2015_03_31 Probe autoinduction conditions for ACS purification - vary time at RT, percent glycerol

Goals:

- Understand how to grow ACS L641P better
    - does adding more glycerol help? The paper
- Figure out what max OD is at RT
    - how is this a function of oxygen availability?
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Summary:

- Added O2 really helps growth *and* specific production.
- Addition of glycerol didn't have large effects.
    - Could test addition of glycerol *and* trace elements.

Background reading:
Stable Expression Clones and Auto-Induction for Protein Production in E . coli (Sturdier 2014)

- Culture:air ratios:
    - Seed stocks for larger scale autoinduction are grown in Erlenmeyer fl asks, the culture occupying approximately 5–10 % of the flask volume. Moderate-scale auto-induction can use 400–500 ml of culture in 1.8-l baffled Fernbach flasks (Bellco).

Overnight:

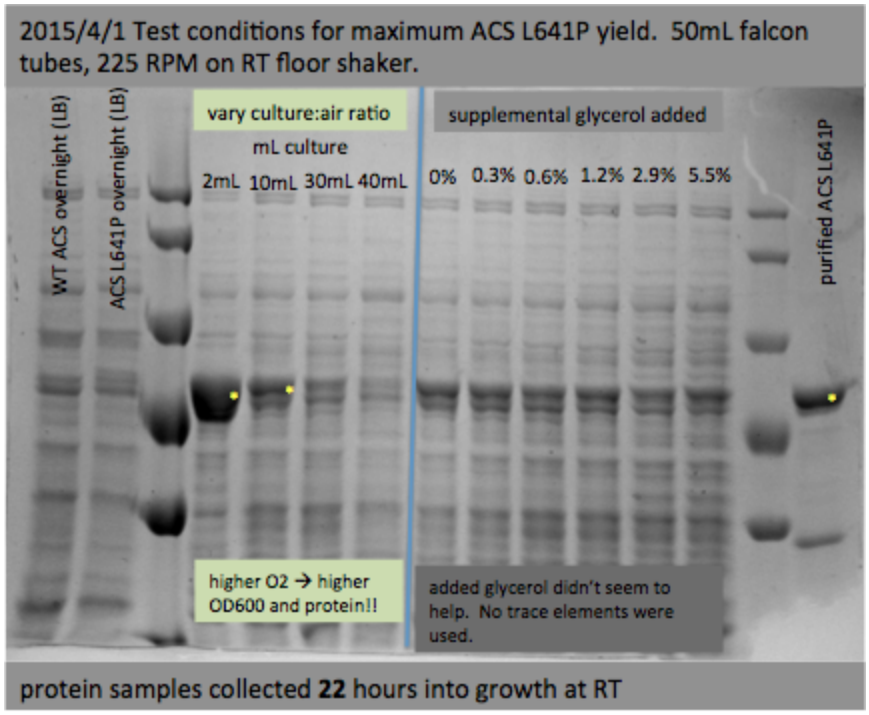
Notes about what happened:

- Used plates that were >1 month old to make the overnights.
- Overnights were done in 10mL of LB + Km
- Made a mixture of culture + pre-made autoinduction media, and put it in the tubes.
- Added glycerol to tubes 6-10
- Put on the room temp shaker. 2 falcon tubes per holder position.
    - 225 RPM. Wanted to try faster but it seemed like I was working the motor too hard.
- I measured the OD at the end of the day the cultures were inoculated into autoinduction, but it was about zero.
- Measured OD the next day (1 day of growth) & collected protein samples.
- Forgot to measure OD again at the end of the day.
- Ran PAGE samples that showed the 2mL sample (maximum O2) grew several fold higher than the next sample.
- I also measured the OD and collected samples 2 days after the autoinduction media was started.

    - Samples hadn't grown much.
- measured pH to see if it droped due to fermentation. Samples didn't have pH away from ~6 (for all samples checked.)

RESULTS:

High oxgen:culture ratio produced more cells and more protein per cell.



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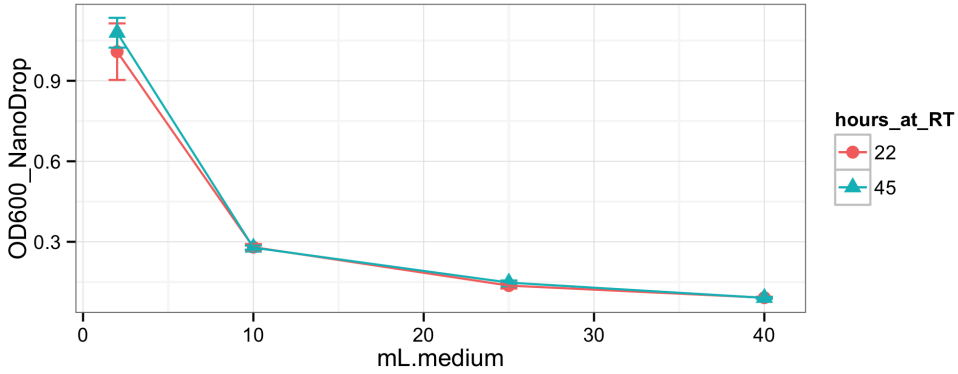
    - This is a protein normalized gel, so that dark band for the 2mL of culture is extra impressive.

less culture volume --> better oxygenation --> higher turbidity

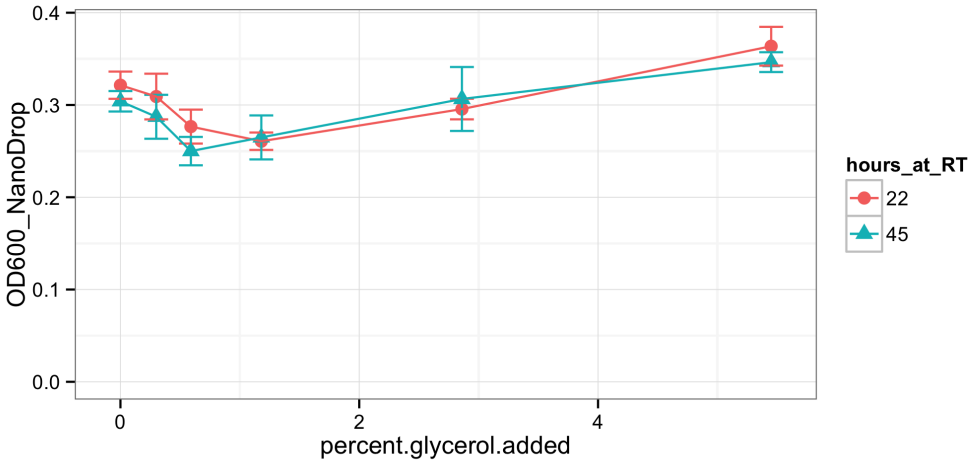
150402: pET ACS L641P in autoinduction media. 50mL falcon tubes @ RT, 225 RPM.



    -
- Adding glycerol didn't help. But it might when we add glycerol + trace elements.

addition of glycerol didn't impact final growth or protein yield

150402: 10mL cultures in 50mL falcon tubes. 225 RPM, RT shaker



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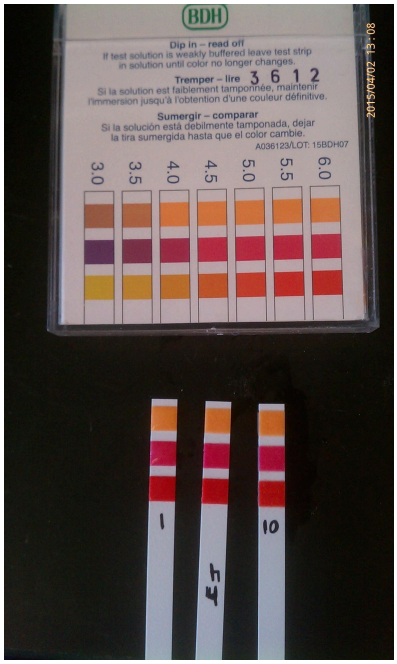
pH values for select tubes 45 hrs into growth:

- 

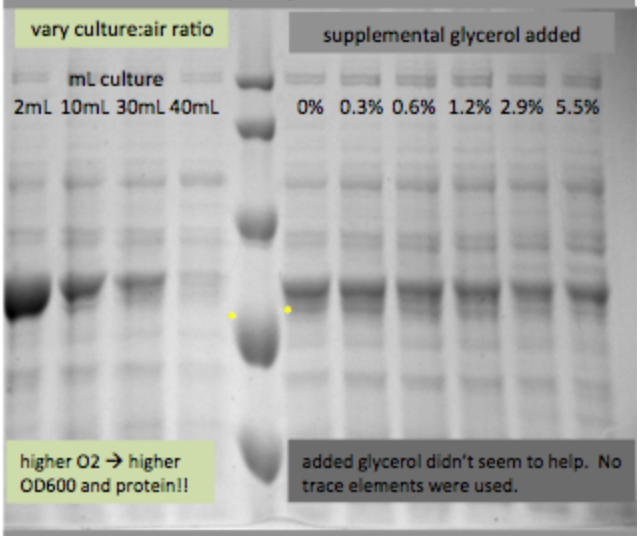
tube name is written on strip.

Amanda checked the 40mL culture tube after I put it in a big flask at RT for another day

- OD droped below 0.3, so the cultures did not grow with the addition of more O2 after the 2 days of incubation without much.

Gel from cultures grown for 2 days looks about the same:

2015/4/2 Test conditions for maximum ACS L641P yield. 50mL falcon tubes, 225 RPM on RT floor shaker. After 2 days of growth



protein samples collected 45 hours into growth at RT