Ethanol Limitation Carboy: 30

This protocol results in media containing 30 mM Ethanol, 60 mM Carbon

Directions for one carboy:

1. Autoclave carboy

2. Measure ~7L Milli-Q water into a "measuring" carboy

3. Add the following to ~900ml in a 1L graduated cylinder and mix well:
   a. 1000x vitamins 10.0 mL
   b. 1000x metals 10.0 mL
   c. Ethanol, 95% 18.3 mL

4. Add contents of cylinder to "measuring" carboy

5. Measure out 1L of 10X MDS into the cylinder and add to the "measuring" carboy

6. Rinse cylinder with Milli-Q water into carboy

7. Adjust volume to 10L

8. Mix for ~5min with magnetic stirrer. Filter sterilize into the autoclaved carboy.

Directions for making 20L stock 10X MDS, in case you need them:

- Fill plastic carboy with approx. 17L MilliQ H₂O
- Add with constant stirring:
  - CaCl₂·2H₂O 20g
  - NaCl 20g
- MgSO$_4$·7 H$_2$O 100g
- KH$_2$PO$_4$ (monobasic) 200g
- (NH$_4$)$_2$SO$_4$ 1000g

Bring up to 20L with MilliQ H$_2$O

Aliquot into 2L media bottles

Autoclave