

Procedure for Making Media and Connecting to Reactor

Preparing the media

- 1) Fill clean bottle with 5 L deionized water. Add a large magnetic stir bar to the bottle.
- 2) Measure out the following, and add to bottle:
 - a. 2 g NH_4Cl
 - b. 60.2 g Na_2HPO_4
 - c. 10.25 g KH_2PO_4
 - d. 13.6 g $\text{NaC}_2\text{H}_3\text{O}_2 \cdot 3\text{H}_2\text{O}$ (i.e., 50 mM acetate)
 - e. 50 mL trace minerals
- 3) Stir until almost completely dissolved.
- 4) Remove stir bar, and cap bottle. Autoclave on 60 min. cycle, and remove carefully using temperature-resistant gloves.
- 5) Allow to cool prior to use in the reactor. The media can be stored for more than a week in a 4°C refrigerator for more than a week.

Installing the media

- 6) Sparge with nitrogen gas for a minimum of 45 minutes. Once sparged, add the following to the media bottle:
 - a. 5 mL $\text{FeCl}_2 \cdot 4\text{H}_2\text{O}$
 - b. 1 mL Na_2S_x
- 7) Sparge again with nitrogen for about 1 minute. (This is more of a mixing step.) Clamp off the gas line and media feed line.
- 8) Fill a Tedlar bag with Nitrogen gas. Connect the bag to the gas line.
- 9) To connect bottle to a reactor that is already running, turn the pump off. Clamp liquid line close to the top of the bottle currently connected to the pump. Then, disconnect the bottle from the pump.
- 10) Place new bottle on high shelf. Without unclamping, connect new bottle to liquid line running through pump. Once securely connected, clamps on liquid line can be removed. Make sure throughout the process that oxygen from the air is not able to enter the bottle.
- 11) Turn pump back on, and ensure that liquid is moving through the line.