are dissolved in the sequence indicated. Solution 1 is boiled for a few minutes, cooled to room temperature, gassed with 80% N<sub>2</sub> + 20% CO<sub>2</sub> gas mixture to reach a pH of around 6, then autoclaved anaerobically under the same gas. Solutions 2, 3, 5, 9, and 10 are autoclaved separately under nitrogen at 121 C for 15 min, solution 3 is filter-sterilized and flushed with 80% N<sub>2</sub> + 20% CO<sub>2</sub> to remove dissolved oxygen. Solution 6 is filter-sterilized and outgassed with N<sub>2</sub>. Solutions 7 and 8 are combined before sterilization at 121 C for 15 min. Solutions 2 to 10 are added to the sterile cooled solution A in the sequence as indicated. The completed medium is distributed anaerobically under 80% N<sub>2</sub> + 20% CO<sub>2</sub> into appropriate vessels.

## **97. DESULFONEMA MAGNUM MEDIUM**

*Solution 1:*

Na<sub>2</sub>SO<sub>4</sub> 3.0 g

NaCl 21.0 g/l

MgCl<sub>2</sub> x 6 H<sub>2</sub>O 5.5 g/l

CaCl<sub>2</sub> x 2 H<sub>2</sub>O 1.35 g/l

KCl 0.5 g

KH<sub>2</sub>PO<sub>4</sub> 0.2 g

NH<sub>4</sub>Cl 0.3 g

Distilled water 850.0 ml

*Solution 2:*

Trace element solution (see below) 1.0 ml

*Solution 3:*

NaHCO<sub>3</sub> 2.5 g/l

Distilled water 100.0 ml

*Solution 4:*

Na-benzoate 0.6 g

Distilled water 10.0 ml

*Solution 5:*

Disodium succinate 0.1 g

Distilled water 1.0 ml

*Solution 6:*

Vitamin solution (see below) 5.0 ml

*Solution 7:*

AlCl<sub>3</sub> x 6 H<sub>2</sub>O 245.0 mg

Distilled water 5.0 ml

*Solution 8:*

Na<sub>2</sub>CO<sub>3</sub> 170.0 mg

Distilled water 1.6 ml

*Solution 9:*

Rumen fluid, clarified 20.0 ml

*Solution 10:*

Na<sub>2</sub>S x 9 H<sub>2</sub>O 0.4 mg

Distilled water 10.0 ml

*Trace element solution:*

HCl (25%; 7.7 M) 10.0 ml

FeCl<sub>3</sub> x 4 H<sub>2</sub>O 1.5 g

ZnCl<sub>2</sub> 70.0 mg

MnCl<sub>2</sub> x 4 H<sub>2</sub>O 100.0 mg

H<sub>3</sub>BO<sub>3</sub> 6.0 mg

CoCl<sub>2</sub> x 6 H<sub>2</sub>O 190.0 mg

CuCl<sub>2</sub> x 2 H<sub>2</sub>O 2.0 mg

NiCl<sub>2</sub> x 6 H<sub>2</sub>O 24.0 mg

Na<sub>2</sub>MoO<sub>4</sub> x 2 H<sub>2</sub>O 36.0 mg

Na<sub>2</sub>SeO<sub>4</sub> 3.0 mg

Distilled water 990.0 ml

*Vitamin solution:*

Biotin 2.0 mg

Folic acid 2.0 mg

Pyridoxine-HCl 10.0 mg

Thiamine-HCl 5.0 mg

Riboflavin 5.0 mg

Nicotinic acid 5.0 mg

Ca DL-pantothenate 5.0 mg

Vitamin B<sub>12</sub> 0.1 mg

p-Aminobenzoic acid 5.0 mg

Lipoic acid 5.0 mg

Vitamin B<sub>12</sub> 50.0 mg

Distilled water 1000.0 ml

pH 7.6

*The trace element solution preparation:* FeCl<sub>3</sub> x 4 H<sub>2</sub>O is dissolved firstly in HCl, and then is mixed with water and other salts are dissolved in the sequence indicated. Solution 1 is boiled for a few minutes, cooled to room temperature, gassed with 80% N<sub>2</sub> + 20% CO<sub>2</sub> gas mixture to reach a pH of around 6, then autoclaved anaerobically under the same gas. Solutions 2, 3, 5, 9, and 10 are autoclaved



separately under nitrogen at 121°C for 15 min, solution 3 is filter-sterilized and flushed with 80% N<sub>2</sub> + 20% CO<sub>2</sub> to remove dissolved oxygen. Solution 6 is filter-sterilized and outgassed with N<sub>2</sub>. Solutions 7 and 8 are combined before sterilization at 121°C for 15 min. Solutions 2 to 10 are added to the sterile cooled solution A in the sequence as indicated. The completed medium is distributed anaerobically under 80% N<sub>2</sub> + 20% CO<sub>2</sub> into appropriate vessels.

#### **Ac 98. WORT AGAR 7 B WITH 2% CaCO<sub>3</sub>**

Malt extract Balling 7 degrees 1000.0 ml

CaCO<sub>3</sub> 20 g

Agar 20.0 g

Sterilize at 111 C for 30 min.

#### **B 99. MEDIUN YE**

Yeast extract 30.0 g

Ethanol 20.0 ml

Agar 20.0 g

Distilled water 1000.0 ml

pH 5.0-6.0

Sterilize at 121 C for 15 min without ethanol.

Apply filter sterilize ethanol (0.1 ml/test tube) onto the surface of agar slants.

#### **100. SAP-2 AGAR**

Tryptone 1.0 g

Yeast extract 1.0 g

Agar 20.0 g

Sea water 1000.0 g

pH 7.4

Sterilize at 121 C for 15 min.

#### **B 101. SOIL AGAR**

Yeast extract 2.0 g

Tryptone 1.0 g

Na-acetate 1.0 g

Soil extract 50.0 ml

Agar 20.0 g

Distilled water add to 1000.0 ml

pH 7.4

Sterilize at 121 C for 15 min.

*Preparation of soil extract:* dry garden soil, rich in organic material, in the air by spreading in a thin layer, comminuting and stirring. Then sieve through a rough sieve, and mix 400 g of soil with 960 ml of tap water. Autoclave at 121 C for 1 hour at the end of the day but leave the autoclave open overnight. Filter the cooled extract through filter paper, autoclave 300 ml of filtrate at 121 C for 20 min and allow to stay for 2 weeks or longer to settle the sediment. Decant the clear supernatant liquid and use to prepare the medium.

#### **102. DESULFOBACTER POSTGATEI MEDIUM**

*Solution 1:*

Na<sub>2</sub>SO<sub>4</sub> 3.0 g

KH<sub>2</sub>PO<sub>4</sub> 0.2 g

NH<sub>4</sub>Cl 0.3 g

NaCl 7.0 g

MgCl<sub>2</sub> x 6 H<sub>2</sub>O 1.3 g

KCl 0.5 g

CaCl<sub>2</sub> x 2 H<sub>2</sub>O 0.15 g

Distilled water 870.0 ml

*Solution 2:*

Trace element solution SL-10 (see below) 1.0 ml

*Solution 3:*

NaHCO<sub>3</sub> 5.0 g

Distilled water 100.0 ml

*Solution 4:*

Na-acetate x 3 H<sub>2</sub>O 2.5 g

Distilled water 10.0 ml

*Solution 5:*

Vitamin solution (see below) 10.0 ml

*Solution 6:*

Na<sub>2</sub>S x 9 H<sub>2</sub>O 0.4 g

Distilled water 10.0 ml

*Trace element solution SL-10:*

HCl (25%; 7.7 M) 10.0 ml

FeCl<sub>3</sub> x 4 H<sub>2</sub>O 1.5 g

ZnCl<sub>2</sub> 70.0 mg

MnCl<sub>2</sub> x 4 H<sub>2</sub>O 100.0 mg

H<sub>3</sub>BO<sub>3</sub> 6.0 mg

CoCl<sub>2</sub> x 6 H<sub>2</sub>O 190.0 mg

CuCl<sub>2</sub> x 2 H<sub>2</sub>O 2.0 mg

NiCl<sub>2</sub> x 6 H<sub>2</sub>O 24.0 mg

Na<sub>2</sub>MoO<sub>4</sub> x 2 H<sub>2</sub>O 36.0 mg

Distilled water 990.0 ml

*Vitamin solution:*

Biotin 2.0 mg

Folic acid 2.0 mg

Pyridoxine-HCl 10.0 mg

Thiamine-HCl 5.0 mg

Riboflavin 5.0 mg

Nicotinic acid 5.0 mg

Ca DL-pantothenate 5.0 mg

Vitamin B<sub>12</sub> 0.1 mg

p-Aminobenzoic acid 5.0 mg

Lipoic acid 5.0 mg

Distilled water 1000.0 ml

pH 7.1-7.4

Solution 1 is boiled before sterilization for a few minutes being flushed with gas mixture of 80% N<sub>2</sub> and 20% CO<sub>2</sub> with sodium bicarbonate added until an equilibrium pH of 6.9-7.1. Solution 1 is sterilized under this gas mixture at 121 C for 15 min. Solution 2 is sterilized under 100% N<sub>2</sub> at 121 C for 15 min. Vitamin solution is filter sterilized.

### **B 103. DESULFOBULBUS MEDIUM**

*Solution 1:*

Na<sub>2</sub>SO<sub>4</sub> 3.0 g

KH<sub>2</sub>PO<sub>4</sub> 0.2 g

NH<sub>4</sub>Cl 0.3 g

NaCl 1.0 g

MgCl<sub>2</sub> x 6 H<sub>2</sub>O 0.4 g

KCl 0.5 g

CaCl<sub>2</sub> x 2 H<sub>2</sub>O 0.15 g

Distilled water 870.0 ml

*Solution 2:*

Trace element solution SL-10 (see below) 1.0 ml

*Solution 3:*

NaHCO<sub>3</sub> 5.0 g

Distilled water 100.0 ml

*Solution 4:*

Na-propionate 1.5 g

Distilled water 10.0 ml

*Solution 5:*

Vitamin solution (see below) 10.0 ml

*Solution 6:*

Na<sub>2</sub>S x 9 H<sub>2</sub>O 0.4 g

Distilled water 10.0 ml

*Trace element solution SL-10:*

HCl (25%; 7.7 M) 10.0 ml

FeCl<sub>3</sub> x 4 H<sub>2</sub>O 1.5 g

ZnCl<sub>2</sub> 70.0 mg

MnCl<sub>2</sub> x 4 H<sub>2</sub>O 100.0 mg

H<sub>3</sub>BO<sub>3</sub> 6.0 mg

CoCl<sub>2</sub> x 6 H<sub>2</sub>O 190.0 mg

CuCl<sub>2</sub> x 2 H<sub>2</sub>O 2.0 mg

NiCl<sub>2</sub> x 6 H<sub>2</sub>O 24.0 mg

Na<sub>2</sub>MoO<sub>4</sub> x 2 H<sub>2</sub>O 36.0 mg

Distilled water 990.0 ml

*Vitamin solution:*

Biotin 2.0 mg

Folic acid 2.0 mg

Pyridoxine-HCl 10.0 mg

Thiamine-HCl 5.0 mg

Riboflavin 5.0 mg

Nicotinic acid 5.0 mg

Ca DL-pantothenate 5.0 mg

Vitamin B<sub>12</sub> 0.1 mg

p-Aminobenzoic acid 5.0 mg

Lipoic acid 5.0 mg

Distilled water 1000.0 ml

pH 7.1-7.4

Solution 1 is boiled before sterilization for a few minutes being flushed with gas mixture of 80% N<sub>2</sub> and 20% CO<sub>2</sub> with sodium bicarbonate added until an equilibrium pH of 6.9-7.1. Solution 1 is sterilized under this gas mixture at 121 C for 15 min. Solution 2 is sterilized under 100% N<sub>2</sub> at 121 C for 15 min. Vitamin solution is filter sterilized.

#### **104. MACROMONAS MEDIUM 1**

Na-acetate 1.0 g

CaCl<sub>2</sub> x 2 H<sub>2</sub>O 0.1 g

Casein acidic hydrolysate 0.1 g

Yeast extract 0.1 g

FeS or CaS 0.2 g

Agar 1.0 g

Distilled water 1000.0 ml

pH 7.2-7.4

Prepare suspension of FeS separately from the equimolar solutions of Na<sub>2</sub>S x 9 H<sub>2</sub>O and FeSO<sub>4</sub>, wash with freshly boiled distilled water under the flow of inert gas, sterilize separately from the main medium under inert gas at 111 C for 30 min. Main medium sterilize at 121 C for 15 min.

### 105. MACROMONAS MEDIUM 2

(NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub> 0.5 g  
MgSO<sub>4</sub> x 7 H<sub>2</sub>O 0.2 g  
Casein acidic hydrolysate 1.0 g  
Na-acetate 1.0 g  
or succinate, or benzoate 0.5 g  
Na<sub>2</sub>S<sub>2</sub>O<sub>3</sub> x 5 H<sub>2</sub>O 0.5 g  
Catalase 2.0 mg  
Vitamin solution (see below) 1.0 ml  
Distilled water 1000.0 ml

*Vitamin solution:*

Biotin 200.0 mg  
Folic acid 20.0 mg  
Pyridoxine-HCl 100.0 mg  
Thiamine-HCl 50.0 mg  
Riboflavin 100.0 mg  
Nicotinic acid 50.0 mg  
DL-Pantothenic acid 50.0 mg  
Vitamin B<sub>12</sub> 1.0 mg  
p-Aminobenzoic acid 50.0 mg  
Distilled water 1000.0 ml  
pH 7.2-7.4

Sterilize at 121 C for 15 min.

Sterilize catalase and vitamins separately from the main medium by filtration. Thiosulfate should also better be sterilized separately – at 121 C for 15 min.

### 106. BEGGIATOIA MEDIUM 1

(NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub> 500.0 mg  
MgSO<sub>4</sub> x 7 H<sub>2</sub>O 100.0 mg  
CaCl<sub>2</sub> x 2 H<sub>2</sub>O 50.0 mg  
Na-lactate 500.0 mg  
Na<sub>2</sub>S<sub>2</sub>O<sub>3</sub> x 5 H<sub>2</sub>O 500.0 mg  
K<sub>2</sub>HPO<sub>4</sub> 110.0 mg  
KH<sub>2</sub>PO<sub>4</sub> 85.0 mg  
FeCl<sub>3</sub> x 6 H<sub>2</sub>O 2.0 mg  
EDTA 3.0 mg  
Vitamin solution (see below) 1.0 ml  
Buffer HEPES 0.01 m  
Distilled water 1000.0 ml

*Vitamin solution:*

Biotin 200.0 mg  
Folic acid 20.0 mg  
Pyridoxine-HCl 100.0 mg  
Thiamine-HCl 50.0 mg  
Riboflavin 100.0 mg  
Nicotinic acid 50.0 mg  
DL-Pantothenic acid 50.0 mg  
Vitamin B<sub>12</sub> 1.0 mg  
p-Aminobenzoic acid 50.0 mg  
Distilled water 1000.0 ml  
pH 7.2-7.5 (adjust with NaOH)

Sterilize at 121 C for 15 min.

Sterilize thiosulfate (at 121 C for 15 min), lactate (at 121 C for 15 min) and vitamins (by filtration) each separately and add into the main medium prior to inoculation.

## **B 107. DESULFOSARCINA MEDIUM**

### *Solution 1:*

Na<sub>2</sub>SO<sub>4</sub> 3.0 g  
KH<sub>2</sub>PO<sub>4</sub> 0.2 g  
NH<sub>4</sub>Cl 0.3 g  
NaCl 13.5 g  
MgCl<sub>2</sub> x 6 H<sub>2</sub>O 2.0 g  
KCl 0.5 g  
CaCl<sub>2</sub> x 2 H<sub>2</sub>O 0.15 g  
Distilled water 870.0 ml

### *Solution 2:*

Trace element solution (see below) 1.0 ml

### *Solution 3:*

NaHCO<sub>3</sub> 5.0 g  
Distilled water 100.0 ml

### *Solution 4:*

Na-benzoate 0.6 g  
Na-lactate 1.0 g  
Distilled water 10.0 ml

### *Solution 5:*

Vitamin solution (see below) 10.0 ml

### *Solution 6:*

Na<sub>2</sub>S x 9 H<sub>2</sub>O 0.4 g  
Distilled water 10.0 ml

### *Trace element solution:*

HCl (25%; 7.7 M) 10.0 ml  
FeCl<sub>3</sub> x 4 H<sub>2</sub>O 1.5 g  
ZnCl<sub>2</sub> 70.0 mg  
MnCl<sub>2</sub> x 4 H<sub>2</sub>O 100.0 mg  
H<sub>3</sub>BO<sub>3</sub> 6.0 mg  
CoCl<sub>2</sub> x 6 H<sub>2</sub>O 190.0 mg  
CuCl<sub>2</sub> x 2 H<sub>2</sub>O 2.0 mg  
NiCl<sub>2</sub> x 6 H<sub>2</sub>O 24.0 mg  
Na<sub>2</sub>MoO<sub>4</sub> x 2 H<sub>2</sub>O 36.0 mg  
Na<sub>2</sub>SeO<sub>4</sub> x 5 H<sub>2</sub>O 3.0 mg  
Distilled water 990.0 ml

### *Vitamin solution:*

Biotin 2.0 mg  
Folic acid 2.0 mg  
Pyridoxine-HCl 10.0 mg  
Thiamine-HCl 5.0 mg  
Riboflavin 5.0 mg  
Nicotinic acid 5.0 mg  
Ca DL-pantothenate 5.0 mg  
Vitamin B<sub>12</sub> 0.1 mg  
p-Aminobenzoic acid 5.0 mg  
Lipoic acid 5.0 mg  
Distilled water 1000.0 ml  
pH 7.1-7.4

Solution 1 is boiled before sterilization for a few minutes being flushed with gas mixture of 80% N<sub>2</sub> and 20% CO<sub>2</sub> with sodium bicarbonate added until an equilibrium pH of 6.9-7.1. Solution 1 is sterilized under this gas mixture at 121 C for 15 min. Solution 2 is sterilized under 100% N<sub>2</sub> at 121 C for 15 min. Vitamin solution is filter sterilized.

### 108. BEGGIATOIA MEDIUM 2

MgCl<sub>2</sub> x 7 H<sub>2</sub>O 50.0 mg  
CaCl<sub>2</sub> x 2 H<sub>2</sub>O 30.0 mg  
Na-lactate 500.0 mg  
Na<sub>2</sub>S<sub>2</sub>O<sub>3</sub> x 5 H<sub>2</sub>O 500.0 mg  
NaH<sub>2</sub>PO<sub>4</sub> 125.0 mg  
KCl 125.0 mg  
NaHCO<sub>3</sub> 125.0 mg  
Na<sub>2</sub>SO<sub>4</sub> 500.0 mg  
NaNO<sub>3</sub> 620.0 mg  
Vitamin solution (see below) 1.0 ml  
Trace element solution according to Hogland: (see below) 1.0 ml  
Distilled water 1000.0 ml

#### *Vitamin solution:*

Biotin 200.0 mg  
Folic acid 20.0 mg  
Pyridoxine-HCl 100.0 mg  
Thiamine-HCl 50.0 mg  
Riboflavin 100.0 mg  
Nicotinic acid 50.0 mg  
DL-Pantothenic acid 50.0 mg  
Vitamin B<sub>12</sub> 1.0 mg  
p-Aminobenzoic acid 50.0 mg  
Distilled water 1000.0 ml

#### *Trace element solution according to Hogland:*

EDTA 5.0 g  
FeSO<sub>4</sub> x 7 H<sub>2</sub>O 2.0 g  
ZnSO<sub>4</sub> x 7 H<sub>2</sub>O 100.0 mg  
MnCl<sub>2</sub> x 4 H<sub>2</sub>O 30.0 mg  
H<sub>3</sub>BO<sub>3</sub> 300.0 mg  
CoCl<sub>2</sub> x 6 H<sub>2</sub>O 200.0 mg  
CuCl<sub>2</sub> 10.0 mg  
NiCl<sub>2</sub> x 6 H<sub>2</sub>O 20.0 mg  
Na<sub>2</sub>MoO<sub>4</sub> x 2 H<sub>2</sub>O 20.0 mg  
Distilled water 1000.0 ml  
pH 7.5 (adjust with 1% HCl)

To prepare the trace element solution, preliminarily acidify water to pH 3.0-4.0 with HCl. Sterilize thiosulfate, lactate, bicarbonate, trace elements and vitamins separately and add to the main medium prior to inoculation. Lactate and thiosulfate can be more conveniently prepared as 10% solutions; bicarbonate, as 5% solution. Sterilize the vitamin solution by filtration, others solutions and base medium at 121 C for 15 min.

### B 109. DESULFOVIBRIO MEDIUM

#### *Solution 1:*

K<sub>2</sub>HPO<sub>4</sub> 0.5 g  
NH<sub>4</sub>Cl 1.0 g  
Na<sub>2</sub>SO<sub>4</sub> 1.0 g  
CaCl<sub>2</sub> x 2 H<sub>2</sub>O 0.1 g  
MgSO<sub>4</sub> x 7 H<sub>2</sub>O 2.0 g  
Na DL-lactate 2.0 g  
Yeast extract 1.0 g  
Resazurin 1.0 g  
Distilled water 980.0 ml

*Solution 2:*

FeSO<sub>4</sub> x 7 H<sub>2</sub>O 0.5 g  
Distilled water 10.0 ml

*Solution 3:*

Na-thioglycollate 0.1 g  
Ascorbic acid 0.1 g  
Distilled water 10.0 ml

*Solution 4:*

Trace element solution (see below) 1.0 ml

*Trace element solution:*

FeCl<sub>3</sub> x 4 H<sub>2</sub>O 1.5 g  
ZnCl<sub>2</sub> 68.0 mg  
MnCl<sub>2</sub> x 4 H<sub>2</sub>O 100.0 ml  
H<sub>3</sub>BO<sub>3</sub> 62.0 mg  
CoCl<sub>2</sub> x 6 H<sub>2</sub>O 120.0 ml  
Na<sub>2</sub>MoO<sub>4</sub> x 2 H<sub>2</sub>O 24.0 mg  
HCl, 0.05 M 1000.0 ml  
pH 7.4-7.8

Sterilization at 121 C for 15 min under an atmosphere of N<sub>2</sub>. Solution 1 is boiled before sterilization, being blown with N<sub>2</sub>.

**B 110. DESULFOVIBRIO MEDIUM WITH 2% NaCl**

*Solution 1:*

K<sub>2</sub>HPO<sub>4</sub> 0.5 g  
NH<sub>4</sub>Cl 1.0 g  
NaCl 20 g  
Na<sub>2</sub>SO<sub>4</sub> 1.0 g  
CaCl<sub>2</sub> x 2 H<sub>2</sub>O 0.1 g  
MgSO<sub>4</sub> x 7 H<sub>2</sub>O 2.0 g  
Na DL-lactate 2.0 g  
Yeast extract 1.0 g  
Resazurin 1.0 g  
Distilled water 980.0 ml

*Solution 2:*

FeSO<sub>4</sub> x 7 H<sub>2</sub>O 0.5 g  
Distilled water 10.0 ml

*Solution 3:*

Na-thioglycollate 0.1 g  
Ascorbic acid 0.1 g  
Distilled water 10.0 ml

*Solution 4:*

Trace element solution (see below) 1.0 ml

*Trace element solution:*

FeCl<sub>3</sub> x 4 H<sub>2</sub>O 1.5 g  
ZnCl<sub>2</sub> 68.0 mg  
MnCl<sub>2</sub> x 4 H<sub>2</sub>O 100.0 ml  
H<sub>3</sub>BO<sub>3</sub> 62.0 mg  
CoCl<sub>2</sub> x 6 H<sub>2</sub>O 120.0 ml  
Na<sub>2</sub>MoO<sub>4</sub> x 2 H<sub>2</sub>O 24.0 mg  
HCl, 0.05 M 1000.0 ml  
pH 7.4-7.8

Sterilization at 121 C for 15 min under an atmosphere of N<sub>2</sub>. Solution 1 is boiled before sterilization, being blown with N<sub>2</sub>.

### **111. DESULFOVIBRIO MEDIUM WITH 3% NaCl**

#### *Solution 1:*

K<sub>2</sub>HPO<sub>4</sub> 0.5 g  
NH<sub>4</sub>Cl 1.0 g  
NaCl 30 g  
Na<sub>2</sub>SO<sub>4</sub> 1.0 g  
CaCl<sub>2</sub> x 2 H<sub>2</sub>O 0.1 g  
MgSO<sub>4</sub> x 7 H<sub>2</sub>O 2.0 g  
Na DL-Lactate 2.0 g  
Yeast extract 1.0 g  
Resazurin 1.0 g  
Distilled water 980.0 ml

#### *Solution 2:*

FeSO<sub>4</sub> x 7 H<sub>2</sub>O 0.5 g  
Distilled water 10.0 ml

#### *Solution 3:*

Na-thioglycollate 0.1 g  
Ascorbic acid 0.1 g  
Distilled water 10.0 ml

#### *Solution 4:*

Trace element solution (see below) 1.0 ml

#### *Trace element solution:*

FeCl<sub>3</sub> x 4 H<sub>2</sub>O 1.5 g  
ZnCl<sub>2</sub> 68.0 mg  
MnCl<sub>2</sub> x 4 H<sub>2</sub>O 100.0 ml  
H<sub>3</sub>BO<sub>3</sub> 62.0 mg  
CoCl<sub>2</sub> x 6 H<sub>2</sub>O 120.0 ml  
Na<sub>2</sub>MoO<sub>4</sub> x 2 H<sub>2</sub>O 24.0 mg  
HCl, 0.05 M 1000.0 ml  
pH 7.4-7.8

Sterilization at 121 C for 15 min under an atmosphere of N<sub>2</sub>. Solution 1 is boiled before sterilization, being blown with N<sub>2</sub>.

### **B 112. GLUCOSE YEAST EXTRACT AGAR**

Glucose 20.0 g  
Yeast extract 10.0 g  
CaCO<sub>3</sub> 20.0 g  
Agar 17.0 g  
Distilled water 1000.0 ml  
Sterilize at 111 C for 30 min.

### **B 113. DESULFOVIBRIO BAARSII MEDIUM**

#### *Solution 1:*

Na<sub>2</sub>SO<sub>4</sub> 3.0 g  
KH<sub>2</sub>PO<sub>4</sub> 0.2 g  
NH<sub>4</sub>Cl 0.3 g  
NaCl 1.0 g  
MgCl<sub>2</sub> x 6 H<sub>2</sub>O 0.4 g  
KCl 0.5 g  
CaCl<sub>2</sub> x 2 H<sub>2</sub>O 0.15 g  
Distilled water 870.0 ml

#### *Solution 2:*

Trace element solution SL-10 (see below) 1.0 ml

#### *Solution 3:*



NaHCO<sub>3</sub> 5.0 g  
Distilled water 100.0 ml

*Solution 4:*

Na-butyrate 0.7 g  
Na-caproate 0.3 g  
Na-octanoate 0.15 g  
Distilled water 10.0 ml

*Solution 5:*

Vitamin solution (see below) 10.0 ml

*Solution 6:*

Na<sub>2</sub>S x 9 H<sub>2</sub>O 0.4 g  
Distilled water 10.0 ml

*Trace element solution SL-10:*

HCl (25%; 7.7 M) 10.0 ml  
FeCl<sub>3</sub> x 4 H<sub>2</sub>O 1.5 g  
ZnCl<sub>2</sub> 70.0 mg  
MnCl<sub>2</sub> x 4 H<sub>2</sub>O 100.0 mg  
H<sub>3</sub>BO<sub>3</sub> 6.0 mg  
CoCl<sub>2</sub> x 6 H<sub>2</sub>O 190.0 mg  
CuCl<sub>2</sub> x 2 H<sub>2</sub>O 2.0 mg  
NiCl<sub>2</sub> x 6 H<sub>2</sub>O 24.0 mg  
Na<sub>2</sub>MoO<sub>4</sub> x 2 H<sub>2</sub>O 36.0 mg  
Distilled water 990.0 ml

*Vitamin solution:*

Biotin 2.0 mg  
Folic acid 2.0 mg  
Pyridoxine-HCl 10.0 mg  
Thiamine-HCl 5.0 mg  
Riboflavin 5.0 mg  
Nicotinic acid 5.0 mg  
Ca DL-pantothenate 5.0 mg  
Vitamin B<sub>12</sub> 0.1 mg  
p-Aminobenzoic acid 5.0 mg  
Lipoic acid 5.0 mg  
Distilled water 1000.0 ml  
pH 6.8-7.0

Solution 1 is boiled before sterilization for a few minutes being flushed with gas mixture of 80% N<sub>2</sub> and 20% CO<sub>2</sub> with sodium bicarbonate added until an equilibrium pH of 6.9-7.1. Solution 1 is sterilized under this gas mixture at 121 C for 15 min. Solution 2 is sterilized under 100% N<sub>2</sub> at 121 C for 15 min. Vitamin solution is filter sterilized.

#### **114. AZOSPIRILLUM BRASILIENSE MEDIUM 2**

Ca-malate 10.0 g or  
glucose 20.0 g  
K<sub>2</sub>HPO<sub>4</sub> 0.1 g  
KH<sub>2</sub>PO<sub>4</sub> 0.2 g  
MgSO<sub>4</sub> x 7 H<sub>2</sub>O 0.5 g  
FeCl<sub>3</sub> x 6 H<sub>2</sub>O 0.1 g  
CaCl<sub>2</sub> x 2 H<sub>2</sub>O 0.02 g  
Na<sub>2</sub>MoO<sub>4</sub> x 2 H<sub>2</sub>O 0.02 mg  
Yeast extract 0.1 g  
Agar 15.0 g  
Distilled water 1000.0 ml  
pH 6.9

Sterilize at 111 C for 30 min.

### **B 115. METHANOBACTERIUM MEDIUM**

KH<sub>2</sub>PO<sub>4</sub> 0.5 g  
MgSO<sub>4</sub> x 7 H<sub>2</sub>O 0.4 g  
NaCl 0.4 g  
NH<sub>4</sub>Cl 0.4 g  
CaCl<sub>2</sub> x 2 H<sub>2</sub>O 0.05 g  
Trace element solution SL-10 (see below) 1.0 ml  
Vitamin solution (see below) 5.0 ml

Yeast extract 1.0 g  
Na-acetate 1.0 g  
Na-formate 2.0 g  
NaHCO<sub>3</sub> 4.0 g  
Resazurin 1.0 mg  
Cystein-HCl 0.5 g  
Na<sub>2</sub>S x 9 H<sub>2</sub>O 0.5 g  
Fatty acid mixture (see below) 20.0 ml  
Sludge water: (see below) 50.0 ml  
Distilled water 940.0 ml

#### *Trace element solution SL-10:*

HCl (25%; 7.7 M) 10.0 ml  
FeCl<sub>3</sub> x 4 H<sub>2</sub>O 1.5 g  
ZnCl<sub>2</sub> 70.0 mg  
MnCl<sub>2</sub> x 4 H<sub>2</sub>O 100.0 mg  
H<sub>3</sub>BO<sub>3</sub> 6.0 mg  
CoCl<sub>2</sub> x 6 H<sub>2</sub>O 190.0 mg  
CuCl<sub>2</sub> x 2 H<sub>2</sub>O 2.0 mg  
NiCl<sub>2</sub> x 6 H<sub>2</sub>O 24.0 mg  
Na<sub>2</sub>MoO<sub>4</sub> x 2 H<sub>2</sub>O 36.0 mg  
Distilled water 990.0 ml

#### *Vitamin solution:*

Biotin 2.0 mg  
Folic acid 2.0 mg  
Pyridoxine (B<sub>2</sub>) 0.1 mg  
Riboflavin (B<sub>1</sub>) 5.0 mg  
Pantotenoic acid 5.0 mg  
p-Aminobenzoic acid 5.0 mg  
Thiamine-HCl 5.0 mg  
Nicotinic acid 5.0 mg  
Cyanocobalamin (B<sub>12</sub>) 0.1 mg  
Lipoic (tioctoic) acid 5.0 mg  
Distilled water 1000.0 ml

#### *Fatty acid mixture:*

Valeric acid 0.5 g  
Isovaleric acid 0.5 g  
p-Methylbutyric acid 0.5 g  
Isobutyric acid 0.5 g  
Distilled water 20.0 ml

pH 7.5 (adjust with concentrated NaOH)

*Sludge water:* to sludge from an anaerobic digester add 0.4% yeast extract and after gassing with N<sub>2</sub> gas for a few minutes incubate it at 37 C for 24 hours. Then centrifuge the sludge at 13,000 g and autoclave the resulting clear supernatant under N<sub>2</sub> gas at 121 C for 15 min (pH 6.7-7.0). Base medium and trace element solution sterilize at 121 C for 15 min. Vitamin

solution is filter sterilized. Prepare the medium anaerobically under a gas atmosphere of 80% H<sub>2</sub> and 20% CO<sub>2</sub>.

### **B 116. THERMUS RUBER MEDIUM**

Pepton 5.0 g  
Yeast extract 1.0 g  
Starch (soluble) 1.0 g  
Agar 12.0 g  
Distilled water 1000.0 ml  
pH 8.0  
Sterilize at 121 C for 15 min.

### **B 117. METHANOSARCINA MEDIUM**

#### *Solution 1:*

NaCl 0.9 g  
MgCl<sub>2</sub> x 7 H<sub>2</sub>O 0.2 g  
CaCl<sub>2</sub> x 2 H<sub>2</sub>O 0.1 g  
NH<sub>4</sub>Cl 1.0 g  
Yeast extract 2.0 g  
Resazurin 0.001 g  
Methanol 10% 10.0 ml  
Trace element solution (see below) 10.0 ml  
Vitamin solution (see below) 5.0 ml  
Distilled water 965.0 ml

#### *Solution 2 (reducing agents):*

Cystein-HCl 0.5 g  
Na<sub>2</sub>S x 9 H<sub>2</sub>O 0.5 g  
Distilled water 10.0 ml

#### *Buffer solutions:*

a) K<sub>2</sub>HPO<sub>4</sub> 29.0 g  
Distilled water 100.0 ml  
b) KH<sub>2</sub>PO<sub>4</sub> 15.0 g  
Distilled water 100.0 ml

#### *Trace element solution:*

Nitilotriacetic acid 12.8 mg  
FeSO<sub>4</sub> x 7 H<sub>2</sub>O 0.1 mg  
MnCl<sub>2</sub> x 6 H<sub>2</sub>O 0.1 mg  
CoCl<sub>2</sub> x 2 H<sub>2</sub>O 0.17 mg  
CaCl<sub>2</sub> x 2 H<sub>2</sub>O 0.1 mg  
ZnCl<sub>2</sub> 0.1 mg  
CuCl<sub>2</sub> 0.02 mg  
H<sub>3</sub>BO<sub>3</sub> 0.01 mg  
Na<sub>2</sub>MoO<sub>4</sub> x 2 H<sub>2</sub>O 0.01 mg  
NaCl 1.0 mg  
Na<sub>2</sub>SeO<sub>4</sub> 0.017 mg  
Distilled water 1000.0 ml

#### *Vitamin solution:*

Biotin 2.0 mg  
Folic acid 2.0 mg  
Pyridoxine (B<sub>2</sub>) 0.1 mg  
Riboflavin (B<sub>1</sub>) 5.0 mg  
Pantotenoic acid 5.0 mg  
p-Aminobenzoic acid 5.0 mg  
Thiamine-HCl 5.0 mg

Nicotinic acid 5.0 mg  
Cyanocobalamin (B<sub>12</sub>) 0.1 mg  
Lipoic (tioctoic) acid 5.0 mg  
Distilled water 1000.0 ml  
pH 7.2-7.4

Prepare medium in anaerobic conditions, blowing through with N<sub>2</sub> without O<sub>2</sub> up to sterilization. Solutions of reducing agents (10 ml) and of buffer (per 1 ml) add to base medium after separate sterilization. Base medium, trace element, buffer solutions and reducing agents sterilize at 121 C for 15 min/ Vitamin solution is filter sterilized.

#### **118. CLARK AGARIZED MEDIUM**

Peptone 5.0 g  
Glucose 5.0 g  
K<sub>2</sub>HPO<sub>4</sub> 5.0 g  
Agar 2.0 g  
Distilled water 1000.0 ml  
pH 6.9-7.0

Sterilize at 111 C for 20 min. Reagents for VP test: 6% alcoholic solution of p-naphthol and 40% aqueous solution of KOH. Reagent for MR test: 0.02% alcoholic-aqueous solution of methyl red.

#### **119. NITROSOLOBUS MEDIUM 2**

(NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub> 0.5 g  
KH<sub>2</sub>PO<sub>4</sub> 0.2 g  
CaCl<sub>2</sub> x 2 H<sub>2</sub>O 0.02 g  
MgSO<sub>4</sub> x 7 H<sub>2</sub>O 0.04 g  
EDTA FeNa 3.8% 0.1 ml  
Phenol red 0.05% 2.0 ml  
Distilled water 1000.0 ml  
pH 8.0 (adjust with 6% Na<sub>2</sub>CO<sub>3</sub>)  
Sterilize at 121 C for 15 min.

#### **120. MANNITOL AGAR WITH PEPTONE**

Yeast extract 5.0 g  
Mannitol 25.0 g  
Peptone 3.0 g  
Agar 15.0 g  
Distilled water 1000.0 ml  
pH 5.0-6.0  
Sterilize at 121 C for 15 min.

#### **121. THIOBACILLUS THIOOXIDANS MEDIUM (WAKSMAN MEDIUM)**

(NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub> 300.0 mg  
MgSO<sub>4</sub> x 7 H<sub>2</sub>O 500.0 mg  
KH<sub>2</sub>PO<sub>4</sub> 3.5 g  
CaCl<sub>2</sub> x 2 H<sub>2</sub>O 250.0 mg  
FeSO<sub>4</sub> x 7 H<sub>2</sub>O 18.0 mg  
Finely dispersed sulfur 5.0 g  
Distilled water 1000.0 ml  
Sterilize at 121 C for 15 min.

#### **122. THIOBACILLUS FERROOXIDANS MEDIUM (LEATHEN MEDIUM)**

(NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub> 150.0 mg  
KCl 50.0 mg  
MgSO<sub>4</sub> x 7 H<sub>2</sub>O 500.0 mg

KH<sub>2</sub>PO<sub>4</sub> 100.0 mg  
Ca(NO<sub>3</sub>)<sub>2</sub> x 4 H<sub>2</sub>O 10.0 mg  
Distilled water 1000.0 ml  
pH 4.0 (adjust after sterilization and addition of iron solution)  
Sterilize at 121 C for 15 min.

After sterilization of the medium, add 10 ml of 10% FeSO<sub>4</sub> x 7 H<sub>2</sub>O preliminarily acidified to pH 3.5 and sterilized separately. Sterilize this solution of iron in sealed ampoules under nitrogen or with minimal content of air by boiling on a water bath at 121 C for 15 min.

### **123. MEDIUM FOR MARINE NITROBACTER**

Solution 1 (see below) 0.5 ml  
Solution 2 (see below) 0.5 ml  
Solution 3 (see below) 1.0 ml  
Solution 4 (see below) 0.5 ml  
Solution 5 (see below) 0.5 ml  
Solution 6 (see below) 0.1 ml  
Distilled water 300.0 ml  
Sea water 700.0 ml

#### *Solution 1:*

CaCl<sub>2</sub> 2.0 g  
Distilled water 100.0 ml

#### *Solution 2:*

MgSO<sub>4</sub> x 7 H<sub>2</sub>O 20.0 g  
Distilled water 100.0 ml

#### *Solution 3:*

Chelated iron 0.1 g  
Distilled water 100.0 ml

#### *Solution 4:*

Na<sub>2</sub>MoO<sub>4</sub> 0.1 g  
MnCl<sub>2</sub> x 4 H<sub>2</sub>O 0.2 g  
CoCl<sub>2</sub> x 6 H<sub>2</sub>O 0.002 g  
ZnSO<sub>4</sub> x 7 H<sub>2</sub>O 0.1 g  
CuSO<sub>4</sub> x 5 H<sub>2</sub>O 0.02 g  
Distilled water 1000.0 ml

#### *Solution 5:*

NaNO<sub>3</sub> 41.4 g  
Distilled water 100.0 ml

#### *Solution 6:*

K<sub>2</sub>HPO<sub>4</sub> 1.74 g  
Distilled water 100.0 ml  
pH 8.6 (adjust with NaOH or KOH)

Sterilize all solutions separately at 121 C for 15 min. Leave the medium to stand for 2 – 3 days so that pH can adjust itself to pH 7.4 - 7.6.

### **B 124. AZOSPIRILLUM MEDIUM**

#### *Solution 1:*

Yeast extract 0.05 g  
FeSO<sub>4</sub> x 7 H<sub>2</sub>O 0.01 g  
K<sub>2</sub>HPO<sub>4</sub> 0.25 g  
Na<sub>2</sub>MoO<sub>4</sub> x 2 H<sub>2</sub>O 1.0 mg  
MnSO<sub>4</sub> x H<sub>2</sub>O 2.0 mg  
MgSO<sub>4</sub> x 7 H<sub>2</sub>O 0.2 g  
NaCl 0.1 g  
CaCl<sub>2</sub> x 2 H<sub>2</sub>O 0.02 g

(NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub> 1.0 g

Biotin 0.1 mg

Bromothymol blue 25.0 mg

Distilled water 950.0 ml

*Solution 2:*

Glucose 20% 25.0 ml

*Solution 3:*

Na-malate 20% 25.0 ml

Dissolve bromothymol blue in diluted KOH before adding into the medium.

pH 7.1

Sterilize solutions 1 and 3 separately at 121 C for 15 min, solution 2 at 111 C for 30 min.

### **125. NITROSOLOBUS MEDIUM 3**

(NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub> 2.0 g

CaCl<sub>2</sub> x 2 H<sub>2</sub>O 20.0 mg

MgSO<sub>4</sub> x 7 H<sub>2</sub>O 200.0 mg

Chelated iron 1.0 mg

Na<sub>2</sub>MoO<sub>4</sub> x 2 H<sub>2</sub>O 100.0 µg

MnCl<sub>2</sub> x 4 H<sub>2</sub>O 200.0 µg

CoCl<sub>2</sub> x 6 H<sub>2</sub>O 2.0 µg

ZnSO<sub>4</sub> x 7 H<sub>2</sub>O 100.0 µg

K<sub>2</sub>HPO<sub>4</sub> 15.9 mg

CuSO<sub>4</sub> x 5 H<sub>2</sub>O 20.0 µg

Distilled water 1000.0 ml

pH 7.5-7.8

Sterilize at 121 C for 15 min.

### **126. DESULFOCOCCUS NIACINI MEDIUM**

*Solution 1:*

Na<sub>2</sub>SO<sub>4</sub> 3.0 g

KH<sub>2</sub>PO<sub>4</sub> 0.2 g

NH<sub>4</sub>Cl 0.3 g

NaCl 13.5 g

MgCl<sub>2</sub> x 6 H<sub>2</sub>O 2.2 g

KCl 0.5 g

CaCl<sub>2</sub> x 2 H<sub>2</sub>O 0.15 g

Distilled water 870.0 ml

*Solution 2:*

Trace element solution SL-10 (see below) 1.0 ml

*Solution 3:*

NaHCO<sub>3</sub> 5.0 g

Distilled water 100.0 ml

*Solution 4:*

Na-nicotinate 5.0 mM

*Solution 5:*

Vitamin solution (see below) 10.0 ml

*Solution 6:*

Na<sub>2</sub>S x 9 H<sub>2</sub>O 0.4 g

Distilled water 10.0 ml

*Trace element solution SL-10:*

HCl (25%; 7.7 M) 10.0 ml

FeCl<sub>3</sub> x 4 H<sub>2</sub>O 1.5 g

ZnCl<sub>2</sub> 70.0 mg

MnCl<sub>2</sub> x 4 H<sub>2</sub>O 100.0 mg  
H<sub>3</sub>BO<sub>3</sub> 6.0 mg  
CoCl<sub>2</sub> x 6 H<sub>2</sub>O 190.0 mg  
CuCl<sub>2</sub> x 2 H<sub>2</sub>O 2.0 mg  
NiCl<sub>2</sub> x 6 H<sub>2</sub>O 24.0 mg  
Na<sub>2</sub>MoO<sub>4</sub> x 2 H<sub>2</sub>O 36.0 mg  
Na<sub>2</sub>SeO<sub>4</sub> x 5 H<sub>2</sub>O 40.0 mg  
Distilled water 990.0 ml

*Vitamin solution:*

Biotin 2.0 mg  
Folic acid 2.0 mg  
Pyridoxine-HCl 10.0 mg  
Thiamine-HCl 5.0 mg  
Riboflavin 5.0 mg  
Nicotinic acid 5.0 mg  
Ca DL-pantothenate 5.0 mg  
Vitamin B<sub>12</sub> 0.1 mg  
p-Aminobenzoic acid 5.0 mg  
Lipoic acid 5.0 mg  
Distilled water 1000.0 ml

Solution 1 is boiled before sterilization for a few minutes being flushed with gas mixture of 80% N<sub>2</sub> and 20% CO<sub>2</sub> with sodium bicarbonate added until an equilibrium pH of 6.9-7.1. Solution 1 is sterilized under this gas mixture at 121 C for 15 min. Solution 2 is sterilized under 100% N<sub>2</sub> at 121 C for 15 min. Trace element solution is sterilized at 121 C for 15 min. Vitamin solution is filter sterilized. Final pH of the complete medium 7.4

### **127. SELENITE CONTROL MEDIUM**

*Solution 1:*

Peptone 5.0 g  
Na<sub>2</sub>HPO<sub>4</sub> 7.0 g  
NaH<sub>2</sub>PO<sub>4</sub> 3.0 g  
Lactose 4.0 g  
Distilled water 960.0 ml  
pH 6.9-7.1

*Solution 2:*

10% Na<sub>2</sub>SeO<sub>4</sub> x 5 H<sub>2</sub>O 40.0 ml  
Sterilize at 111 C for 30 min.

### **B 128. HIRSCH MEDIUM**

KH<sub>2</sub>PO<sub>4</sub> 1.36 g  
Na<sub>2</sub>HPO<sub>4</sub> 2.15 g  
MgSO<sub>4</sub> x 7 H<sub>2</sub>O 0.2 g  
MnSO<sub>4</sub> x 5 H<sub>2</sub>O 1.05 g  
(NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub> 0.5 g  
CaCl<sub>2</sub> x 2 H<sub>2</sub>O 5.97 mg  
Na<sub>2</sub>MoO<sub>4</sub> x 2 H<sub>2</sub>O 1.5 mg  
Methanol 5.0 ml  
Distilled water to 1000.0 ml  
pH 7.0

Sterilize at 121 C for 15 min. Sterilize methanol by filtration and add to the medium after autoclaving.

### **129. CONTROL MEDIUM C-1**

KH<sub>2</sub>PO<sub>4</sub> 2.0 g  
K<sub>2</sub>HPO<sub>4</sub> 2.0 g

MgSO<sub>4</sub> x 7 H<sub>2</sub>O 0.2 g  
NaCl 5.0 g  
Raffinose 2.0 g  
Bromothymol blue (1.6% alkaline) 2.0 ml  
Crystal violet (0.01%) 20.0 ml  
50% Urea 4.0 ml  
Distilled water 975.0 ml  
Sterilize at 111 C for 30 min. Urea solution is filter sterilized.

### **130. POTATO – GLUCOSE AGAR**

Potato 200.0 g  
Glucose 10.0 g  
Agar 20.0 g  
Tap water 1000.0 ml  
pH 7.0  
Boil 200g scrubbed and sliced potatoes in 1000.0 ml water for 1 hour, filter cold through a cotton-gauze filter, add water to the initial volume, add glucose and agar. Do not use new potatoes.  
Sterilize at 111 C for 30 min.

### **Ac 131. MEDIUM Q MOD FOR FRANKIA**

K<sub>2</sub>HPO<sub>4</sub> 300.0 mg  
NaH<sub>2</sub>PO<sub>4</sub> 200.0 mg  
MgSO<sub>4</sub> x 7 H<sub>2</sub>O 200.0 mg  
KCl 200.0 mg  
Yeast extract 500.0 mg  
Peptone 5.0 g  
Glucose 10.0 g  
Fe-citrate 1.0 ml  
Trace element solution (see below) 1.0 ml  
CaCO<sub>3</sub> 100.0 mg  
Tween-80 2.0 g  
Agar 15.0 g  
Distilled water 1000.0 ml  
*Trace element solution:*  
H<sub>3</sub>BO<sub>3</sub> 1.5 g  
MnSO<sub>4</sub> x 7 H<sub>2</sub>O 0.8 g  
ZnSO<sub>4</sub> x 7 H<sub>2</sub>O 0.6 g  
CuSO<sub>4</sub> x 7 H<sub>2</sub>O 0.1 g  
(NH<sub>4</sub>)<sub>6</sub>Mo<sub>7</sub>O<sub>24</sub> x 4 H<sub>2</sub>O 0.2 g  
CoSO<sub>4</sub> 0.001 g  
Distilled water 1000.0 ml  
pH 7.0  
Sterilize at 111 C for 30 min.

### **132. THIOBACILLUS FERROOXIDANS MEDIUM 9K**

#### *Solution 1:*

KCl 100.0 mg  
MgSO<sub>4</sub> x 7 H<sub>2</sub>O 500.0 mg  
K<sub>2</sub>HPO<sub>4</sub> 500.0 mg  
Ca(NO<sub>3</sub>)<sub>2</sub> x 4 H<sub>2</sub>O 10.0 mg  
Distilled water 700.0 ml

#### *Solution 2:*

(NH<sub>4</sub>)<sub>2</sub>Fe(SO<sub>4</sub>)<sub>2</sub> 63.0 g  
H<sub>2</sub>SO<sub>4</sub> (10 N) 1.0 ml



Distilled water 300.0 ml

pH 3.5

Sterilize solution 1 at 121 C for 15 min, solution 2 at 111 C 30 min. Mix the solutions before inoculation.

### **133. COLBY AND ZATMAN MEDIUM WITH METHANOL**

$K_2HPO_4$  1.2 g

$KH_2PO_4$  0.62 g

$CaCl_2 \times 6 H_2O$  0.05 g

$MgSO_4 \times 7 H_2O$  0.2 g

NaCl 0.1 g

$FeCl_3 \times 6 H_2O$  1.0 mg

$(NH_4)_2SO_4$  0.5 g

$CuSO_4 \times 5 H_2O$  5.0 mkg

$MnSO_4 \times 5 H_2O$  10.0 mkg

$Na_2MoO_4 \times 2 H_2O$  10.0 mkg

$H_3BO_3$  10.0 mkg

$ZnSO_4 \times 7 H_2O$  70.0 mkg

$CoCl_2 \times 6 H_2O$  5.0 mkg

Purified agar 15.0 g

Methanol 1.0 ml

Distilled water 1000.0 ml

pH 6.8

Sterilize at 121 C for 15 min. Methanol is filter sterilized.

### **B 134. METHYLOTROPH MEDIUM 1**

$KH_2PO_4$  2.0 g

$(NH_4)_2SO_4$  2.0 g

$MgSO_4 \times 7 H_2O$  0.025 g

NaCl 0.5 g

$FeSO_4 \times 7 H_2O$  0.002 g

Methanol 5.0 ml

Distilled water 1000.0 ml

pH 7.0

Sterilize at 121 C for 15 min. Methanol is filter sterilized. Methanol may be replacement 30 ml 10 % solution of methylamine, sterilize at 111 C for 30 min.

### **B 135. METHYLOTROPH MEDIUM 2**

$KH_2PO_4$  0.8 g

$Na_2HPO_4 \times 12 H_2O$  3.0 g

$(NH_4)_2SO_4$  0.8 g

$MgSO_4 \times 7 H_2O$  0.1 g

$FeSO_4 \times 7 H_2O$  10.0 mg

Methanol 5.0 ml

Trace element solution (see below) 1.0 ml

Distilled water 1000.0 ml

*Trace element solution:*

$CaCl_2 \times 2 H_2O$  1.25 g

$CoCl_2 \times 6 H_2O$  1.25 g

$MnSO_4 \times 4 H_2O$  0.1 g

$ZnSO_4 \times 7 H_2O$  1.25 g

$Na_2MoO_4 \times 2 H_2O$  50.0 mg

$CuCl_2 \times 2 H_2O$  250.0 mg

Distilled water 250.0 ml

pH 7.0-7.2

Sterilize at 121 C for 15 min. Methanol is filter sterilized. Methanol may be replacement 30 ml 10 % solution of methylamine, sterilize at 111 C for 30 min.

### **136. MEDIUM FOR PERCHLORATE-REDUCING BACTERIA**

*Solution 1:*

NH<sub>4</sub>Cl 0.1 g  
NaCl 0.02 g  
KH<sub>2</sub>PO<sub>4</sub> 0.1 g  
K<sub>2</sub>HPO<sub>4</sub> 0.4 g  
MgSO<sub>4</sub> x 7 H<sub>2</sub>O 0.1 g  
Tap water 1000.0 ml

*Solution 2:*

HCl, to dissolve the precipitate in solution 1

*Solution 3:*

pH 6.9-7.2 (adjust with 5% NaOH)

*Solution 4:*

5% NH<sub>4</sub>ClO<sub>4</sub> 5.0 ml

*Solution 5:*

5% Na-acetate 5.0 ml

*Solution 6:*

Trace element solution according to Hogland (see below) 0.5 ml

*Solution 7:*

Vitamin B<sub>12</sub> (dispensary~~made~~ solution) 0.2 ml

*Solution 8:*

96° ethanol 0.5 ml

*Trace element solution according to Hogland:*

EDTA 5.0 g  
FeSO<sub>4</sub> x 7 H<sub>2</sub>O 2.0 g  
ZnSO<sub>4</sub> x 7 H<sub>2</sub>O 100.0 mg  
MnCl<sub>2</sub> x 4 H<sub>2</sub>O 30.0 mg  
H<sub>3</sub>BO<sub>3</sub> 300.0 mg  
CoCl<sub>2</sub> x 6 H<sub>2</sub>O 200.0 mg  
CuCl<sub>2</sub> 10.0 mg  
NiCl<sub>2</sub> x 6 H<sub>2</sub>O 20.0 mg  
Na<sub>2</sub>MoO<sub>4</sub> x 2 H<sub>2</sub>O 20.0 mg  
Distilled water 1000.0 ml

Sterilize ethanol by filtration, others solutions separately at 121 C for 15 min. Add solutions and additions to the main medium in the order of their enumeration.

### **137. MEDIUM FOR CHROMATE-REDUCING BACTERIA**

*Solution 1:*

NH<sub>4</sub>Cl 0.3 g  
NaCl 0.1 g  
KH<sub>2</sub>PO<sub>4</sub> 0.5 g  
K<sub>2</sub>HPO<sub>4</sub> 0.3 g  
MgSO<sub>4</sub> x 7 H<sub>2</sub>O 0.1 g  
CaCO<sub>3</sub> 0.05 g  
FeCl<sub>3</sub> x 6 H<sub>2</sub>O 0.05 g  
K<sub>2</sub>CrO<sub>4</sub> 0.1 g  
Paper-filtered pond water 900.0 ml

*Solution 2:*

Peptone meat broth 100.0 ml

*Solution 3:*

5% Na-acetate 5.0 ml

*Solution 4:*

Trace element solution according to Hogland (see below) 0.5 ml

*Solution 5:*

Vitamin B<sub>12</sub> (dispensarymade solution) 0.2 ml

*Trace element solution according to Hogland:*

EDTA 5.0 g

FeSO<sub>4</sub> x 7 H<sub>2</sub>O 2.0 g

ZnSO<sub>4</sub> x 7 H<sub>2</sub>O 100.0 mg

MnCl<sub>2</sub> x 4 H<sub>2</sub>O 30.0 mg

H<sub>3</sub>BO<sub>3</sub> 300.0 mg

CoCl<sub>2</sub> x 6 H<sub>2</sub>O 200.0 mg

CuCl<sub>2</sub> 10.0 mg

NiCl<sub>2</sub> x 6 H<sub>2</sub>O 20.0 mg

Na<sub>2</sub>MoO<sub>4</sub> x 2 H<sub>2</sub>O 20.0 mg

Distilled water 1000.0 ml

pH 7.0-7.2

Sterilize solutions separately at 121 C for 15 min. Add solutions and additions to the main medium in the order of their enumeration.

### **138. LARSEN PHOTOTROPH MEDIUM**

NH<sub>4</sub>Cl 0.5 g

KH<sub>2</sub>PO<sub>4</sub> 1.0 g

MgCl<sub>2</sub> 0.5 g

CaCl<sub>2</sub> 0.1 g

Trace element solution SL-12B (see below) 1.0 ml

NaHCO<sub>3</sub> 5.0 g

Na-acetate 2.0 g

Na<sub>2</sub>S<sub>2</sub>O<sub>3</sub> 0.4-0.6 g

Na<sub>2</sub>S 0.1 g

Fe-citrate Traces

Distilled water 1000.0 ml

*Trace element solution SL-12B:*

Ethylenediaminetetraacetate (EDTA) Na 3.0 g

FeSO<sub>4</sub> x 7 H<sub>2</sub>O 1.1 g

CoCl<sub>2</sub> x 6 H<sub>2</sub>O 190.0 mg

MnCl<sub>2</sub> x 2 H<sub>2</sub>O 50.0 mg

ZnCl<sub>2</sub> 42.0 mg

NiCl<sub>2</sub> x 6 H<sub>2</sub>O 24.0 mg

Na<sub>2</sub>MoO<sub>4</sub> x 2 H<sub>2</sub>O 18.0 mg

H<sub>3</sub>BO<sub>3</sub> 300.0 mg

CuCl<sub>2</sub> x 2 H<sub>2</sub>O 2.0 mg

Distilled water 1000.0 ml

pH of the trace element solution 6.0

pH 8.4

Sterilize solutions separately at 121 C for 15 min.

### **B 139. POSTGATE MEDIUM B FOR SULFATE REDUCERS**

*Solution 1:*

NaCl 1.0 g

KH<sub>2</sub>PO<sub>4</sub> 0.5 g

NH<sub>4</sub>Cl 1.0 g

CaSO<sub>4</sub> x 2 H<sub>2</sub>O 1.0 g

MgSO<sub>4</sub> x 7 H<sub>2</sub>O 2.0 g

Na-lactate 3.5 g  
Yeast extract 1.0 g  
Tap water 980.0 ml  
Dissolve above and gas with O<sub>2</sub> free N<sub>2</sub> for 10-15 minutes

*Solution 2:*

Ascorbic acid 1.0 g  
Thioglycolic acid 1.0 g  
Distilled water 10.0 ml

*Solution 3:*

FeSO<sub>4</sub> x 7 H<sub>2</sub>O 0.5 g  
Distilled water 10.0 ml  
pH 7.4

Sterilize solution 1 at 121 C for 15 min, solutions 2 and 3 separately at 111 C for 30 min.

#### **140. GLYCEROL-FUCHSIN BROTH**

*Solution 1:*

Hottinger broth (see below) 1000.0 ml

*Solution 2:*

Basic fuchsin, 10% alcoholic saturated solution 2.5 ml

*Solution 3:*

10% Na<sub>2</sub>SO<sub>4</sub> 16.6 ml

*Solution 4:*

Glycerol 10.0 ml

pH 6.8–7.2

Sterilize at 111 C for 15 min.

#### **141. CITRATE AGAR**

NaCl 5.0 g

MgSO<sub>4</sub> x 7 H<sub>2</sub>O 0.2 g

NH<sub>4</sub>H<sub>2</sub>PO<sub>4</sub> 1.0 g

K<sub>2</sub>HPO<sub>4</sub> 1.0 g

Na-citrate 3.0 g

Bromothymol blue 0.08 g

Agar 20.0 g

Distilled water 1000.0 ml

pH 7.2

Sterilize at 121 C for 15 min.

#### **B 142. PPYA**

Potato decoction (see below) 200.0 ml

Peptone 5.0 g

Yeast extract 1.0 g

Agar 25.0 g

Distilled water to 1000.0 ml

pH 8.0.

Sterilize at 121 C for 15 min

*Preparation of potato decoction:* boil 200 g scrubbed and sliced potatoes in 1000.0 ml water for 1 hour, filter cold through a cotton-gauze filter, add water to the initial volume, add glucose and agar. Do not use new potatoes.

#### **143. PEPTONE MEAT AGAR WITH 1% GLYCEROL**

Peptone 10.0 g

Beef extract 3.0 g

NaCl 5.0 g

Glycerol 10.0 ml  
Agar 20.0 g  
Distilled water 1000.0 ml  
pH 7.0-7.2  
Sterilize at 121 C for 15 min.

**144. PEPTONE MEAT AGAR WITH 0.5% GLUCOSE**

Peptone 10.0 g  
Beef extract 3.0 g  
NaCl 5.0 g  
Glucose 5.0 g  
Agar 20.0 g  
Distilled water 1000.0 ml  
pH 7.0-7.2  
Sterilize at 111 C for 30 min

**B 145. PSEUDOMONAS SP. (ARTHROBACTER GLOBIFORMIS) MEDIUM**

Glucose 10.0 g  
Maize extract 10.0 g  
Tap water 1000.0 ml  
pH 7.8 (adjust with  $\text{NH}_4\text{OH}$ ); paper filter.  
Sterilize at 111 C for 30 min.

**146. AGAR ENDO**

Beef extract 5.0 g  
Peptone 10.0 g  
 $\text{Na}_2\text{HPO}_4$  3.5 g  
Lactose 10.0 g  
 $\text{Na}_2\text{SO}_3$  2.5 g  
Basic fuchsin 0.5 g  
Agar 15.0 g  
Tap water 1000.0 ml

Prepare the basic medium without fuchsin and sulfite, pH of the medium 7.4-7.6. Prepare separately 10% fuchsin solution in 90 degree ethanol, filter, add sulfite (possible as 10-20% aqueous solution) to bright green staining. Autoclave 15 min at 121 C. Pour plates. The plates are clear and pale pink. On exposure to  $\text{O}_2$  the plated culture medium gradually becomes red due to the oxidation of sulfite and can thus no longer be used. It can only be kept for a few days even if it is stored in the dark and at refrigerator temperature.

Not sterilize!

**147. HYPHOMICROBIUM MEDIUM**

$\text{NaNO}_3$  1.0 g  
NaCl 0.5 g  
 $\text{K}_2\text{HPO}_4$  1.0 g  
 $\text{MgSO}_4 \times 7 \text{H}_2\text{O}$  0.2 g  
Methanol 5.0-10.0 ml  
Distilled water 1000.0 ml  
pH 6.8-7.0

Sterilize at 121 C for 15 min. Methanol is filter sterilized.  
Grow in an exsiccator in the presence of methanol vapors.

**B 148. GLUCOSE YEAST CHALK MEDIUM**

Beef extract 3.0 g  
Yeast extract 10.0 g

Glucose 50.0 g  
CaCO<sub>3</sub> 30 g  
Agar 25 g  
Distilled water 1000.0 ml  
Sterilize at 121 C for 15 min.

#### **149. CLAVIBACTER XYLI MEDIUM**

*Solution 1:*

Flour agar 5.0 g  
Papaya hydrolysate of soybean meal 8.0 g  
K<sub>2</sub>HPO<sub>4</sub> 1.0 g  
KH<sub>2</sub>PO<sub>4</sub> 1.0 g  
MgSO<sub>4</sub> x 7 H<sub>2</sub>O 0.2 g  
Distilled water 965.0 ml

*Solution 2:*

Bovine hemine chloride (0.1% in 0.05 N NaOH) 15.0 ml

*Solution 3:*

Bovine serum albumin, fraction 5 (20%) 10.0 ml

*Solution 4:*

Glucose (50%) 1.0 ml

*Solution 5:*

Cysteine (10%) 10.0 ml

pH 6.6

Sterilize solution 1 at 121 C for 15 min, cool to 50 C and add the filter-sterilized solutions 2-5.

#### **150. GETCHINSON MEDIUM WITH FILTER PAPER**

K<sub>2</sub>HPO<sub>4</sub> 1.3 g  
MgSO<sub>4</sub> x 7 H<sub>2</sub>O 0.3 g  
CaCl<sub>2</sub> x 6 H<sub>2</sub>O 0.1 g  
FeCl<sub>3</sub> x 6 H<sub>2</sub>O 0.01 g  
NaNO<sub>3</sub> 2.5 g  
Distilled water 1000.0 ml  
pH 7.2-7.3.

Sterilize at 121 C for 15 min. Cut filter paper into strips, sterilize by dry heat and immerse into the medium so that they are not completely in the liquid medium.

#### **151. MUNZ MEDIUM FOR METHANE-OXIDIZING BACTERIA**

K<sub>2</sub>HPO<sub>4</sub> 0.5 g  
KH<sub>2</sub>PO<sub>4</sub> 0.5 g  
MgSO<sub>4</sub> x 7 H<sub>2</sub>O 0.5 g  
NH<sub>4</sub>Cl 1.0 g  
Tap water 1000.0 ml  
pH 6.8

Sterilize at 121 C for 15 min.

Cultivate in the mixed atmosphere of air and methane (2:1).

#### **B 152. TRYPTONE THIOGLYCOLLATE MEDIUM**

*Solution 1:*

K<sub>2</sub>HPO<sub>4</sub> 5.45 g  
KH<sub>2</sub>PO<sub>4</sub> 1.20 g  
MgSO<sub>4</sub> x 7 H<sub>2</sub>O 0.025 g  
CaCl<sub>2</sub> x 2 H<sub>2</sub>O 0.015 g  
FeSO<sub>4</sub> x 7 H<sub>2</sub>O 0.01 g  
MnCl<sub>2</sub> x 4 H<sub>2</sub>O 2.0 mg

CoCl<sub>2</sub> x 6 H<sub>2</sub>O 2.5 mg  
Na<sub>2</sub>MoO<sub>4</sub> x 2 H<sub>2</sub>O 2.5 mg  
Peptone 2.0 g  
Tryptone 2.0 g  
Yeast extract 6.0 g  
Na-thioglycollate 0.5 g  
Distilled water 950.0 ml

*Solution 2:*

Glucose 20.0 g  
Distilled water 50.0 ml

pH 7.5

Sterilize solution 1 at 121 C for 15 min., solution 2 at 111 C for 30 min.

**B 153. MEDIUM P-2 FOR THERMOPHILIC ANAEROBIC BACTERIA**

K<sub>2</sub>HPO<sub>4</sub> 3.0 g  
KH<sub>2</sub>PO<sub>4</sub> 2.0 g  
NH<sub>4</sub>Cl 2.0 g  
MgCl<sub>2</sub> x 6 H<sub>2</sub>O 0.2 g  
CaCl<sub>2</sub> x 6 H<sub>2</sub>O 0.05 g  
Tryptone 10.0 g  
Glucose 5.0 g  
Yeast extract 5.0 g  
Resazurin 0.001 g  
Distilled water 1000.0 ml

pH 7.0-7.2

Prepare medium anaerobically under N<sub>2</sub> and sterilize under N<sub>2</sub> at 121 C for 15 min. Sterilize glucose separately under N<sub>2</sub> at 111 C for 30 min.

**B 154. DESULFOVIBRIO SULFODISMUTANS MEDIUM**

*Solution 1:*

KH<sub>2</sub>PO<sub>4</sub> 0.2 g  
NH<sub>4</sub>Cl 0.3 g  
NaCl 1.0 g  
MgCl<sub>2</sub> x 6 H<sub>2</sub>O 0.4 g  
KCl 0.5 g  
CaCl<sub>2</sub> x 2 H<sub>2</sub>O 0.15 g  
Distilled water 920.0 ml

*Solution 2:*

Trace element solution SL-10 (see below) 1.0 ml

*Solution 3:*

NaHCO<sub>3</sub> 2.5 g  
Distilled water 50.0 ml

*Solution 4:*

Na-acetate x 3 H<sub>2</sub>O 0.3 g  
Distilled water 10.0 ml

*Solution 5:*

D(+)-Biotin 10.0 µg  
Ca-D(+)-Pantothenate 50.0 µg  
Distilled water 1.0 ml

*Solution 6:*

Na<sub>2</sub>S x 9 H<sub>2</sub>O 0.4 g  
Distilled water 10.0 ml

*Solution 7:*

Na<sub>2</sub>S<sub>2</sub>O<sub>3</sub> (0.5 M) 10.0 ml

pH 7.5-8.0 (adjust with NaOH)

*Trace element solution SL-10:*

HCl (25%; 7.7 M) 10.0 ml

FeCl<sub>3</sub> x 4 H<sub>2</sub>O 1.5 g

ZnCl<sub>2</sub> 70.0 mg

MnCl<sub>2</sub> x 4 H<sub>2</sub>O 100.0 mg

H<sub>3</sub>BO<sub>3</sub> 6.0 mg

CoCl<sub>2</sub> x 6 H<sub>2</sub>O 190.0 mg

CuCl<sub>2</sub> x 2 H<sub>2</sub>O 2.0 mg

NiCl<sub>2</sub> x 6 H<sub>2</sub>O 24.0 mg

Na<sub>2</sub>MoO<sub>4</sub> x 2 H<sub>2</sub>O 36.0 mg

Distilled water 990.0 ml

pH 7.1-7.4

Solution 1 is prepared and autoclaved anaerobically at under 80% N<sub>2</sub> + 20% CO<sub>2</sub> at 121 C for 15 min.

Solutions 2, 4, 5 and 6 are gassed with N<sub>2</sub> and sterilized separately at 121 C for 15 min. Solution 3

(gassed with N<sub>2</sub> + CO<sub>2</sub>) and solution 7 (gassed with N<sub>2</sub>) are filter-sterilized. Solutions with 2 to 7 are added to the sterile, cooled solution 1 in the sequence as indicated. When growth has started feed culture again with same amount of solution 7. After a further two days repeat feeding once more.

### **155. SCHATZ AND BOVELL MEDIUM FOR HYDROGEN-OXIDIZING BACTERIA**

KH<sub>2</sub>PO<sub>4</sub> 1.0 g

NH<sub>4</sub>NO<sub>2</sub> 1.0 g

MgSO<sub>4</sub> x 7 H<sub>2</sub>O 0.2 g

FeSO<sub>4</sub> x 7 H<sub>2</sub>O 0.01 g

CaCl<sub>2</sub> x 2 H<sub>2</sub>O 0.01 g

NaHCO<sub>3</sub> 0.5 g

Agar 15.0 g

Distilled water 1000.0 ml

pH 6.8-7.2

Sterilize at 121 C for 15 min. Cultivate in a gas mixture of carbon dioxide, air and hydrogen (1:3:6).

### **156. MEDIUM FOR MIXOBACTERIA**

Casein hydrolysate 2.5 g

Asparagin 2.5 g

K<sub>2</sub>HPO<sub>4</sub> 2.0 g

NaCl 1.0 g

MgSO<sub>4</sub> x 7 H<sub>2</sub>O 0.1 g

FeSO<sub>4</sub> x 7 H<sub>2</sub>O 0.003 g

CaCl<sub>2</sub> x 2 H<sub>2</sub>O 0.01 g

Distilled water 1000.0 ml

pH 7.4

Sterilize at 121 C for 15 min.

### **Ac, B 157. EMERSON STARCH YEAST EXTRACT AGAR**

Yeast extract 4.0 g

Starch (soluble) 15.0 g

KH<sub>2</sub>PO<sub>4</sub> 1.0 g

MgSO<sub>4</sub> x 7 H<sub>2</sub>O 0.5 g

Agar 20.0 g

Distilled water 1000.0 ml

pH 7.0

Sterilize at 121 C for 15 min.

### **Ac, B 158. GLYCEROL YEAST AGAR**



Yeast extract 5.0 g  
Glycerol 50.0 g  
CaCO<sub>3</sub> 1.0 g  
Agar 20.0 g  
Distilled water 1000.0 ml  
pH 7.0-7.2  
Sterilize at 121 C for 15 min.

#### **159. AGAR WITH GLUCOSE AND YEAST EXTRACT**

Glucose 20.0 g  
Yeast extract 10.0 g  
CaCO<sub>3</sub> 20.0 g  
Agar 17.0 g  
Distilled water 1000.0 ml  
pH 7.0-7.2  
Sterilize at 111 C for 30 min.

#### **160. MEAT GLUCOSE MEDIUM**

Peptone 10.0 g  
Glucose 10.0 g  
Beef extract 3.0 g  
NaCl 5.0 g  
Distilled water 1000.0 ml  
pH 7.2-7.4  
Sterilize at 111 C for 30 min.

#### **B 161. RHIZOBIUM MEDIUM**

Yeast extract 1.0 g  
Mannitol 10.0 g  
Agar 15.0 g  
Soil extract (see below) 200.0 ml  
Distilled water 800.0 ml  
*Soil extract:*  
Air-dried garden soil 80.0 g  
Na<sub>2</sub>CO<sub>3</sub> 0.2 g  
Distilled water 200.0 ml  
Autoclave at 121 C for 1 h. filter and make up to 200 ml.  
pH 7.2  
Sterilize at 121 C for 15 min.

#### **B 162. ANCYLOBACTER-SPIROSOMA MEDIUM**

Glucose 1.0 g  
Peptone 1.0 g  
Yeast extract 1.0 g  
Agar 15.0 g  
Distilled water 1000 0 ml  
pH 7.0-7.2  
Sterilize at 111 C for 30 min.

#### **163. MICROCYCLUS MEDIUM**

Glucose 5.0 g  
Peptone 5.0 g  
Yeast extract 5.0 g

Agar 15.0 g  
Distilled water 1000.0 ml  
pH 6.8  
Sterilize at 111 C for 30 min.

#### **B 164. PD BROTH FOR FLEXIBACTER**

Peptone 1.0 g  
KNO<sub>3</sub> 100.0 mg  
Yeast extract 100.0 mg  
K<sub>2</sub>HPO<sub>4</sub> 66.7 mg  
MgSO<sub>4</sub> x 7 H<sub>2</sub>O 33.3 mg  
Trace element solution (see below) 1.0 ml  
Distilled water 1000.0 ml  
*Trace element solution:*  
Zn SO<sub>4</sub> x 7 H<sub>2</sub>O 22.0 mg  
MnSO<sub>4</sub> x 7 H<sub>2</sub>O 1.81 g  
CuSO<sub>4</sub> x 5 H<sub>2</sub>O 79.0 mg  
Na<sub>3</sub>BO<sub>3</sub> x 4 H<sub>2</sub>O 1.0 g  
(NH<sub>4</sub>)<sub>6</sub>Mo<sub>7</sub>O<sub>24</sub> x 4 H<sub>2</sub>O 9.3 g  
FeSO<sub>4</sub> x 7 H<sub>2</sub>O 20.0 mg  
Co(NO<sub>3</sub>)<sub>2</sub> x H<sub>2</sub>O 20.0 mg  
Trilon B 10.0 g  
Distilled water 1000.0 ml  
pH 7.0  
Sterilize at 111 C for 30 min.

#### **165. INDICATOR MEDIUM WITH MALONATE**

Yeast extract 1.0 g  
(NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub> 2.0 g  
KH<sub>2</sub>PO<sub>4</sub> 0.4 g  
K<sub>2</sub>HPO<sub>4</sub> 0.6 g  
NaCl 2.0 g  
Na-malonate 3.0 g  
Glucose 0.25 g  
Bromothymol blue (0.2%) 12.0 ml  
Distilled water 1000.0 ml  
Dissolve components of the medium in boiling water in the specified sequence (except the indicator). Then filter through a cotton wool-gauze filter to remove the possible precipitate, bring to the initial volume, cool, adjust pH 6.7, add the indicator. Sterilize at 111 C for 30 min.

#### **Ac 166. CORYNEBACTERIUM AGAR**

Casein peptone, tryptic digest 10.0 g  
Yeast extract 5.0 g  
Glucose 5.0 g  
NaCl 5.0 g  
Agar 15.0 g  
Distilled water 1000.0 ml  
pH 7.2-7.4  
Sterilize at 111 C for 30 min.

#### **B 167. PEPTONE MEAT AGAR WITH 3% NaCl**

Peptone 10.0 g  
Beef extract 3.0 g  
NaCl 30.0 g

Agar 20.0 g  
Distilled water 1000.0 ml  
pH 7.2-7.4  
Sterilize at 121 C for 15 min.

**Ac 168. CORYNEBACTERIUM MEDIUM WITH SALT**

Casein peptone, tryptic digest 10.0 g  
Yeast extract 5.0 g  
Glucose 5.0 g  
NaCl 60.0 g  
Agar 15.0 g  
Distilled water 1000.0 ml  
pH 7.2-7.4  
Sterilize at 121 C for 15 min.

**B 169. ALCALIGENES PARADOXUS MEDIUM**

*Solution 1:*

KH<sub>2</sub>PO<sub>4</sub> 2.3 g  
Na<sub>2</sub>HPO<sub>4</sub> x 2 H<sub>2</sub>O 2.9 g  
NH<sub>4</sub>Cl 1.0 g  
MgSO<sub>4</sub> x 7 H<sub>2</sub>O 0.5 g  
NaHCO<sub>3</sub> 0.5 g  
CaCl<sub>2</sub> x 2 H<sub>2</sub>O 0.5 g  
Trace element solution SL-6 (see below) 5.0 ml  
Agar (if necessary) 15.0 g  
Distilled water 980.0 ml

*Solution 2:*

Fe(NH<sub>4</sub>)-citrate 0.05 g  
Distilled water 20.0 ml

*Trace element solution SL-6:*

ZnSO<sub>4</sub> x 7 H<sub>2</sub>O 0.1 g  
MnCl<sub>2</sub> x 4 H<sub>2</sub>O 0.03 g  
H<sub>3</sub>BO<sub>3</sub> 0.3 g  
CoCl<sub>2</sub> x 6 H<sub>2</sub>O 0.2 g  
CuCl<sub>2</sub> x 2 H<sub>2</sub>O 0.01 g  
NiCl<sub>2</sub> x 6 H<sub>2</sub>O 0.02 g  
Na<sub>2</sub>MoO<sub>4</sub> 0.03 g  
Distilled water 1000.0 ml  
pH 6.8

Sterilize solutions separately at 121 C for 15 min.

**B 170. CYTOPHAGA MEDIUM**

Yeast extract 10.0 g  
NH<sub>4</sub>Cl 1.0 g  
MgSO<sub>4</sub> x 7 H<sub>2</sub>O 1.0 g  
K<sub>2</sub>HPO<sub>4</sub> 0.2 g  
NaCl 20.0 g  
FeCl<sub>3</sub> x 6 H<sub>2</sub>O Traces  
Agar (if necessary) 2.0-3.0 g  
Distilled water 1000.0 ml  
pH 7.5  
Sterilize at 121 C for 15 min.

**B 171. YEAST AGAR**

K<sub>2</sub>HPO<sub>4</sub> 1.0 g  
MgSO<sub>4</sub> x 7 H<sub>2</sub>O 0.5 g  
Yeast extract 10.0 g  
Agar 20.0 g  
Tap water 1000.0 ml  
pH 7.0-7.2  
Sterilize at 121 C for 15 min.

**B 172. PEPTONE SUCCINATE AGAR**

(NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub> 1.0 g  
MgSO<sub>4</sub> x 7 H<sub>2</sub>O 1.0 g  
MnSO<sub>4</sub> x 5 H<sub>2</sub>O 2.0 mg  
FeCl<sub>3</sub> x 6 H<sub>2</sub>O 2.0 mg  
Succinic acid 1.68 g  
Peptone 5.0 g  
Agar 1.5 g  
Distilled water 1000.0 ml  
pH 7.0  
Sterilize at 121 C for 15 min.

**B 173. SPIRILLUM GRACILLE MEDIUM**

Peptone 5.0 g  
Yeast extract 0.5 g  
Tween 80 0.02 g  
K<sub>2</sub>HPO<sub>4</sub> 0.1 g  
Agar (if needed) 15.0 g  
Tap water 1000.0 ml  
pH to 7.2  
Sterilize at 121 C for 15 min.

**174. MEDIUM FOR DENTRIFYING BACTERIA (GILTAY MEDIUM)**

*Solution 1:*

KNO<sub>3</sub> 1.0 g  
Asparagin 1.0 g  
Distilled water 250.0 ml

*Solution 2:*

Ca-citrate 8.5 g  
KH<sub>2</sub>PO<sub>4</sub> 1.0 g  
Distilled water 500.0 ml

*Solution 3:*

MgSO<sub>4</sub> x 7 H<sub>2</sub>O 1.0 g  
FeCl<sub>3</sub> x 4 H<sub>2</sub>O traces  
CaCl<sub>2</sub> x 2 H<sub>2</sub>O 0.2 g  
Distilled water 250.0 ml

Sterilize solutions separately at 121 C for 15 min and mix aseptically.

**175. PEPTONE MEAT AGAR WITH 1% GLUCOSE**

Peptone 10.0 g  
Beef extract 3.0 g  
NaCl 5.0 g  
Glucose 10.0 g  
Agar 20.0 g  
Distilled water 1000.0 ml  
pH 7.2-7.4

Sterilize at 111 C for 30 min.

### **B 176. METHANOBACTERIUM MEDIUM**

#### *Solution 1:*

NaCl 0.9 g  
MgCl<sub>2</sub> x 7 H<sub>2</sub>O 0.2 g  
CaCl<sub>2</sub> x 2 H<sub>2</sub>O 0.1 g  
NH<sub>4</sub>Cl 1.0 g  
Yeast extract 2.0 g  
Resazurin 0.001 g  
Trace element solution (see below) 10.0 ml  
Vitamin solution (see below) 5.0 ml  
Buffer solution a (see below) 10.0 ml  
Buffer solution b (see below) 10.0 ml  
Distilled water 965.0 ml

#### *Solution 2 (reducing agents):*

Cystein-HCl 0.5 g  
Na<sub>2</sub>S x 9 H<sub>2</sub>O 0.5 g  
Distilled water 10.0 ml

#### *Buffer solutions:*

a) K<sub>2</sub>HPO<sub>4</sub> 29.0 g  
Distilled water 100.0 ml  
b) KH<sub>2</sub>PO<sub>4</sub> 15.0 g  
Distilled water 100.0 ml

#### *Trace element solution:*

Nitrilotriacetic acid 12.8 mg  
FeSO<sub>4</sub> x 7 H<sub>2</sub>O 0.1 mg  
MnCl<sub>2</sub> x 6 H<sub>2</sub>O 0.1 mg  
CoCl<sub>2</sub> x 2 H<sub>2</sub>O 0.17 mg  
CaCl<sub>2</sub> x 2 H<sub>2</sub>O 0.1 mg  
ZnCl<sub>2</sub> 0.1 mg  
CuCl<sub>2</sub> 0.02 mg  
H<sub>3</sub>BO<sub>3</sub> 0.01 mg  
Na<sub>2</sub>MoO<sub>4</sub> x 2 H<sub>2</sub>O 0.01 mg  
NaCl 1.0 mg  
Na<sub>2</sub>SeO<sub>4</sub> 0.017 mg  
Distilled water 1000.0 ml

#### *Vitamin solution:*

Biotin 2.0 mg  
Folic acid 2.0 mg  
Pyridoxine (B<sub>2</sub>) 0.1 mg  
Riboflavin (B<sub>1</sub>) 5.0 mg  
Pantotenoic acid 5.0 mg  
p-Aminobenzoic acid 5.0 mg  
Thiamine-HCl 5.0 mg  
Nicotinic acid 5.0 mg  
Cyanocobalamin (B<sub>12</sub>) 0.1 mg  
Lipoic (tioctoic) acid 5.0 mg  
Distilled water 1000.0 ml  
pH 7.2-7.4

Prepare medium in anaerobic conditions, blowing through with N<sub>2</sub> without O<sub>2</sub> up to sterilization. Solutions of reducing agents (10 ml) and others solutions add to base medium after separate sterilization at 121 C for 15 min. Sterilize vitamin solution by filtration.

Cultivate in a gas mixture of 80% H<sub>2</sub> and 20% CO<sub>2</sub>.

**Ac 177.**

Starch (soluble) 10.0 g  
Yeast extract 1.0 g  
Beef extract 1.0 g  
Pancreatic casein hydrolysate 2.0 g  
CoCl<sub>2</sub> x 7 H<sub>2</sub>O 0.02 g  
Agar 20.0 g  
Distilled water 1000.0 ml  
pH 7.3  
Sterilize at 121 C for 15 min.

**178. MEDIUM FOR MARINE CYTOPHAGA***Solution 1:*

Yeast extract 1.0 g  
Tryptone 1.0 g  
KCl 0.7 g  
NaCl 24.7 g  
MgSO<sub>4</sub> x 7 H<sub>2</sub>O 6.3 g  
MgCl<sub>2</sub> x 6 H<sub>2</sub>O 4.6 g  
Agar 15.0 g  
Distilled water 950.0 ml

*Solution 2:*

CaCl<sub>2</sub> x 2 H<sub>2</sub>O 1.2 g  
Distilled water 25.0 ml

*Solution 3:*

NaHCO<sub>3</sub> 0.2 g  
Distilled water 25.0 ml  
pH 7.2

Sterilize solutions separately at 121 C for 15 min and mix aseptically.

**179.**

Glucose 1.0 g  
Trypticase 5.0 g  
KH<sub>2</sub>PO<sub>4</sub> 1.0 g  
Na-acetate 4.0 g  
Yeast extract 2.0 g  
n-Valeric acid 0.1 ml  
Resazurin 1.0 mg  
Na<sub>2</sub>CO<sub>3</sub> 4.0 g  
Cystein-HCl 0.5 g  
Distilled water 1000.0 ml  
pH 7.0  
Sterilize at 111 C for 30 min.  
Gas atmosphere: 100% CO<sub>2</sub>.

**B 180. DESULFOVIBRIO CARBINOLICUS MEDIUM***Solution 1:*

Na<sub>2</sub>SO<sub>4</sub> 3.0 g  
KH<sub>2</sub>PO<sub>4</sub> 0.2 g  
NH<sub>4</sub>Cl 0.3 g  
NaCl 1.0 g  
MgCl<sub>2</sub> x 6 H<sub>2</sub>O 0.4 g  
KCl 0.5 g

CaCl<sub>2</sub> x 2 H<sub>2</sub>O 0.15 g  
Distilled water 870.0 ml

*Solution 2:*

Trace element solution SL-10 (see below) 1.0 ml

*Solution 3:*

NaHCO<sub>3</sub> 5.0 g  
Distilled water 100.0 ml

*Solution 4:*

Ethanol 0.7 g  
Casamino acids 0.1 g  
Yeast extract 0.1 g  
Distilled water 10.0 ml

*Solution 5:*

Vitamin solution (see below) 10.0 ml

*Solution 6:*

Na<sub>2</sub>S x 9 H<sub>2</sub>O 0.4 g  
Distilled water 10.0 ml

*Trace element solution SL-10:*

HCl (25%; 7.7 M) 10.0 ml  
FeCl<sub>3</sub> x 4 H<sub>2</sub>O 1.5 g  
ZnCl<sub>2</sub> 70.0 mg  
MnCl<sub>2</sub> x 4 H<sub>2</sub>O 100.0 mg  
H<sub>3</sub>BO<sub>3</sub> 6.0 mg  
CoCl<sub>2</sub> x 6 H<sub>2</sub>O 190.0 mg  
CuCl<sub>2</sub> x 2 H<sub>2</sub>O 2.0 mg  
NiCl<sub>2</sub> x 6 H<sub>2</sub>O 24.0 mg  
Na<sub>2</sub>MoO<sub>4</sub> x 2 H<sub>2</sub>O 36.0 mg  
Distilled water 990.0 ml

*Vitamin solution:*

Biotin 2.0 mg  
Folic acid 2.0 mg  
Pyridoxine-HCl 10.0 mg  
Thiamine-HCl 5.0 mg  
Riboflavin 5.0 mg  
Nicotinic acid 5.0 mg  
Ca DL-pantothenate 5.0 mg  
Vitamin B<sub>12</sub> 0.1 mg  
p-Aminobenzoic acid 5.0 mg  
Lipoic acid 5.0 mg  
Distilled water 1000.0 ml

Solution 1 is boiled before sterilization for a few minutes being flushed with gas mixture of 80% N<sub>2</sub> and 20% CO<sub>2</sub> with sodium bicarbonate added until an equilibrium pH of 6.9-7.1. Solution 1 is sterilized under this gas mixture at 121 C for 15 min. Solution 2 is sterilized under 100% N<sub>2</sub> at 121 C for 15 min. Vitamin solution is filter sterilized. Others solutions sterilize at 121 C for 15 min and add to base medium aseptically.

Final pH of the complete medium 7.1-7.4

**Ac 181. CASEIN-CITRATE AGAR**

Casein hydrolysate 7.5 g  
Yeast extract 10.0 g  
MgSO<sub>4</sub> x 7 H<sub>2</sub>O 20.0 g  
Na-citrate 3.0 g  
KCl 2.0 g  
NaCl 200.0 g

4.98% FeSO<sub>4</sub> in 0.001 N HCl 1.0 ml  
Agar 20.0 g  
Distilled water 1000.0 ml  
pH 7.4  
Sterilize at 121 C for 15 min.

### **B 182. DESULFOBACTERIUM MEDIUM**

#### *Solution 1:*

Na<sub>2</sub>SO<sub>4</sub> 3.0 g  
KH<sub>2</sub>PO<sub>4</sub> 0.2 g  
NH<sub>4</sub>Cl 0.3 g  
NaCl 21.0 g  
MgCl<sub>2</sub> x 6 H<sub>2</sub>O 3.0 g  
KCl 0.5 g  
CaCl<sub>2</sub> x 2 H<sub>2</sub>O 0.15 g  
Resazurin 1.0 mg  
Distilled water 930.0 ml

#### *Solution 2:*

Trace element solution SL-10 (see below) 1.0 ml

#### *Solution 3:*

Vitamin solution (see below) 10.0 ml

#### *Solution 4:*

NaHCO<sub>3</sub> 2.5 g  
Distilled water 50.0 ml

#### *Solution 5:*

Na<sub>2</sub>SeO<sub>4</sub> x 5 H<sub>2</sub>O (3 mg in 1000.0 ml 0.01 M NaOH) 1.0 ml

#### *Solution 6:*

Na<sub>2</sub>S x 9 H<sub>2</sub>O 0.4 g  
Distilled water 10.0 ml

#### *Solution 7:*

Substrate - depending on the species of bacteria:

25% Na-acetate 10.0 ml or indole 0.3 g

NaCl 2.1 g  
MgCl<sub>2</sub> x 6 H<sub>2</sub>O 0.3 g  
Distilled water 100.0 ml

Phenol 40.0 mg  
or Na-benzoate 400.0 mg  
Distilled water 4.0 ml

#### *Trace element solution SL-10:*

HCl (25%; 7.7 M) 10.0 ml

FeCl<sub>3</sub> x 4 H<sub>2</sub>O 1.5 g

ZnCl<sub>2</sub> 70.0 mg

MnCl<sub>2</sub> x 4 H<sub>2</sub>O 100.0 mg

H<sub>3</sub>BO<sub>3</sub> 6.0 mg

CoCl<sub>2</sub> x 6 H<sub>2</sub>O 190.0 mg

CuCl<sub>2</sub> x 2 H<sub>2</sub>O 2.0 mg

NiCl<sub>2</sub> x 6 H<sub>2</sub>O 24.0 mg

Na<sub>2</sub>MoO<sub>4</sub> x 2 H<sub>2</sub>O 36.0 mg

Distilled water 990.0 ml

#### *Vitamin solution:*

p-Aminobenzoic acid 4.0 mg

D(+)-Biotin 1.0 mg

Thiamine-HCl 10.0 mg

Distilled water 100.0 ml



pH 7.1-7.4

Solution 1 is prepared and autoclaved anaerobically under 80% N<sub>2</sub> + 20% CO<sub>2</sub> at 121 C for 15 min. Solutions 2, 4, 5 and 6 are gassed with N<sub>2</sub> and sterilized separately at 121 C for 15 min. Solution 3 (gassed with N<sub>2</sub> + CO<sub>2</sub>) and solution 7 (gassed with N<sub>2</sub>) are filter-sterilized.

#### **Ac 183. BENETT MEDIUM**

Yeast extract 1.0 g

Meat extract 1.0 g

Fermentative casein hydrolysate 2.0 g

Glucose 10.0 g

Agar 15.0 g

Distilled water 1000.0 ml

pH 7.2

Sterilize at 111 C for 30 min.

#### **Ac 184. ISP 2 MEDIUM**

Glucose 4.0 g

Yeast extract 4.0 g

Malt extract 10.0 g

Agar 15.0 g

Distilled water 1000.0 ml

pH 7.2

Sterilize at 111 C for 30 min.

#### **B 185. HALOBACTERIUM MEDIUM 4**

Yeast extract 5.0 g

Casamino acids 5.0 g

Na-glutamate 1.0 g

KCl 2.0 g

Na-citrate 3.0 g

MgSO<sub>4</sub> x 7 H<sub>2</sub>O 20.0 g

NaCl 200.0 g

FeCl<sub>3</sub> x 4 H<sub>2</sub>O 36.0 mg

MnCl<sub>2</sub> x 4 H<sub>2</sub>O 0.36 mg

Agar 20.0 g

Distilled water to 1000.0 ml

pH 7.0-7.2

Sterilize at 111 C for 30 min.

#### **186. HALOBACTERIUM MEDIUM 5**

*Solution 1:*

Casamino acids 7.5 g

Yeast extract 10.0 g

Na-citrate 3.0 g

KCl 2.0 g

MgSO<sub>4</sub> x 7 H<sub>2</sub>O 20.0 g

FeSO<sub>4</sub> x 7 H<sub>2</sub>O 0.05 g

MnSO<sub>4</sub> x H<sub>2</sub>O 0.2 g

NaCl 250.0 g

Distilled water 750.0 ml

*Solution 2:*

Agar 20.0 g

Distilled water 250.0 ml

pH 7.4

Sterilize separately at 121 C for 15 min and mix aseptically.

### **187. HALOCOCCUS MEDIUM**

*Solution 1:*

Skim milk 50.0 g  
Distilled water 500.0 ml

*Solution 2:*

MgSO<sub>4</sub> x 7 H<sub>2</sub>O 10.0 g  
KNO<sub>3</sub> 2.0 g  
NaCl 200.0 g  
Fe-citrate traces  
Distilled water 100.0 ml

*Solution 3:*

Neopeptone 5.0 g  
Glycerol 10.0 g  
Agar 25.0 g  
Distilled water 400.0 ml

Sterilization of solution 1 at 111 C for 15 min. Mix together heated solutions 2 and 3, adjust pH of the mixture to 8.4 and sterilize at 121 C for 15 min.

### **B 188. NATRONOBACTERIA MEDIUM**

*Solution 1:*

KH<sub>2</sub>PO<sub>4</sub> 1.0 g  
KCl 1.0 g  
NH<sub>4</sub>Cl 1.0 g  
MgSO<sub>4</sub> x 7 H<sub>2</sub>O 0.24 g  
CaSO<sub>4</sub> x 2 H<sub>2</sub>O 0.17 g  
Trace element solution SL-10 (see below) 1.0 ml  
NaCl 200.0 g  
Glutamate 1.0 g  
Yeast extract 5.0 g  
Casamini acids 5.0 g  
Agar, if necessary (heat and dissolve it before adding sodium chloride) 20.0 g  
Distilled water to 1000.0 ml  
pH 6.5

*Solution 2:*

Na<sub>2</sub>CO<sub>3</sub> 5.0 g  
Distilled water 50.0 ml

*Trace element solution SL-10:*

HCl (25%; 7.7 M) 10.0 ml  
FeCl<sub>3</sub> x 4 H<sub>2</sub>O 1.5 g  
ZnCl<sub>2</sub> 70.0 mg  
MnCl<sub>2</sub> x 4 H<sub>2</sub>O 100.0 mg  
H<sub>3</sub>BO<sub>3</sub> 6.0 mg  
CoCl<sub>2</sub> x 6 H<sub>2</sub>O 190.0 mg  
CuCl<sub>2</sub> x 2 H<sub>2</sub>O 2.0 mg  
NiCl<sub>2</sub> x 6 H<sub>2</sub>O 24.0 mg  
Na<sub>2</sub>MoO<sub>4</sub> x 2 H<sub>2</sub>O 36.0 mg  
Distilled water 990.0 ml  
pH 9.0-9.5

Sterilize separately at 121 C for 15 min and mix aseptically.

### **189. HALOBACTERIUM MEDIUM 6**

NaCl 156.0 g

MgCl<sub>2</sub> x 6 H<sub>2</sub>O 13.0 g  
MgSO<sub>4</sub> x 7 H<sub>2</sub>O 20.0 g  
CaCl<sub>2</sub> x 6 H<sub>2</sub>O 1.0 g  
KCl 4.0 g  
NaHCO<sub>3</sub> 0.2 g  
NaBr 0.5 g  
Yeast extract 5.0 g  
Glucose 1.0 g  
Distilled water 1000.0 ml  
pH 7.0  
Sterilize at 111 C for 30 min.

**Ac 190. PRAUSER MEDIUM 79**

Glucose 10.0 g  
Peptone 10.0 g  
Yeast extract 2.0 g  
Casamino acids 2.0 g  
NaCl 6.0 g  
Agar 20.0 g  
Tap water 1000.0 ml  
pH 7.5  
Sterilize at 111 C for 30 min.

**Ac 191. STARCH-YEAST AGAR**

Yeast extract 2.0 g  
Starch (soluble) 10.0 g  
Agar 20.0 g  
Tap water 1000.0 ml  
pH 7.3  
Sterilize at 121 C for 15 min.

**192. MYA-AGAR**

Glucose 2.0 g  
L-Asparagin 1.0 g  
K<sub>2</sub>HPO<sub>4</sub> 0.5 g  
MgSO<sub>4</sub> x 7 H<sub>2</sub>O 0.5 g  
Trace element solution (see below) 1.0 ml  
Agar 20.0 g

Distilled water 1000.0 ml

*Trace element solution:*

FeSO<sub>4</sub> x 7 H<sub>2</sub>O 0.1 g  
CuSO<sub>4</sub> x 5 H<sub>2</sub>O 0.1 g  
ZnSO<sub>4</sub> x 7 H<sub>2</sub>O 0.1 g  
Distilled water 100.0 ml

pH 7.4

Sterilize base medium at 111 C for 30 min, trace element solution at 121 C for 15 min.

**193. ACETATE AGAR**

NaCl 5.0 g  
K<sub>2</sub>HPO<sub>4</sub> 1.0 g  
NH<sub>4</sub>H<sub>2</sub>PO<sub>4</sub> 1.0 g  
MgSO<sub>4</sub> x 7 H<sub>2</sub>O 0.2 g  
Na-acetate 2.0 g  
Bromothymol blue (0.2%) 40.0 ml

Agar 20.0 g

Distilled water 1000.0 ml

pH 7.2

Add the indicator last, after pH is set and the possible precipitate is separated by filtration through a cotton wool-gauze filter.

Sterilize at 121 C for 15 min.

### **B 194. INMI MEDIUM 3**

NaCl 250.0 g

KCl 2.0 g

Na-citrate 3.0 g

MgSO<sub>4</sub> x 7 H<sub>2</sub>O 20.0 g

Casamino acids 5.0 g

Yeast extract 2.5 g

Agar 20.0 g

Distilled water 1000.0 ml

pH 7.2

Sterilize at 121 C for 15 min.

### **B 195. INMI MEDIUM 4**

Yeast extract 2.5 g

Casamino acids 5.0 g

pH 9.5

Sterilize at 121 C for 15 min.

### **196. MEDIUM FOR PURPLE BACTERIA (VAN NIEL MEDIUM)**

NH<sub>4</sub>Cl 1.0 g

K<sub>2</sub>HPO<sub>4</sub> 0.5 g

MgCl<sub>2</sub> x 6 H<sub>2</sub>O 0.2 g

NaHCO<sub>3</sub> 1.0 g

Na<sub>2</sub>S x 9 H<sub>2</sub>O 1.0 g

Tap water 1000.0 ml

pH 7.6

Sterilize base medium and sulfide separately at 121 C for 15 min.

### **B 197. MEDIUM FOR RHODOSPIRILLUM (PFENNIG MEDIUM)**

NH<sub>4</sub>Cl 0.4 g

KH<sub>2</sub>PO<sub>4</sub> 0.5 g

MgSO<sub>4</sub> x 7 H<sub>2</sub>O 0.2 g

NaCl 0.4 g

CaCl<sub>2</sub> x 2 H<sub>2</sub>O 0.05 g

Acetate, or butyrate, or propionate, or succinate 1.0 g

Yeast extract 0.2 g

Fe-citrate (0.1%) 5.0 ml

Trace element solution according to Pfennig (see below) 1.0 ml

Vitamin B<sub>12</sub> (dispensary solution, 0,01%) 1.0 ml

Distilled water 1000.0 ml

*Trace element solution according to Pfennig:*

EDTA 1.5 g

Trace element solution according to Hogland (see below) 6.0 ml

FeSO<sub>4</sub> x 7 H<sub>2</sub>O 0.2 g

MnCl<sub>2</sub> x 4 H<sub>2</sub>O 0.02 g

ZnSO<sub>4</sub> x 7 H<sub>2</sub>O 0.1 g

Distilled water 1000.0 ml

*Trace element solution according to Hogland:*

EDTA 5.0 g  
FeSO<sub>4</sub> x 7 H<sub>2</sub>O 2.0 g  
ZnSO<sub>4</sub> x 7 H<sub>2</sub>O 100.0 mg  
MnCl<sub>2</sub> x 4 H<sub>2</sub>O 30.0 mg  
H<sub>3</sub>BO<sub>3</sub> 300.0 mg  
CoCl<sub>2</sub> x 6 H<sub>2</sub>O 200.0 mg  
CuCl<sub>2</sub> 10.0 mg  
NiCl<sub>2</sub> x 6 H<sub>2</sub>O 20.0 mg  
Na<sub>2</sub>MoO<sub>4</sub> x 2 H<sub>2</sub>O 20.0 mg  
Distilled water 1000.0 ml

Sterilize base medium and trace element solution separately at 121 C for 15 min.

### **198. THIOBACILLUS DENITRIFICANS MEDIUM (TAYLOR MEDIUM)**

KNO<sub>3</sub> 2.0 g  
NH<sub>4</sub>Cl 1.0 g  
KH<sub>2</sub>PO<sub>4</sub> 2.0 g  
NaHCO<sub>3</sub> 2.0 g  
MgSO<sub>4</sub> x 7 H<sub>2</sub>O 0.8 g  
Na<sub>2</sub>S<sub>2</sub>O<sub>3</sub> x 5 H<sub>2</sub>O 5.0 g  
Trace element solution according to Pfennig (see below) 1.0 ml  
Distilled water 1000.0 ml

*Trace element solution according to Pfennig:*

EDTA 1.5 g  
Trace element solution according to Hogland (see below) 6.0 ml  
FeSO<sub>4</sub> x 7 H<sub>2</sub>O 0.2 g  
MnCl<sub>2</sub> x 4 H<sub>2</sub>O 0.02 g  
ZnSO<sub>4</sub> x 7 H<sub>2</sub>O 0.1 g  
Distilled water 1000.0 ml

*Trace element solution according to Hogland:*

EDTA 5.0 g  
FeSO<sub>4</sub> x 7 H<sub>2</sub>O 2.0 g  
ZnSO<sub>4</sub> x 7 H<sub>2</sub>O 100.0 mg  
MnCl<sub>2</sub> x 4 H<sub>2</sub>O 30.0 mg  
H<sub>3</sub>BO<sub>3</sub> 300.0 mg  
CoCl<sub>2</sub> x 6 H<sub>2</sub>O 200.0 mg  
CuCl<sub>2</sub> 10.0 mg  
NiCl<sub>2</sub> x 6 H<sub>2</sub>O 20.0 mg  
Na<sub>2</sub>MoO<sub>4</sub> x 2 H<sub>2</sub>O 20.0 mg  
Distilled water 1000.0 ml

Sterilize base medium and trace element solution separately at 121 C for 15 min.

### **199. THIOBACILLUS DENITRIFICANS MEDIUM (BAALSRUD MEDIUM)**

KNO<sub>3</sub> 2.0 g  
NH<sub>4</sub>Cl 0.5 g  
KH<sub>2</sub>PO<sub>4</sub> 2.0 g  
NaHCO<sub>3</sub> 1.0 g  
MgCl<sub>2</sub> x 6 H<sub>2</sub>O 0.5 g  
Na<sub>2</sub>S<sub>2</sub>O<sub>3</sub> x 5 H<sub>2</sub>O 5.0 g  
FeSO<sub>4</sub> x 7 H<sub>2</sub>O 0.01 g  
Distilled water 1000.0 ml

pH 7.0

Sterilize iron, phosphorus and bicarbonate salts separately at 121 C for 15 min.

## 200. THIOBACILLUS DENITRIFICANS MEDIUM (LIESKE MEDIUM)

KNO<sub>3</sub> 5.0 g  
KH<sub>2</sub>PO<sub>4</sub> 0.2 g  
NaHCO<sub>3</sub> 1.0 g  
MgCl<sub>2</sub> x 6 H<sub>2</sub>O 0.1 g  
Na<sub>2</sub>S<sub>2</sub>O<sub>3</sub> x 5 H<sub>2</sub>O 5.0 g  
CaCl<sub>2</sub> x 6 H<sub>2</sub>O Traces  
FeCl<sub>3</sub> x 6 H<sub>2</sub>O Traces  
Distilled water 1000.0 ml  
Sterilize at 121 C for 15 min.

## 201. MODIFIED BROCK MEDIUM FOR SULFUROXIDIZING BACTERIA

(NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub> 1.3 g  
KH<sub>2</sub>PO<sub>4</sub> 0.37 g  
MgSO<sub>4</sub> x 7 H<sub>2</sub>O 0.25 g  
CaCl<sub>2</sub> x 6 H<sub>2</sub>O 0.07 g  
Distilled water 1000.0 ml  
pH 2.4 (range: 2.3-3.0; adjust with H<sub>2</sub>SO<sub>4</sub>).  
*Additions to the medium (sterilize separately each):*  
Trace element solution according to Pfennig (see below) 1.0 ml  
Yeast extract 0.2 g  
Element sulfur 10.0 g  
Chalk (CaCO<sub>3</sub>) 10.0 g  
For cultivation of heterotrophic representatives of the group the medium after sterilization is to be also supplemented with sterilized as separate solutions at 111 C for 30 min:

Peptone 1.75 g  
Sucrose 0.25 g  
Trace element solution according to Hogland (see below) 6.0 ml  
FeSO<sub>4</sub> x 7 H<sub>2</sub>O 0.2 g  
MnCl<sub>2</sub> x 4 H<sub>2</sub>O 0.02 g  
ZnSO<sub>4</sub> x 7 H<sub>2</sub>O 0.1 g  
Distilled water 1000.0 ml

*Trace element solution according to Pfennig:*

EDTA 1.5 g

*Trace element solution according to Hogland:*

EDTA 5.0 g

FeSO<sub>4</sub> x 7 H<sub>2</sub>O 2.0 g

ZnSO<sub>4</sub> x 7 H<sub>2</sub>O 100.0 mg

MnCl<sub>2</sub> x 4 H<sub>2</sub>O 30.0 mg

H<sub>3</sub>BO<sub>3</sub> 300.0 mg

CoCl<sub>2</sub> x 6 H<sub>2</sub>O 200.0 mg

CuCl<sub>2</sub> 10.0 mg

NiCl<sub>2</sub> x 6 H<sub>2</sub>O 20.0 mg

Na<sub>2</sub>MoO<sub>4</sub> x 2 H<sub>2</sub>O 20.0 mg

Distilled water 1000.0 ml

Sterilize base medium and others components of medium separately at 121 C for 15 min.

## 202. MEDIUM FOR MAGNETIC BACTERIA

Tartaric acid 0.37 g  
Succinic acid 0.37 g  
Na-acetate 0.05 g  
KH<sub>2</sub>PO<sub>4</sub> 0.68 g  
NaNO<sub>3</sub> 0.12 g  
Vitamin solution (see below) 10.0 ml

Trace element solution (see below) 5.0 ml

Fe-quinat 2.0 ml

Na-thioglycolate 0.05 g

Resazurin 0.5 mg

Agar 1.3 g

Bidistilled water 1000.0 ml

*Vitamin solution:*

Biotin 200.0 mg

Folic acid 20.0 mg

Pyridoxine-HCl 100.0 mg

Thiamine-HCl 50.0 mg

Riboflavin 100.0 mg

Nicotinic acid 50.0 mg

DL-Pantothenic acid 50.0 mg

Vitamin B<sub>12</sub> 1.0 mg

p-Aminobenzoic acid 50.0 mg

Distilled water 1000.0 ml

*Trace element solution:*

Nitrilotriacetic acid 12.8 g

FeCl<sub>3</sub> x 4 H<sub>2</sub>O 0.2 g

MnCl<sub>2</sub> x 4 H<sub>2</sub>O 0.1 g

CoCl<sub>2</sub> x 6 H<sub>2</sub>O 0.17 g

CaCl<sub>2</sub> x 2 H<sub>2</sub>O 0.1 g

ZnCl<sub>2</sub> 0.1 g

CuCl<sub>2</sub> 0.02 g

Na<sub>2</sub>MoO<sub>4</sub> x 2 H<sub>2</sub>O 0.01 g

NiCl<sub>2</sub> x 6 H<sub>2</sub>O 0.026 g

NaCl 1.0 g

Na<sub>2</sub>SeO<sub>4</sub> x 5 H<sub>2</sub>O 0.02 g

Distilled water 1000.0 ml

pH 6.75 (adjust with NaOH)

Prior to sterilization the medium is to be blown down by the flow of N<sub>2</sub> and sterilized in the nitrogen atmosphere at 121 C for 15 min. Vitamin solution is filter sterilized. Trace element solution sterilize at 121 C for 15 min and add to base medium aseptically.

### **B 203. MEDIUM FOR FLEXIBACTER (LEWIN MEDIUM)**

Na-glycerophosphate 0.1 g

KNO<sub>3</sub> 0.1 g

MgSO<sub>4</sub> x 7 H<sub>2</sub>O 0.1 g

Trace element solution (see below) 1.0 ml

Vitamin B<sub>12</sub> 1.0 µg

CaCl<sub>2</sub> x 6 H<sub>2</sub>O 0.1 g

Tris buffer 1.0 g

Thiamine 1.0 mg

Casamino acids 1.0 g

Distilled water 1000.0 ml

*Trace element solution:*

Nitrilotriacetic acid 12.8 g

FeCl<sub>3</sub> x 4 H<sub>2</sub>O 0.2 g

MnCl<sub>2</sub> x 4 H<sub>2</sub>O 0.1 g

CoCl<sub>2</sub> x 6 H<sub>2</sub>O 0.17 g

CaCl<sub>2</sub> x 2 H<sub>2</sub>O 0.1 g

ZnCl<sub>2</sub> 0.1 g

CuCl<sub>2</sub> 0.02 g

Na<sub>2</sub>MoO<sub>4</sub> x 2 H<sub>2</sub>O 0.01 g

NiCl<sub>2</sub> x 6 H<sub>2</sub>O 0.026 g

NaCl 1.0 g

Na<sub>2</sub>SeO<sub>4</sub> x 5 H<sub>2</sub>O 0.02 g

Distilled water 1000.0 ml

pH 7.5

Sterilize base medium and trace element solution separately at 121 C for 15 min.

#### **B 204. MEDIUM FOR CLOSTRIDIUM**

Glucose 10.0 g

Peptone 12.0 g

NaCl 2.0 g

Agar 16.0 g

Distilled water 1000.0 ml

The medium can be used with chalk addition as buffer against acidification of the medium during cultivation.

pH to 6.8-7.2

Sterilize at 111 C for 30 min.

#### **Ac 205. OATMEAL AGAR A**

Oatmeal or oat flakes 20.0 g

Agar 20.0 g

Tap water 1000.0 ml

pH to 7.2

Boil oat flakes in water bath for 20 min. filter through a cotton-gauze filter, fill up to 1000,0 ml.

Sterilize at 121 C 15 min.

#### **Ac 206. ISP MEDIUM 3**

Oatmeal 20.0 g

Salt solution A (see below) 1.0 ml

Agar 20.0 g

Distilled water 1000.0 ml

*Salt solution A:*

FeSO<sub>4</sub> 0.1 g

MnCl<sub>2</sub> 0.1 g

ZnSO<sub>4</sub> 0.1 g

Distilled water 100.0 ml

Boil oat flakes in water bath for 20 min filter through a cotton-gauze filter, fill up to 1000.0 ml.

pH 7.2

Sterilize separately at 121 C 15 min.

#### **Ac 207. MINERAL AGAR 1**

Starch (soluble) 20.0 g

K<sub>2</sub>HPO<sub>4</sub> 0.5 g

MgSO<sub>4</sub> x 7 H<sub>2</sub>O 0.5 g

KNO<sub>3</sub> 1.0 g

NaCl 0.5 g

FeSO<sub>4</sub> 0.01 g

Agar 30.0 g

Distilled water 1000.0 ml

pH 7.2-7.4

Sterilize at 121 C 15 min.

#### **208. GLUCOSE ASPARAGIN AGAR**



Glucose 10.0 g  
L-Asparagin 0.5 g  
K<sub>2</sub>HPO<sub>4</sub> 0.5 g  
Agar 20.0 g  
Distilled water 1000.0 ml  
pH 6.8.  
Sterilize at 111 C for 30 min.

**Ac 209. MODIFICATION OF THE CZAPEK MEDIUM WITH STARCH**

Starch (soluble) 20.0 g  
K<sub>2</sub>HPO<sub>4</sub> 0.5 g  
KNO<sub>3</sub> 1.0 g  
NaCl 0.2 g  
MgSO<sub>4</sub> x 7 H<sub>2</sub>O 0.3 g  
CaCO<sub>3</sub> 0.5 g  
FeSO<sub>4</sub> 0.001 g  
Agar 20.0 g  
Distilled water 1000.0 ml  
pH 7.2-7.4  
Sterilize at 121 C 15 min.

**Ac 210. GLYCEROL-ASPARAGIN AGAR**

L-Asparagin 1.0 g  
Glycerol 10.0 g  
K<sub>2</sub>HPO<sub>4</sub> 1.0 g  
Salt solution A (see below) 1.0 ml  
Agar 20.0 g  
Distilled water 1000.0 ml  
*Salt solution A:*  
FeSO<sub>4</sub> 0.1 g  
MnCl<sub>2</sub> 0.1 g  
ZnSO<sub>4</sub> 0.1 g  
Distilled water 100.0 ml  
pH 7.0-7.4  
Sterilize separately at 121 C 15 min.

**Ac 211. OATMEAL AGAR WITH 0.1% YEAST EXTRACT**

Oatmeal 20.0 g  
Yeast extract 1.0 g  
Agar 20.0 g  
Tap water 1000.0 ml  
pH 7.2  
Boil oat flakes in water bath for 20 min filter through a cotton-gauze filter, fill up to 1000.0 ml.  
Sterilize at 121 C 15 min.

**Ac 212. MODIFICATION OF CZAPEK MEDIUM WITH GLUCOSE**

Glucose 20.0 g  
K<sub>2</sub>HPO<sub>4</sub> 0.5 g  
KNO<sub>3</sub> 1.0 g  
NaCl 0.2 g  
MgSO<sub>4</sub> x 7 H<sub>2</sub>O 0.3 g  
CaCO<sub>3</sub> 0.5 g  
FeSO<sub>4</sub> 0.001 g  
Agar 20.0 g

Distilled water 1000.0 ml  
pH 7.2-7.4  
Sterilize at 111 C for 30 min.

**Ac 213. ORGANIC AGAR 2**

Hottinger broth 30.0 ml  
Peptone 5.0 g  
NaCl 5.0 g  
Glucose 10.0 g  
Agar 30.0 g  
Tap water 970.0 ml  
pH 7.0-7.2  
Sterilize at 111 C for 30 min.

**Ac 214. STARCH AMMONIA AGAR**

Starch (soluble) 10.0 g  
K<sub>2</sub>HPO<sub>4</sub> 1.0 g  
MgSO<sub>4</sub> x 7 H<sub>2</sub>O 1.0 g  
NaCl 1.0 g  
(NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub> 2.0 g  
CaCO<sub>3</sub> 2.0 g  
Salt solution A (see below) 1.0 ml  
Agar 20.0 g  
Distilled water 1000.0 ml  
*Salt solution A:*  
FeSO<sub>4</sub> 0.1 g  
MnCl<sub>2</sub> 0.1 g  
ZnSO<sub>4</sub> 0.1 g  
Distilled water 100.0 ml  
pH 7.0-7.4  
Sterilize separately at 121 C 15 min.

**Ac 215. GLYCEROL NITRATE AGAR**

Glycerol 30.0 g  
NaNO<sub>3</sub> 2.0 g  
K<sub>2</sub>HPO<sub>4</sub> 1.0 g  
MgSO<sub>4</sub> x 7 H<sub>2</sub>O 0.5 g  
KCl 0.5 g  
FeSO<sub>4</sub> 0.01 g  
Agar 20.0 g  
Distilled water 1000.0 ml  
pH 7.0-7.2.  
Sterilize at 121 C for 15 min.

**216. TETRATHIONATE BROTH (MULLER MEDIUM)**

*Solution 1:*

Hottinger broth 880.0 ml

*Solution 2:*

Lugol solution (see below) 20.0 ml

*Solution 3:*

50% Na<sub>2</sub>S<sub>2</sub>O<sub>3</sub> x 5 H<sub>2</sub>O 100.0 ml

*Lugol solution:*

KJ 20.0 g

J<sub>2</sub> 25.0 g

Distilled water 100.0 ml

pH 7.2 - 7.4

Sterilize solution 3 at 121 C 15 min. Lugol solution is filter sterilized. Pour the medium into sterile vials with CaCO<sub>3</sub> (25 g CaCO<sub>3</sub> per 1000.0 ml of medium). Sterilize the flasks with CaCO<sub>3</sub> with dry heat (170 C for 60 min).

#### **Ac 217. MEDIUM FOR MICROMONOSPORES**

Glucose 10.0 g

Starch (soluble) 20.0 g

Yeast extract 5.0 g

Fermentative casein hydrolysate 5.0 g

CaCO<sub>3</sub> 1.0 g

Agar 20.0 g

Distilled water 1000.0 ml

pH 7.2

Sterilize at 111 C for 30 min.

#### **218. PYA WITH MARINE WATER**

Peptone 5.0 g

Yeast extract 3.0 g

Agar 12.0 g

Distilled water 250.0 ml

Aged filtered sea water 750.0 ml

pH 7.5-7.6

Sterilize at 121 C 15 min.

#### **Ac 219. MEDIUM FOR HALOPHILIC BACILLI**

NaCl 100.0 g

NaHCO<sub>3</sub> 10.0 g

Na<sub>2</sub>CO<sub>3</sub> 10.0 g

Nutrient broth 1000.0 ml

pH 9.5

Sterilize at 121 C 15 min.

#### **Ac 220. PYEA MEDIUM**

Peptone 10.0 g

Yeast extract 10.0 g

NaCl 5.0 g

Agar 15.0 g

Distilled water 1000.0 ml

pH 7.2

Sterilize at 121 C 15 min.

#### **Ac 221. MEDIUM FOR METHANOTROPHIC BACTERIA**

KNO<sub>3</sub> 1.0 g

KH<sub>2</sub>PO<sub>4</sub> 0.7 g

MgSO<sub>4</sub> x 7 H<sub>2</sub>O 0.2 g

CaCl<sub>2</sub> 0.02 g

Na<sub>2</sub>HPO<sub>4</sub> x 5 H<sub>2</sub>O 1.5 g

Trilon B 5.0 mg

FeSO<sub>4</sub> x 7 H<sub>2</sub>O 2.0 mg

ZnSO<sub>4</sub> x 7 H<sub>2</sub>O 0.1 mg

MnCl<sub>2</sub> x 4 H<sub>2</sub>O 0.03 mg

CoCl<sub>2</sub> x 6 H<sub>2</sub>O 0.2 mg

CuCl<sub>2</sub> x 5 H<sub>2</sub>O 0.1 mg  
NiCl<sub>2</sub> x 6 H<sub>2</sub>O 0.02 mg  
Na<sub>2</sub>MoO<sub>4</sub> 0.03 mg  
Distilled water 1000.0 ml  
pH 6.7-7.1  
Sterilize at 121 C 15 min.  
Cultivation under mixture of methane and air (1:1).

### **Ac 222. MEDIUM FOR MARINE METHYLOTROPHIC BACTERIA**

KH<sub>2</sub>PO<sub>4</sub> 2.0 g  
(NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub> 2.0 g  
NaCl 30.0 g  
MgSO<sub>4</sub> x 7 H<sub>2</sub>O 0.025 g  
FeSO<sub>4</sub> x 7 H<sub>2</sub>O 0.002 g  
Yeast extract 0.1 g  
Methanol 5.0 ml  
Biotine 0.01 mg  
Distilled water 1000.0 ml  
pH 7.0  
Sterilize at 121 C 15 min. After autoclaving add filter sterilize methanol and biotine.

### **223. MEDIUM FOR OLIGOCARBOPHILIC BACTERIA.**

*Solution 1 (basic solution):*

Peptone 0.25 g  
Yeast extract 0.25 g  
Agar 5.0 g  
Solution 2 20.0 ml  
Distilled water 965.0 ml  
Sterilize 15 min. at 121 C. After cooling add the following solutions:  
Glucose (2.5 %) 10.0 ml  
Solution 3 5.0 ml  
pH 7.5

*Solution 2 (trace elements):*

Nitritotriacetate 10.0 g  
MgSO<sub>4</sub> x 7 H<sub>2</sub>O 29.7 g  
CaCl<sub>2</sub> x 2 H<sub>2</sub>O 3.34 g  
Na<sub>2</sub>MoO<sub>4</sub> x 2 H<sub>2</sub>O 12.67 mg  
FeSO<sub>4</sub> x 7 H<sub>2</sub>O 350.0 mg  
Na-EDTA 125.0 mg  
ZnSO<sub>4</sub> x 7 H<sub>2</sub>O 548.0 mg  
MnSO<sub>4</sub> x H<sub>2</sub>O 77.0 mg  
CuSO<sub>4</sub> x 5 H<sub>2</sub>O 20.0 mg  
Co(NO<sub>3</sub>)<sub>2</sub> x 6 H<sub>2</sub>O 12.4 mg  
Na<sub>2</sub>B<sub>4</sub>O<sub>7</sub> x 10 H<sub>2</sub>O 8.8 mg  
Distilled water 950.0 ml  
Dissolve nitritotriacetate first by neutralizing with KOH, then add other salts. Adjust volume to 1000 ml.  
Adjust pH to 7.2

*Solution 3 (vitamin solution):*

Biotin 4.0 mg  
Folic acid 4.0 mg  
Pyridoxine-HCl 20.0 mg  
Riboflavine 10.0 mg  
Thiamine-HCl 10.0 mg

Nicotin amide 10.0 mg  
Calcium D-pantothenate 10.0 mg  
Vitamin B<sub>12</sub> 0.2 mg  
p-aminobenzoic acid 10.0 mg  
Distilled water 1000.0 ml  
pH 6.8–7.0  
Sterilize glucose at 111 C for 30 min, solution 2 at 121 C 15 min. Vitamin solution is filter sterilized.  
Store in refrigerator at +5 C.

## **224. MEDIUM FOR DESULFOTOMACULUM ALKALIPHILUM**

*Solution 1 (basic solution):*

Solution 2 (see below) 10.0 ml  
Na<sub>2</sub>CO<sub>3</sub> 0.5 g  
Na<sub>2</sub>SO<sub>4</sub> 5.0 g  
NaCl 5.0 g  
Na formate 5.0 g  
Yeast extract 1.0 g  
Solution 3 (see below) 2.0 ml  
Solution 4 (see below) 1.0 ml  
Rezazurine traces  
Distilled water 1000.0 ml  
NaHCO<sub>3</sub> final concentration 8.0 g /1000.0ml  
Na<sub>2</sub>S x 9 H<sub>2</sub>O final concentration 0.5 g/1000.0 ml

*Solution 2:*

KH<sub>2</sub>PO<sub>4</sub> 0.2 g  
MgCl<sub>2</sub> x 6 H<sub>2</sub>O 0.1 g  
NH<sub>4</sub>Cl 1.0 g  
KCl 0.2 g  
Distilled water 1000.0 ml

*Solution 3 (vitamin solution):*

Biotin 10.0 mg  
Folic acid 10.0 mg  
Pyridoxine-HCl 50.0 mg  
Riboflavine 25.0 mg  
Thiamine-HBr 25.0 mg  
Nicotin amide 25.0 mg  
D-pantothenate 25.0 mg  
Vitamin B<sub>12</sub> 0.5 mg  
p-aminobenzoic acid 25.0 mg  
Thioctic acid 25.0 mg  
Distilled water 500.0 ml  
Store in refrigerator at +5°C.

*Solution 4 (trace elements):*

Na<sub>2</sub>MoO<sub>4</sub> x 2 H<sub>2</sub>O 20.0 mg  
FeSO<sub>4</sub>(NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub> x 6 H<sub>2</sub>O 400.0 mg  
FeSO<sub>4</sub> x 7 H<sub>2</sub>O 200.0 mg  
ZnSO<sub>4</sub> x 7 H<sub>2</sub>O 200.0 mg  
MnCl<sub>2</sub> x 4 H<sub>2</sub>O 720.0 mg  
NiCl<sub>2</sub> 100.0 mg  
CuSO<sub>4</sub> x 5 H<sub>2</sub>O 20.0 mg  
AlK(SO<sub>4</sub>)<sub>2</sub> x 12 H<sub>2</sub>O 20.0 mg  
CoCl<sub>2</sub> x 6 H<sub>2</sub>O 200.0 mg  
H<sub>3</sub>BO<sub>3</sub> 20.0 mg  
HCl 5.0 ml

Distilled water 200.0 ml

pH of the autoclaved medium is 8.7-9.0

Vitamin solution is filter sterilized. Others solutions sterilize separately at 121 C for 15 min and add to the Solution 1 after cooling.

#### **Ac, B 225. 1/5 STARCH-YEAST AGAR**

Yeast extract 0.4 g

Soluble starch 2.0 g

Agar 20.0 g

Distilled water 1000.0 ml

pH 7.3

Sterilize at 121 C 15 min.

#### **Ac 226. MEDIUM FOR ACTINOPOLYSPORA MORTIVALLIS**

Bacto vitamin assay casamino acids 7.5 g

Yeast extract 10.0 g

MgSO<sub>4</sub> x 7 H<sub>2</sub>O 20.0 g

Trisodium citrate x 2 H<sub>2</sub>O 3.0 g

KCl 2.0 g

NaCl 150.0 g

4.98% FeSO<sub>4</sub> in 0.001 N HCl 1.0 ml

Agar 20.0 g

Distilled water 1000.0 ml

pH 7.4

Sterilize at 121 C 15 min.

#### **Ac 227. MICROLUNATUS MEDIUM**

Glucose 0.5 g

Peptone 0.5 g

Yeast extract 0.5 g

Monosodium glutamate 0.5 g

KH<sub>2</sub>PO<sub>4</sub> 0.44 g

(NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub> 0.1 g

MgSO<sub>4</sub> x 7H<sub>2</sub>O 0.1 g

Distilled water 1000.0 ml

pH 7.0

Sterilize at 111 C for 30 min.

#### **Ac 228. MICROCOCCUS HALOPHILUS MEDIUM**

Peptone 10.0 g

Yeast extract 5.0 g

Malt extract 5.0 g

Casamino acids 5.0 g

Meat extract 2.0 g

Glycerol 2.0 g

Tween-80 50.0 mg

MgSO<sub>4</sub> x 7 H<sub>2</sub>O 1.0 g

NaCl 50.0 g

Agar 20.0 g

Distilled water 1000.0 ml

pH 7.2

Sterilize at 121 C 15 min.

#### **Ac 229. ALKALIBACTER MEDIUM**

(NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub> 1.00 g  
NH<sub>4</sub>Cl 0.40 g  
Na<sub>2</sub>S<sub>2</sub>O<sub>3</sub> x 5 H<sub>2</sub>O 0.10 g  
K<sub>2</sub>HPO<sub>4</sub> 0.50 g  
MgSO<sub>4</sub> x 7 H<sub>2</sub>O 0.10 g  
CaCl<sub>2</sub> x 2 H<sub>2</sub>O 0.05 g  
NaCl 10.00 g  
FeSO<sub>4</sub> x 7 H<sub>2</sub>O 2.00 mg  
Trace element solution (see below) 10.00 ml  
Resazurin 0.001 g  
Yeast extract 0.25 g  
Tryptone 2.0 g  
Glucose 5.0 g  
Vitamin solution (see below) 10.00 ml

L-cysteine 0.50 g  
Na<sub>2</sub>CO<sub>3</sub> (5% w/v) 50.00 ml  
Distilled water 930.00 ml

*Trace element solution:*

Nitrilotriacetic acid 12.8 mg  
FeSO<sub>4</sub> x 7 H<sub>2</sub>O 0.1 mg  
MnCl<sub>2</sub> x 6 H<sub>2</sub>O 0.1 mg  
CoCl<sub>2</sub> x 2 H<sub>2</sub>O 0.17 mg  
CaCl<sub>2</sub> x 2 H<sub>2</sub>O 0.1 mg  
ZnCl<sub>2</sub> 0.1 mg  
CuCl<sub>2</sub> 0.02 mg  
H<sub>3</sub>BO<sub>3</sub> 0.01 mg  
Na<sub>2</sub>MoO<sub>4</sub> x 2 H<sub>2</sub>O 0.01 mg  
NaCl 1.0 mg  
Na<sub>2</sub>SeO<sub>4</sub> 0.017 mg  
Distilled water 1000.0 ml

*Vitamin solution:*

Biotin 2.0 mg  
Folic acid 2.0 mg  
Pyridoxine (B<sub>2</sub>) 0.1 mg  
Riboflavin (B<sub>1</sub>) 5.0 mg  
Pantotenoic acid 5.0 mg  
p-Aminobenzoic acid 5.0 mg  
Thiamine-HCl 5.0 mg  
Nicotinic acid 5.0 mg  
Cyanocobalamin (B<sub>12</sub>) 0.1 mg  
Lipoic (tioctoic) acid 5.0 mg  
Distilled water 1000.0 ml

Basal medium sterilize at 111 C for 30 min. Vitamin solution is filter sterilized. Others solutions sterilize at 121 C for 15 min (Na<sub>2</sub>CO<sub>3</sub> is sterilized under 100% N<sub>2</sub>).

Final pH 9.0

**B 230. CALDITHRIX ABYSSI MEDIUM**

Sea salt 37.9 g  
NH<sub>4</sub>Cl 0.33 g  
KH<sub>2</sub>PO<sub>4</sub> 0.33 g  
Resazurin 0.50 mg  
Yeast extract (20% w/v) 15 ml  
Trace element solution (see below) 10.00 ml  
Vitamin solution (see below) 10.00 ml

Na<sub>2</sub>S x 9 H<sub>2</sub>O (3% w/v) 20.00 ml

Selenite-tungstate solution (see below) 1.00 ml

Distilled water 900.00 ml

Prepare medium anaerobically under 80% N<sub>2</sub> + 20% CO<sub>2</sub> gas mixture. After autoclaving add from separately prepared, sterile anaerobic stock solutions NaHCO<sub>3</sub> (5% w/v) 50.00 ml

*Trace element solution:*

Nitrilotriacetic acid 12.8 mg

FeSO<sub>4</sub> x 7 H<sub>2</sub>O 0.1 mg

MnCl<sub>2</sub> x 6 H<sub>2</sub>O 0.1 mg

CoCl<sub>2</sub> x 2 H<sub>2</sub>O 0.17 mg

CaCl<sub>2</sub> x 2 H<sub>2</sub>O 0.1 mg

ZnCl<sub>2</sub> 0.1 mg

CuCl<sub>2</sub> 0.02 mg

H<sub>3</sub>BO<sub>3</sub> 0.01 mg

Na<sub>2</sub>MoO<sub>4</sub> x 2 H<sub>2</sub>O 0.01 mg

NaCl 1.0 mg

Na<sub>2</sub>SeO<sub>4</sub> 0.017 mg

Distilled water 1000.0 ml

*Vitamin solution:*

Biotin 2.0 mg

Folic acid 2.0 mg

Pyridoxine-HCl 10.0 mg

Thiamine-HCl x 2 H<sub>2</sub>O 5.0 mg

Riboflavin 5.0 mg

Nicotinic acid 5.0 mg

D-Ca-pantothenate 5.0 mg

Vitamin B<sub>12</sub> 0.1 mg

p-Aminobenzoic acid 5.0 mg

Lipoic acid 5.0 mg

Distilled water 1000.0 ml

*Selenite-tungstate solution:*

NaOH 0.5 g

Na<sub>2</sub>SeO<sub>3</sub> x 5 H<sub>2</sub>O 3 mg

Na<sub>2</sub>WO<sub>4</sub> x 2 H<sub>2</sub>O 4 mg

Distilled water 1000.0 ml

Vitamin solution is filter sterilized. Others solutions sterilize separately at 121 C for 15 min.

Final pH of the medium to 6.8

### **B 231. CARBOXYDOCELLA SPOROPRODUCENS MEDIUM**

KCl 0.33 g

MgCl<sub>2</sub> x 6 H<sub>2</sub>O 0.52 g

CaCl<sub>2</sub> x 2 H<sub>2</sub>O 0.29 g

NH<sub>4</sub>Cl 0.33 g

KH<sub>2</sub>PO<sub>4</sub> 0.33 g

NaHCO<sub>3</sub> 1.00 g

Trace element solution SL-4 (see below) 10.00 ml

Resazurin 0.50 mg

Vitamin solution (see below) 10.00 ml

Pyruvate 2.5 g

Yeast extract 0.05 g

Na<sub>2</sub>S x 9 H<sub>2</sub>O 0.30 g

Distilled water 1000.00 ml

*Trace element solution SL-4:*

EDTA 0.5 g



FeSO<sub>4</sub> x 7H<sub>2</sub>O 0.2 g  
Trace element solution SL-6 (see below) 100.0 ml  
Distilled water 900.0 ml

*Vitamin solution:*

Biotin 2.0 mg  
Folic acid 2.0 mg  
Pyridoxine (B<sub>2</sub>) 0.1 mg  
Riboflavin (B<sub>1</sub>) 5.0 mg  
Pantotenoic acid 5.0 mg  
p-Aminobenzoic acid 5.0 mg  
Thiamine-HCl 5.0 mg  
Nicotinic acid 5.0 mg  
Cyanocobalamin (B<sub>12</sub>) 0.1 mg  
Lipoic (tioctoic) acid 5.0 mg  
Distilled water 1000.0 ml

*Trace element solution SL-6:*

ZnSO<sub>4</sub> x 7 H<sub>2</sub>O 0.1 g  
MnCl<sub>2</sub> x 4 H<sub>2</sub>O 0.03 g  
H<sub>3</sub>BO<sub>3</sub> 0.3 g  
CoCl<sub>2</sub> x 6 H<sub>2</sub>O 0.2 g  
CuCl<sub>2</sub> x 2 H<sub>2</sub>O 0.01 g  
NiCl<sub>2</sub> x 6 H<sub>2</sub>O 0.02 g  
Na<sub>2</sub>MoO<sub>4</sub> 0.03 g  
Distilled water 1000.0 ml

Dissolve ingredients except bicarbonate, yeast extract, vitamins, pyruvate and sulfide, boil medium for 1 min, then cool to room temperature under N<sub>2</sub> gas atmosphere. Dispense medium under same gas atmosphere in tubes or serum bottles and autoclave at 121 C for 15 min. Add vitamins (sterilized by filtration), yeast extract, pyruvate and sulfide from sterile anoxic stock solutions prepared under N<sub>2</sub> gas atmosphere and bicarbonate from a sterile anoxic solution prepared under 80% N<sub>2</sub> and 20% CO<sub>2</sub> gas mixture (all solutions sterilize separately at 121 C 15 min). Adjust pH to 7.0 with a sterile anoxic solution of 10% (w/v) NaHCO<sub>3</sub>. Inoculated vessels are pressurized with carbon monoxide gas to 2 bar overpressure.

**B 232. CARBOXYDOTHERMUS FERRIREDUCTENS MEDIUM**

KH<sub>2</sub>PO<sub>4</sub> 0.33 g  
NH<sub>4</sub>Cl 0.33 g  
KCl 0.33 g  
MgCl x 6 H<sub>2</sub>O 0.33 g  
CaCl<sub>2</sub> x 2 H<sub>2</sub>O 0.33 g  
NaHCO<sub>3</sub> 2.00 g  
Glycerol (87%) 3.00 ml  
Vitamin solution (see below) 10.00 ml  
Trace element solution SL-10 (see below) 1.00 ml  
NiCl<sub>2</sub> x 6 H<sub>2</sub>O 200.00 µg  
Na<sub>2</sub>SeO<sub>3</sub> x 5 H<sub>2</sub>O 120.00 µg  
Na<sub>2</sub>WO<sub>4</sub> x 2 H<sub>2</sub>O 30.00 µg  
Yeast extract 1.00 g  
Na<sub>2</sub>-9,10-anthraquinone-2,6-disulfonate (Sigma A9706) 8.25 g  
Distilled water 1000.00 ml

*Vitamin solution:*

Biotin 2.0 mg  
Folic acid 2.0 mg  
Pyridoxine (B<sub>2</sub>) 0.1 mg  
Riboflavin (B<sub>1</sub>) 5.0 mg

Pantotenoic acid 5.0 mg  
p-Aminobenzoic acid 5.0 mg  
Thiamine-HCl 5.0 mg  
Nicotinic acid 5.0 mg  
Cyanocobalamin (B<sub>12</sub>) 0.1 mg  
Lipoic (tioctoic) acid 5.0 mg  
Distilled water 1000.0 ml

*Trace element solution SL-10:*

HCl (25%; 7.7 M) 10.0 ml  
FeCl<sub>3</sub> x 4 H<sub>2</sub>O 1.5 g  
ZnCl<sub>2</sub> 70.0 mg  
MnCl<sub>2</sub> x 4 H<sub>2</sub>O 100.0 mg  
H<sub>3</sub>BO<sub>3</sub> 6.0 mg  
CoCl<sub>2</sub> x 6 H<sub>2</sub>O 190.0 mg  
CuCl<sub>2</sub> x 2 H<sub>2</sub>O 2.0 mg  
NiCl<sub>2</sub> x 6 H<sub>2</sub>O 24.0 mg  
Na<sub>2</sub>MoO<sub>4</sub> x 2 H<sub>2</sub>O 36.0 mg  
Distilled water 990.0 ml

Dissolve ingredients (except CaCl<sub>2</sub> x 2 H<sub>2</sub>O, NaHCO<sub>3</sub>, and vitamins), boil medium for some minutes to dissolve the anthraquinone, then cool under 80% N<sub>2</sub> and 20% CO<sub>2</sub> gas atmosphere to room temperature. Add solid NaHCO<sub>3</sub> and adjust medium pH to 6.8 with NaOH. Dispense medium in tubes or bottles under same gas. Autoclave at 121 C for 15 min. Before use, add CaCl<sub>2</sub> and vitamins from anoxic, sterile stock solutions. Vitamin solution is filter sterilized. CaCl<sub>2</sub> and trace element solution sterilize at 121 C for 15 min

**B 233. CLOSTRIDIUM ALKALICELLULOSI MEDIUM**

NH<sub>4</sub>Cl 0.5 g  
KH<sub>2</sub>PO<sub>4</sub> 0.2 g  
MgCl<sub>2</sub> x 6 H<sub>2</sub>O 0.1 g  
KCl 0.2 g  
Trace element solution SL-10 (see below) 1.0 ml  
Selenite-tungstate solution (see below) 1.00 ml  
Na<sub>2</sub>CO<sub>3</sub> 1.0 g  
NaHCO<sub>3</sub> 7.6 g  
NaCl 10.0 g  
Yeast extract 0.2 g  
Cellobiose 3.0 g  
Na<sub>2</sub>S x 9 H<sub>2</sub>O 0.5 g

*Trace element solution SL-10:*

HCl (25%; 7.7 M) 10.0 ml  
FeCl<sub>3</sub> x 4 H<sub>2</sub>O 1.5 g  
ZnCl<sub>2</sub> 70.0 mg  
MnCl<sub>2</sub> x 4 H<sub>2</sub>O 100.0 mg  
H<sub>3</sub>BO<sub>3</sub> 6.0 mg  
CoCl<sub>2</sub> x 6 H<sub>2</sub>O 190.0 mg  
CuCl<sub>2</sub> x 2 H<sub>2</sub>O 2.0 mg  
NiCl<sub>2</sub> x 6 H<sub>2</sub>O 24.0 mg  
Na<sub>2</sub>MoO<sub>4</sub> x 2 H<sub>2</sub>O 36.0 mg  
Distilled water 990.0 ml

*Selenite-tungstate solution:*

NaOH 0.5 g  
Na<sub>2</sub>SeO<sub>3</sub> x 5 H<sub>2</sub>O 3 mg  
Na<sub>2</sub>WO<sub>4</sub> x 2 H<sub>2</sub>O 4 mg  
Distilled water 1000.0 ml

Dissolve ingredients except cellobiose and sulfide, flush medium with N<sub>2</sub> gas for 30–60 min, dispense under N<sub>2</sub> gas atmosphere and autoclave at 121 C 15 min. Add cellobiose after autoclaving from an anoxic stock solution sterilized by filtration and sulfide from a sterile (sterilize at 121 C 15 min), anoxic stock solution prepared under N<sub>2</sub>.

Final pH of the medium 8.8-9.0.

#### **B 234. DESULFOHALOBIUM UTAHENSE MEDIUM**

NaCl 100.0 g  
MgSO<sub>4</sub> x 7 H<sub>2</sub>O 10.0 g  
KCl 6.0 g  
CaCl<sub>2</sub> x 2 H<sub>2</sub>O 0.4 g  
NH<sub>4</sub>Cl 1.0 g  
KH<sub>2</sub>PO<sub>4</sub> 0.1 g  
Yeast extract 0.5 g  
Trace element solution SL-10 (see below) 1.0 ml  
Selenite-tungstate solution (see below) 1.00 ml  
NaHCO<sub>3</sub> 4.0 g  
Na-(L)-lactate 2.5 g  
Resazurin 0.5 mg  
Na<sub>2</sub>S x 9 H<sub>2</sub>O 0.3 g  
Distilled water 1000.0 ml

*Trace element solution SL-10:*

HCl (25%; 7.7 M) 10.0 ml  
FeCl<sub>3</sub> x 4 H<sub>2</sub>O 1.5 g  
ZnCl<sub>2</sub> 70.0 mg  
MnCl<sub>2</sub> x 4 H<sub>2</sub>O 100.0 mg  
H<sub>3</sub>BO<sub>3</sub> 6.0 mg  
CoCl<sub>2</sub> x 6 H<sub>2</sub>O 190.0 mg  
CuCl<sub>2</sub> x 2 H<sub>2</sub>O 2.0 mg  
NiCl<sub>2</sub> x 6 H<sub>2</sub>O 24.0 mg  
Na<sub>2</sub>MoO<sub>4</sub> x 2 H<sub>2</sub>O 36.0 mg  
Distilled water 990.0 ml

*Selenite-tungstate solution:*

NaOH 0.5 g  
Na<sub>2</sub>SeO<sub>3</sub> x 5 H<sub>2</sub>O 3 mg  
Na<sub>2</sub>WO<sub>4</sub> x 2 H<sub>2</sub>O 4 mg  
Distilled water 1000.0 ml

Dissolve ingredients (except lactate, bicarbonate and sulfide), boil medium for 1 min, then cool to room temperature under 80% N<sub>2</sub> and 20% CO<sub>2</sub> gas mixture. Dispense under same gas atmosphere in culture vessels and autoclave at 121 C 15 min. Add sodium lactate and sulfide from sterile anoxic stock solutions prepared under N<sub>2</sub> and bicarbonate from a sterile stock solution prepared under 80% N<sub>2</sub> and 20% CO<sub>2</sub> (all solutions sterilize separately at 121 C 15 min).

Final pH of the medium 7.0-7.2

#### **B 235. DESULFONATRONUM COOPERATIVUM MEDIUM**

KH<sub>2</sub>PO<sub>4</sub> 0.2 g  
MgCl<sub>2</sub> x 6 H<sub>2</sub>O 0.1 g  
NH<sub>4</sub>Cl 1.0 g  
KCl 0.2 g  
Na<sub>2</sub>SO<sub>4</sub> 5.0 g  
NaCl 10.0 g  
Na<sub>2</sub>CO<sub>3</sub> 3.5 g  
Trace element solution SL-10 (see below) 1.0 ml  
Vitamin solution (see below) 10.0 ml

Yeast extract 1.0 g  
Resazurin 0.5 mg  
Na-formate 4.0 g  
Na<sub>2</sub>S x 9 H<sub>2</sub>O 0.5 g  
Distilled water 1000.0 ml  
*Trace element solution SL-10:*  
HCl (25%; 7.7 M) 10.0 ml  
FeCl<sub>3</sub> x 4 H<sub>2</sub>O 1.5 g  
ZnCl<sub>2</sub> 70.0 mg  
MnCl<sub>2</sub> x 4 H<sub>2</sub>O 100.0 mg  
H<sub>3</sub>BO<sub>3</sub> 6.0 mg  
CoCl<sub>2</sub> x 6 H<sub>2</sub>O 190.0 mg  
CuCl<sub>2</sub> x 2 H<sub>2</sub>O 2.0 mg  
NiCl<sub>2</sub> x 6 H<sub>2</sub>O 24.0 mg  
Na<sub>2</sub>MoO<sub>4</sub> x 2 H<sub>2</sub>O 36.0 mg  
Distilled water 990.0 ml

*Vitamin solution:*

Biotin 2.0 mg  
Folic acid 2.0 mg  
Pyridoxine (B<sub>2</sub>) 0.1 mg  
Riboflavin (B<sub>1</sub>) 5.0 mg  
Pantotenoic acid 5.0 mg  
p-Aminobenzoic acid 5.0 mg  
Thiamine-HCl 5.0 mg  
Nicotinic acid 5.0 mg  
Cyanocobalamin (B<sub>12</sub>) 0.1 mg  
Lipoic (tioctoic) acid 5.0 mg  
Distilled water 1000.0 ml

Dissolve the ingredients (except formate, vitamin solution and sulfide) and flush medium with 100 % N<sub>2</sub> for 30 min. Add the sodium sulfide, adjust the pH to 8.8-9.0, dispense in Hungate tubes under N<sub>2</sub>, and autoclave at 121 C 15 min. Before use add sodium formate and vitamin solution from a sterile, anaerobic stock solution (sterilize sodium formate 121 C 15 min and vitamine solution by filtration).

**B 236. DESULFOTOMACULUM CARBOXYDIVORANS MEDIUM**

**95. DESULFOTOMACULUM ACETOXIDANS MEDIUM**

*Solution 1:*

NaCl 1.17 g  
MgCl<sub>2</sub> x 6 H<sub>2</sub>O 0.4 g  
KCl 0.3 g  
CaCl<sub>2</sub> x 2 H<sub>2</sub>O 0.15 g  
NH<sub>4</sub>Cl 0.27 g  
KH<sub>2</sub>PO<sub>4</sub> 0.2 g  
Na<sub>2</sub>SO<sub>4</sub> 2.84 g  
Na-acetate 1.4 g  
Na-butyrate 1.4 g  
Yeast extract 0.5 g  
Vitamin solution (see below) 1.0 ml  
Trace element solution 1.0 ml (see below)  
Distilled water 940.0 ml

*Solution 2:*

Na<sub>2</sub>S x 9 H<sub>2</sub>O 0.36 g  
Distilled water 10.0 ml

*Sodium bicarbonate for alkalization:*

NaHCO<sub>3</sub> 4.5 g

Distilled water 50.0 g

*Vitamin solution:*

p-Aminobenzoic acid 4.0 mg

D(+)-Biotin 1.0 mg

Thiamine-HCl 10.0 mg

Distilled water 100.0 ml

*Trace element solution:*

FeCl<sub>3</sub> x 4 H<sub>2</sub>O 1.5 g

ZnCl<sub>2</sub> 68.0 mg

MnCl<sub>2</sub> x 4 H<sub>2</sub>O 100.0 ml

H<sub>3</sub>BO<sub>3</sub> 62.0 mg

CoCl<sub>2</sub> x 6 H<sub>2</sub>O 120.0 ml

Na<sub>2</sub>MoO<sub>4</sub> x 2 H<sub>2</sub>O 24.0 mg

HCl, 0.05 M 1000.0 ml

pH 7.0-7.2

Solution 1 is boiled before sterilization for a few minutes being flushed with gas mixture of 80% N<sub>2</sub> and 20% CO<sub>2</sub> with sodium bicarbonate added until an equilibrium pH of 6.9-7.1. Solution 1 is sterilized under this gas mixture at 121 C for 15 min. Solution 2 is sterilized under 100% N<sub>2</sub> at 121 C for 15 min. Vitamin solution is filter sterilized.

Use medium 95 but lower the amount of yeast extract to 0.5 g/1000.0 ml Na-acetate and Na-butyrate is replaced by 2.2 g/1000.0 ml Na-pyruvate added from an anoxic stock solution (sterilized by filtration) after autoclaving.

### **B 237. DESULFUROCOCCUS FERMENTANS MEDIUM**

NH<sub>4</sub>Cl 0.33 g

KH<sub>2</sub>PO<sub>4</sub> 0.33 g

KCl 0.33 g

CaCl<sub>2</sub> x 2 H<sub>2</sub>O 0.44 g

MgCl<sub>2</sub> x 6 H<sub>2</sub>O 0.7 g

NaCl 0.50 g

Trace elements SL-10 (see below) 1.0 ml

Vitamin solution (see below) 10.0 ml

Yeast extract 0.2 g

Starch 5.0 g

Resazurin 1.0 mg

NaHCO<sub>3</sub> 0.8 g

Na<sub>2</sub>S x 9 H<sub>2</sub>O 0.5 g

Distilled water 1000.0 ml

*Trace element solution SL-10:*

HCl (25%; 7.7 M) 10.0 ml

FeCl<sub>3</sub> x 4 H<sub>2</sub>O 1.5 g

ZnCl<sub>2</sub> 70.0 mg

MnCl<sub>2</sub> x 4 H<sub>2</sub>O 100.0 mg

H<sub>3</sub>BO<sub>3</sub> 6.0 mg

CoCl<sub>2</sub> x 6 H<sub>2</sub>O 190.0 mg

CuCl<sub>2</sub> x 2 H<sub>2</sub>O 2.0 mg

NiCl<sub>2</sub> x 6 H<sub>2</sub>O 24.0 mg

Na<sub>2</sub>MoO<sub>4</sub> x 2 H<sub>2</sub>O 36.0 mg

Distilled water 990.0 ml

*Vitamin solution:*

Biotin 2.0 mg

Folic acid 2.0 mg

Pyridoxine (B<sub>2</sub>) 0.1 mg

Riboflavin (B<sub>1</sub>) 5.0 mg  
Pantotenoic acid 5.0 mg  
p-Aminobenzoic acid 5.0 mg  
Thiamine-HCl 5.0 mg  
Nicotinic acid 5.0 mg  
Cyanocobalamin (B<sub>12</sub>) 0.1 mg  
Lipoic (tioctoic) acid 5.0 mg  
Distilled water 1000.0 ml

Dissolve ingredients (except vitamins, bicarbonate and sulfide), boil medium for 1 min, then cool to room temperature under 80% N<sub>2</sub> and 20% CO<sub>2</sub> gas atmosphere. Adjust pH to 6.2-6.4 and autoclave at 121 C 15 min. After autoclaving add vitamins from an anoxic stock solution sterilized by filtration and bicarbonate from a sterile stock solution prepared under 80% N<sub>2</sub> and 20% CO<sub>2</sub> gas mixture (sterilize at 121 C 15 min). Prior to inoculation reduce medium by adding sulfide from a sterile, anoxic stock solution prepared under N<sub>2</sub> (sterilize at 121 C 15 min).

### **B 238. AQUASPIRILLUM MEDIUM**

(NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub> 1.0 g  
MgSO<sub>4</sub> x 7 H<sub>2</sub>O 1.0 g  
CaCl<sub>2</sub> x 6 H<sub>2</sub>O 30.0 mg  
Na<sub>2</sub>HPO<sub>4</sub> 10.0 mg  
Casamino acids 1.5 g  
Sodium succinate (10% solution) 10.0 ml  
Na<sub>2</sub>S<sub>2</sub>O<sub>3</sub> x 5 H<sub>2</sub>O (10% solution) 1.0 ml  
Vitamin solution (see below) 5.0 ml  
Trace element solution SL-10 (see below) 1.0 ml

#### *Vitamin solution:*

Biotin 200.0 mg  
Folic acid 20.0 mg  
Pyridoxine-HCl 100.0 mg  
Thiamine-HCl 50.0 mg  
Riboflavin 100.0 mg  
Nicotinic acid 50.0 mg  
DL-Pantothenic acid 50.0 mg  
Vitamin B<sub>12</sub> 1.0 mg  
p-Aminobenzoic acid 50.0 mg  
Distilled water 1000.0 ml

#### *Trace element solution SL-10:*

HCl (25%; 7.7 M) 10.0 ml  
FeCl<sub>3</sub> x 4 H<sub>2</sub>O 1.5 g  
ZnCl<sub>2</sub> 70.0 mg  
MnCl<sub>2</sub> x 4 H<sub>2</sub>O 100.0 mg  
H<sub>3</sub>BO<sub>3</sub> 6.0 mg  
CoCl<sub>2</sub> x 6 H<sub>2</sub>O 190.0 mg  
CuCl<sub>2</sub> x 2 H<sub>2</sub>O 2.0 mg  
NiCl<sub>2</sub> x 6 H<sub>2</sub>O 24.0 mg  
Na<sub>2</sub>MoO<sub>4</sub> x 2 H<sub>2</sub>O 36.0 mg  
Distilled water 990.0 ml  
Agar 0.5 g  
Distilled water 985.0 ml  
pH 7.5

Sterilize separately at 121 C 15 min. Vitamine solution sterilize by filtration.

### **B 239. METHYLOPHAGA ALCALICA MEDIUM**

KH<sub>2</sub>PO<sub>4</sub> 1.0 g

KNO<sub>3</sub> 1.0 g  
MgSO<sub>4</sub> x 7 H<sub>2</sub>O 0.22 g  
NaCl 30.0 g  
Na<sub>2</sub>CO<sub>3</sub> 5.0 g  
Trace element solution (see below) 1.0 ml  
Distilled water 1000.0 ml  
Final pH 9.5

*Trace elements solution:*

Ferric citrate 30.0 mg  
CaCl<sub>2</sub> x 2 H<sub>2</sub>O 30.0 mg  
MgCl<sub>2</sub> x 4 H<sub>2</sub>O 5.0 mg  
ZnSO<sub>4</sub> x 7 H<sub>2</sub>O 5.0 mg  
CuSO<sub>4</sub> x 5 H<sub>2</sub>O 0.5 g  
Distilled water 1000.0 ml

Prepare the medium without the Na<sub>2</sub>CO<sub>3</sub>. Basal medium, trace element and Na<sub>2</sub>CO<sub>3</sub> sterilize separately at 121 C 15 min. 10 ml/1000.0 ml filter-sterilized methanol is added to the cooled medium. When preparing liquid media cool the mineral salts solution and Na<sub>2</sub>CO<sub>3</sub> to room temperature before mixing. When preparing agar add 2.0 % agar to the mineral salts solution and autoclave. Cool the Na<sub>2</sub>CO<sub>3</sub> stock solution and agar to 50-55 C before mixing.

**B 240. METHYLOTHERMUS THERMALIS MEDIUM**

KNO<sub>3</sub> 0.25 g  
NH<sub>4</sub>Cl 0.25 g  
KH<sub>2</sub>PO<sub>4</sub> 0.13 g  
Na<sub>2</sub>HPO<sub>4</sub> x 12H<sub>2</sub>O 0.358 g  
MgSO<sub>4</sub> x 7H<sub>2</sub>O 0.4 g  
CaCl<sub>2</sub> 0.1 g  
Agar 20.0 g  
Distilled water 1000.00 ml  
pH 6.8  
The gas phase methane/air mixture (4:1)  
Sterilize at 121 C 15 min.

**B 241. OCEANITHERMUS PROFUNDUS MEDIUM**

NH<sub>4</sub>Cl 0.33 g  
MgCl<sub>2</sub> x 6 H<sub>2</sub>O 0.33 g  
CaCl<sub>2</sub> x 2 H<sub>2</sub>O 0.33 g  
KCl 0.33 g  
KNO<sub>3</sub> 0.33 g  
NaCl 30.00 g  
HEPES 2.38 g  
Yeast extract 0.2 g  
Tryptone 1.0 g  
Sucrose 2.0 g  
Vitamin solution (see below) 1.0 ml  
Trace elements (see below) 1.0 ml  
Distilled water 1000.0 ml  
*Vitamin solution:*  
Biotin 2.0 mg  
Folic acid 2.0 mg  
Pyridoxine (B<sub>2</sub>) 0.1 mg  
Riboflavin (B<sub>1</sub>) 5.0 mg  
Pantotenoic acid 5.0 mg  
p-Aminobenzoic acid 5.0 mg

Thiamine-HCl 5.0 mg  
Nicotinic acid 5.0 mg  
Cyanocobalamin (B<sub>12</sub>) 0.1 mg  
Lipoic (tioctoic) acid 5.0 mg  
Distilled water 1000.0 ml

*Trace element solution:*

Nitrilotriacetic acid 12.8 mg  
FeSO<sub>4</sub> x 7 H<sub>2</sub>O 0.1 mg  
MnCl<sub>2</sub> x 6 H<sub>2</sub>O 0.1 mg  
CoCl<sub>2</sub> x 2 H<sub>2</sub>O 0.17 mg  
CaCl<sub>2</sub> x 2 H<sub>2</sub>O 0.1 mg  
ZnCl<sub>2</sub> 0.1 mg  
CuCl<sub>2</sub> 0.02 mg  
H<sub>3</sub>BO<sub>3</sub> 0.01 mg  
Na<sub>2</sub>MoO<sub>4</sub> x 2 H<sub>2</sub>O 0.01 mg  
NaCl 1.0 mg  
Na<sub>2</sub>SeO<sub>4</sub> 0.017 mg  
Distilled water 1000.0 ml

Prepare the medium anaerobically, under N<sub>2</sub>, omitting the CaCl<sub>2</sub>, MgCl<sub>2</sub>, KNO<sub>3</sub>, tryptone, yeast extract, vitamins and sucrose. The pH should be 7.0-7.5. Dispense the medium into vessels suitable for anaerobic growth (Hungate tubes or serum bottles) under an atmosphere of N<sub>2</sub> and autoclave at 121 C 15 min. To the sterile, cooled medium add, from sterile stock solutions the CaCl<sub>2</sub>, MgCl<sub>2</sub>, KNO<sub>3</sub>, tryptone, yeast extract, vitamins and sucrose. The CaCl<sub>2</sub>, MgCl<sub>2</sub>, KNO<sub>3</sub>, tryptone, and yeast extract, stock solutions should be autoclaved at 121 C 15 min, sucrose at 111 C 30 min, while the vitamin solution is sterile filtered.

#### **B 242. PSYCHROBACTER MEDIUM**

Peptone 5.0 g  
Yeast extract 1.0 g  
Sea salts 17.0 g  
Distilled water 1000.0 ml  
pH 7.2  
Sterilize at 121 C 15 min.

#### **B 243. ROSEICYCLUS MEDIUM**

KH<sub>2</sub>PO<sub>4</sub> 0.3 g  
MgSO<sub>4</sub> 2.0 g  
NH<sub>4</sub>Cl 0.3 g  
KCl 0.3 g  
CaCl<sub>2</sub> x 2H<sub>2</sub>O 0.05 g  
Na<sub>2</sub>SO<sub>4</sub> 15.0 g  
NaHCO<sub>3</sub> 0.5 g  
Na-acetate 1.0 g  
Na-malate 1.0 g  
Yeast extract 1.0 g  
Peptone 0.5 g  
Agar 20.0 g/l  
Distilled water 1000.0 ml  
pH 7.8-8.0  
Sterilize at 121 C 15 min.

#### **B 244. SULFOBACILLUS MEDIUM**

*Solution A:*

(NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub> 3.0 g



KCl 0.1 g  
K<sub>2</sub>HPO<sub>4</sub> 0.5 g  
MgSO<sub>4</sub> x 7 H<sub>2</sub>O 0.5 g  
Ca(NO<sub>3</sub>)<sub>2</sub> 0.01 g  
Distilled water 680.0 ml  
pH 2.0-2.2 (adjust with sulfuric acid)

*Solution B:*

FeSO<sub>4</sub> x 7 H<sub>2</sub>O 44.2 g  
Distilled water 300.0 ml  
H<sub>2</sub>SO<sub>4</sub> (10 N) 1.0 ml

*Solution C:*

Yeast extract (1% w/v in water) 20.0 ml  
pH 1.9-2.4

After autoclaving at 121 C 15 min, combine the three solutions.

**B 245. THERMINCOLA MEDIUM**

NH<sub>4</sub>Cl 1.0 g  
MgCl<sub>2</sub> x 6 H<sub>2</sub>O 0.33 g  
CaCl<sub>2</sub> x 2 H<sub>2</sub>O 0.1 g  
KCl 0.33 g  
KH<sub>2</sub>PO<sub>4</sub> 0.5 g  
Resazurin 0.5 mg  
Wolfe's mineral elixir (see below) 1.0 ml  
Vitamin solution (see below) 20.0 ml

NaHCO<sub>3</sub> 0.5 g  
Na<sub>2</sub>CO<sub>3</sub> 0.5 g  
Na-acetate 0.2 g  
Yeast extract 0.2 g  
Na<sub>2</sub>S x 9 H<sub>2</sub>O 1.0 g  
Distilled water 1000.0 ml

*Wolfe's mineral elixir:*

MgSO<sub>4</sub> x 7 H<sub>2</sub>O 30.0 g  
MnSO<sub>4</sub> x H<sub>2</sub>O 5.0 g  
NaCl 10.0 g  
FeSO<sub>4</sub> x 7 H<sub>2</sub>O 1.0 g  
CoCl<sub>2</sub> x 6 H<sub>2</sub>O 1.8 g  
CaCl<sub>2</sub> x 2 H<sub>2</sub>O 1.0 g  
ZnSO<sub>4</sub> x 7 H<sub>2</sub>O 1.8 g  
CuSO<sub>4</sub> x 5 H<sub>2</sub>O 0.1 g  
KAl(SO<sub>4</sub>)<sub>2</sub> x 12 H<sub>2</sub>O 0.18 g  
H<sub>3</sub>BO<sub>3</sub> 0.10 g  
Na<sub>2</sub>MoO<sub>4</sub> x 2 H<sub>2</sub>O 0.1 g  
(NH<sub>4</sub>)<sub>2</sub>Ni(SO<sub>4</sub>)<sub>2</sub> x 6 H<sub>2</sub>O 2.8 g  
Na<sub>2</sub>WO<sub>4</sub> x 2 H<sub>2</sub>O 0.1 g  
Na<sub>2</sub>SeO<sub>4</sub> 0.1 g  
Distilled water 1000.0 ml

*Vitamin solution:*

Biotin 2.0 mg  
Folic acid 2.0 mg  
Pyridoxine (B<sub>2</sub>) 0.1 mg  
Riboflavin (B<sub>1</sub>) 5.0 mg  
Pantotenoic acid 5.0 mg  
p-Aminobenzoic acid 5.0 mg  
Thiamine-HCl 5.0 mg

Nicotinic acid 5.0 mg  
Cyanocobalamin (B<sub>12</sub>) 0.1 mg  
Lipoic (tioctoic) acid 5.0 mg  
Distilled water 1000.0 ml

First adjust pH to 1.0 with diluted H<sub>2</sub>SO<sub>4</sub>, then add and dissolve the salts. Dissolve ingredients except carbonates, vitamine solution, yeast extract and sulfide, boil medium for 1 min, then cool to room temperature under N<sub>2</sub> gas atmosphere. Add carbonates and sulfide to the medium, dispense under CO gas atmosphere in culture vessels (e.g., 10 ml medium in 50 ml serum bottles) and autoclave at 121 C 15 min. Prior to inoculation add yeast extract from a sterile, anoxic stock solution prepared under N<sub>2</sub> and adjust pH of final medium to 8.0 with a sterile, anoxic solution of 1 N HCl (this solutions sterilize at 121 C 15 min). Add filter-sterilization vitamine solution.

#### **B 246. VULCANITHERMUS MEDIUM**

NH<sub>4</sub>Cl 0.33 g  
MgCl<sub>2</sub> x 6 H<sub>2</sub>O 0.33 g  
CaCl<sub>2</sub> x 2 H<sub>2</sub>O 0.33 g  
KCl 0.33 g  
KNO<sub>3</sub> 0.33 g  
NaCl 25.0 g  
PIPES 3.6 g  
Yeast extract 0.5 g  
Tryptone 1.0 g  
Sucrose 1.0 g  
Vitamin (see below) 1.0 ml  
Trace elements (see below) 1.0 ml  
Distilled water 1000.0 ml

##### *Vitamin solution:*

Biotin 2.0 mg  
Folic acid 2.0 mg  
Pyridoxine (B<sub>2</sub>) 0.1 mg  
Riboflavin (B<sub>1</sub>) 5.0 mg  
Pantotenoic acid 5.0 mg  
p-Aminobenzoic acid 5.0 mg  
Thiamine-HCl 5.0 mg  
Nicotinic acid 5.0 mg  
Cyanocobalamin (B<sub>12</sub>) 0.1 mg  
Lipoic (tioctoic) acid 5.0 mg  
Distilled water 1000.0 ml

##### *Trace element solution:*

Nitrilotriacetic acid 12.8 mg  
FeSO<sub>4</sub> x 7 H<sub>2</sub>O 0.1 mg  
MnCl<sub>2</sub> x 6 H<sub>2</sub>O 0.1 mg  
CoCl<sub>2</sub> x 2 H<sub>2</sub>O 0.17 mg  
CaCl<sub>2</sub> x 2 H<sub>2</sub>O 0.1 mg  
ZnCl<sub>2</sub> 0.1 mg  
CuCl<sub>2</sub> 0.02 mg  
H<sub>3</sub>BO<sub>3</sub> 0.01 mg  
Na<sub>2</sub>MoO<sub>4</sub> x 2 H<sub>2</sub>O 0.01 mg  
NaCl 1.0 mg  
Na<sub>2</sub>SeO<sub>4</sub> 0.017 mg  
Distilled water 1000.0 ml

pH 6.8

Prepare the medium anaerobically, under N<sub>2</sub>, omitting the CaCl<sub>2</sub>, MgCl<sub>2</sub>, KNO<sub>3</sub>, tryptone, yeast extract, vitamins and sucrose. Dispense the medium into vessels suitable for anaerobic growth (Hungate

tubes or serum bottles) under an atmosphere of N<sub>2</sub> and autoclave at 121 C 15 min. To the sterile, cooled medium add, from sterile stock solutions the CaCl<sub>2</sub>, MgCl<sub>2</sub>, KNO<sub>3</sub>, tryptone, yeast extract, vitamins and sucrose. The CaCl<sub>2</sub>, MgCl<sub>2</sub>, KNO<sub>3</sub>, tryptone, and yeast extract, stock solutions should be autoclaved at 121 C 15 min, sucrose at 111 C 30 min, while the vitamin solution is sterile filtered.

#### **B 247. RHODOBLASTUS MEDIUM**

Yeast extract 0.1 g  
Na<sub>2</sub>-succinate 1.0 g  
KH<sub>2</sub>PO<sub>4</sub> 0.5 g  
MgSO<sub>4</sub> x 7 H<sub>2</sub>O 0.4 g  
NaCl 0.4 g  
NH<sub>4</sub>Cl 0.4 g  
CaCl<sub>2</sub> x 2 H<sub>2</sub>O 0.05 g  
Trace element solution SL-6 (see below) 1.0 ml  
Methanol 0.01-1%  
Distilled water 1000.0 ml  
pH 5.7

*Trace element solution SL-6:*

ZnSO<sub>4</sub> x 7 H<sub>2</sub>O 0.1 g  
MnCl<sub>2</sub> x 4 H<sub>2</sub>O 0.03 g  
H<sub>3</sub>BO<sub>3</sub> 0.3 g  
CoCl<sub>2</sub> x 6 H<sub>2</sub>O 0.2 g  
CuCl<sub>2</sub> x 2 H<sub>2</sub>O 0.01 g  
NiCl<sub>2</sub> x 6 H<sub>2</sub>O 0.02 g  
Na<sub>2</sub>MoO<sub>4</sub> x 2 H<sub>2</sub>O 0.03 g  
Distilled water 1000.0 ml

Sterilize basal medium and trace element solution separately at 121 C 15 min, methanol sterilize by filtration.

#### **B 248. PFENNIG'S MEDIUM**

KH<sub>2</sub>PO<sub>4</sub> 0.34 g  
NH<sub>4</sub>Cl 0.34 g  
MgSO<sub>4</sub> x 7 H<sub>2</sub>O 0.5 g  
CaCl<sub>2</sub> x 2 H<sub>2</sub>O 0.05 g  
KCl 0.34 g  
Trace element solution SLA (see below) 1 ml  
Cyanocobalamin (B<sub>12</sub>) 20 µg  
NaHCO<sub>3</sub> 1.5 g  
Na<sub>2</sub>S x 7-9 H<sub>2</sub>O 0.4 g  
Na<sub>2</sub>S<sub>2</sub>O<sub>3</sub> x 5 H<sub>2</sub>O 0.5 g  
NaCl 15.0 g  
MgCl<sub>2</sub> x 6 H<sub>2</sub>O 2.5 g.  
pH 7.5

*Trace element solution SLA:*

FeCl<sub>2</sub> x 4 H<sub>2</sub>O 1.8 mg  
CoCl<sub>2</sub> x 6 H<sub>2</sub>O 250 mg  
NiCl<sub>2</sub> x 6 H<sub>2</sub>O 10 mg  
CuCl<sub>2</sub> x 2 H<sub>2</sub>O 10 mg  
MnCl<sub>2</sub> x 4 H<sub>2</sub>O 70 mg  
ZnCl<sub>2</sub> 100 mg  
H<sub>3</sub>BO<sub>3</sub> 500 mg  
Na<sub>2</sub>MoO<sub>4</sub> x 2 H<sub>2</sub>O 30 mg  
Na<sub>2</sub>SeO<sub>3</sub> x 5 H<sub>2</sub>O 10 mg  
Distilled water 1000 ml

Sterilize basal medium and trace element solution separately at 121 C 15 min, cyanocobalamin (B<sub>12</sub>) sterilize by filtration.

**B 249. MODIFIED METHYLOTROPHMEDIUM 1 WITH 3.0% METHYLAMINE**

KH<sub>2</sub>PO<sub>4</sub> 2.0 g  
(NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub> 2.0 g  
MgSO<sub>4</sub> x 7 H<sub>2</sub>O 0.025 g  
NaCl 0.5 g  
FeSO<sub>4</sub> x 7 H<sub>2</sub>O 0.002 g  
Methanol 5.0 ml or  
Methylamine 30.0 g  
Distilled water 1000.0 ml  
pH 7.0

Sterilize at 121 C 15 min, methanol and methylamine sterilize by filtration.

**B 250. MODIFIED MEDIUM FOR MARINEMETHYLOTROPHIC BACTERIA WITHOUT NaCl AND WITH METHANOL**

KH<sub>2</sub>PO<sub>4</sub> 2.0 g  
(NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub> 2.0 g  
MgSO<sub>4</sub> x 7 H<sub>2</sub>O 0.025 g  
FeSO<sub>4</sub> x 7 H<sub>2</sub>O 0.002 g  
Yeast extract 0.1 g  
Methanol 10.0 ml  
Biotine 0.01 mg  
Distilled water 1000.0 ml  
pH 7.0

Sterilize at 121 C 15 min, methanol and biotine sterilize by filtration.

**B 251. MODIFIED METHYLOTROPH MEDIUM 1 WITHOUT METHYLAMINE**

KH<sub>2</sub>PO<sub>4</sub> 2.0 g  
(NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub> 2.0 g  
MgSO<sub>4</sub> x 7 H<sub>2</sub>O 0.025 g  
NaCl 0.5 g  
FeSO<sub>4</sub> x 7 H<sub>2</sub>O 0.002 g  
Methanol 5.0 ml  
Distilled water 1000.0 ml  
pH 7.0

Sterilize at 121 C 15 min, methanol sterilize by filtration.

**B 252. MODIFIED METHYLOTROPH MEDIUM 1 WITH KCNS**

KH<sub>2</sub>PO<sub>4</sub> 2.0 g  
(NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub> 2.0 g  
MgSO<sub>4</sub> x 7 H<sub>2</sub>O 0.025 g  
NaCl 0.5 g  
KCNS 0.24 g  
FeSO<sub>4</sub> x 7 H<sub>2</sub>O 0.002 g  
Methanol 5.0 ml or  
Methylamine 3.0 g  
Distilled water 1000.0 ml  
pH 7.0

Sterilize at 121 C 15 min, methanol and methylamine sterilize by filtration.

**B 253. MODIFIED MEDIUM FOR METHANOTROPHIC BACTERIA WITH ETHANOL**

KNO<sub>3</sub> 1.0 g

KH<sub>2</sub>PO<sub>4</sub> 0.7 g  
MgSO<sub>4</sub> x 7 H<sub>2</sub>O 0.2g  
CaCl<sub>2</sub> 0.02 g  
Na<sub>2</sub>HPO<sub>4</sub> x 5 H<sub>2</sub>O 1.5 g  
Trylon B 5.0 mg  
FeSO<sub>4</sub> x 7 H<sub>2</sub>O 2.0mg  
ZnSO<sub>4</sub> x 7 H<sub>2</sub>O 0.1mg  
MnCl<sub>2</sub> x 4 H<sub>2</sub>O 0.03mg  
CoCl<sub>2</sub> x 6 H<sub>2</sub>O 0.2mg  
CuCl<sub>2</sub> x 5 H<sub>2</sub>O 0.1mg  
NiCl<sub>2</sub> x 6 H<sub>2</sub>O 0.02mg  
Na<sub>2</sub>MoO<sub>4</sub> 0.03 mg  
Ethanol 5.0 ml  
Distilled water 1000.0 ml  
pH 6.7 - 7.1  
Sterilize at 121 C 15 min, ethanol sterilize by filtration.  
Cultivation under mixture of methane and air (1:1).

**B 254. MODIFICATION OF TWEEN-80 MEDIUM FOR MILK-ACID BACTERIA (10% NaCl)**

Beef extract 1.2 g  
Yeast extract 5.0 g  
Glucose 2.5 g  
Tween-80 1.0 ml  
K<sub>2</sub>HPO<sub>4</sub> 2.0 g  
Na-acetate 5.0 g  
NaCl 60.0 g  
NH<sub>4</sub>-citrate 2.0 g  
MgSO<sub>4</sub> x 7 H<sub>2</sub>O 0.2g  
MnSO<sub>4</sub> x 4 H<sub>2</sub>O 0.05g  
Agar 5.0 g  
Distilled water 1000.0 ml  
pH 6.0-6.5  
Sterilize at 111 C for 30 min.

**B 255. MODIFIED METHYLOTROPH MEDIUM 1 WITHOUT METHANOL**

KH<sub>2</sub>PO<sub>4</sub> 2.0 g  
(NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub> 2.0 g  
MgSO<sub>4</sub> x 7 H<sub>2</sub>O 0.025 g  
NaCl 0.5 g  
FeSO<sub>4</sub> x 7 H<sub>2</sub>O 0.002 g  
Methylamine 3.0 g  
Distilled water 1000.0 ml  
pH 7.0  
Sterilize at 121 C 15 min, methylamine sterilize by filtration.

**B 256. MODIFIED METHYLOTROPH MEDIUM 1 WITH YEAST AUTOLYZATE**

KH<sub>2</sub>PO<sub>4</sub> 2.0 g  
(NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub> 2.0 g  
MgSO<sub>4</sub> x 7 H<sub>2</sub>O 0.025 g  
NaCl 0.5 g  
FeSO<sub>4</sub> x 7 H<sub>2</sub>O 0.002 g  
Methanol 5.0 ml or Methylamine 3.0 g  
Yeast autolizate 1.0 g

Distilled water 1000.0 ml

pH 7.0

Sterilize at 121 C 15 min, methanol and methylamine sterilize by filtration.

#### **Ac 257. MODIFIED STARCH AMMONIA AGAR (10% NaCl)**

Starch (soluble) 10.0 g

K<sub>2</sub>HPO<sub>4</sub> 1.0 g

MgSO<sub>4</sub> x 7 H<sub>2</sub>O 1.0g

NaCl 100.0 g

(NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub> 2.0 g

CaCO<sub>3</sub> 2.0 g

Salt solution A (see below) 1.0 ml

Agar 20.0 g

Distilled water 1000.0 ml

*Salt solution A:*

FeSO<sub>4</sub> 0.1 g

MnCl<sub>2</sub> 0.1 g

ZnSO<sub>4</sub> 0.1 g

Distilled water 100.0 ml

pH 7.0-7.4

Sterilize separately at 121 C 15 min.

#### **Ac 258. NOCCARDIODES AQUATICUS MEDIUM**

Base medium (see below) 600 ml

Artificial sea water (see below) 400.0 ml

pH 7.5

Sterilize Base medium ( except glucose solution and vitamin solution) and Artificial sea water separately at 121 C 15 min.

After cooling to 60 C aseptically add to the Base medium glucose solution (sterilize by filtration) and vitamine solution (sterilize by filtration). Store vitamine solution in the dark and cold (5 C).

Add 600 ml Base medium to 400 ml Artificial sea water and mix thoroughly.

*Base medium:*

Peptone Bacto 0.25 g

Yeast extract Bacto 0.25 g

Glucose solution (2.5%) 10.00 ml

Vitamin solution, double concentration (see below) 5.00 ml

Mineral salt solution (see below) 20 ml

Agar Bacto 15.00 g

Distilled water 965.00 ml

*Artificial sea water (AWS):*

NaCl 23.477 g

Na<sub>2</sub>SO<sub>4</sub> 3.917 g

MgCl<sub>2</sub> x 6 H<sub>2</sub>O 4.981 g

CaCl<sub>2</sub> 1.102 g

NaHCO<sub>3</sub> 192.000 mg

KCl 664.000 mg

KBr 6.000 mg

H<sub>3</sub>BO<sub>3</sub> 26.000 mg

SrCl<sub>2</sub> 24.000 g

NaF 3.000 mg

Distilled water 1000.000 ml

*Mineral salt solution:*

Nitrilotriacetic acid (NTA) 10.00 g

MgSO<sub>4</sub> x 7 H<sub>2</sub>O 29.70 g

CaCl<sub>2</sub> x 2 H<sub>2</sub>O 3.34 g  
Na<sub>2</sub>MoO<sub>4</sub> x 2H<sub>2</sub>O 12.67 mg  
FeSO<sub>4</sub> x 7 H<sub>2</sub>O 99.00 mg  
Metal salt solution "44" (see below) 50 ml  
Dissolve NTA first by neutralizing with KOH, then add other salts.  
pH 7.2 (adjust with KOH or H<sub>2</sub>SO<sub>4</sub>).  
Adjust volume to 1000.00 ml with distilled water.

*Metal solution "44":*

Na-EDTA – 250.00 mg  
ZnSO<sub>4</sub> x 7 H<sub>2</sub>O 1095.00 mg  
FeSO<sub>4</sub> x 7 H<sub>2</sub>O 500.00 mg  
MnSO<sub>4</sub> x 7 H<sub>2</sub>O 154.00 mg  
CuSO<sub>4</sub> x 5 H<sub>2</sub>O 39.00 mg  
Co(NO<sub>3</sub>)<sub>2</sub> x 6 H<sub>2</sub>O 24.80 mg  
Na<sub>2</sub>B<sub>4</sub>O<sub>7</sub> x 10 H<sub>2</sub>O 17.70 mg  
Distilled water 1000.00 ml  
Dissolve Na-EDTA and add a few drops of concentrated H<sub>2</sub>SO<sub>4</sub> to retard precipitation of heavy metal ions.

*Vitamin solution, double concentration:*

Biotin 4.00 mg  
Folic acid 4.00 mg  
Pyridoxine-HCl 20.00 mg  
Riboflavine 10.00 mg  
Thiamine-HCl x 2 H<sub>2</sub>O 10.00 mg  
Nicotinamide 10.00 mg  
D-Ca-pantothenate 10.00 mg  
Vitamin B12 0.20 mg  
p-Aminobenzoic acid 10.00 mg  
Distilled water 1000.00 ml

#### **AC 259. ISP-2 MEDIUM WITH 5% NaCl**

Yeast extract 4.0 g  
Malt extract 10.0 g  
Dextrose(d-glucose) 4.0 g  
Agar 15.0-20.0 g  
NaCl 50.0 g  
Distilled water 1000.0 ml  
pH 7.2  
Sterilize at 111 C 30 min.

#### **F 260. V-8**

Commercial V8 vegetable juice 175.0 ml  
CaCO<sub>3</sub> 3.0 g  
Agar 20.0 g  
Distilled water to 1000.0 ml  
pH ca 6.4  
Sterilize at 110 C for 30 min.

#### **B 261. ROSEINATRONOBACTER MONICUS MEDIUM**

NH<sub>4</sub>Cl 0.4 g  
KH<sub>2</sub>PO<sub>4</sub> 0.5 g  
MgCl<sub>2</sub> x 6 H<sub>2</sub>O 0.2 g  
Na<sub>2</sub>SO<sub>4</sub> 0.5 g  
NaNO<sub>3</sub> 0.4 g

NaCl 40.0 g  
KCl 0.5 g  
NaHCO<sub>3</sub> 10.0 g  
Na<sub>2</sub>CO<sub>3</sub> 5.0 g  
Na<sub>2</sub>S<sub>2</sub>O<sub>3</sub> x 5 H<sub>2</sub>O 1.0 g  
Vitamin B12 10.0 µg  
Sodium pyruvate 1.0 g  
Sodium acetate 1.0 g  
Peptone 1.0 g  
Yeast extract 1.0 g  
Trace element solution (SL10) 1.0 ml

Distilled water 1000.0 ml

*Trace element solution SL-10:*

HCl (25%; 7.7 M) 10.0 ml

FeCl<sub>3</sub> x 4 H<sub>2</sub>O 1.5 g

ZnCl<sub>2</sub> 70.0 mg

MnCl<sub>2</sub> x 4 H<sub>2</sub>O 100.0 mg

H<sub>3</sub>BO<sub>3</sub> 6.0 mg

CoCl<sub>2</sub> x 6 H<sub>2</sub>O 190.0 mg

CuCl<sub>2</sub> x 2 H<sub>2</sub>O 2.0 mg

NiCl<sub>2</sub> x 6 H<sub>2</sub>O 24.0 mg

Na<sub>2</sub>MoO<sub>4</sub> x 2 H<sub>2</sub>O 36.0 mg

Distilled water 990.0 ml

*The trace element solution preparation:* FeCl<sub>3</sub> x 4 H<sub>2</sub>O is dissolved firstly in HCl, and then is mixed with water and other salts are dissolved in the sequence indicated.

Autoclave the trace element solution, NaHCO<sub>3</sub> and Na<sub>2</sub>CO<sub>3</sub> separately at 121 C for 15 min and add to the medium. Add the vitamin B12 solution from a filter sterilised stock solution to the autoclaved, cooled medium.

Final pH 9.0 - 9.5.

Sterilize at 121 C for 15 min.

### **B 262. MINERAL MEDIUM**

KNO<sub>3</sub> 250.00 mg

KH<sub>2</sub>PO<sub>4</sub> 100.00 mg

MgSO<sub>4</sub> x 7 H<sub>2</sub>O 50.00 mg

CaCl<sub>2</sub> x 2 H<sub>2</sub>O 10.00 mg

Trace elements 1.00 ml

Distilled water 1000.00 ml

*Trace elements:*

EDTA 5.00 g

CuCl<sub>2</sub> x 5 H<sub>2</sub>O 0.10 g

FeSO<sub>4</sub> x 7 H<sub>2</sub>O 2.00 g

ZnSO<sub>4</sub> x 7 H<sub>2</sub>O 0.10 g

NiCl<sub>2</sub> x 6 H<sub>2</sub>O 0.02 g

CoCl<sub>2</sub> x 6 H<sub>2</sub>O 0.20 g

Na<sub>2</sub>MoO<sub>4</sub> 0.03 g

MnCl<sub>2</sub> x 4 H<sub>2</sub>O 0.03 g

Distilled water 1000.00 ml

Final pH 5.5-6.0.

Sterilize at 121 C for 15 min.

### **B 263. SINGULISPHAERA MEDIUM**

N-acetylglucosamine 1.0 g

KH<sub>2</sub>PO<sub>4</sub> 0.1 g



Hutners basal salts 20.0 ml

Peptone 0.1 g

Yeast extract 0.1 g

Agar-agar (Difco) 18.0 g

Distilled water 1000.0 ml

Adjust pH to 5.8.

Sterilize at 121 C 15 min.

*Hutners basal salts*

Nitrilotriacetic acid (NTA) 10.0 g

MgSO<sub>4</sub> x 7 H<sub>2</sub>O 29.7 g

CaCl<sub>2</sub> x 2 H<sub>2</sub>O 3.34 g

Na<sub>2</sub>MoO<sub>4</sub> x 2H<sub>2</sub>O 12.67 mg

FeSO<sub>4</sub> x 7 H<sub>2</sub>O 99.0 mg

Metal salt solution "44" (see below) 50 ml

Dissolve NTA first by neutralizing with KOH, then add other salts.

pH 7.2 (adjust with KOH or H<sub>2</sub>SO<sub>4</sub>).

Adjust volume to 1000.0 ml with distilled water.

*Metal solution "44":*

Na-EDTA – 250.0 mg

ZnSO<sub>4</sub> x 7 H<sub>2</sub>O 1095.0 mg

FeSO<sub>4</sub> x 7 H<sub>2</sub>O 500.0 mg

MnSO<sub>4</sub> x 7 H<sub>2</sub>O 154.0 mg

CuSO<sub>4</sub> x 5 H<sub>2</sub>O 39.0 mg

Co(NO<sub>3</sub>)<sub>2</sub> x 6 H<sub>2</sub>O 24.8 mg

Na<sub>2</sub>B<sub>4</sub>O<sub>7</sub> x 10 H<sub>2</sub>O 17.7 mg

Distilled water 1000.0 ml

Dissolve Na-EDTA and add a few drops of concentrated H<sub>2</sub>SO<sub>4</sub> to retard precipitation of heavy metal ions.

#### **B 264. ROSEOCOCCUS SUDUNTUYENSIS MEDIUM**

NH<sub>4</sub>Cl 0.4 g

KH<sub>2</sub>PO<sub>4</sub> 0.5 g

MgCl<sub>2</sub> x 6 H<sub>2</sub>O 0.2 g

Na<sub>2</sub>SO<sub>4</sub> 0.5 g

NaCl 2.0 g

KCl 0.5 g

NaHCO<sub>3</sub> 5.0 g

Na<sub>2</sub>CO<sub>3</sub> 0.5 g

Na<sub>2</sub>S<sub>2</sub>O<sub>3</sub> x 5 H<sub>2</sub>O 1.0 g

Vitamin B<sub>12</sub> 10.0 µg

Sodium pyruvate 1.0 g

Sodium acetate 1.0 g

Peptone 1.0 g

Yeast extract 1.0 g

Trace element solution (SL10) 1.0 ml

Distilled water 1000.0 ml

*Trace element solution SL-10:*

HCl (25%; 7.7 M) 10.0 ml

FeCl<sub>3</sub> x 4 H<sub>2</sub>O 1.5 g

ZnCl<sub>2</sub> 70.0 mg

MnCl<sub>2</sub> x 4 H<sub>2</sub>O 100.0 mg

H<sub>3</sub>BO<sub>3</sub> 6.0 mg

CoCl<sub>2</sub> x 6 H<sub>2</sub>O 190.0 mg

CuCl<sub>2</sub> x 2 H<sub>2</sub>O 2.0 mg

NiCl<sub>2</sub> x 6 H<sub>2</sub>O 24.0 mg  
Na<sub>2</sub>MoO<sub>4</sub> x 2 H<sub>2</sub>O 36.0 mg  
Distilled water 990.0 ml

*The trace element solution preparation:* FeCl<sub>3</sub> x 4 H<sub>2</sub>O is dissolved firstly in HCl, and then is mixed with water and other salts are dissolved in the sequence indicated.

Autoclave the trace element solution, NaHCO<sub>3</sub> and Na<sub>2</sub>CO<sub>3</sub> separately at 121 C for 15 min and add to the medium. Add the vitamin B12 solution from a filter sterilised stock solution to the autoclaved, cooled medium.

Final pH 8.5.

Sterilize at 121 C for 15 min.

### **B 265. ECTOTHIORHODOSPIRA VARIABILIS MEDIUM**

NaCl 50.00 g  
Na<sub>2</sub>CO<sub>3</sub> 5.00 g  
NaHCO<sub>3</sub> 15.00 g  
KCl 0.10 g  
K<sub>2</sub>HPO<sub>4</sub> 0.50 g  
MgCl<sub>2</sub> x 6 H<sub>2</sub>O 0.20 g  
NH<sub>4</sub>Cl 0.50 g  
Na<sub>2</sub>S x 9 H<sub>2</sub>O 0.50 g  
Na<sub>2</sub>S<sub>2</sub>O<sub>3</sub> x 5 H<sub>2</sub>O 0.50 g  
Yeast extract 0.10 g  
Na acetate 0.50 g  
Trace element solution (SL-10) 1.00 ml  
Vitamin B<sub>12</sub> 20.00 µg  
Distilled water 1000.00 ml  
Final pH 9.0 – 9.5

*Trace element solution SL-10:*

HCl (25%; 7.7 M) 10.0 ml  
FeCl<sub>3</sub> x 4 H<sub>2</sub>O 1.5 g  
ZnCl<sub>2</sub> 70.0 mg  
MnCl<sub>2</sub> x 4 H<sub>2</sub>O 100.0 mg  
H<sub>3</sub>BO<sub>3</sub> 6.0 mg  
CoCl<sub>2</sub> x 6 H<sub>2</sub>O 190.0 mg  
CuCl<sub>2</sub> x 2 H<sub>2</sub>O 2.0 mg  
NiCl<sub>2</sub> x 6 H<sub>2</sub>O 24.0 mg  
Na<sub>2</sub>MoO<sub>4</sub> x 2 H<sub>2</sub>O 36.0 mg  
Distilled water 990.0 ml

*The trace element solution preparation:* FeCl<sub>3</sub> x 4 H<sub>2</sub>O is dissolved firstly in HCl, and then is mixed with water and other salts are dissolved in the sequence indicated.

Prepare and boil the medium in the absence of Na<sub>2</sub>S x 9 H<sub>2</sub>O, NaHCO<sub>3</sub>, Vitamin B<sub>12</sub>, CaCl<sub>2</sub> x 2 H<sub>2</sub>O, MgCl<sub>2</sub> x 6 H<sub>2</sub>O. Cool under a stream of nitrogen and add the remaining components, except vitamin B<sub>12</sub> and Na<sub>2</sub>S<sub>2</sub>O<sub>3</sub> x 5 H<sub>2</sub>O. Dispense into tubes or bottle fitted with rubber stoppers and autoclave. Add the vitamin B<sub>12</sub> (filter sterilised) and Na<sub>2</sub>S<sub>2</sub>O<sub>3</sub> x 5 H<sub>2</sub>O from sterile stock solutions.

Sterilize at 121 C for 15 min.

### **B 266. ACIDOSOMA MEDIUM**

KH<sub>2</sub>PO<sub>4</sub> 0.1 g  
(NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub> 0.25 g  
CaCl<sub>2</sub> x 2 H<sub>2</sub>O 0.05 g  
MgSO<sub>4</sub> x 7 H<sub>2</sub>O 0.02 g  
Trace elements (see below) 1.00 ml  
Yeast extract 0.10 g

Na gluconate 0.50 g  
Distilled water 1000.00 ml  
Adjust to pH 5.0 – 5.5. Agar may be added at 15 g/l for solid media.

*Trace elements:*

EDTA 5.00 g  
CuCl<sub>2</sub> x 5 H<sub>2</sub>O 0.10 g  
FeSO<sub>4</sub> x 7 H<sub>2</sub>O 2.00 g  
ZnSO<sub>4</sub> x 7 H<sub>2</sub>O 0.10 g  
NiCl<sub>2</sub> x 6 H<sub>2</sub>O 0.02 g  
CoCl<sub>2</sub> x 6 H<sub>2</sub>O 0.20 g  
Na<sub>2</sub>MoO<sub>4</sub> 0.03 g  
Distilled water 1000.00 ml

Autoclave the trace base medium and element solution separately at 121 C for 15 min

**B 267. GRANULICELLA MEDIUM**

Glucose 0.50 g  
Yeast extract 0.10 g  
Casamino acids 0.10 g  
MgSO<sub>4</sub> x 7 H<sub>2</sub>O 0.04 g  
CaCl<sub>2</sub> x 2 H<sub>2</sub>O 0.02 g  
Distilled water 1000.00 ml  
The medium may be solidified with 15 g/l agar.  
Final pH 4.5 – 5.2.  
Sterilize at 121 C for 15 min.

**B 268. ZAVARZINELLA FORMOSA MEDIUM**

KH<sub>2</sub>PO<sub>4</sub> 0.1 g  
MgSO<sub>4</sub> x 7 H<sub>2</sub>O 0.1 g  
CaCl<sub>2</sub> x 6 H<sub>2</sub>O 0.05 g  
NaCl 0.01 g  
N-acetylglucosamine 1.0 g  
Glucose 0.5 g  
Peptone 0.1 g  
Yeast extract 0.1 g  
Casamino acids 0.1 g  
“Metals 44” 1.0 ml  
Agar (for solid media) 15.0 g  
Distilled water 1000.0 ml  
*Metal solution “44”:*  
Na-EDTA – 250.0 mg  
ZnSO<sub>4</sub> x 7 H<sub>2</sub>O 1095.0 mg  
FeSO<sub>4</sub> x 7 H<sub>2</sub>O 500.0 mg  
MnSO<sub>4</sub> x 7 H<sub>2</sub>O 154.0 mg  
CuSO<sub>4</sub> x 5 H<sub>2</sub>O 39.0 mg  
Co(NO<sub>3</sub>)<sub>2</sub> x 6 H<sub>2</sub>O 24.8 mg  
Na<sub>2</sub>B<sub>4</sub>O<sub>7</sub> x 10 H<sub>2</sub>O 17.7 mg  
Distilled water 1000.0 ml  
Dissolve Na-EDTA and add a few drops of concentrated H<sub>2</sub>SO<sub>4</sub> to retard precipitation of heavy metal ions.  
Final pH 5.8 – 6.0  
Sterilize at 121 C for 15 min.

**B 269. THIOTHRIX MEDIUM**

NH<sub>4</sub>Cl 0.20 g  
K<sub>2</sub>HPO<sub>4</sub> 0.01 g  
MgSO<sub>4</sub> x 7 H<sub>2</sub>O 0.01 g  
CaSO<sub>4</sub> (saturated solution) 20.0 ml  
Trace element solution (see below) 5.0 ml  
Na-acetate 0.10 g  
Agar (if necessary) 12.0 g  
Na<sub>2</sub>S x 9 H<sub>2</sub>O 10% (w/v) solution 3.0 ml  
Distilled water 1000.0 ml

*Trace element solution:*

EDTA 0.200 g  
FeSO<sub>4</sub> x 7 H<sub>2</sub>O 0.7 g  
ZnSO<sub>4</sub> x 7 H<sub>2</sub>O 0.01 g  
MnSO<sub>4</sub> x 4 H<sub>2</sub>O 0.002 g  
CuSO<sub>4</sub> x 5 H<sub>2</sub>O 5.0 µg  
H<sub>3</sub>BO<sub>3</sub> 10.000 mg  
Co(NO<sub>3</sub>)<sub>2</sub> 1.000 mg  
Na<sub>2</sub>MoO<sub>4</sub> x 2 H<sub>2</sub>O 1.0 mg  
Distilled water 1000.0 ml

Adjust pH to 7.5 before autoclaving.

Basal medium and Na<sub>2</sub>S x 9 H<sub>2</sub>O solution sterilize separately at 121 C 15 min.

#### **B 270. TRYPTICASE SOY BROTH AGAR**

Trypticase Soy Broth (BBL 11768, Oxoid CM129 or Merck 5459) 30.0 g or  
Pancreatic digest of casein 18.0 g  
Papaic digest of soyabean 6.0 g  
NaCl 6.0 g  
Agar 15.0 g  
Distilled water 1000.0 ml  
pH 7.3  
Autoclave at 121°C for 15 min.

#### **B 271. MARINE SPIROCHETE MEDIUM**

Tryptone 2.0 g  
Yeast extract 1.0 g  
Na-Thioglycolate 1.0 g  
Resazurin 0.5 mg  
Charcoal-filtered, natural seawater 800.0 ml  
Distilled water 200.0 ml  
Dissolve ingredients (except thioglycolate), boil medium for 3 min., then cool to room temperature under N<sub>2</sub> gas atmosphere. Add thioglycolate and adjust pH of medium to 7.5 with 10 N KOH. Dispense under N<sub>2</sub> gas atmosphere in culture vessels and autoclave at 121°C for 15 min.  
Prepare 10% cellobiose solution (10.0 g in 100 ml distilled water) under nitrogen atmosphere, filter-sterilize and add 0.2 ml to 10 ml autoclaved medium.

#### **B 272. ECTOTHIORHODOSPIRA MAGNA MEDIUM**

KH<sub>2</sub>PO<sub>4</sub> 0.5 g  
NaCl 30.0 g  
NH<sub>4</sub>Cl 0.5 g  
MgCl<sub>2</sub> x 6 H<sub>2</sub>O 0.2 g  
Na-acetate 1.0 g  
Na<sub>2</sub>S<sub>2</sub>O<sub>3</sub> 0.5 g  
Na<sub>2</sub>S x 9 H<sub>2</sub>O 0.5 g  
NaHCO<sub>3</sub> 5.0 g

Na<sub>2</sub>CO<sub>3</sub> 5.0 g  
Yeast extract 0.1 g  
Vitamin B<sub>12</sub> 10.0 µg  
Trace elements solution (see below) 1.0 ml  
Distilled water 1000.0 ml  
pH 9.0-9.5

Prepare the medium without the NaHCO<sub>3</sub>, Na<sub>2</sub>S x 9 H<sub>2</sub>O, Na<sub>2</sub>CO<sub>3</sub> and vitamin B<sub>12</sub>, under a nitrogen atmosphere.

Autoclave at 121°C for 15 min and add the vitamin B<sub>12</sub> from a filter-sterilised stock solution and the NaHCO<sub>3</sub> (autoclaved in sealed, half full vessels), Na<sub>2</sub>S x 9 H<sub>2</sub>O, Na<sub>2</sub>CO<sub>3</sub> from sterilized at 121°C for 15 min stock solutions. The final pH of the medium should be 9.0-9.5.

*Trace elements solution*

EDTA 5.0 g  
FeSO<sub>4</sub> x 6 H<sub>2</sub>O 2.2 g  
ZnSO<sub>4</sub> x 7 H<sub>2</sub>O 0.1 g  
MnCl<sub>2</sub> x 4 H<sub>2</sub>O 0.03 g  
H<sub>3</sub>BO 0.03 g  
CoCl<sub>2</sub> x 6 H<sub>2</sub>O 0.2 g  
CuCl<sub>2</sub> x 2 H<sub>2</sub>O 0.03 g  
NiCl<sub>2</sub> x 6H<sub>2</sub>O 0.03 g  
Na<sub>2</sub>MoO<sub>4</sub> x 2 H<sub>2</sub>O 0.03 g  
Distilled water 1000.0 ml  
pH 3.0-4.0

**B 273. LAMPROBACTER MODESTOHALOPHILUS MEDIUM**

KH<sub>2</sub>PO<sub>4</sub> 0.5 g  
NaCl 40.0 g  
NH<sub>4</sub>Cl 0.5 g  
MgCl<sub>2</sub> x 6 H<sub>2</sub>O 0.2 g  
Na-acetate 0.5 g  
Na<sub>2</sub>S<sub>2</sub>O<sub>3</sub> 0.5 g  
Na<sub>2</sub>S x 9 H<sub>2</sub>O 0.5 g  
NaHCO<sub>3</sub> 1.5 g  
Yeast extract 0.1 g  
Vitamin B<sub>12</sub> 20.0 µg  
Trace elements solution (see below) 1.0 ml  
Distilled water 1000.0 ml  
pH 7.5

Prepare the medium without the NaHCO<sub>3</sub>, Na<sub>2</sub>S x 9 H<sub>2</sub>O, Na<sub>2</sub>CO<sub>3</sub> and vitamin B<sub>12</sub>, under a nitrogen atmosphere.

Autoclave at 121°C for 15 min and add the vitamin B<sub>12</sub> from a filter-sterilised stock solution and the NaHCO<sub>3</sub> (autoclaved in sealed, half full vessels), Na<sub>2</sub>S x 9 H<sub>2</sub>O, Na<sub>2</sub>CO<sub>3</sub> from sterilized at 121°C for 15 min stock solutions. The final pH of the medium should be 9.0-9.5.

*Trace elements solution*

EDTA 5.0 g  
FeSO<sub>4</sub> x 6 H<sub>2</sub>O 2.2 g  
ZnSO<sub>4</sub> x 7 H<sub>2</sub>O 0.1 g  
MnCl<sub>2</sub> x 4 H<sub>2</sub>O 0.03 g  
H<sub>3</sub>BO 0.03 g  
CoCl<sub>2</sub> x 6 H<sub>2</sub>O 0.2 g  
CuCl<sub>2</sub> x 2 H<sub>2</sub>O 0.03 g  
NiCl<sub>2</sub> x 6H<sub>2</sub>O 0.03 g  
Na<sub>2</sub>MoO<sub>4</sub> x 2 H<sub>2</sub>O 0.03 g  
Distilled water 1000.0 ml

pH 3.0-4.0

### **B 274. GRANULICELLA PALUDICOLA MEDIUM**

Fructose 0.5 g

Yeast extract 0.05 g

Casamino acids 0.05 g

MgSO<sub>4</sub> x 7 H<sub>2</sub>O 0.04 g

CaCl<sub>2</sub> x 2 H<sub>2</sub>O 0.02 g

KH<sub>2</sub>PO<sub>4</sub> 0.1 g

(NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub> 0.1 g

Distilled water 1000.0 ml

The medium may be solidified with 15 g/l agar. Adjust to pH 4.0 – 5.0 with alginic acid.

Autoclave at 121°C for 15 min.

### **B 275. TELMATOCOLA MEDIUM**

#### **Mineral Salts Solution:**

KH<sub>2</sub>PO<sub>4</sub> 0.1 g

(NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub> 0.1 g

MgSO<sub>4</sub> x 7 H<sub>2</sub>O 0.1 g

CaCl<sub>2</sub> x 2 H<sub>2</sub>O 0.02 g

Trace Elements Solution 44 1.0 ml

Staley's Vitamin Solution 1.0 ml

Yeast Extract 0.1 g

Glucose (1% v/v) 10.0 ml

Distilled Water 1000.0 ml

#### **Trace Elements Solution 44:**

##### **Hutner's salts:**

Nitrilotriacetic acid (NTA) 10.0 g

MgSO<sub>4</sub> x 7 H<sub>2</sub>O 29.7 g

CaCl<sub>2</sub> x 2 H<sub>2</sub>O 3.335 g

(NH<sub>4</sub>)<sub>6</sub>MoO<sub>7</sub>O<sub>24</sub> x 4 H<sub>2</sub>O 9.25 mg

FeSO<sub>4</sub> x 7 H<sub>2</sub>O 99.0 mg

"Metals 44" 50.0 ml

Distilled water 950.0 ml

Dissolve the nitrilotriacetic acid, adjust the pH to 7.0 with KOH (about 7.3 g). Dissolve other salts separately, combine and adjust the pH to 6.8.

##### **"Metals 44":**

Na-EDTA 250.0 mg

ZnSO<sub>4</sub> x 7 H<sub>2</sub>O 1095.0 mg

FeSO<sub>4</sub> x 7 H<sub>2</sub>O 500.0 mg

MnSO<sub>4</sub> x H<sub>2</sub>O 154.0 mg

CuSO<sub>4</sub> x 5 H<sub>2</sub>O 39.2 mg

Co(NO<sub>3</sub>)<sub>2</sub> x 6 H<sub>2</sub>O 24.8 mg

Na<sub>2</sub>B<sub>4</sub>O<sub>7</sub> x 10 H<sub>2</sub>O 17.7 mg

Distilled water 1000.0 ml

Dissolve the EDTA and add a few drops of concentrated H<sub>2</sub>SO<sub>4</sub> to retard precipitation of the heavy metal ions.

##### **Staley's Vitamin Solution:**

Vitamin B<sub>12</sub> 0.2 mg

Biotin 4.0 mg

Thiamine-HCl x 2 H<sub>2</sub>O 10.0 mg

Ca-pantothenate 10.0 mg

Folic acid 1.0 mg

Riboflavin 10.0 mg  
Nicotinamide 10.0  
p-Aminobenzoic acid 10.0 mg  
Pyridoxine hydrochloride 10.0 mg  
Distilled water 1000.0 ml

Prepare the liquid medium. Adjust to pH 4.8 - 5.5. Dispense the medium into serum bottles under gassing with CO<sub>2</sub> (5% v/v). Sterilize by autoclaving at 121°C for 15 min. Before inoculation add from filter-sterilised Staley's Vitamin Solution and autoclaving at 121°C for 15 min glucose.

### **B 276. PROTEINIVORAX MEDIUM**

KH<sub>2</sub>PO<sub>4</sub> 0.30 g  
MgSO<sub>4</sub> x 7 H<sub>2</sub>O 0.12 g  
CaCl<sub>2</sub> x 2 H<sub>2</sub>O 0.03 g  
NaCl 20.0 g  
Tryptone 3.0 g  
Trace element solution SL-10 1.0 ml  
Na<sub>2</sub>CO<sub>3</sub> 60.0 g  
NaHCO<sub>3</sub> 50.0 g  
Na-Thioglycolate 1.0 g  
Distilled water 1000.0 ml

Dissolve ingredients (except carbonates and thioglycolate), boil medium for 1 min., then cool to room temperature under N<sub>2</sub> gas atmosphere. Add carbonates and adjust pH to 8.5 – 9.0. Dispense under same gas atmosphere in culture vessels and autoclave. After autoclaving at 121°C for 15 min add thioglycolate from a sterile anoxic stock solution sterilized by filtration.

*Trace element solution SL-10:*

HCl (25%; 7.7 M) 10.0 ml  
FeCl<sub>3</sub> x 4 H<sub>2</sub>O 1.5 g  
ZnCl<sub>2</sub> 70.0 mg  
MnCl<sub>2</sub> x 4 H<sub>2</sub>O 100.0 mg  
H<sub>3</sub>BO<sub>3</sub> 6.0 mg  
CoCl<sub>2</sub> x 6 H<sub>2</sub>O 190.0 mg  
CuCl<sub>2</sub> x 2 H<sub>2</sub>O 2.0 mg  
NiCl<sub>2</sub> x 6 H<sub>2</sub>O 24.0 mg  
Na<sub>2</sub>MoO<sub>4</sub> x 2 H<sub>2</sub>O 36.0 mg  
Distilled water 990.0 ml

*The trace element solution preparation:* FeCl<sub>3</sub> x 4 H<sub>2</sub>O is dissolved firstly in HCl, and then is mixed with water and other salts are dissolved in the sequence indicated.

### **B-277. MS1 MEDIUM**

NH<sub>4</sub>Cl 2.0 g  
MgSO<sub>4</sub> x 7 H<sub>2</sub>O 0.2 g  
K<sub>2</sub>SO<sub>4</sub> 0.5 g  
FeSO<sub>4</sub> x 7 H<sub>2</sub>O 2.5 mg  
CaCl<sub>2</sub> x 2 H<sub>2</sub>O 10.0 mg  
CuSO<sub>4</sub> x 5 H<sub>2</sub>O 2.0 mg  
H<sub>3</sub>BO<sub>3</sub> 0.06 mg  
ZnCl<sub>2</sub> 20.0 mg  
MnSO<sub>4</sub> x H<sub>2</sub>O 1.0 mg  
NiCl<sub>2</sub> x 6 H<sub>2</sub>O 0.05 mg  
Na<sub>2</sub>MoO<sub>4</sub> x 2 H<sub>2</sub>O 0.3 mg  
Na-glutamate 10.0 g  
Distilled water 1000.0 ml  
pH 7.0-7.5  
Sterilize at 121 C for 15 min.

### **B 278. MODIFIED S MEDIUM**

$\text{KH}_2\text{PO}_4$  0.14 g

$\text{MgSO}_4 \times 7 \text{H}_2\text{O}$  0.4 g

$\text{CaCl}_2 \times 2 \text{H}_2\text{O}$  0.1 g

$\text{NH}_4\text{Cl}$  0.25 g

$\text{KCl}$  0.36 g

$\text{NaCl}$  6.0

Yeast extract 0.1 g

Autoclave at 121 C 15 min. Add filter-sterilization vitamine solution.

*Trace element solution according to Kevbrin and Zavarzin, 1992*

$(\text{NH}_4)_2\text{SO}_4\text{FeSO}_4 \times 6\text{H}_2\text{O}$  (Mohr's salt) 784.0 mg

$\text{CoCl}_2 \times \text{H}_2\text{O}$  238.0 mg

$(\text{NH}_4)_2\text{SO}_4\text{NiSO}_4 \times 6\text{H}_2\text{O}$  395.0 mg

$\text{Na}_2\text{MoO}_4 \times \text{H}_2\text{O}$  24.0 mg

$\text{Na}_2\text{WO}_4 \times 2\text{H}_2\text{O}$  33.0 mg

$\text{ZnSO}_4 \times 7\text{H}_2\text{O}$  144.0 mg

$\text{CuCl}_2 \times 2\text{H}_2\text{O}$  2.0 mg

$\text{Na}_2\text{SeO}_4$  94.0 mg

$\text{HBO}_3$  6.0 mg

$\text{MnCl}_2 \times 4\text{H}_2\text{O}$  99.0 mg

Mohr's salt is dissolved firstly in concentrated HCl, and then is mixed with water and other salts are dissolved in the sequence indicated.

*Vitamin solution according to Wolin et al., 1963:*

Biotin 20.0 mg

Folic acid 20.0 mg

Pyridoxine ( $\text{B}_2$ ) 0.1 mg

Riboflavin ( $\text{B}_1$ ) 50.0 mg

Pantotenoic acid 50.0 mg

p-Aminobenzoic acid 50.0 mg

Thiamine-HCl 50.0 mg

Nicotinic acid 50.0 mg

Cyanocobalamin ( $\text{B}_{12}$ ) 1.0 mg

Lipoic (tioctoic) acid 50.0 mg

Distilled water 1000.0 ml

### **B 279. M3 MEDIUM**

$\text{KH}_2\text{PO}_4$  0.1 g

$\text{NH}_4\text{Cl}$  0.2 g

$\text{MgSO}_4 \times 7\text{H}_2\text{O}$  0.1 g

$\text{CaCl}_2 \times 2\text{H}_2\text{O}$  0.02 g

Yeast extract 0.1 g

Glucose (or malate) 0.5 g

*Trace element solution 'SLA'* 1 ml

Distilled water 1000 ml

pH 5.8-6.0

Sterilize basal medium and trace element solution separately at 121 C 15 min.

*Trace element solution SLA:*

$\text{FeCl}_2 \times 4 \text{H}_2\text{O}$  1.8 mg

$\text{CoCl}_2 \times 6 \text{H}_2\text{O}$  250 mg

$\text{NiCl}_2 \times 6 \text{H}_2\text{O}$  10 mg

$\text{CuCl}_2 \times 2 \text{H}_2\text{O}$  10 mg

$\text{MnCl}_2 \times 4 \text{H}_2\text{O}$  70 mg

$\text{ZnCl}_2$  100 mg

$\text{H}_3\text{BO}_3$  500 mg



Na<sub>2</sub>MoO<sub>4</sub> x 2 H<sub>2</sub>O 30 mg  
Na<sub>2</sub>SeO<sub>3</sub> x 5 H<sub>2</sub>O 10 mg  
Distilled water 1000 ml

### **B 279. PALUDIBACULUM FERMENTANS MEDIUM**

KH<sub>2</sub>PO<sub>4</sub> 0.1 g  
(NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub> 0.1 g  
MgSO<sub>4</sub> × 7H<sub>2</sub>O 0.05 g  
CaCl<sub>2</sub> × 2H<sub>2</sub>O 0.01 g  
Yeast extract 0.1 g  
Glucose 0.5 g  
Distilled water 1000 ml  
pH 5.5-6.0  
Autoclave at 121 C 15 min.

### **B 280. PLANCTOMYCES MEDIUM**

KH<sub>2</sub>PO<sub>4</sub> 0.1 g  
(NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub> 0.2 g  
MgSO<sub>4</sub> x 7 H<sub>2</sub>O 0.1 g  
CaCl<sub>2</sub> x 6 H<sub>2</sub>O 0.02 g  
NaCl 0.01 g  
N-acetylglucosamine 1.0 g  
Glucose 0.5 g  
Yeast extract 0.2 g  
Hutners basal salts (see below) 1.0 ml  
Staley's vitamin solution (see below) 1.0 ml  
Agar (for solid media) 18.0 g  
Distilled water 1000.0 ml  
Adjust pH to 5.8-6.5  
Autoclave at 121 C 15 min. Add filter-sterilization vitamine solution.

#### *Hutners basal salts*

Nitilotriacetic acid (NTA) 10.0 g  
MgSO<sub>4</sub> x 7 H<sub>2</sub>O 29.7 g  
CaCl<sub>2</sub> x 2 H<sub>2</sub>O 3.34 g  
Na<sub>2</sub>MoO<sub>4</sub> x 2H<sub>2</sub>O 12.67 mg  
FeSO<sub>4</sub> x 7 H<sub>2</sub>O 99.0 mg  
Metal salt solution "44" (see below) 50 ml  
Dissolve NTA first by neutralizing with KOH, then add other salts.  
pH 7.2 (ajust with KOH or H<sub>2</sub>SO<sub>4</sub>).  
Ajust volume to 1000.0 ml with distelled water.

#### *Metal solution "44":*

Na-EDTA – 250.0 mg  
ZnSO<sub>4</sub> x 7 H<sub>2</sub>O 1095.0 mg  
FeSO<sub>4</sub> x 7 H<sub>2</sub>O 500.0 mg  
MnSO<sub>4</sub> x 7 H<sub>2</sub>O 154.0 mg  
CuSO<sub>4</sub> x 5 H<sub>2</sub>O 39.0 mg  
Co(NO<sub>3</sub>)<sub>2</sub> x 6 H<sub>2</sub>O 24.8 mg  
Na<sub>2</sub>B<sub>4</sub>O<sub>7</sub> x 10 H<sub>2</sub>O 17.7 mg  
Distilled water 1000.0 ml  
Dissolve Na-EDTA and add a few drops of concentrated H<sub>2</sub>SO<sub>4</sub> to retard precipitation of heavy metal ions.

#### *Vitamin solution (double conc.):*

Biotin 4.0 mg  
Folic acid 4.0 mg

Pyridoxine-HCl 20.0 mg  
Riboflavine 10.0 mg  
Thiamine-HCl x 2 H<sub>2</sub>O 10.0 mg  
Nicotinamide 10.0 mg  
D-Ca-pantothenate 10.0 mg  
Vitamin B<sub>12</sub> 0.2 mg  
p-Aminobenzoic acid 10.0 mg  
Distilled water 1000.0 ml  
Store in the dark and cold (5°C).

#### **B 281. AZOSPIRILLUM AMAZONENSE MEDIUM**

K<sub>2</sub>HPO<sub>4</sub> 0.2 g  
KH<sub>2</sub>PO<sub>4</sub> 0.6 g  
CaCl<sub>2</sub> x 2 H<sub>2</sub>O 0.02 g  
MgSO<sub>4</sub> x 7 H<sub>2</sub>O 0.2 g  
Na<sub>2</sub>MoO<sub>4</sub> x 2 H<sub>2</sub>O 0.002 g  
FeCl<sub>3</sub> 0.01 g  
Bromothymol blue (0.5% in 0.2N KOH) 5.0 ml  
Sucrose 5.0 g  
Distilled water 1000.0 ml  
Adjust pH to 6.0  
For semisolid medium, add 0.5 g of agar; for solid medium, add 15 g of agar.  
Autoclave at 105°C for 30 min.

#### **B 282. AZOSPIRILLUM HALOPRAEFERENS MEDIUM**

Beef extract 1.0 g  
Yeast extract 2.0 g  
Peptone 5.0 g  
NaCl 5.0 g  
Agar 15.0 g  
Distilled water 1000.0 ml  
pH 7.4  
Autoclave at 121 C 15 min.

#### **B 283. Azospirillum VM medium**

Döbereiner basic 10 ml  
Fe<sub>3</sub> EDTA 0.66% (w/v) 10.0 ml  
NH<sub>4</sub>Cl 0.5 g  
NaCl 1.0 g  
Yeast extract 1.0 g  
Peptone 3.0 g  
Phosphate buffer pH 6.8 3.0 ml  
Agar 15.0 g  
Distilled water 1000.0 ml  
Döbereiner basic:  
MgSO<sub>4</sub> 20.0 g  
NaCl 10.0 g  
CaCl<sub>2</sub> 2.64 g  
Na<sub>2</sub>MoO<sub>4</sub> x 2H<sub>2</sub>O 0.2 g  
MnSO<sub>4</sub> x 2H<sub>2</sub>O 1.0 g  
Distilled water 1000.0 ml  
Phosphate buffer pH 6.8:  
KH<sub>2</sub>PO<sub>4</sub> 0.6 g  
K<sub>2</sub>HPO<sub>4</sub> 0.4 g

Distilled water 3 ml

Autoclave basal medium, Fe<sub>3</sub> EDTA solution, Döbereiner basic and phosphate buffer separately at 121 C 15 min.