**Bacteria culture Day**

* Setup traveling tray with:
  + Loops, culture tubes, pipettes, pipette bulb, marker, tube rack, media broth
  + Gloves, bleach, and EtOH, sample containers
* Clean biohood with bleach then EtOH, wiping off the walls of the hood first and then the work surface. Next, clean everything with EtOH before placing it into the hood (including gloves)
* Retrieve microbe:
  + Obtain desired bacteria for testing from glycerol tube.
    - Remove glycerol stock from -80C freezer and transport to biohood frozen (dry ice or liquid N2) 🡪 make sure the glycerol tube is submerged up to the cap in liquid N2 to ensure that it remains at optimal temperature
  + Inoculate selected microbe into liquid media (LB/MRS/TSB, dependent upon which one it was isolated on originally).
    - Prepare culture tube with 6mL of broth media 🡪 first rinse the pipette with broth media before distribution
    - With a loop, scrape the glycerol stock tube and place loop into culture tube (repeat as needed).
    - Return stock tube to freezing conditions **AS QUICKLY AS POSSIBLE** (the sample should not begin to thaw)
    - Before removing the loop from each culture tube, gently twist/shake in broth media to ensure that all the sample is off the loop
    - Place the lids loosely on each of the culture tubes (past the first stop) & label each culture tube appropriately
  + Incubate the sample in the shaking incubator for a 24-hour period at 32℃.
  + Prepare inoculation schedule and sampling schedule

**Inoculation Day**

* After 24-hours, aliquot 200uL from each culture tube into 4 wells of a 96well plate to obtain an absorbance reading.
* Prepare container intended for bacterial inoculation (sampling container)
* Pellet bacteria and resuspend in saline solution
  + Centrifuge culture tubes at 6400 rcf for 10 minutes.
  + Remove supernatant
  + Add \*resuspension liquid into the culture tube
  + Use pipet and mix bacteria pellet of each sample.
* Add the total volume of each resuspended culture tube to the sample container
* Add 6 ml of treatment liquid into the sample container
* Incubate sample container in a 32C incubator, and sample at 2 hours, 24 hours, and 48 hours after initial inoculation.

\*resuspension media could be saline, liquid media, juice, etc. The goal is to resuspend the pellet, so it can be transferred into the treatment container.

**VOC Collection**

1. Remove desired treatment jar from the incubator. Once under the hood, carefully remove the lid and start the 10 second timer. After 10 seconds, replace the lid. Then immediately start the 10-minute timer.

\*wear appropriate personal protective equipment when handling the treatment jar under the hood

1. Place the treatment jar in the incubator for the remainder of the 10 minutes.
2. After 10 minutes, carefully insert the SPME through the small hole in the lid of the jar, leaving the jar in the incubator. Then start the 5-minute timer.
3. While the timer is running, set-up the run on Mass-Hunter.
4. After 5 minutes, remove the SPME from the treatment jar and insert directly into the GC system & begin the run. Start the timer for 6 minutes.
5. After 6 minutes, remove the SPME from the GC system and allow the run to continue. Repeat the above steps for each treatment jar.

**Data Mining:**

1. Opening data
   1. File 🡪 Load data file (and/or overlay data files)🡪
   2. File 🡪 Integrate using default integration parameters🡪
   3. File 🡪 select Percent Report🡪
   4. Spectrum 🡪 Select Library
      1. Choose library databases for ion search
   5. Spectrum 🡪 select Spectrum then “Library Search Report” 🡪
      1. “Screen” report only, select correct integration parameter (the default one: event16), and select “Apex” 🡪”ok”
   6. File🡪export to csv file🡪 “current file with current data” 🡪generate library search results🡪”ok” and take note of “appended” message
2. Building database
   1. Open file explorer and open the “Results.csv” file you just made.
      1. If you overlayed TICs, the “Results.csv” file is located in the folder of the first data file you opened
   2. Compare the retention times between the library search results and the integration results for the first data file only
   3. Copy and paste “RT”, “Area”, and “Library” columns to the end of the integration results section and remove the remaining columns in lower results
   4. Save main database as a separate file, in .xlxs extension, and in a different location. As more data is collected it will be added to this main database.
3. Walking through peaks:
   1. These steps apply to the results generated on the initial MSD and the verification MSD (DB-WAX and the DB-1)
   2. Zoom into a region containing peaks. Make a small “left click” box inside the peak to select the peak then make a small “left click” box in a region that does not contain the peak and SUBTRACT the non-peak area.
   3. Double “right click” in the “Scan” window to obtain the identification result of the peak. Repeat this step **several** times before annotating the peak in the database you built.
      1. If the identified peak has a higher match rate than 50 then add the chemical name in the database you built. Also add peak information for all peaks that were manually integrated and identified
   4. Identify two or three of the largest ion signals. The largest ion peak is the parent ion and the two smaller peaks are the qualifier ions. Add this information to the database you built
4. MassHunter Quantitation for GCMS (B.08)
   1. Open a batch of samples you would like to analyze
   2. Navigate to a sample
   3. Edit the method
   4. Input information
      1. Compound name
         1. Copy and paste compound names, retention times, and MZ (parent ion) from the database you built the appropriate fields into MSD Quant
      2. Retention times
         1. Setup the left and right delta
      3. Qualifier setup
         1. Add qualifier information from the database you built into MSD Quant
      4. Validate
         1. Select validate and correct any issues found
      5. Save Method
         1. Save the method you created using a unique identifier
      6. Exit
         1. Select Analyze to process the batch of files you opened.
5. Calculating RI’s: DO NOT SAVE OVER MACROS! EVER!
   1. Complete these steps on the DB-WAX and the DB-1
   2. Open “Huge list of VOCs” file🡪navigate to ”WAX” sheet🡪open main database🡪file🡪open🡪browse🡪navigate to DB-WAX macro, highlight it, and select open🡪”enable” it once the file opens as needed then minimize it
   3. Navigate to database🡪make an empty column next to chemical name column🡪select all the RT in question🡪press ctrl + g to calculate the RI values