A guide on how to grow Synechocystis

General notes on starting a liquid culture

Synechocystis is a very sensitive organism that gains robustness as it increases in concentration. This is most likely due to the fact that as the concentration of cells increases the cells themselves are more protected from light (as light penetration is reduced at higher OD). This means that one doesn't want to start a culture at too low concentrations, this can be solved in two ways: use small volumes of media or inoculate a large amount of cells from plate.

When it comes to light the cells are not only sensitive to intensities that are too high, but they are also dependent on having light. Growing them in the dark has generally resulted in major failure (it is supposedly possible to grow the WT in complete darkness with glucose if that is ever needed). Generally speaking the light on the desk on the right side in the 30 C room seems to be the best available to us.

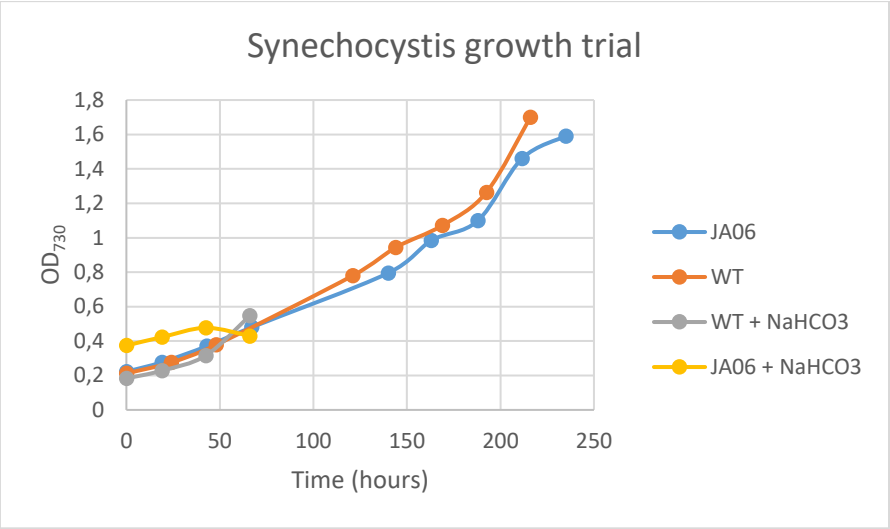
JA06 has throughout the project been a lot harder to grow healthily. It seems that when inoculating JA06 from plate it is sensitive many different conditions, for example it seems as if it's somewhat sensitive to carbonate (from added sodium-bicarbonate) when recently inoculated. The best choices for inoculating JA06 seems to be using only BG-11 or BG-11 with added air flow (just regular atmospheric air), none of these conditions have however had a 100 % success rate so it's advisable to inoculate multiple cultures simultaneously. It's worth noting that the cultures with added air flow seem to have a higher success rate.

Once a liquid culture has started growing it can be diluted into a lot of different conditions while stile maintaining healthy growing cells.

Starting liquid culture – Protocol

1. Prepare media in suitable container according to tables below.
2. Take 1-2 loops with a lot of cells from plates and suspend in media (I usually scrape both sides of the loop with quite large amounts of cells).
3. If JA06: add chloramphenicol – 25 µg/ml.
4. Place on shaker at 130 or 200 RPM (both seem to work, but 130 is recommended) at appropriate lighting (shaker on right side in 30 C room is recommended).

Expected growth curves



In this figure the cultures 'WT' and 'JA06' were grown using only BG-11 (and chloramphenicol in the case of JA06). The cultures 'WT + NaHCO3' and 'JA06 + NaHCO3' also had HEPES and NaHCO3 added according to media recipe (found in separate file).

Other notes on Synechocystis

On pelleting the cells: Synechocystis can be somewhat hard to pellet by centrifugation. When pelleting Synechocystis one can notice that the pellet is quite loose and that you get a green smear across the side of the eppendorf/falcon tube where the pellet is formed. It is unclear whether this is due to the cells not pelleting properly or if this is simply extracellular chlorophyll that doesn't pellet with the cells. Another interesting thing to note about this behavior is that it only seems to occur with Synechocystis that is actively growing, once they reach the stationary phase they usually pellet properly without the smear.