Wednesday Lab Notebook

Morning: Data Analysis Course with Jonathan

Explanation of why Github is useful, how to use it
Integrating a curve : using interpolation

Our absorbance values from yesterday are constant over the 7 hours of measurements no matter the concentration (including in normal LB). We therefore think that our night culture contained no bacteria. In fact, we had noticed that the falcon containing the night cultures contained a translucent solution instead of an opaque one, therefore evidence that not many bacteria had grown. We therefore had to prepare a new protocol for our preliminary experiment and to get ready for our lab session.

Morning: New protocol and lab session preparation

→ **REDOING THE PRELIMINARY EXPERIMENT (Wednesday)**

Prepare :

- LB solutions with different concentrations of 1% SDS
  S1, S2, S3, S4, S5, S6

Serial dilution from 1%; 0,1; 0,01; 0,001; 0,0001; 0% with Vtot eppendorfs 1mL
Prepare LB in 6 eppendorfs: S1: no LB; S2, S3, S4, S5, S6 : 900 uL LB,
S1: 1mL SDS → take 100mL and put it in S2 and resuspend, repeat for other solutions
(Adèle)

- LB solutions with 96% ethanol (considering it as 99% ethanol):
  E0, E1, E2, E3, E4, E5, E6

E0: 1000 uL EtOH
E1: 700 uL EtOH + 300 uL LB
E2: 600uL EtOH + 400uL LB
E3: 500 uL EtOH + 500 uL LB
E4: 400 uL EtOH + 600 uL LB
E5: 300 uL EtOH + 700 uL LB
E6: 1000 uL LB
(Pauline)

Inoculate with 10 ul of E.coli bacteria to dilute the cultures.

Incubate the eppendorfs for 5 minutes in static incubator near hood at 37°C. Then remove them quickly and centrifuge at 9.5 rpm for 1 minute to separate cells from the solution. Pipette out the 1 mL of solution (surnageant) while being careful not to damage the cells at the bottom of the eppendorf. Add 1 new mL of normal LB. Resuspend!
Put back in the incubator at 37°C for 1h. Remove from the incubator.

Take 6,6 ul of each concentration for each counting chamber. (Clara) Observe with the microscope, take a picture and analyse data with Image J. (Pauline + Alex)

Put the different biological and technical replicates in the 96-well plate. (Alex) Measure absorbance of the wells with the TECAN spectrophotometer for continuous measurements for 1h30. Analyse the data to see what the threshold concentration for death was and choose the one just under. Compare for SDS and for ethanol and make sure the threshold concentration is not too different.

Afternoon: Lab session

We did exactly the same protocol as indicated above. We noticed several potential problems: first of all, we observed that some eppendorfs had no visible cell mass at the bottom: this might be normal because of the high concentration of detergent in some tubes but it is important to note anyways.
Another error to take into account is the fact that Pauline's hand might have passed over the open tube of bacteria, allowing some contaminations to enter the tube.

All the manipulations before preparing the 96-well plate took us an hour. The 96-well plate preparation took us 20 minutes. We then put the plate in the TECAN spectrophotometer for continuous measurements over 1h30 minutes.

TECAN might be having problems so data might be false (because it has trouble moving from one well to another).

Evening: rewriting the protocol for Thursday

→ EXPERIMENT (Thursday)

Prepare LB solutions with 1 optimal concentration and different ratios of triclosan and ethanol :

4 different ratios: 0:100, 20:80, 50:50, 80:20, 100:0

Put 1 mL of each solution in 3 eppendorfs. Inoculate with 10 ul of E.coli bacteria to dilute the cultures.
Incubate the eppendorfs for 5 minutes at 37°C. Then remove them quickly and centrifuge at 9.5 rpm for 1 minute to separate cells from the solution. Pipette out the 1 mL of solution while being careful not to damage the cells at the bottom of the eppendorf. Add 1 new mL of normal LB. Put back in the incubator at 37°C for 1h. Remove from the incubator.

Take 6,6 ul of each concentration for each counting chamber. Observe with the microscope, take a picture and analyse data with Image J.

Put the different biological and technical replicates in the 96-well plate. Measure absorbance of the wells with the TECAN spectrophotometer. Analyse the data to compare the different ratios of SDS and ethanol and make sure the threshold concentration is not too different.