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Methangen H-cell set up

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Protocol status: In development
We are still developing and optimizing this protocol

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Abstract

This protocol outlines set up and use of H-cells in a three electrode set up. This protocol was developed for the work performed in Rowe et al. 2019. It was adapted from technques learned in P. Girgius's lab at Harvard.

Attachments

DOCX

Rowe_HcellSetup.docx

119KB

Guidelines

This requires equipment for anaerobic culturing including a gas station equipped with a 80% N2; 20% CO2 mix and an anaerobic chamber.

1 Assembly of Reactors: Each side of the h-cell reactor resembles a ~100 mL corning glass bottle. On one side of each component is an open circle flatten port for supporting a proton exchange membrane between the two sides. One side has two sample-ports; the side I generally use for the working electrode. You will also need a clean proton exchange membrane, white plastic fitting complete with O-ring, and a plastic chain clamp. Assemble as shown below, prior to attaching clamp. Red caps and stoppers for side ports should also be attached. 30m

2 PARTS: Glass ware: All glass ware can be cleaned with soap and DI water, (periodic acid washing). Proton exchange membrane should be cleaned and protonated according to MFC protocol. 10m

3 PARTS: PEM cleaning, briefly: • Clean dirty PEMs with Etoh • Low Boil (~80C) for 30min-1hr with DI water • Low Boil (~80C) for 1hr with 3% H2O2 • Low Boil (~80C) for 1hr with 0.5M H2SO4 • Store in DI water til use 3h

4 PARTS Electrodes: In a separate covered bin autoclave working and counter electrodes for each reactor. Working electrode is attached through a titanium wire across a thick black high pressure anaerobic stopper. Carbon cloth threaded through titanium wire. White stoppers with potential for three holes (only one open, to be used for the reference electrode) used for titanium/platinum counter electrode. Titanium should be flamed to remove organics between use. As a note, using an H-cell with crimp sealable ports one can also configure a port of a reference electrode to be compatible with the working chamber. I have not noticed any differences in the potentials monitored with either configuration, but there is higher likely hood (especially in high current density systems) of not properly referencing the working electrode environment. The trade-off is leaking headspace in higher pressure systems, which can through off Coulombic efficiency measurements. Reference electrodes are ethanol and UV sterilized. 30m

5 Autoclaving and Purging of reactors: Once reactors assembled (minus caps and electrodes) I autoclave with media in the reactor (note I separate media into two components for autoclaving and I only autoclave with component A). This is different than other protocols I have seen but I feel it is more anaerobic and better for the PEMs. Autoclave with ¾ volume of component A (cover with a loose normal bottle cap) in the reactors. Autoclave component B Separately (usually autoclave all in first round but can aliquot in case of time restrictions. All reactors gassed with N2/CO2 80/20. Usually takes around 10 min to be anaerobic. Post purge Attach stoppers and open screw caps (red anaerobic for working electrode) and blue (smaller hole punch) for the counter/reference. Place in the anaerobic chamber overnight. Run abiotic CV's DPV's etc. 6:Inoculating reactors: I perform the equivalent of a 10% biomass transfer for electrochemical experiments. From stationary phase methanol grown cultures (~OD600 1.0) I centrifuge 10 mL to add to electrochemical cells. I use a centrifuge in an anaerobic chamber. I do 6 2-mL aliquots and save one 2 ml sample for protein analysis. Each 2-mL aliquot is centrifuges at 8,000 G for 5 min. Spent media is removed. (This spent media 4h

can be added to a reactor as a spent media control. As a not I recommend centrifuging media longer as the cells do not all pellet in 5 min though the majority do, I have had a few spent media controls make methane) Cells are re-suspended in media from each electrochemical cell (I remove prior to resuspension with a syringe through one of the septa) and then reinjected via septa into the working electrode chamber. I have washed cells in the past, but have found that one wash is sufficient to remove spent media and additional washes usually result in stress on cells and/or loss of biomass. From this point I generally run chronoamperometry ~-500 mV SHE for at least 48 hours before performing any electrochemical experiments. If you are quantifying methane I suggest taking a time zero sample to control for background methane in chamber or dissolved in spent media.