**Tube preparation, which can happen the day before:**

Label a set of 8 50mL tubes (1-8) to take the original samples.

Label top of 4 sets of 8 epis with the following –

Trial #: T46

Hour: 24

Reactor #: 1

Type: VFA (or MTX)

* One set of VFA tubes can just be labelled 1-8, since we are only taken the supernatant.

Label a second set of 50mL tubes, with the trial, hour and reactor number for DNA extraction.

Can also label the plastic bags that the tubes end up going in (one VFA, one MTX)

**Steps to start reactor:**

1. Turn the pump off (green switch)
2. Close the line from the pumps to the reactors
3. Close the valve on reactor (line form the pump)
4. Pull the piston pin
5. Take the liquid sample (50mL syringe). Attach needle, open valve on the reactor. Draw 20 mL back and forth a few times before taking the 50 mL.
6. Turn off the stirring (either red button)
7. Take the solid sample bags (except on first day):
   1. Take off the clamp carefully
   2. Tip the lid and lift it carefully, and hang
   3. Squeeze the bag over the reactor
   4. The bags will go into the washing bucket
8. As the lid is up, put in the mycotoxin
9. Replace the feed bag. Christian lies them out in the same orientation as the reactors, as to not make any mistakes.
10. Close the system.
11. Put the solid samples in the washer bucket. Attach the bottom hose to the faucet on the sink, the other tubes just hangs into the sink. There is a mark on the tap for how much to turn on the water. The setting is range 1, speed 4 for 30 mins. Start this right away before of the time it takes.
12. After closing the system, turn on the stirring right away.
13. Pipet the liquid from the 50 mL sample into the epi tubes. Centrifuge one of the VFA tubes to get the supernatant. 16600 RCF, 30 min. Take supernatant when its done.
14. Take the pH of the 50mL samples and record it in the log book.
15. Put the samples in baggies and freeze them all as soon as they are done.
16. Add the MgCa. They add it separately because it precipitates in the buffer. Take 30mL, attach to the reactor (its always on the inlet that does not going into the headspace, the gas one), and put it in.
17. To refill the buffer. Usually we make the buffer (they need to show me where the water is). Use the normal stirrer and set it to range 2 speed 7 for 10 mins.
18. Close all valves. Put the line in the bucket. Open the middle valve (where the buffer will do in). The system is uncoupled so it moves up and down. Use the remote control and take 700 mL. Go up a bit further to get the air out, back down to 700 mL. Actually can go more than this, for example we need 950 mL when we increase the flow rate.
19. Close valve from buffer bucket. Open valve to reactors. **When opening and closing valves, don’t have 2 open at the same time.**
20. Recouple the piston.
21. Open the valves on the reactors.
22. Turn on the pump, green swtich. Wait until you hear a noise, then hit the quite button.
23. Turn on the stirring (red button), make sure everything is stirring properly.
24. After the bags are done cleaning, squeeze them out. Put in the large plastic bags and freeze them.