

## 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 alkalized, 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.

### 1. ALLOMONAS ENTERICA MEDIUM

Peptone 10.0 g  
NaCl 20.0 g  
Meat extract 5.0 g  
or yeast extract 3.0 g  
Distilled water 1000.0 ml

### 2. YEAST WATER

Pressed yeast 200.0 g  
Tap water 1000.0 ml  
Twice filter hot through a paper filter or centrifuge. Sterilize at 121°C for 15 min.

### 3. POTATO AGAR

Potato 200.0 g  
Agar 20.0 g  
Tap water 1000.0 ml  
Boil potatoes for 1 h, filter cold through a cotton wool-gauze filter. Sterilize at 121°C for 30 min.

### 4. WORT AGAR

Wort extract (malt extract) 20.0 g  
Agar 20.0 g  
Water 1000.0 ml  
Sterilize at 121°C for 30 min.

### 5. PEPTONE MEAT AGAR

Peptone 10.0 g  
NaCl 5.0 g

Agar 20.0 g

Meat water 1000.0 ml

*Preparation of meat water:* comminute 500 g of meat free of bones, fat and tendons, add 1000.0 ml of tap water and leave for 12 h at room temperature or in a thermostat at 30°C, or for 2 h at 37°C. Then squeeze the meat through gauze or cloth and boil the filtrate for 5 min. The proteins are denatured. Filter the cooled down mass through a cotton-wool filter and add water to the initial volume. Sterilize at 121°C for 30 min.

## **6. PEPTONE MEAT BROTH**

Peptone 10.0 g

NaCl 5.0 g

Meat water 1000.0 ml

pH 7.2 - 7.4. Sterilize at 121°C for 30 min.

*Preparation of meat water:* comminute 500 g of meat free of bones, fat and tendons, add 1000.0 ml of tap water and leave for 12 h at room temperature or in a thermostat at 30°C, or for 2 h at 37°C. Then squeeze the meat through gauze or cloth and boil the filtrate for 5 min. The proteins are denatured. Filter the cooled down mass through a cotton-wool filter and add water to the initial volume. Sterilize at 121°C for 30 min.

## **7. OATMEAL AGAR**

Oats 30.0 g

Agar 15.0 g

Tap water 1000.0 ml

Keep oats on a water bath at 58°C for 1 h, filter through 2 layers of gauze, dilute to 1000.0 ml and add 15.0 g agar. Sterilize at 121°C for 30 min.

## **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 30 min.

## **9. WORT AGAR 7 B**

Malt wort 7 B 1000.0 ml

Agar 20.0 g

Sterilize at 105°C for 30 min.

*Preparation of malt wort:* mix 250 g of ground malt in 1000.0 ml of tap water, heat to 55-60°C and keep at this temperature for 1.5-2 h at periodic stirring. Then increase the temperature to 80°C and keep for 10 min. Then cool the wort, squeeze through a linen bag, adjust the concentration of sugars to 7 B.

## **10. WORT AGAR 2-3 B**

Malt wort 2-3 B 1000.0 ml

Agar 20.0 g

Sterilize at 105°C for 30 min.

*Preparation of malt wort:* mix 250 g of ground malt in 1000.0 ml of tap water, heat to 55-60°C and keep at this temperature for 1.5-2 h at periodic stirring. Then increase the temperature to 80°C and keep for 10 min. Then cool the wort, squeeze through a linen bag, adjust the concentration of sugars to 2-3 B.

### **11. WORT AGAR 3.5 B (MALT AGAR)**

Malt wort 3.5 B 1000.0 ml

Agar 20.0 g

Sterilize at 105°C for 30 min.

*Preparation of malt wort:* mix 250 g of ground malt in 1000.0 ml of tap water, heat to 55-60°C and keep at this temperature for 1.5-2 h at periodic stirring. Then increase the temperature to 80°C and keep for 10 min. Then cool the wort, squeeze through a linen bag, adjust the concentration of sugars to 3.5B.

### **12. CZAPEK DOX MEDIUM**

NaNO<sub>2</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.

### **13. POTATO-GLUCOSE AGAR 1**

Grated potato 200.0 g

Glucose 20.0 g

Agar 20.0 g

Tap water 1000.0 ml

Boil potatoes for 1 h in 1000.0 ml of water, filter through gauze, add water to the initial volume, add glucose and agar. Sterilize at 105°C for 30 min.

### **14. POTATO-CARROT AGAR**

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, add water to the initial volume, adjust pH to 7.0 - 7.1 and add agar. Sterilize at 121°C for 30 min.

### **15. LB MEDIUM**

Tryptone 10.0 g

Yeast extract 5.0 g

NaCl 10.0 g

Tap water 1000.0 ml

### **16. YT MEDIUM**

Tryptone 8.0 g

Yeast extract 5.0 g

NaCl 5.0 g

Tap water 1000.0 ml

### **17. GLUCOSE PEPTONE AGAR WITH YEAST WATER**

Glucose 40.0 g

Peptone 5.0 g

Agar 20.0 g

10% yeast water 1000.0 ml

Sterilize by steam.

*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.

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

Glucose 40.0 g

Peptone 5.0 g

NaCl 50.0 g

Agar 20.0 g

10% yeast water 1000.0 ml

Sterilize by steam.

*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.

### **19. WORT AGAR 7 B WITH 5% NaCl**

Malt wort 7 B 1000.0 ml

NaCl 50.0 g

Agar 20.0 g

Sterilize at 105°C for 30 min.

*Preparation of malt wort:* mix 250 g of ground malt in 1000.0 ml of tap water, heat to 55-60°C and keep at this temperature for 1.5-2 h at periodic stirring. Then increase the temperature to 80°C and keep for 10 min. Then cool the wort, squeeze through a linen bag, adjust the concentration of sugars to 7 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.

### **22. MALT AGAR WITH 60% SUCROSE**

Malt wort 3.5 B 1000.0 ml

Sucrose 600.0 g

Agar 20.0 g

Sterilize at 121°C for 20 min.

*Preparation of malt wort:* mix 250 g of ground malt in 1000.0 ml of tap water, heat to 55-60°C and keep at this temperature for 1.5-2 h at periodic stirring. Then increase the temperature to 80°C and keep for 10 min. Then cool the wort, squeeze through a linen bag, adjust the concentration of sugars to 3.5B.

### **23. MALT AGAR WITH 40% SUCROSE**

Malt wort 3.5 B 1000.0 ml

Sucrose 400.0 g

Agar 20.0 g

Sterilize at 121°C for 20 min.

*Preparation of malt wort:* mix 250 g of ground malt in 1000.0 ml of tap water, heat to 55-60°C and keep at this temperature for 1.5-2 h at periodic stirring. Then increase the temperature to 80°C and keep for 10 min. Then cool the wort, squeeze through a linen bag, adjust the concentration of sugars to

3.5B.

**24. MALT AGAR WITH 20% SUCROSE**

Malt wort 3.5 B 1000.0 ml

Sucrose 200.0 g

Agar 20.0 g

Sterilize at 121°C for 20 min.

*Preparation of malt wort:* mix 250 g of ground malt in 1000.0 ml of tap water, heat to 55-60°C and keep at this temperature for 1.5-2 h at periodic stirring. Then increase the temperature to 80°C and keep for 10 min. Then cool the wort, squeeze through a linen bag, adjust the concentration of sugars to 3.5B.

**25. MALT AGAR WITH FILTER PAPER**

Malt wort 3.5 B 1000.0 ml

Agar 20.0 g

Sterilize at 105°C for 30 min.

*Preparation of malt wort:* mix 250 g of ground malt in 1000.0 ml of tap water, heat to 55-60°C and keep at this temperature for 1.5-2 h at periodic stirring. Then increase the temperature to 80°C and keep for 10 min. Then cool the wort, squeeze through a linen bag, adjust the concentration of sugars to 3.5B.

Sterilize filter paper strips with dry heat and soak it with sterile medium.

**26. MANURE AGAR**

Horse manure 100-125 g

Agar 25.0 g

Distilled water 1000.0 ml

Boil manure in 1000.0 ml of water for 10 min, then keep for 16-20 h, 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 105°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 105°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 30 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 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.

### **32. CURD DECOCTION**

Pour 9.0 l 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.

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

Peptone 10.0 g

NaCl 5.0 g

Urea 2.0 g

Agar 20.0 g

Meat water 1000.0 ml

Sterilize urea at 105°C for 30 min to 1 h.

*Preparation of meat water:* comminute 500 g of meat free of bones, fat and tendons, add 1000.0 ml of tap water and leave for 12 h at room temperature or in a thermostat at 30°C, or for 2 h at 37°C. Then squeeze the meat through gauze or cloth and boil the filtrate for 5 min. The proteins are denatured. Filter the cooled down mass through a cotton-wool filter and add water to the initial volume. Sterilize at 121°C for 30 min.

### **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 h 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

### **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.

### **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  
Sterilize at 121°C for 30 min.  
Sterilize filter paper strips by dry heat and soak with sterile medium.

### **38. WORT AGAR WITH 12% NaCl**

Malt wort 7 B 1000.0 ml  
NaCl 120.0 g  
Agar 20.0 g  
Sterilize at 105°C for 30 min.

*Preparation of malt wort:* mix 250 g of ground malt in 1000.0 ml of tap water, heat to 55-60°C and keep at this temperature for 1.5-2 h at periodic stirring. Then increase the temperature to 80°C and keep for 10 min. Then cool the wort, squeeze through a linen bag, adjust the concentration of sugars to 7 B.

### **39. WORT AGAR WITH 1% NaCl**

Malt wort 7 B 1000.0 ml

NaCl 10.0 g

Agar 20.0 g

Sterilize at 105°C for 30 min.

*Preparation of malt wort:* mix 250 g of ground malt in 1000.0 ml of tap water, heat to 55-60°C and keep at this temperature for 1.5-2 h at periodic stirring. Then increase the temperature to 80°C and keep for 10 min. Then cool the wort, squeeze through a linen bag, adjust the concentration of sugars to 7 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. Sterilization at 105°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.

### **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 1000.0 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

### **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.

#### **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

#### **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

#### **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:*

0.01 N HCl 100.0 ml

FeSO<sub>4</sub> x 7 H<sub>2</sub>O 4.98 g

Adjust pH of medium to 7.4 with 1N NaOH.

#### **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  
Adjust pH to 7.4 with NaOH.

#### **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.

#### **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 121°C for 15 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.

#### **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.

#### **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.

#### **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

### **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

### **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.

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

Peptone 10.0 g  
NaCl 5.0 g  
Urea 10.0 g  
Agar 20.0 g  
Meat water 1000.0 ml  
pH 8.0.

*Preparation of meat water:* comminute 500 g of meat free of bones, fat and tendons, add 1000.0 ml of tap water and leave for 12 h at room temperature or in a thermostat at 30°C, or for 2 h at 37°C. Then squeeze the meat through gauze or cloth and boil the filtrate for 5 min. The proteins are denatured. Filter the cooled down mass through a cotton-wool filter and add water to the initial volume. Sterilize at 121°C for 30 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 at 121°C for 15 min.

**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

**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 105°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.

**62. MANURE TINCTURE**

Cow manure (fresh) 1.0 kg  
Distilled water 3000.0 ml  
Boil, squeeze through gauze into a bottle and dilute to 3.0 l.

**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 9.0 l 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 3.0 l.

#### **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.

#### **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.

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

Peptone 5.0 g  
Meat extract 3.0 g  
Agar 15.0 g  
Soil extract 250.0 ml  
Tap water 750.0 ml  
pH 7.0.

*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 h 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**

Yeast autolysate 2.0 g  
Trace element solution (see below) 1.0 ml  
Agar 20.0 g  
Peptone meat broth (see below) 1000.0 ml

*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

*Peptone meat broth:*

Peptone 10.0 g  
NaCl 5.0 g  
Meat water 1000.0 ml  
pH 7.2 - 7.4. Sterilize at 121°C for 30 min.

*Preparation of meat water:* comminute 500 g of meat free of bones, fat and tendons, add 1000.0 ml of tap water and leave for 12 h at room temperature or in a thermostat at 30°C, or for 2 h at 37°C. Then

squeeze the meat through gauze or cloth and boil the filtrate for 5 min. The proteins are denatured. Filter the cooled down mass through a cotton-wool filter and add water to the initial volume. Sterilize at 121 °C for 30 min.

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

Peptone 10.0 g  
NaCl 5.0 g  
Sea salt 30.0 g  
Agar 20.0 g  
Meat water 1000.0 ml

*Preparation of meat water:* comminute 500 g of meat free of bones, fat and tendons, add 1000.0 ml of tap water and leave for 12 h at room temperature or in a thermostat at 30 °C, or for 2 h at 37 °C. Then squeeze the meat through gauze or cloth and boil the filtrate for 5 min. The proteins are denatured. Filter the cooled down mass through a cotton-wool filter and add water to the initial volume. Sterilize at 121 °C for 30 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 1000.0 ml

Solution 2:

Glucose 2.0 g  
Distilled water 20.0 ml  
pH 7.0.

#### **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  
Sterilize at 105 °C for 20 min. Vitamin B<sub>12</sub> sterilize separately with filtration. pH of the medium 7.2 (adjust with KOH before adding agar).

#### **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  
Adjust final pH to 7.5 - 7.8 with 1 N HCl.

## 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

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. Sterilize at 121 °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.

## 74. HOTTINGER BROTH

Boil meat (1-2 cm pieces) (without fat or tendons) in 2.0 l 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 alkalize 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 h 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.

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

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

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

Meat water 400.0 ml

Distilled water 600.0 ml

pH 6.0-6.5.

*Preparation of meat water:* comminute 500 g of meat free of bones, fat and tendons, add 1000.0 ml of tap water and leave for 12 h at room temperature or in a thermostat at 30°C, or for 2 h at 37°C. Then squeeze the meat through gauze or cloth and boil the filtrate for 5 min. The proteins are denatured. Filter the cooled down mass through a cotton-wool filter and add water to the initial volume. Sterilize at 121°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

*Preparation of potato decoction:* boil 200.0 g potatoes in 1.0 l of tap water for 1 h, filter cold through a cotton wool-gauze filter. Sterilize at 121°C for 30 min.

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

Peptone 10.0 g

NaCl 5.0 g

Agar 20.0 g

Yeast extract 1.0 g

Glucose 1.0 g

B<sub>12</sub> 2.0 mg

B<sub>1</sub> 2.0 mg

Meat water 1000.0 ml

Sterilize at 105°C for 30 min.

*Preparation of meat water:* comminute 500 g of meat free of bones, fat and tendons, add 1000.0 ml of tap water and leave for 12 h at room temperature or in a thermostat at 30°C, or for 2 h at 37°C. Then squeeze the meat through gauze or cloth and boil the filtrate for 5 min. The proteins are denatured. Filter the cooled down mass through a cotton-wool filter and add water to the initial volume. Sterilize at 121°C for 30 min.

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

Peptone 10.0 g

NaCl 5.0 g

Soluble starch 20.0 g

Agar 20.0 g

Meat water 1000.0 ml

*Preparation of meat water:* comminute 500 g of meat free of bones, fat and tendons, add 1000.0 ml of tap water and leave for 12 h at room temperature or in a thermostat at 30°C, or for 2 h at 37°C. Then squeeze the meat through gauze or cloth and boil the filtrate for 5 min. The proteins are denatured. Filter the cooled down mass through a cotton-wool filter and add water to the initial volume. Sterilize at 121°C for 30 min.

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

Peptone 10.0 g

NaCl 5.0 g

Soluble starch 10.0 g

Agar 20.0 g

Meat water 1000.0 ml

pH 7.2

*Preparation of meat water:* comminute 500 g of meat free of bones, fat and tendons, add 1000.0 ml of tap water and leave for 12 h at room temperature or in a thermostat at 30°C, or for 2 h at 37°C. Then squeeze the meat through gauze or cloth and boil the filtrate for 5 min. The proteins are denatured. Filter the cooled down mass through a cotton-wool filter and add water to the initial volume. Sterilize at 121°C for 30 min.

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

Peptone 10.0 g

NaCl 60.0 g

Agar 20.0 g

Meat water 1000.0 ml

*Preparation of meat water:* comminute 500 g of meat free of bones, fat and tendons, add 1000.0 ml of tap water and leave for 12 h at room temperature or in a thermostat at 30°C, or for 2 h at 37°C. Then squeeze the meat through gauze or cloth and boil the filtrate for 5 min. The proteins are denatured. Fil-

ter the cooled down mass through a cotton-wool filter and add water to the initial volume. Sterilize at 121°C for 30 min.

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

Peptone 10.0 g

NaCl 5.0 g

Sea salt 18.0 g

Agar 20.0 g

Meat water 1000.0 ml

*Preparation of meat water:* comminute 500 g of meat free of bones, fat and tendons, add 1000.0 ml of tap water and leave for 12 h at room temperature or in a thermostat at 30°C, or for 2 h at 37°C. Then squeeze the meat through gauze or cloth and boil the filtrate for 5 min. The proteins are denatured. Filter the cooled down mass through a cotton-wool filter and add water to the initial volume. Sterilize at 121°C for 30 min.

### **82. POTATO AGAR WITH 2% GLUCOSE**

Potato 200.0 g

Agar 20.0 g

Glucose 20.0 g

Tap water 1000.0 ml

Boil potatoes for 1 h, filter cold through a cotton wool-gauze filter. Add glucose.

Sterilize at 105°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

Water 1000.0 ml

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

Casein hydrolysate 10.0 g

Glucose 5.0 g

p-Aminobenzoic acid 5.0 µg

Agar 20.0 g

Water 1000.0 ml

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

Peptone 10.0 g

Yeast autolysate 10.0 g

Hottinger broth 10.0 ml

Phosphate solution (see below) 0.5 ml

Salt solution (see below) 0.5 ml

Glucose 5.0 g

Water 950.0 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

*Preparation of Hottinger broth:* boil meat (1 - 2 cm pieces) (without fat or tendons) in 2.0 l 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 alkalize 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 h 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.

#### **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  
Adjust pH to 7.5 with 0.1 M Na<sub>2</sub>CO<sub>3</sub>.

#### **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  
Adjust pH to 7.5 - 7.8 with 1 N HCl.

#### **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.

#### **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.

### **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 (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

Sterilize solutions 1 and 3 under nitrogen. Filter sterilize vitamin solution and add 5 ml of the solution to 1 l of basal medium. Solution 2 is not to be kept for long. pH of the medium 7.2.

### **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

$\text{Na}_2\text{HPO}_4 \times 12 \text{ H}_2\text{O}$  2.0 g

$\text{FeSO}_4 \times 7 \text{ H}_2\text{O}$  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:

$\text{Na}_2\text{S} \times 9 \text{ H}_2\text{O}$  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

$\text{FeCl}_3 \times 4 \text{ H}_2\text{O}$  0.2 g

$\text{MnCl}_2 \times 4 \text{ H}_2\text{O}$  0.1 g

$\text{CoCl}_2 \times 6 \text{ H}_2\text{O}$  0.17 g

$\text{CaCl}_2 \times 2 \text{ H}_2\text{O}$  0.1 g

$\text{ZnCl}_2$  0.1 g

$\text{CuCl}_2$  0.02 g

$\text{Na}_2\text{MoO}_4 \times 2 \text{ H}_2\text{O}$  0.01 g

$\text{NiCl}_2 \times 6 \text{ H}_2\text{O}$  0.026 g

$\text{NaCl}$  1.0 g

$\text{Na}_2\text{SeO}_4 \times 5 \text{ H}_2\text{O}$  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

Solutions 1, 3, 4 and 5 sterilize under N<sub>2</sub>. Solution 6 is filter sterilized. Before use, neutralize solution 5 by dropwise of 1 N HCl. pH of the medium 6.8 - 7.0.

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

Solution 1:

$\text{K}_2\text{HPO}_4$  0.5 g

$\text{NH}_4\text{Cl}$  1.0 g

$\text{CaCl}_2 \times 6 \text{ H}_2\text{O}$  0.1 g

$\text{MgSO}_4 \times 7 \text{ H}_2\text{O}$  2.0 g

$\text{Na}_2\text{SO}_4$  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

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. Sterilize in atmosphere of this gas mixture. Solution 3 sterilize in atmosphere of N<sub>2</sub>. pH of the medium 6.8.

### **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.

### **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 SL6 (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. 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 1000.0 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

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. Solution 2 is sterilized under 100% N<sub>2</sub>. Vitamin solution is filter sterilized. Final pH of the complete medium 7.0-7.2.

## **96. DESULFONEMA LIMICOLA MEDIUM**

Solution 1:

Na<sub>2</sub>SO<sub>4</sub> 3.0 g  
NaCl 13.0 g  
MgCl<sub>2</sub> x 6 H<sub>2</sub>O 2.2 g  
CaCl<sub>2</sub> x 2 H<sub>2</sub>O 0.15 g  
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> 5.0 g  
Distilled water 100.0 ml

Solution 4:

Na-acetate x 3 H<sub>2</sub>O 2.5 g  
Distilled water 100.0 ml

Solution 5:

Disodium succinate 0.1 g  
Distilled water 1.0 ml

Solution 6:

Vitamin solution (see below) 5.0 ml

Solution 7:

$\text{AlCl}_3 \times 6 \text{H}_2\text{O}$  245.0 mg

Distilled water 5.0 ml

Solution 8:

$\text{Na}_2\text{CO}_3$  170.0 mg

Distilled water 1.6 ml

Solution 9:

Rumen fluid, clarified 20.0 ml

Solution 10:

$\text{Na}_2\text{S} \times 9 \text{H}_2\text{O}$  0.4 mg

Distilled water 10.0 ml

*Trace element solution SL-10:*

HCl (25%; 7.7 M) 10.0 ml

$\text{FeCl}_3 \times 4 \text{H}_2\text{O}$  1.5 g

$\text{ZnCl}_2$  70.0 mg

$\text{MnCl}_2 \times 4 \text{H}_2\text{O}$  100.0 mg

$\text{H}_3\text{BO}_3$  6.0 mg

$\text{CoCl}_2 \times 6 \text{H}_2\text{O}$  190.0 mg

$\text{CuCl}_2 \times 2 \text{H}_2\text{O}$  2.0 mg

$\text{NiCl}_2 \times 6 \text{H}_2\text{O}$  24.0 mg

$\text{Na}_2\text{MoO}_4 \times 2 \text{H}_2\text{O}$  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

The trace element solution preparation:  $\text{FeCl}_3 \times 4 \text{H}_2\text{O}$  is dissolved firstly in HCl, then is mixed with water and other salts are dissolved in the sequence indicated. For the preparation and sterilization of the medium see medium 95. Solutions 7 and 8 are combined before sterilization. pH of the medium 7.6.

## **97. DESULFONEMA MAGNUM MEDIUM**

Solution 1:

$\text{Na}_2\text{SO}_4$  3.0 g

NaCl 21.0 g/l

$\text{MgCl}_2 \times 6 \text{H}_2\text{O}$  5.5 g/l

$\text{CaCl}_2 \times 2 \text{H}_2\text{O}$  1.35 g/l

KCl 0.5 g

$\text{KH}_2\text{PO}_4$  0.2 g

$\text{NH}_4\text{Cl}$  0.3 g

Solution 2:

Trace element solution (see below) 1.0 ml

Solution 3:

$\text{NaHCO}_3$  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:

$\text{AlCl}_3 \times 6 \text{H}_2\text{O}$  245.0 mg

Distilled water 5.0 ml

Solution 8:

$\text{Na}_2\text{CO}_3$  170.0 mg

Distilled water 1.6 ml

Solution 9:

Rumen fluid, clarified 20.0 ml

Solution 10:

$\text{Na}_2\text{S} \times 9 \text{H}_2\text{O}$  0.4 mg

Distilled water 10.0 ml

*Trace element solution:*

HCl (25%; 7.7 M) 10.0 ml

$\text{FeCl}_3 \times 4 \text{H}_2\text{O}$  1.5 g

$\text{ZnCl}_2$  70.0 mg

$\text{MnCl}_2 \times 4 \text{H}_2\text{O}$  100.0 mg

$\text{H}_3\text{BO}_3$  6.0 mg

$\text{CoCl}_2 \times 6 \text{H}_2\text{O}$  190.0 mg

$\text{CuCl}_2 \times 2 \text{H}_2\text{O}$  2.0 mg

$\text{NiCl}_2 \times 6 \text{H}_2\text{O}$  24.0 mg

$\text{Na}_2\text{MoO}_4 \times 2 \text{H}_2\text{O}$  36.0 mg

$\text{Na}_2\text{SeO}_4$  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

The trace element solution preparation:  $\text{FeCl}_3 \times 4 \text{H}_2\text{O}$  is dissolved firstly in HCl, then is mixed with water and other salts are dissolved in the sequence indicated. For the preparation and sterilization of the medium see medium 95. Solutions 7 and 8 are combined before sterilization. pH of the medium 7.6.

### **98. WORT AGAR 7 B WITH 2% $\text{CaCO}_3$**

Malt wort 7 B 1000.0 ml

$\text{CaCO}_3$  20 g

Agar 20.0 g

Sterilize at 105°C for 30 min.

*Preparation of malt wort:* mix 250 g of ground malt in 1000.0 ml of tap water, heat to 55-60°C and keep at this temperature for 1.5-2 h at periodic stirring. Then increase the temperature to 80°C and keep for 10 min. Then cool the wort, squeeze through a linen bag, adjust the concentration of sugars to 7 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. Apply sterile 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.

#### **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.

*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 h 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 (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

Solutin 6:

$\text{Na}_2\text{S} \times 9 \text{H}_2\text{O}$  0.4 g

Distilled water 10.0 ml

*Trace element solution SL-10:*

HCl (25%; 7.7 M) 10.0 ml

$\text{FeCl}_3 \times 4 \text{H}_2\text{O}$  1.5 g

$\text{ZnCl}_2$  70.0 mg

$\text{MnCl}_2 \times 4 \text{H}_2\text{O}$  100.0 mg

$\text{H}_3\text{BO}_3$  6.0 mg

$\text{CoCl}_2 \times 6 \text{H}_2\text{O}$  190.0 mg

$\text{CuCl}_2 \times 2 \text{H}_2\text{O}$  2.0 mg

$\text{NiCl}_2 \times 6 \text{H}_2\text{O}$  24.0 mg

$\text{Na}_2\text{MoO}_4 \times 2 \text{H}_2\text{O}$  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. Solution 2 is sterilized under 100% N<sub>2</sub>. Vitamin solution is filter sterilized. Final pH of the complete medium 7.1-7.4.

### **103. DESULFOBULBUS MEDIUM**

Solution 1:

$\text{Na}_2\text{SO}_4$  3.0 g

$\text{KH}_2\text{PO}_4$  0.2 g

$\text{NH}_4\text{Cl}$  0.3 g

NaCl 1.0 g

$\text{MgCl}_2 \times 6 \text{H}_2\text{O}$  0.4 g

KCl 0.5 g

$\text{CaCl}_2 \times 2 \text{H}_2\text{O}$  0.15 g

Distilled water 870.0 ml

Solution 2:

Trace element solution (see below) 1.0 ml

Solution 3:

$\text{NaHCO}_3$  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

Solutin 6:

$\text{Na}_2\text{S} \times 9 \text{H}_2\text{O}$  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. Solution 2 is sterilized under 100% N<sub>2</sub>. Vitamin solution is filter sterilized. Final pH of the complete medium 7.1-7.4.

**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 105°C.

**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

Sterilize catalase and vitamins separately from the main medium by filtration. Thiosulfate should also better be sterilized separately – at 105°C for 30 min.

#### **106. BEGGIATO A 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

Sterilize thiosulfate, lactate and vitamins each separately and add into the main medium prior to inoculation. Sterilize the vitamin solution by filtration. pH of the medium 7.2-7.5 (adjust with NaOH).

#### **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

Solutin 6:  
 $\text{Na}_2\text{S} \times 9 \text{H}_2\text{O}$  0.4 g  
Distilled water 10.0 ml  
*Trace element solution:*  
HCl (25%; 7.7 M) 10.0 ml  
 $\text{FeCl}_3 \times 4 \text{H}_2\text{O}$  1.5 g  
ZnCl<sub>2</sub> 70.0 mg  
 $\text{MnCl}_2 \times 4 \text{H}_2\text{O}$  100.0 mg  
H<sub>3</sub>BO<sub>3</sub> 6.0 mg  
 $\text{CoCl}_2 \times 6 \text{H}_2\text{O}$  190.0 mg  
 $\text{CuCl}_2 \times 2 \text{H}_2\text{O}$  2.0 mg  
 $\text{NiCl}_2 \times 6 \text{H}_2\text{O}$  24.0 mg  
 $\text{Na}_2\text{MoO}_4 \times 2 \text{H}_2\text{O}$  36.0 mg  
 $\text{Na}_2\text{SeO}_4 \times 5 \text{H}_2\text{O}$  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

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. Solution 2 is sterilized under 100% N<sub>2</sub>. Vitamin solution is filter sterilized. Final pH of the complete medium 7.1-7.4.

### **108. BEGGIATO A MEDIUM 2**

$\text{MgCl}_2 \times 7 \text{H}_2\text{O}$  50.0 mg  
 $\text{CaCl}_2 \times 2 \text{H}_2\text{O}$  30.0 mg  
Na-lactate 500.0 mg  
 $\text{Na}_2\text{S}_2\text{O}_3 \times 5 \text{H}_2\text{O}$  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 (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

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. pH of the medium 7.5 (adjust with 1% HCl).

#### **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>.

### **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>.

### **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

### **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 (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

Solutin 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. Solution 2 is sterilized under 100% N<sub>2</sub>. Vitamin solution is filter sterilized. Final pH of the complete medium 6.8-7.0.

#### **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.

#### **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 (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 fluid (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

Adjust pH to 7.5 with conc. NaOH.

Sludge water: to sludge from an anaerobic digester add 0.4% yeast extract and after gassing with nitrogen 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 nitrogen gas. pH of the medium, 6.7-7.0. Prepare the medium anaerobically under a gas atmosphere of 80% H<sub>2</sub> and 20% CO<sub>2</sub>.

### **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.

### **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)  $\text{K}_2\text{HPO}_4$  29.0 g

Distilled water 100.0 ml

b)  $\text{KH}_2\text{PO}_4$  15.0 g

Distilled water 100.0 ml

*Trace element solution:*

Nitrilotriacetic acid 12.8 mg

$\text{FeSO}_4 \times 7 \text{H}_2\text{O}$  0.1 mg

$\text{MnCl}_2 \times 6 \text{H}_2\text{O}$  0.1 mg

$\text{CoCl}_2 \times 2 \text{H}_2\text{O}$  0.17 mg

$\text{CaCl}_2 \times 2 \text{H}_2\text{O}$  0.1 mg

$\text{ZnCl}_2$  0.1 mg

$\text{CuCl}_2$  0.02 mg

$\text{H}_3\text{BO}_3$  0.01 mg

$\text{Na}_2\text{MoO}_4 \times 2 \text{H}_2\text{O}$  0.01 mg

$\text{NaCl}$  1.0 mg

$\text{Na}_2\text{SeO}_4$  0.017 mg

Distilled water 1000.0 ml

*Vitamin solution:*

Biotin 2.0 mg

Folic acid 2.0 mg

Pyridoxine ( $\text{B}_2$ ) 0.1 mg

Riboflavin ( $\text{B}_1$ ) 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 ( $\text{B}_{12}$ ) 0.1 mg

Lipoic (tioctoic) acid 5.0 mg

Distilled water 1000.0 ml

Prepare medium in anaerobic conditions, blowing through with  $\text{N}_2$  without  $\text{O}_2$  up to sterilization. Solutions of reducing agents (10 ml) and of buffer (per 1 ml) add to base medium after separate sterilization. pH 7.2-7.4.

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

Peptone 5.0 g

Glucose 5.0 g

$\text{K}_2\text{HPO}_4$  5.0 g

Agar 2.0 g

Distilled water 1000.0 ml

pH 6.9-7.0. Sterilize at  $112^\circ\text{C}$  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**

$(\text{NH}_4)_2\text{SO}_4$  0.5 g

$\text{KH}_2\text{PO}_4$  0.2 g

$\text{CaCl}_2 \times 2 \text{H}_2\text{O}$  0.02 g

$\text{MgSO}_4 \times 7 \text{H}_2\text{O}$  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%  $\text{Na}_2\text{CO}_3$ ).

**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.

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

$(\text{NH}_4)_2\text{SO}_4$  300.0 mg  
 $\text{MgSO}_4 \times 7 \text{H}_2\text{O}$  500.0 mg  
 $\text{KH}_2\text{PO}_4$  3.5 g  
 $\text{CaCl}_2 \times 2 \text{H}_2\text{O}$  250.0 mg  
 $\text{FeSO}_4 \times 7 \text{H}_2\text{O}$  18.0 mg  
Finely dispersed sulfur 5.0 g  
Distilled water 1000.0 ml

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

$(\text{NH}_4)_2\text{SO}_4$  150.0 mg  
KCl 50.0 mg  
 $\text{MgSO}_4 \times 7 \text{H}_2\text{O}$  500.0 mg  
 $\text{KH}_2\text{PO}_4$  100.0 mg  
 $\text{Ca}(\text{NO}_3)_2 \times 4 \text{H}_2\text{O}$  10.0 mg  
Distilled water 1000.0 ml

After sterilization of the medium, add 10 ml of 10%  $\text{FeSO}_4 \times 7 \text{H}_2\text{O}$  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. pH of the medium 4.0 (adjust after sterilization and addition of iron solution).

**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:

$\text{CaCl}_2$  2.0 g  
Distilled water 100.0 ml

Solution 2:

$\text{MgSO}_4 \times 7 \text{H}_2\text{O}$  20.0 g  
Distilled water 100.0 ml

Solution 3:

Chelated iron 0.1 g  
Distilled water 100.0 ml

Solution 4:

$\text{Na}_2\text{MoO}_4$  0.1 g  
 $\text{MnCl}_2 \times 4 \text{H}_2\text{O}$  0.2 g  
 $\text{CoCl}_2 \times 6 \text{H}_2\text{O}$  0.002 g  
 $\text{ZnSO}_4 \times 7 \text{H}_2\text{O}$  0.1 g  
 $\text{CuSO}_4 \times 5 \text{H}_2\text{O}$  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

#### **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 of the medium 7.1.

#### **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.

#### **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 (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. Solution 2 is sterilized under 100% N<sub>2</sub>. 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 112°C for 30 min.

### **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.

### **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 1000.0 ml

### **130. GLUCOSE POTATO AGAR**

Potato 200.0 g  
Agar 20.0 g  
Glucose 10.0 g  
Tap water 1000.0 ml  
Boil potatoes for 1 h, filter cold through a cotton wool-gauze filter. Add agar and glucose. Sterilize at 121°C for 30 min. pH 7.0.

### **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  
Sterilize at 105°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

Sterilize solution 1 at 121°C for 15 min, solution 2, at 105°C. Mix the solutions before inoculation. pH of the medium 3.5.

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

K<sub>2</sub>HPO<sub>4</sub> 1.2 g  
KH<sub>2</sub>PO<sub>4</sub> 0.62 g  
CaCl<sub>2</sub> x 6 H<sub>2</sub>O 0.05 g  
MgSO<sub>4</sub> x 7 H<sub>2</sub>O 0.2 g  
NaCl 0.1 g  
FeCl<sub>3</sub> x 6 H<sub>2</sub>O 1.0 mg  
(NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub> 0.5 g  
CuSO<sub>4</sub> x 5 H<sub>2</sub>O 5.0 µg  
MnSO<sub>4</sub> x 5 H<sub>2</sub>O 10.0 µg  
Na<sub>2</sub>MoO<sub>4</sub> x 2 H<sub>2</sub>O 10.0 µg  
H<sub>3</sub>BO<sub>3</sub> 10.0 µg  
ZnSO<sub>4</sub> x 7 H<sub>2</sub>O 70.0 µg  
CoCl<sub>2</sub> x 6 H<sub>2</sub>O 5.0 µg  
Purified agar 15.0 g  
Methanol 1.0 ml  
Distilled water 1000.0 ml  
pH 6.8.

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

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  
Distilled water 1000.0 ml  
pH 7.0.

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

KH<sub>2</sub>PO<sub>4</sub> 0.8 g  
Na<sub>2</sub>HPO<sub>4</sub> x 12 H<sub>2</sub>O 3.0 g  
(NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub> 0.8 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 10.0 mg  
Trace element solution (see below) 1.0 ml  
Distilled water 1000.0 ml  
*Trace element solution:*  
CaCl<sub>2</sub> x 2 H<sub>2</sub>O 1.25 g  
CoCl<sub>2</sub> x 6 H<sub>2</sub>O 1.25 g  
MnSO<sub>4</sub> x 4 H<sub>2</sub>O 0.1 g  
ZnSO<sub>4</sub> x 7 H<sub>2</sub>O 1.25 g

Na<sub>2</sub>MoO<sub>4</sub> x 2 H<sub>2</sub>O 50.0 mg  
CuCl<sub>2</sub> x 2 H<sub>2</sub>O 250.0 mg  
Distilled water 250.0 ml  
pH of the medium, 7.0-7.2. Sterilize at 121°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:

5% NaOH, to adjust pH 6.9-7.2

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 (see below) 0.5 ml

Solution 7:

Vitamin B<sub>12</sub> (dispensarymade 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

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 (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

Add solutions and additions to the main medium in the order of their enumeration. pH 7.0-7.2.

### **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 (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 of the medium 8.4.

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

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

Ascorbic acid 1.0 g

Thioglycolic acid 1.0 g

FeSO<sub>4</sub> x 7 H<sub>2</sub>O 0.5 g  
Tap water 1000.0 ml

#### **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

Sterilize at 112°C for 15 min.

*Preparation of Hottinger broth:* boil meat (1-2 cm pieces) (without fat or tendons) in 2.0 l 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 alkalize 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 h 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.

#### **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 or at 112°C for 30 min.

#### **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.

*Preparation of potato decoction:* boil 200.0 g potatoes in 1.0 l of tap water for 1 h, filter cold through a cotton wool-gauze filter. Sterilize at 121°C for 30 min.

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

Peptone 10.0 g

NaCl 5.0 g

Glycerol 10.0 ml

Agar 20.0 g

Meat water 1000.0 ml

*Preparation of meat water:* comminute 500 g of meat free of bones, fat and tendons, add 1000.0 ml of tap water and leave for 12 h at room temperature or in a thermostat at 30°C, or for 2 h at 37°C. Then

squeeze the meat through gauze or cloth and boil the filtrate for 5 min. The proteins are denatured. Filter the cooled down mass through a cotton-wool filter and add water to the initial volume. Sterilize at 121°C for 30 min.

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

Peptone 10.0 g  
NaCl 5.0 g  
Glucose 5.0 g  
Agar 20.0 g  
Meat water 1000.0 ml

*Preparation of meat water:* comminute 500 g of meat free of bones, fat and tendons, add 1000.0 ml of tap water and leave for 12 h at room temperature or in a thermostat at 30°C, or for 2 h at 37°C. Then squeeze the meat through gauze or cloth and boil the filtrate for 5 min. The proteins are denatured. Filter the cooled down mass through a cotton-wool filter and add water to the initial volume. Sterilize at 121°C for 30 min.

#### **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 NH<sub>4</sub>OH); paper filter. Sterilize at 105°C for 30 min.

#### **146. FUCHSIN-SULFITE AGAR ENDO**

Meat extract 5.0 g  
Peptone 10.0 g  
NaCl 5.0 g  
Lactose 5.0 g  
Na<sub>2</sub>SO<sub>4</sub> 5.0 g  
Basic fuchsin 0.4 g  
Agar 20.0 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° ethanol, filter, add sulfite (possible as 10-20% aqueous solution) to bright green staining. Mix the melted medium with fuchsin and sulfite immediately before pouring into Petri dishes.

#### **147. HYPHOMICROBIUM MEDIUM**

NaNO<sub>3</sub> 1.0 g  
NaCl 0.5 g  
K<sub>2</sub>HPO<sub>4</sub> 1.0 g  
MgSO<sub>4</sub> x 7 H<sub>2</sub>O 0.2 g  
Methanol 5.0-10.0 ml  
Distilled water 1000.0 ml

Grow in an exsiccator in the presence of ethanol vapors.

#### **148. UROBACTERIA MEDIUM**

Urea 20.0 g  
Gelatine 150.0 g  
or agar 15.0 g  
Meat broth 1000.0 ml

#### **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  
Sterilize solutions 2-5 by filtration.

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

#### **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. Solution 2 sterilize separately.

### 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

### 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 (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

Solution 1 is prepared and autoclaved anaerobically under 80% N<sub>2</sub> + 20% CO<sub>2</sub>. Solutions 2, 4, 5 and 6 are gassed with N<sub>2</sub> and sterilized separately. Solution 3 (gassed with N<sub>2</sub> + CO<sub>2</sub>) and solution 7 (gassed with N<sub>2</sub>) are filter-sterilized. pH of the medium 7.1-7.4.

**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. Cultivate in a gas mixture of carbon dioxide, air and hydrogen (1:3:6).

**156. 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

pH 7.2. Sterilize at 121°C for 1 h.

**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

**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

**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

**160. MEAT GLUCOSE MEDIUM**

Peptone 10.0 g

Glucose 10.0 g

NaCl 5.0 g

Meat water 1000.0 ml

pH 7.2-7.4. Sterilize at 121°C for 15 min.

*Preparation of meat water:* comminute 500 g of meat free of bones, fat and tendons, add 1000.0 ml of tap water and leave for 12 h at room temperature or in a thermostat at 30°C, or for 2 h at 37°C. Then squeeze the meat through gauze or cloth and boil the filtrate for 5 min. The proteins are denatured. Fil-

ter the cooled down mass through a cotton-wool filter and add water to the initial volume. Sterilize at 121 °C for 30 min.

#### **161. 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

#### **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

#### **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.

#### **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 105 °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 112°C for 30 min or at 121°C for 15 min.

#### **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.

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

Peptone 10.0 g  
NaCl 30.0 g  
Agar 20.0 g  
Meat water 1000.0 ml

*Preparation of meat water:* comminute 500 g of meat free of bones, fat and tendons, add 1000.0 ml of tap water and leave for 12 h at room temperature or in a thermostat at 30°C, or for 2 h at 37°C. Then squeeze the meat through gauze or cloth and boil the filtrate for 5 min. The proteins are denatured. Filter the cooled down mass through a cotton-wool filter and add water to the initial volume. Sterilize at 121°C for 30 min.

#### **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.

#### **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 (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  
pH 6.8. Sterilize at 121°C for 15 min.

#### **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.

#### **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.

#### **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.

#### **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  
Adjust pH to 7.2.

#### **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

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

Peptone 10.0 g

NaCl 5.0 g

Glucose 10.0 g

Agar 20.0 g

Meat water 1000.0 ml

*Preparation of meat water:* comminute 500 g of meat free of bones, fat and tendons, add 1000.0 ml of tap water and leave for 12 h at room temperature or in a thermostat at 30°C, or for 2 h at 37°C. Then squeeze the meat through gauze or cloth and boil the filtrate for 5 min. The proteins are denatured. Filter the cooled down mass through a cotton-wool filter and add water to the initial volume. Sterilize at 121°C for 30 min.

### **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

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

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. pH 7.2-7.4.

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

**177.**

Starch (soluble) 10.0 g  
Yeast extract 1.0 g  
Meat 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.

**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.

**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. Gas atmosphere: 100% CO<sub>2</sub>.

### **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 (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. Solution 2 is sterilized under 100% N<sub>2</sub>. Vitamin solution is filter sterilized. Final pH of the complete medium 7.1-7.4.

### **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.

### **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 1 l 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

Solution 1 is prepared and autoclaved anaerobically under 80% N<sub>2</sub> + 20% CO<sub>2</sub>. Solutions 2, 4, 5 and 6 are gassed with N<sub>2</sub> and sterilized separately. Solution 3 (gassed with N<sub>2</sub> + CO<sub>2</sub>) and solution 7 (gassed with N<sub>2</sub>) are filter-sterilized. pH of the medium 7.1-7.4.

**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.

**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.

**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.

**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 at 121°C for 15 min.

### **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 112°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 20 min.

### **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 (see below) 1.0 ml  
Agar, if necessary (heat and dissolve it before adding sodium chloride) 20.0 g  
NaCl 200.0 g  
Glutamate 1.0 g  
Yeast extract 5.0 g  
Casamini acids 5.0 g  
Distilled water to 1000.0 ml  
pH of solution 1 before sterilization, 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 of the medium, 9.0 - 9.5.

**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.

**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.

**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.

**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.

**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 112°C for 15 min.

**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.

**195. INMI MEDIUM 4**

Yeast extract 2.5 g  
Casamino acids 5.0 g  
pH 9.5.

**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  
Sterilize sulfide separately. pH of the medium 7.6.

**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 (see below) 1.0 ml  
Vitamin B<sub>12</sub> (1.0%) 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

**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 (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

**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  
Sterilize iron, phosphorus and bicarbonate salts separately.  
pH 7.0.

**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

## 201. MODIFIED BROCK MEDIUM FOR SULFUROXIDIZING BACTERIA

$(\text{NH}_4)_2\text{SO}_4$  1.3 g

$\text{KH}_2\text{PO}_4$  0.37 g

$\text{MgSO}_4 \times 7 \text{H}_2\text{O}$  0.25 g

$\text{CaCl}_2 \times 6 \text{H}_2\text{O}$  0.07 g

Distilled water 1000.0 ml

pH 2.4 (range: 2.3 - 3.0; adjust with  $\text{H}_2\text{SO}_4$ ).

Additions to the medium (sterilize separately each):

Trace element solution (see below) 1.0 ml

Yeast extract 0.2 g

Element sulfur 10.0 g

Chalk ( $\text{CaCO}_3$ ) 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 105°C:

Peptone 1.75 g

Sucrose 0.25 g

*Trace element solution according to Pfennig:*

EDTA 1.5 g

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

$\text{FeSO}_4 \times 7 \text{H}_2\text{O}$  0.2 g

$\text{MnCl}_2 \times 4 \text{H}_2\text{O}$  0.02 g

$\text{ZnSO}_4 \times 7 \text{H}_2\text{O}$  0.1 g

Distilled water 1000.0 ml

*Trace element solution according to Hogland:*

EDTA 5.0 g

$\text{FeSO}_4 \times 7 \text{H}_2\text{O}$  2.0 g

$\text{ZnSO}_4 \times 7 \text{H}_2\text{O}$  100.0 mg

$\text{MnCl}_2 \times 4 \text{H}_2\text{O}$  30.0 mg

$\text{H}_3\text{BO}_3$  300.0 mg

$\text{CoCl}_2 \times 6 \text{H}_2\text{O}$  200.0 mg

$\text{CuCl}_2$  10.0 mg

$\text{NiCl}_2 \times 6 \text{H}_2\text{O}$  20.0 mg

$\text{Na}_2\text{MoO}_4 \times 2 \text{H}_2\text{O}$  20.0 mg

Distilled water 1000.0 ml

## 202. MEDIUM FOR MAGNETIC BACTERIA

Tartaric acid 0.37 g

Succinic acid 0.37 g

Na-acetate 0.05 g

$\text{KH}_2\text{PO}_4$  0.68 g

$\text{NaNO}_3$  0.12 g

Agar 1.3 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

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 105°C for 20 min.

**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.

**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.

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

Oatmeal 20.0 g  
Agar 20.0 g  
Tap water 1000.0 ml  
pH 7.2.

### **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  
pH 7.2.

### **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.

### **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 105°C for 30 min.

### **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 105°C for 30 min.

### **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.

#### **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.

#### **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.

#### **213. ORGANIC AGAR 2**

Hottinger broth (see below) 30.0 ml  
Peptone 5.0 g  
NaCl 5.0 g  
Glucose 10.0 g  
Agar 30.0 g  
Tap water 1000.0 ml  
pH 7.0-7.2.

*Preparation of Hottinger broth:* boil meat (1-2 cm pieces) (without fat or tendons) in 2.0 l 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 h 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.

#### **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.

### **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 30 min.

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

Solution 1:  
Hottinger broth (see below) 900.0 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 with steam for 30 min. Pour the medium into sterile vials with CaCO<sub>3</sub> (25 g CaCO<sub>3</sub> per 1 liter of medium). Sterilize the flasks with CaCO<sub>3</sub> with dry heat.  
*Preparation of Hottinger broth:* boil meat (1-2 cm pieces) (without fat or tendons) in 2.0 l 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 alkalize 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 h 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.

### **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

#### **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.

#### **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

#### **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

#### **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. Cultivation under mixture of methane and air (1:1).

#### **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

### **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 20 min at 121°C. After cooling add the following solutions:

Glucose (2.5 %) 10.0 ml

Solution 3 5.0 ml

Adjust pH to 7.5.

Solution 2 (trace elements):

Nitrilotriacetate 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 nitrilotriacetate first by neutralizing with KOH, then add other salts. Adjust pH to 7.2. Adjust volume to 1000 ml.

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  
Store in refrigerator at +5°C.

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

Solution 1 (basic solution):

Solution 2 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 2.0 ml

Solution 4 1.0 ml

Rezaurine traces

Distilled water 1000.0 ml

Sterilize 20 min at 121°C. After cooling add the following solutions:

NaHCO<sub>3</sub> final concentration 8.0 g/l

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

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

### **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.

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

Bacto vitamin assay casamino acids 7.5 g

Yeast extract 10.0 g

MgSO<sub>4</sub>x7 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.

**227. MICROLUNATUS MEDIUM**

Glucose 0.5 g  
Peptone 0.5 g  
Yeast extract 0.5 g  
Monosodium glutamate 0.5 g  
 $\text{KH}_2\text{PO}_4$  0.44 g  
 $(\text{NH}_4)_2\text{SO}_4$  0.1 g  
 $\text{MgSO}_4 \times 7\text{H}_2\text{O}$  0.1 g  
Distilled water 1000.0 ml  
pH 7.0.

**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  
 $\text{MgSO}_4 \times 7 \text{H}_2\text{O}$  1.0 g  
NaCl 50.0 g  
Agar 20.0 g  
Distilled water 1000.0 ml  
pH 7.2.

**229. ALKALIBACTER MEDIUM**

$(\text{NH}_4)_2\text{SO}_4$  1.00 g  
 $\text{NH}_4\text{Cl}$  0.40 g  
 $\text{Na}_2\text{S}_2\text{O}_3 \times 5 \text{H}_2\text{O}$  0.10 g  
 $\text{K}_2\text{HPO}_4$  0.50 g  
 $\text{MgSO}_4 \times 7 \text{H}_2\text{O}$  0.10 g  
 $\text{CaCl}_2 \times 2 \text{H}_2\text{O}$  0.05 g  
NaCl 10.00 g  
 $\text{FeSO}_4 \times 7 \text{H}_2\text{O}$  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  
Distilled water 950.00 ml  
*Trace element solution:*  
Nitrilotriacetic acid 12.8 mg  
 $\text{FeSO}_4 \times 7 \text{H}_2\text{O}$  0.1 mg  
 $\text{MnCl}_2 \times 6 \text{H}_2\text{O}$  0.1 mg  
 $\text{CoCl}_2 \times 2 \text{H}_2\text{O}$  0.17 mg  
 $\text{CaCl}_2 \times 2 \text{H}_2\text{O}$  0.1 mg  
 $\text{ZnCl}_2$  0.1 mg  
 $\text{CuCl}_2$  0.02 mg  
 $\text{H}_3\text{BO}_3$  0.01 mg  
 $\text{Na}_2\text{MoO}_4 \times 2 \text{H}_2\text{O}$  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  
After autoclaving, add from sterile anaerobic solution (per liter medium):  
50 ml of 5% w/v Na<sub>2</sub>CO<sub>3</sub>  
Adjust final pH of medium to 9.0.

### **230. CALDITRUX 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  
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  
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 (0.5 g NaOH, 3 mgNa<sub>2</sub>SeO<sub>3</sub> x 5 H<sub>2</sub>O, 4 mgNa<sub>2</sub>WO<sub>4</sub> x 2 H<sub>2</sub>O, 1 l distilled water) 1.00 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  
Adjust final pH of the medium to 6.8.

### **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 (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. 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. 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.

### 232. CARBOXYDOTHERMUS FERRIREDUCENS 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 (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 20 min. Before use, add CaCl<sub>2</sub> and vitamins from anoxic, sterile stock solutions.

### 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 (see below) 1.0 ml  
Selenite-tungstate solution (0.5 g NaOH, 3 mgNa<sub>2</sub>SeO<sub>3</sub> x 5 H<sub>2</sub>O, 4 mgNa<sub>2</sub>WO<sub>4</sub> x 2 H<sub>2</sub>O, 1 l distilled water) 1.0 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

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. Add cellobiose after autoclaving from an anoxic stock solution sterilized by filtration and sulfide from a sterile, anoxic stock solution prepared under N<sub>2</sub>. Adjust final pH of the medium to pH 8.8-9.0.

### **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 (see below) 1.0 ml  
Selenite-tungstate solution (0.5 g NaOH, 3 mgNa<sub>2</sub>SeO<sub>3</sub> x 5 H<sub>2</sub>O, 4 mgNa<sub>2</sub>WO<sub>4</sub> x 2 H<sub>2</sub>O, 1 l distilled water) 1.0 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

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. 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>. Adjust the final pH of the medium to 7.0-7.2.

### 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 (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 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. Before use add sodium formate from a sterile, anaerobic stock solution.

### 236. DESULFOTOMACULUM CARBOXYDIVORANS MEDIUM

Use medium 95 but lower the amount of yeast extract to 0.5 g/l. Na-acetate and Na-butyrate is replaced by 2.2 g/l Na-pyruvate added from an anoxic stock solution (sterilized by filtration) after autoclaving.

### 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 (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. 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. Prior to inoculation reduce medium by adding sulfide from a sterile, anoxic stock solution prepared under N<sub>2</sub>.

**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  
Agar 0.5 g  
Distilled water 1000.0 ml  
pH 7.5

Add after autoclaving sterile solutions:

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 (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

### **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>, which can be sterilised separately by autoclaving. 10 ml/l sterile 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.

### **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)

#### **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:*

Nitritotriacetic 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 nitrogen, 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 nitrogen and autoclave. 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, yeast extract, and sucrose stock solutions should be autoclaved, while the vitamin solution is sterile filtered.

#### **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

### 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.

### 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 700.0 ml  
Adjust pH to 2.0-2.2 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  
After autoclaving, combine the three solutions. Medium pH 1.9-2.4.

### 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, 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. 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.

**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:*

Nitritotriacetic 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 nitrogen, omitting the CaCl<sub>2</sub>, MgCl<sub>2</sub>, KNO<sub>3</sub>, tryptone, yeast extract, vitamins and sucrose. The pH should be 6.8. Dispense the medium into vessels suitable for anaerobic growth (Hungate tubes or serum bottles) under an atmosphere of nitrogen and autoclave. 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, yeast extract, and sucrose stock solutions should be autoclaved, while the vitamin solution is sterile filtered.

#### **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 (see below) 1.0 ml

Distilled water 1000.0 ml

Adjust pH to 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

#### **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

$\text{MgCl}_2 \times 6 \text{H}_2\text{O}$  2.5 g.

The pH was adjusted to 7.5.

*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

$\text{Na}_2\text{MoO}_4 \times 2 \text{H}_2\text{O}$  30 mg

$\text{Na}_2\text{SeO}_3 \times 5 \text{H}_2\text{O}$  10 mg

Distilled water 1000 ml