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 \( \text{10g} \)
  - NaCl \( \text{10g} \)
  - MgSO4-7 H2O \( \text{50g} \)
  - KH2PO4 (monobasic) \( \text{100g} \)
- 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 \( \text{10mL} \)
  - 1000x metals \( \text{10mL} \)
  - Glucose \( \text{50g} \) (final concentration is 0.5 %) or \( \text{200 g} \) (final concentration is 2 %)
- From 100mM N-source stock:
  - [N-source] final = 25uM \( \Rightarrow 2.5\text{mL} \)
  - = 50uM \( \Rightarrow 5\text{mL} \)
  - = 100uM \( \Rightarrow 10\text{mL} \)
  - = 200uM \( \Rightarrow 20\text{mL} \)
  - = 400uM \( \Rightarrow 40\text{mL} \)
  - = 800uM \( \Rightarrow 80\text{mL} \)
  - = 1.6mM \( \Rightarrow 160\text{mL} \)
- 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)

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