Settled Cell Volume - SOP

EXP22000214

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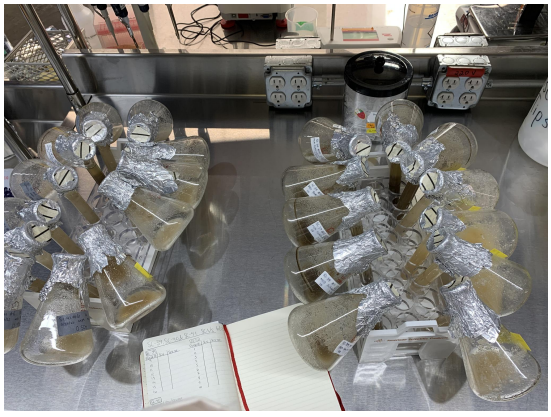
THURSDAY, 6/2/2022

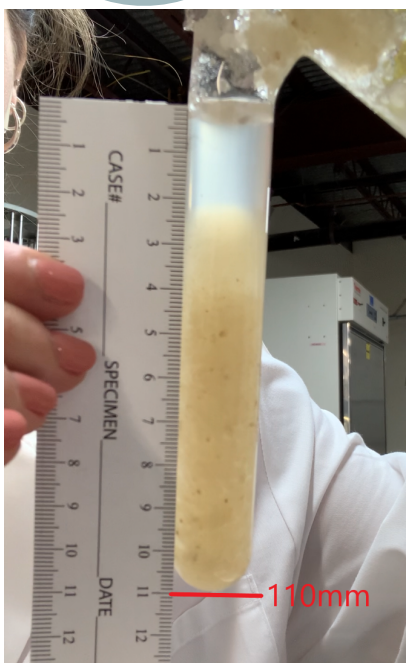
Sidearm Flasks (250mL volume for seed train)

- Grab the SCV tube rack and flasks from the incubator or warm room
- Swirl the contents of the flask until all the cells are evenly suspended in the media, creating a homogenous mixture
- Carefully tip the solution into the sidearm of the flask
    - FIll the sidearm as full as possible without the solution spilling into the rest of the flask
    - Ensure that the contents of the flask do not touch the foam stopper or foil covering the top of the flask to maintain best sterility
- Place the flask, sidearm down, into the SCV rack
- Let the flasks set for ~30 minutes

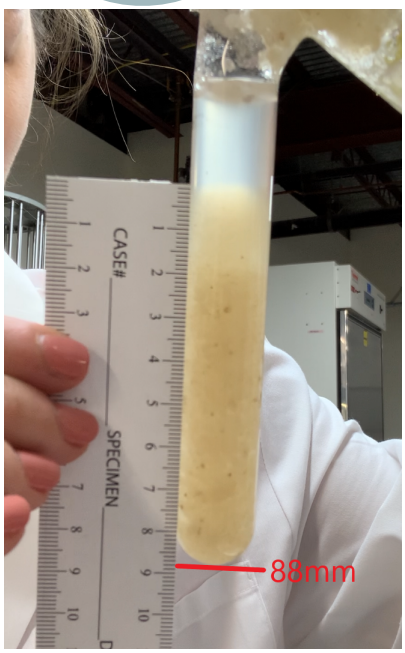


flasks settling in the tube racks for SCVs

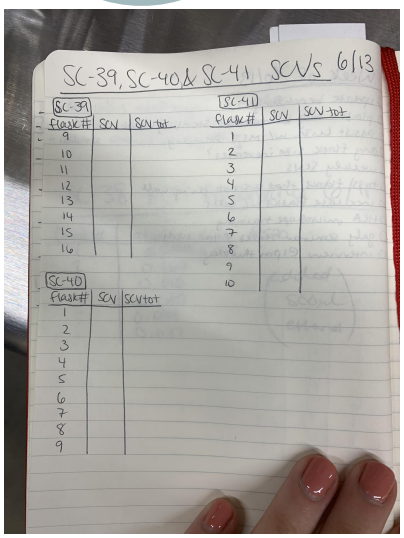
- After 30 min;



To measure the total volume (often denoted SCV tot or SCV total), use a ruler and place the 0mm mark at the top of the total media and measure to the bottom of the flask (measure in mm)



To measure the settled cells (commonly denoted SCV), use a ruler and place the 0mm mark at the top of the settled cells and measure to the bottom of the flask (measure in mm)



Record the data in some sort of lab notebook (physical or electronic or both). Here's an example of the easiest way to do so.

- After the data has been recorded, tip the sidearm and its contents back into the rest of the flask
    - You might need to tip the media/solution back and forth a couple of times to recover the cells stuck to the sides of the sidearms, lightly tapping the glass with your palms or fingers also helps to unstick the cells as well
- Return the flasks to the incubator/warm room and be sure to turn the shakers back on

Flasks Without Sidearms (all other volumes in seed train)

not typically taken, but sometimes are necessary for various experiments

- Ensure sterility throughout the entire procedure
    - Work completely in the hood (BSC or laminar flow) and ensure it has been running for the appropriate amount of time before starting
    - Sterilize the countertop of the hood with a 70% ethanol solution
    - Before placing any items in the hood, spray them down thoroughly with the same ethanol solution
    - Make sure to also wear gloves and to spray your arms and hands with the ethanol solution every time you remove them from the hood before putting them back in the hood
- Carefully break the tip off of a 10 mL pipette, being careful not to puncture the wrapper, and attach it to the pipette controller
    - If the wrapper breaks, it is no longer sterile and it must be disposed of
- Swirl the flask until the cells are evenly distributed within the media, creating a homogeneous solution
- Remove the foil from the flask and the graduated cylinder
- Tip the flask on its side and use the pipette to remove the contents of the flask and dispense them into the graduated cylinder
    - If it is a 50mL flask, all of the contents of the flask can be transferred. If the flask is larger, take ~20mL of the solution

- Replace the aluminum foil covers to the graduated cylinder and flask, ensuring to not switch the two
- Place a piece of tape on the base of the graduated cylinder and label it corresponding to the flask that it has its contents from
    - Don't let the tape obstruct the view of the solution in the cylinder!
- Throw out the pipette and use a new one each time
- Allow the graduated cylinders to sit for 30 minutes before taking measurements
- To measure the total volume of the graduated cylinder, place the 0mm measurement at the base of the graduated cylinder and measure up to the top of the media in mm
- To measure the SCV, place the 0 mm measurement at the base of the graduated cylinder and measure up to the top of the settled cells in mm
- The SCV is the settled cells/total volume
- Return the cells to the flask
    - Swirl the contents of the graduated cylinder
    - Remove the foil covers of both the flask and graduated cylinder
    - Pour the contents of the graduated cylinder into the flask carefully, making sure not to spill anything or let the glassware touch
    - Any spills or drips should be cleaned with ethanol and a Kimwipe
    - If any residual cells are left behind, use a pipette to extract just media from the flask by tipping the flask onto its edge and placing the tip of the pipette into the lowest corner of the flask before pulling the media up into the pipette. Use this media to rinse the inside of the graduated cylinder and pour its contents back into the flask. This can be repeated until the cells are all dispensed back into the flask.
- Clean up the hood
- Return the flasks to the incubator/warm room and be sure to turn the shakers back on

Benchtop Bioreactors

- On the day of inoculation, place a large strip of tape down the side of the vessel of the reactor
- To measure cells, turn off any form of agitation including stirring, aeration, etc. using the reactor software (see benchtop reactor SOP)
- Allow the cells to settle for approx 20 minutes
- Unwrap any coverings over the strip of tape
- Using a flashlight under the reactor to better see the cells, mark the level of the settled cells on the tape along with the date
- Over the course of the experiement, mark the settled cell heights every day on the same piece of tape
- Quantify this data by measuring the distance of each of the heights from each of the subsequent days