MEDIA:

Components of media		
Components (abbreviation)	Compound(s)	Final concentration
Ammonium (N)	NH ₄ Cl	10 μ M
Phosphate (P)	KH ₂ PO ₄	10 μM
Trace metals (TM)	FeCl ₃ ·6H ₂ O	117 nM
	MnCl ₂ ·4H ₂ O	9 nM
	ZnSO ₄ ·7H ₂ O	800 pM
	CoCl ₂ ·6H ₂ O	500 pM
	Na ₂ MoO ₄ ·2H ₂ O	300 pM
	Na ₂ SeO ₃	1 nM
	NiCl ₂ ·6H ₂ O	1 nM
Vitamin mixture (V)	Thiamine·HCl	59 nM
	Niacin	81 nM
	Ca-Pantothenate	84 nM
	Pyridoxine	59 nM
	Biotin	409 pM
	Folic acid	453 pM
	Vitamin B12	70 pM
	Myo-inositol	555 nM
	p-Aminobenzoic Acid	7 nM
Carbon mixture (CM)	Pyruvate	50 μM
	D-Glucose	5 μΜ
	N-Acetyl-D-glucosamine	5 μΜ
	D-Ribose	5 μΜ
	Methyl alcohol	5 μΜ
20 proteinogenic amino acid mixture (AA)	Each amino acid	100 nM, each
Media definition		
Media	Definition	
FAM	0.2 µm-filtered and autoclaved freshwater	
	medium supplemented with N, P, and TM	
FAMV	FAM supplemented with V	
FAMV+CM	FAMV supplemented with CM	

• Inorganic Basal Medium

FAMV+AA FAMV+CM+AA

- o DOM sources: peptone, yeast extract, α -D-Glucose
- Undefined Media
 - Yeast extract
- https://www.researchgate.net/publication/302934940 A recipes for freshw ater and seawater media
 - o COMBO, MW,
 - o Pages 482-486
- https://www.oieau.org/eaudoc/system/files/documents/35/178744/178744_doc.pdf

FAMV supplemented with AA
FAMV supplemented with CM and AA

AUTOCLAVED AND FILTERED LAKE WATER (Possibly supplemented with Trace metals and Vitamins)

Marine Broth from model experiment:

- Peptone 5g/L,
- Yeast extract 1g/L,
- Ferric Citrate 0.1g/L,
- Sodium Chloride 19.45g/L,
- Magnesium Chloride 5.9g/L
- Magnesium Sulfate 3.24g/L,
- Calcium Chloride 1.8g/L,

- Potassium Chloride 0.55g/L,
- Sodium Bicarbonate 0.16g/L,
- Potassium Bromide 0.08g/L,
- Strontium

Link to Tibbles Rawlings media: https://www.jstor.org/stable/4251335?seq=3

MAKE EXCEL DOC

PRACTICE PLAN

- 1. Make Plates
 - a. 10⁻⁴-10⁻⁸ Dilutions
 - i. 5 plates for each sample
 - b. 3 samples→ 15 plates
 - c. Make 0.5 liters of plate media
 - i. 23.5 g TB
 - ii. 2ml glycerol
 - iii. 0.5 liters H2O
 - iv. 7.5 g agar?
- 2. Collect samples
 - a. 1 gram of sediment per sample
- 3. Wash samples in PBS
 - a. Remove non particle associated microbes
- 4. Sonicate with 1 gram sediment in 10 grams sterile solution
 - a. 30 second on 30 seconds off
 - i. 2, 4, 6 times
- 5. Separate bacteria from sediment
 - a. Spin
- 6. Plate supernatant
 - a. DTE