

Supplemental Material

Culture growth and sample staining for *in-situ* imaging.

A continuous-flow system consisting of a culture medium vessel, peristaltic pump, flowcell (35x35x4 mm) and an effluent vessel was assembled in the specified order inside an anaerobic cabinet. Silicon tubing (0.188 x 0.313 x 0.063 in., I.D x O.D x Wall, VWR, ON, Canada) and polycarbonate adapters were used to connect the different components. The flowcell and the tubing were sterilized with 0.6% sodium hypochlorite for 2 hours and washed for 24 hours with sterile water in continuous-flow, prior to using the culture medium. The *Clostridium thermocellum* inoculum (10%-30% v/v , from batch culture source) was introduced directly into the flowcell via sterile syringe and needles (BD size 25G, Fisher Scientific, ON, Canada) and the flow was stopped for one hour to allow cellulose colonization, with incubation at 60°C. Normal flow (10mL/h) was then resumed and culture growth proceeded for one to three days. Syto 9 and WGA-TRITC were prepared as a mixture with ultrapure water to 15 μ M and 15 μ g/mL final concentrations, respectively, as described in their product documentation. The stain mixture was then applied very slowly with syringe and needle in the flowcell influent tubing and sample binding was allowed for 15 minutes in the dark, with the pump turned off. Flow was then resumed for 20 minutes to remove the excess dye and the flowcell was capped at both ends, removed from the anaerobic chamber and set up on the microscope stage. Imaging was done directly through the transparent coverslip window of the flowcell chamber.

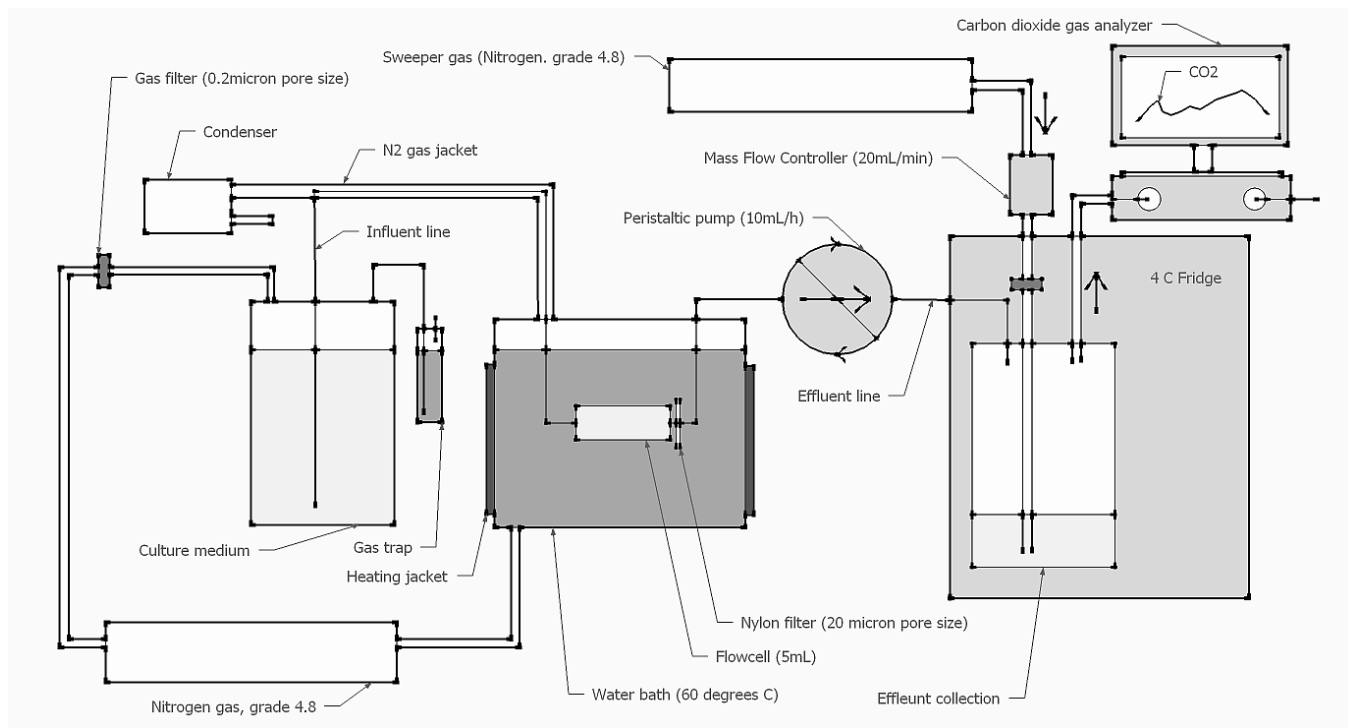


Figure S1. Diagram of the continuous-flow system used for carbon mass balance experiments