Variable Nitrogen Source Limitation Carboy

To make 10L stock 10X Nitrogen limited salts:

- Fill plastic carboy with approx. 7L MilliQ H2O
- Add with constant stirring:
  - CaCl2-2H2O 10g
  - NaCl 10g
  - MgSO4-7 H2O 50g
  - KH2PO4 (monobasic) 100g
- Bring up to 10L with MilliQ H2O

To make 100mM nitrogen source:

- Add to ~90mL Milli-Q water in a beaker and mix well adding: 0.01 moles of Nitrogen source
- Bring to 100mL
- Filter sterilize

For one carboy:

- Autoclave an empty carboy
- Measure ~7L Milli-Q water into a "measuring" carboy
- Add the following to ~900mL Milli-Q water in a 1L glass graduated cylinder and mix well:
  - 1000x vitamins 10mL
  - 1000x metals 10mL
  - Glucose 50g (final concentration is 0.5 %) or 200 g (final concentration is 2 %)
- From 100mM N-source stock:
  - [N-source] final = 25uM => 2.5mL
  - = 50uM => 5mL
  - = 100uM => 10mL
  - = 200uM => 20mL
  - = 400uM => 40mL
  - = 800uM => 80mL
  - = 1.6mM => 160mL
- When glucose is fully dissolved, add contents of cylinder to "measuring" carboy
- Measure out 1L of 10X Nitrogen limited salts into the cylinder and add to the "measuring" carboy
- Rinse cylinder with Milli-Q water into carboy
- Adjust volume to 10L
- Mix for ~5min with magnetic stirrer
- Filter sterilize into autoclaved carboy (Filter-sterilization seems to make more consistent media than autoclave)

*10 L of media supplies a 300 ml chemostat running at a dilution rate of 0.17 hr⁻¹ for about 6 days.