Step Up Your Starters

Insights Into Yeast Propagation for Homebrewers

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Motivation

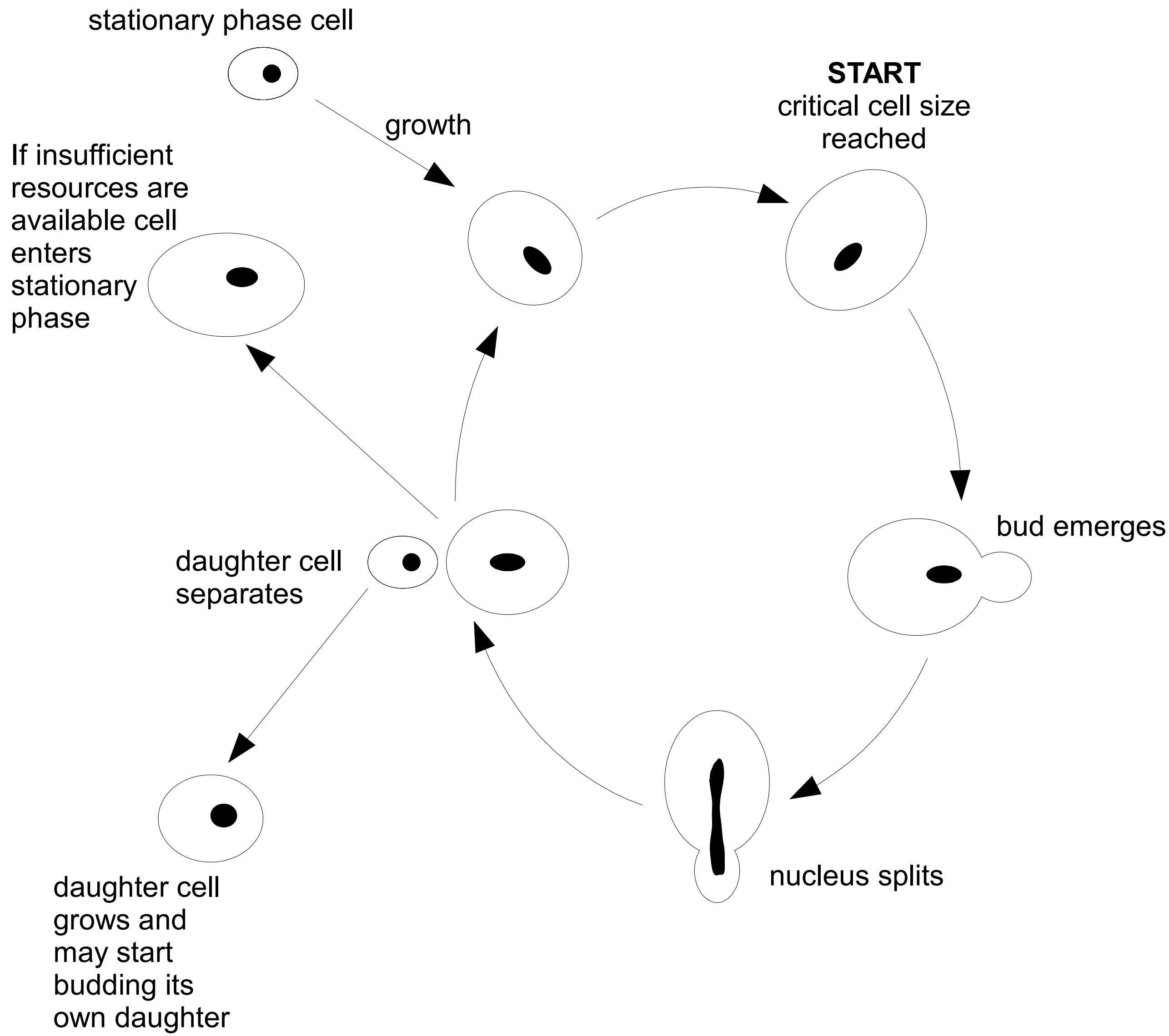
- In recent years yeast growth calculators have gained popularity
- Most of them are based on work done by Jamil Zainasheff and Chris White
- I have been keeping track of yeast propagation data in a “Master Spreadsheet” and the trends I were seeing contradicted these calculators
- I also came across Thomas Kurz's (Weihenstephan) dissertation on the topic of modeling yeast growth, which has a lot of insight into current knowledge on the topic.
- 6 months ago I started looking into yeast growth more systematically
- I also wanted to asses fermentation performance of propagated yeast.
- This presentation shows results and insight I have gathered so far

The Basics

Cell Cycle, Nutrients and Energy

Cell cycle

- After budding or stationary phase cells need to reach critical cell size
- Once the budding process starts it will complete
- Daughter cell needs to grow more than mother cells before they can start budding



Nutrients needed for growth

- **Energy source**
  - Anaerobic or aerobic metabolization of wort sugars.
  - Aerobic metabolization of ethanol. Yes, yeast can grow on ethanol!
- **Building blocks**
  - Carbon source (wort sugar, or even ethanol if oxygen is available)
  - Nitrogen for protein synthesis (from wort amino acids or ammonium ions)
  - Oxygen or unsaturated fatty acids for sterol production
- **Trace elements**
  - Metal ions (Ca2+, Mg2+ Zn2+)
  - Vitamins which the yeast cannot synthesize on its own

Yeast growth energy requirement

- Energy is needed to create new biomass (BM) from the various building blocks
- Aerobic yield on glucose: 3.5 mol BM per mol glucose [Sonnenleiter via Kurz]
- This is about 10 Billion cells per gram of glucose or 6.3 B/g DME[1)]
- Anaerobic growth yields only 0.72 mol BM/mol glucose or 2 B/g glucose (1.3 B/g DME)
- Observation from experiments support the 1.3 B/g DME under anaerobic growth

[1)] yeast BM is 25g/mol, cells per BM are about 20 B/g and DME is about 65% fermentable (glucose equivalents)

Yeast Metabolism Pathways

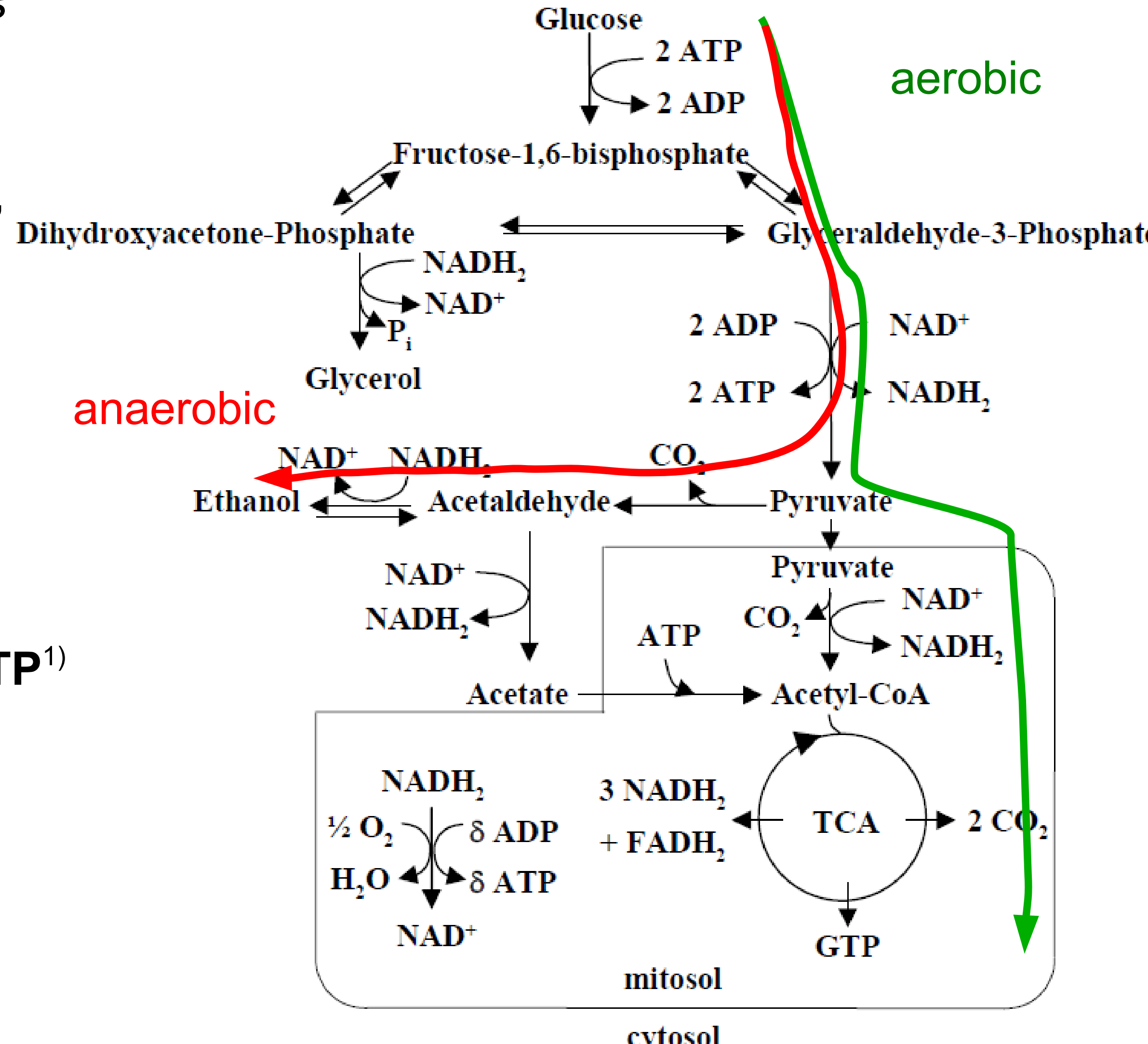
- This is how the yeast generates energy
- Glucose can be produced from other sugars (sucrose, maltose, maltotriose, etc.)
- Anaerobic (fermentative) metabolism:

$C_6H_{12}O_6 \text{-> } 2C_2H_5OH+2CO_2+\mathbf{2ATP}$

- Aerobic metabolism

$C_6H_{12}O_6 + 6O_2 \text{-> } 6CO_2+6H_2O+\mathbf{30\ ATP}$[1)]

Aerobic metabolism is substantially more efficient than anaerobic metabolism

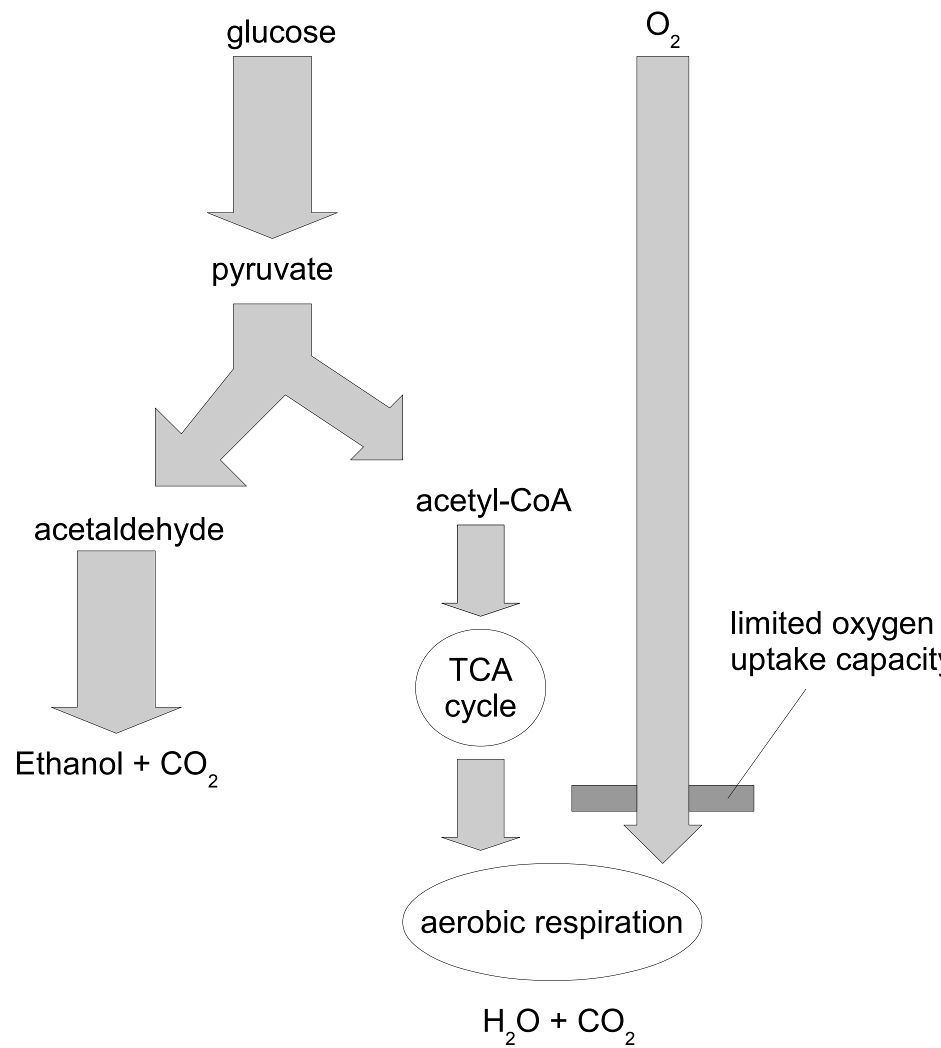


Source: T. Kurz

1) http://en.wikipedia.org/wiki/Cellular_respiration

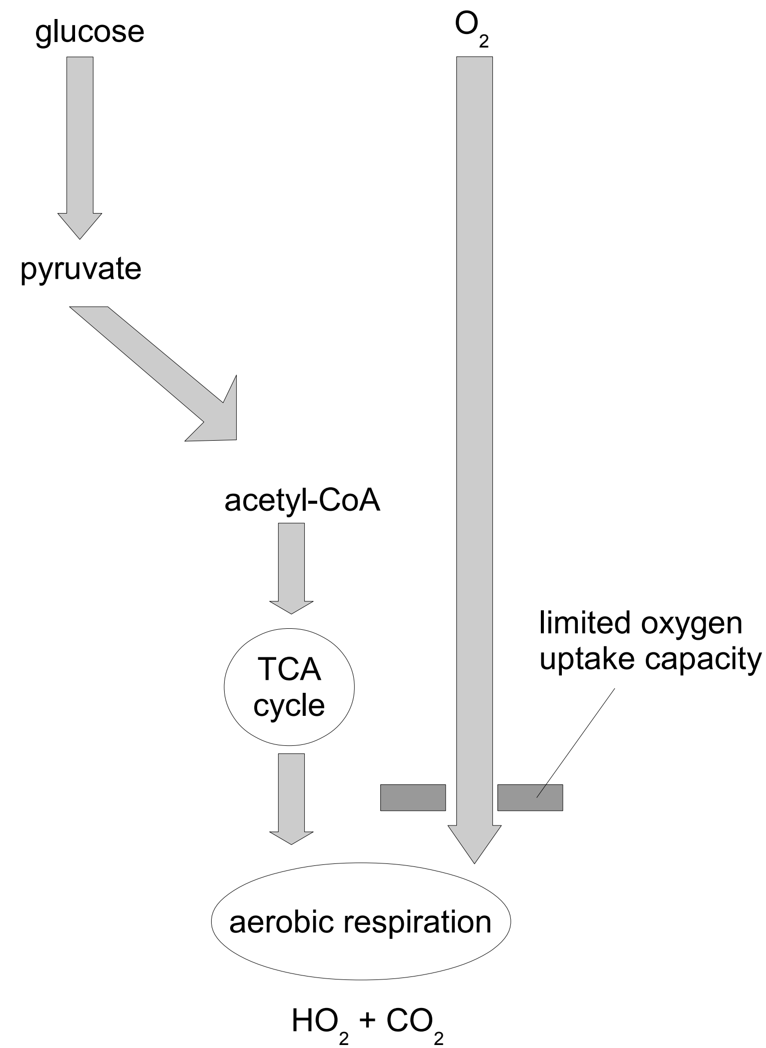
Oxygen Uptake Capacity

- How does the yeast “choose” between aerobic and anaerobic metabolism?
- The rate of oxygen uptake and thus the aerobic respiration rate is limited
- While glucose uptake is also limited, its limit is much higher
- When more glucose than respiratory capacity is present fermentation takes place even in the presence of oxygen → Crabtree Effect



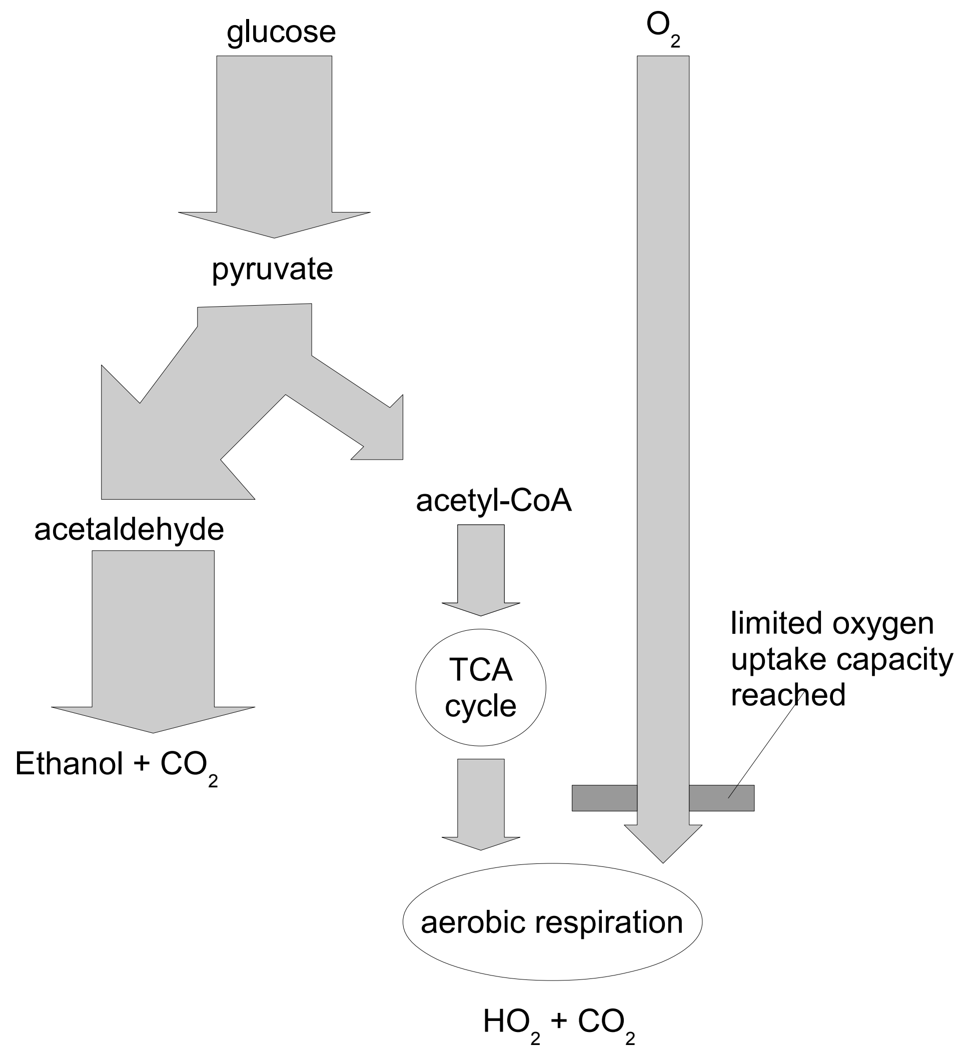
Low Glucose + O_2

- Low glucose concentration leads to low glucose uptake
- In presence of oxygen all glucose can be metabolized aerobically
- Used for bakers yeast propagation



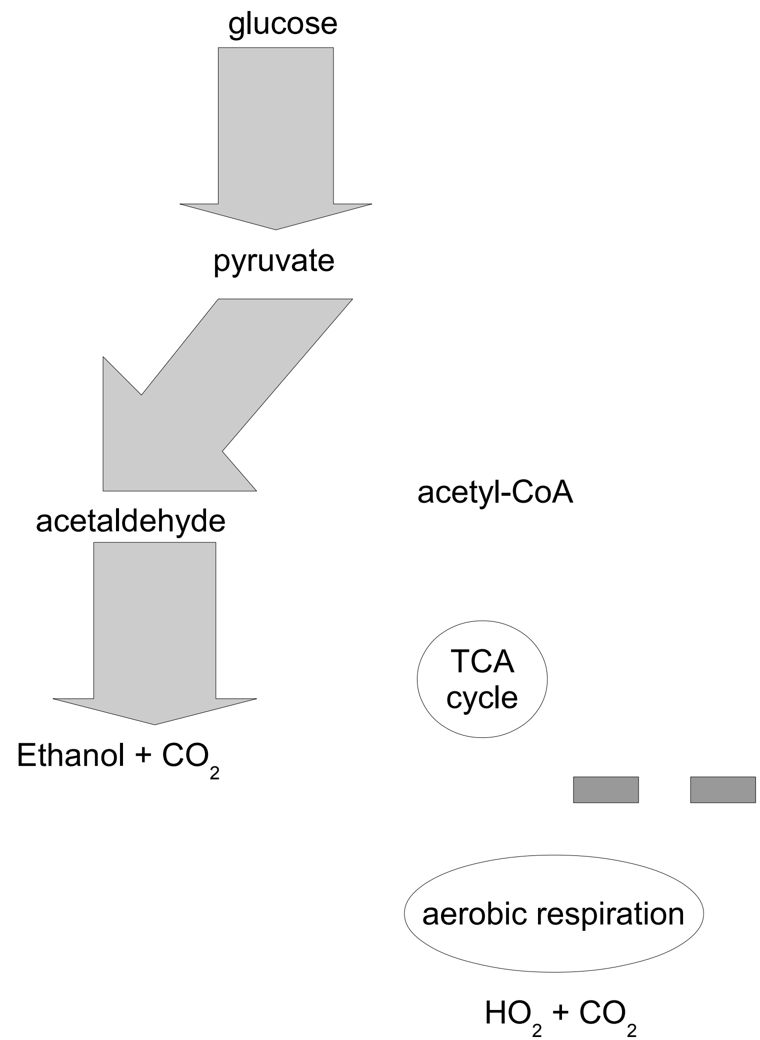
High Glucose + O_2

- Reached capacity of aerobic respiration
- Any additional glucose needs to be metabolized through fermentation
- This is what happens in starters when access to O_2 is available
- More on this later



Glucose but no O_2

- In absence of O_2 no aerobic respiration is possible
- Glucose is fermented to ethanol and CO_2
- This happens during normal beer fermentation and starters with no access to O_2



Crabtree Effect

- Chart below shows the saturation of the oxygen uptake as the available glucose is increased (Barford&Hall 1978)
- Measured in chemostat where nutrients are added at a constant rate.

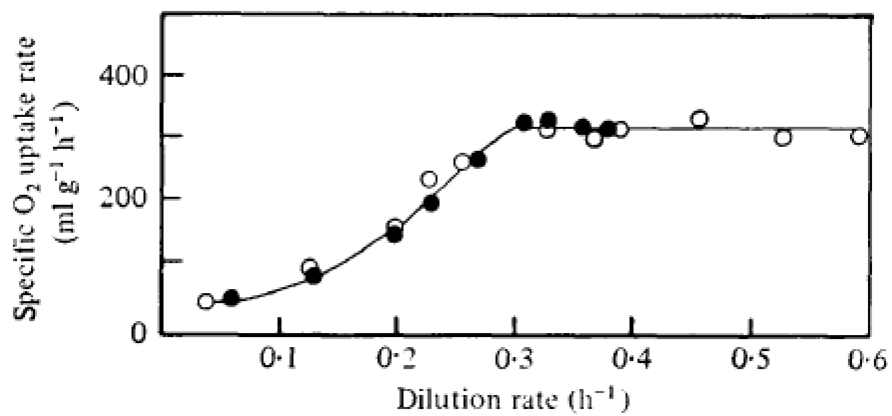


Fig. 3. Combined specific oxygen uptake rate profiles of *S. cerevisiae* grown in continuous culture with glucose and galactose as the limiting substrate. Estimations were specific oxygen uptake rates for glucose (○) and galactose (●).

The Experiments

Wife: “What are you doing?”

Me: “Counting yeast”

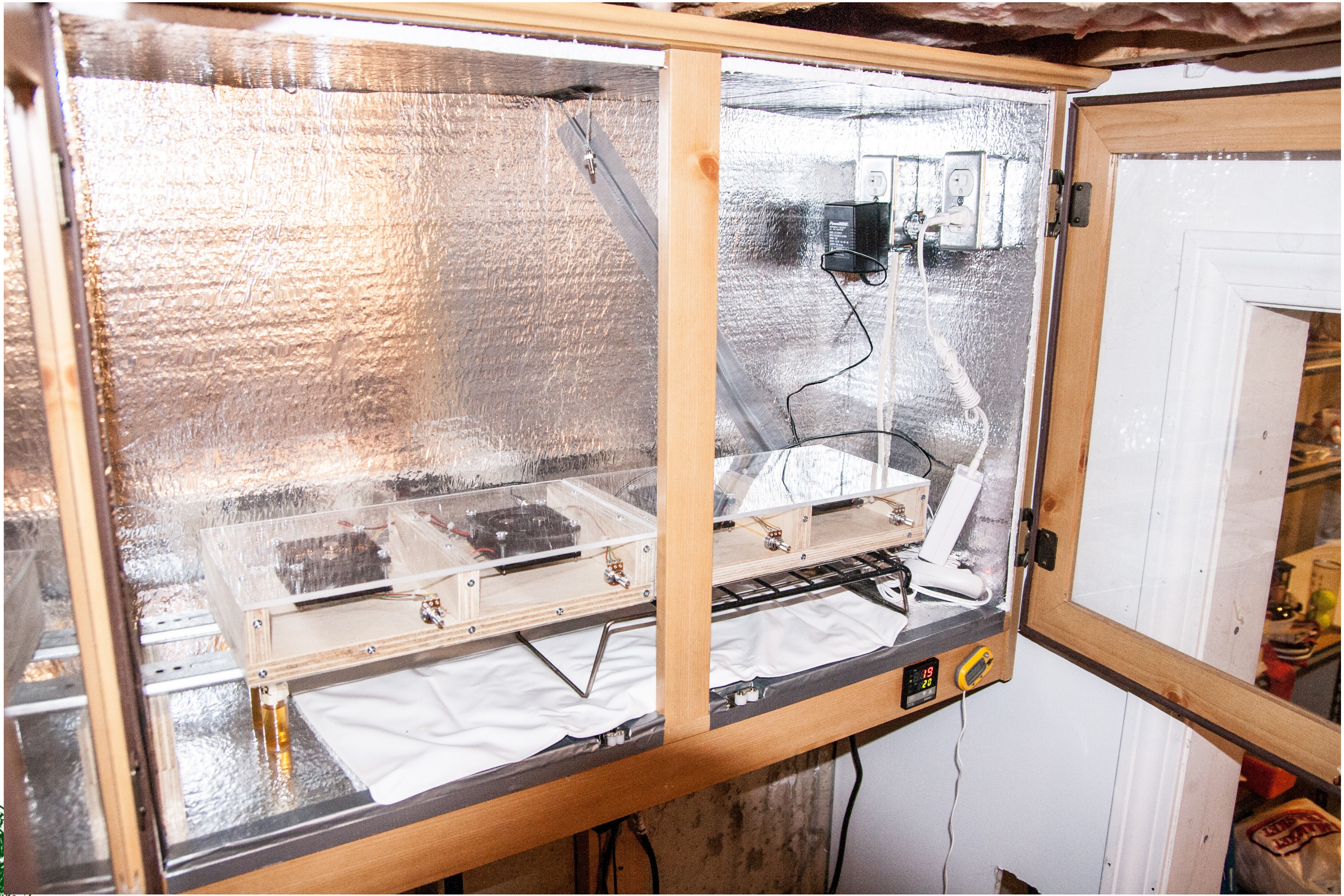
Wife: “That must take a long time”

Experiments done on yeast growth

To evaluate the effect that different starter conditions have on yeast growth many yeast growth experiments were conducted

- Mostly stirred since that's how most brewers propagate their yeast
- 2 double stir plates in incubator allowed 4 experiments side-by side
- 2 yeast strains were used predominantly: WY2042 (Danish Lager) and WLP 036 (Duessedorf Alt). Both are poor flocculators which made counting easier (but they did start flocculating on occasions)
- Default starter size was ~250 ml in 500 ml flasks
- Total cells counted so far: ~210,000

Incubator with double stir plates

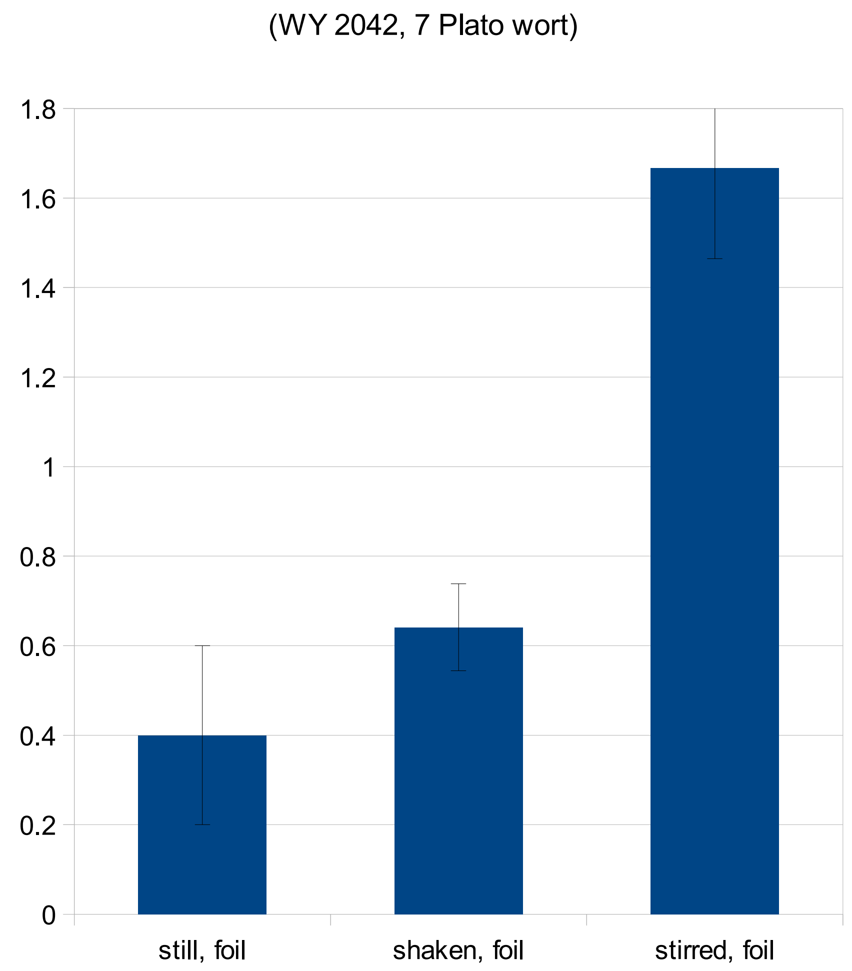


Characterizing Yeast Growth

- For the experiments the primary characteristic that was tracked was yeast growth per extract expressed as **Billion new yeast cells per gram of extract**
- Reflects the fact that the amount of available extract is one of the most significant factors in yeast growth
- Expresses the utilization of a given amount of extract for yeast growth
- Does not take into account attenuation like the Yield Factor proposed by Nielsen
  - Most brewers use DME and the expected attenuation should remain the same
  - The fluctuations in yeast growth are expected to be larger than changes in wort attenuation
  - By ignoring wort fermentability there is one less parameter to worry about.
  - Unless otherwise noted all experiments used Briess Light DME

Agitation

- Keeps yeast in suspension → all yeast cells have equal access to wort nutrients
- Promotes gas exchange → CO_2 concentration is starter is lower and more oxygen is allowed to enter
- Increased O2 uptake allows for more aerobic sugar metabolization → increased yeast growth

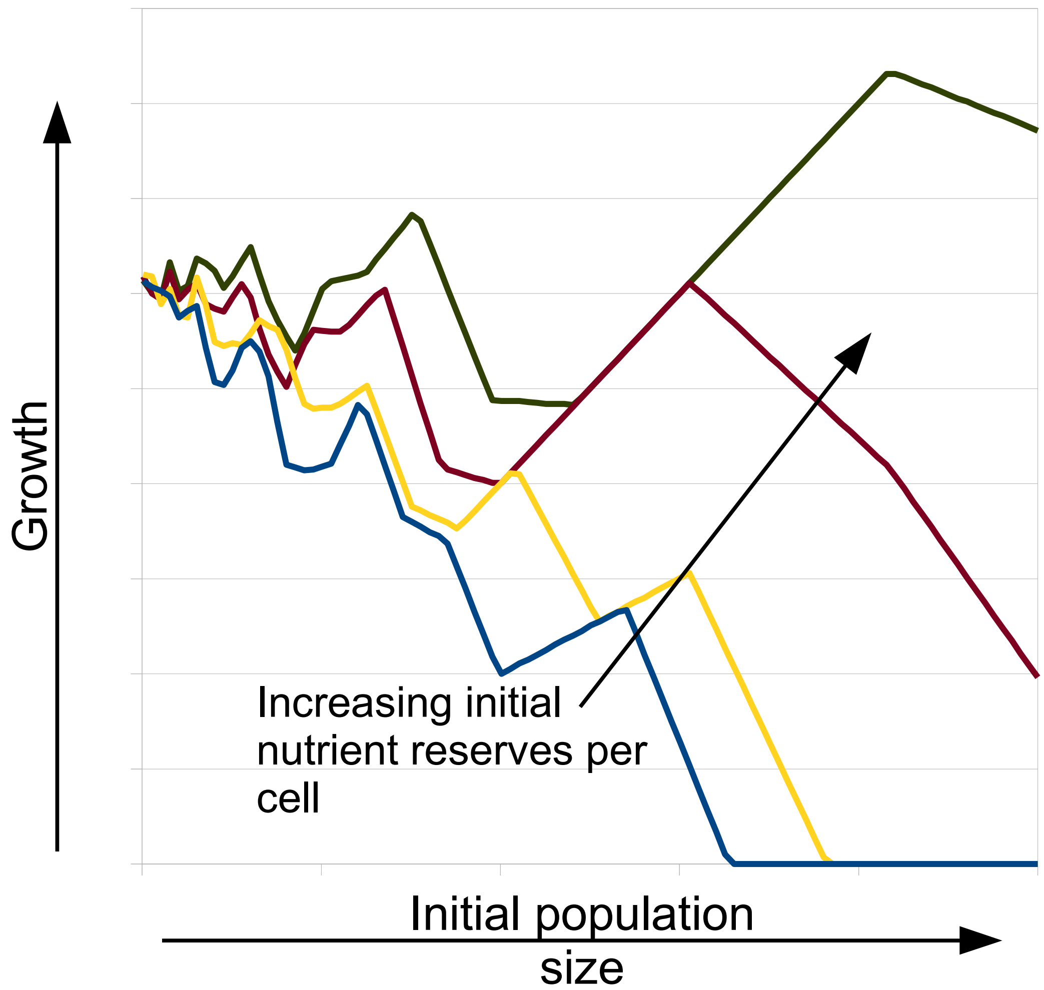


Initial population density

- Initial population size is a very interesting factor in yeast growth.
- Most yeast growth calculators take this into account
- Observations:
  - Agitated and non agitated starters show different growth yields over changing pitching rate
  - Non agitated starters have a distinct optimal pitching rate
  - Agitated starters show fairly constant growth over pitching rate until it drops off. (Unless starter is deficient in a given nutrient in which case more initial yeast can aid growth)
- Just like growth, expressing pitching rate as B/g (billion per gram of initial extract) is more useful than M/ml (million per milliliter) (Billion per Plato and liter would be an equivalent unit)

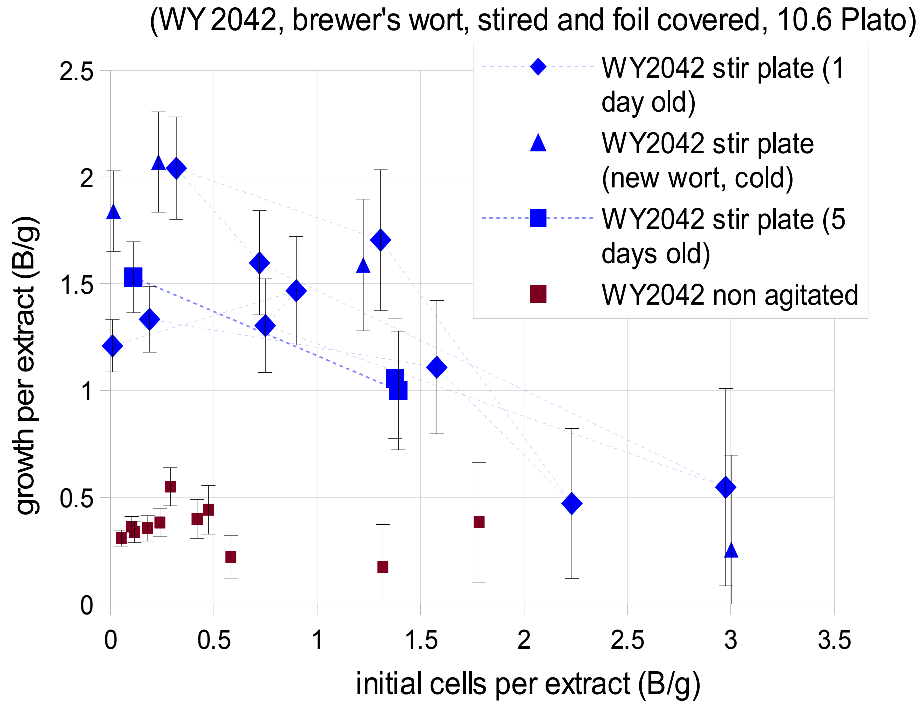
The Theory

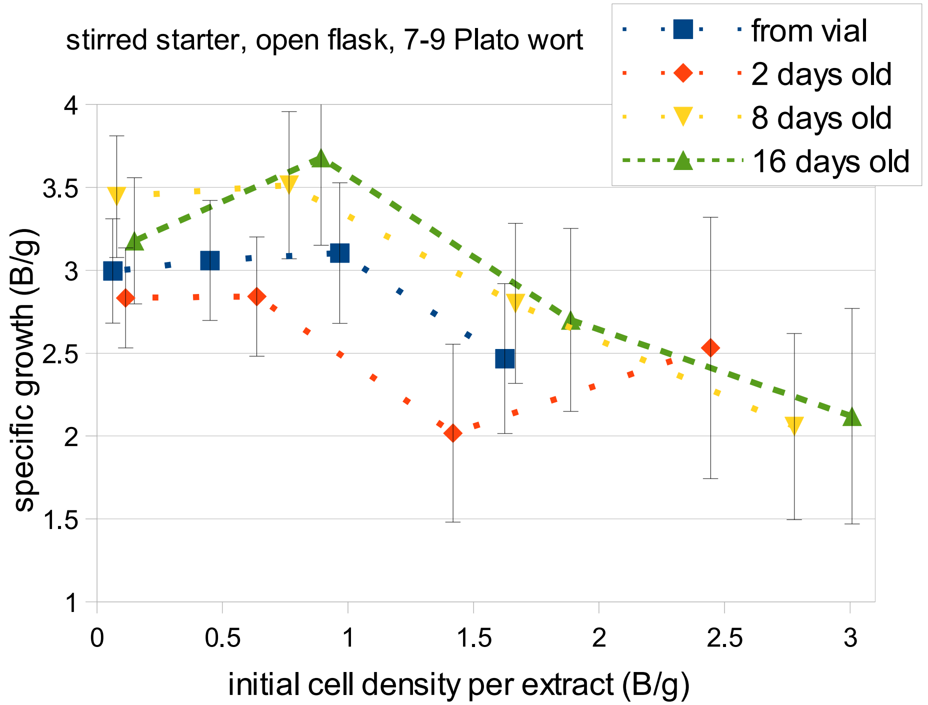
- Chart on the left is the result of a simple yeast growth simulation
- The smaller the initial population the more the wort nutrients affect yeast growth
- Very large populations don't grow well since many cells consume nutrients but only few consume enough for growth.
- The peaks and valleys are result of somewhat synchronized growth cycles



Observations

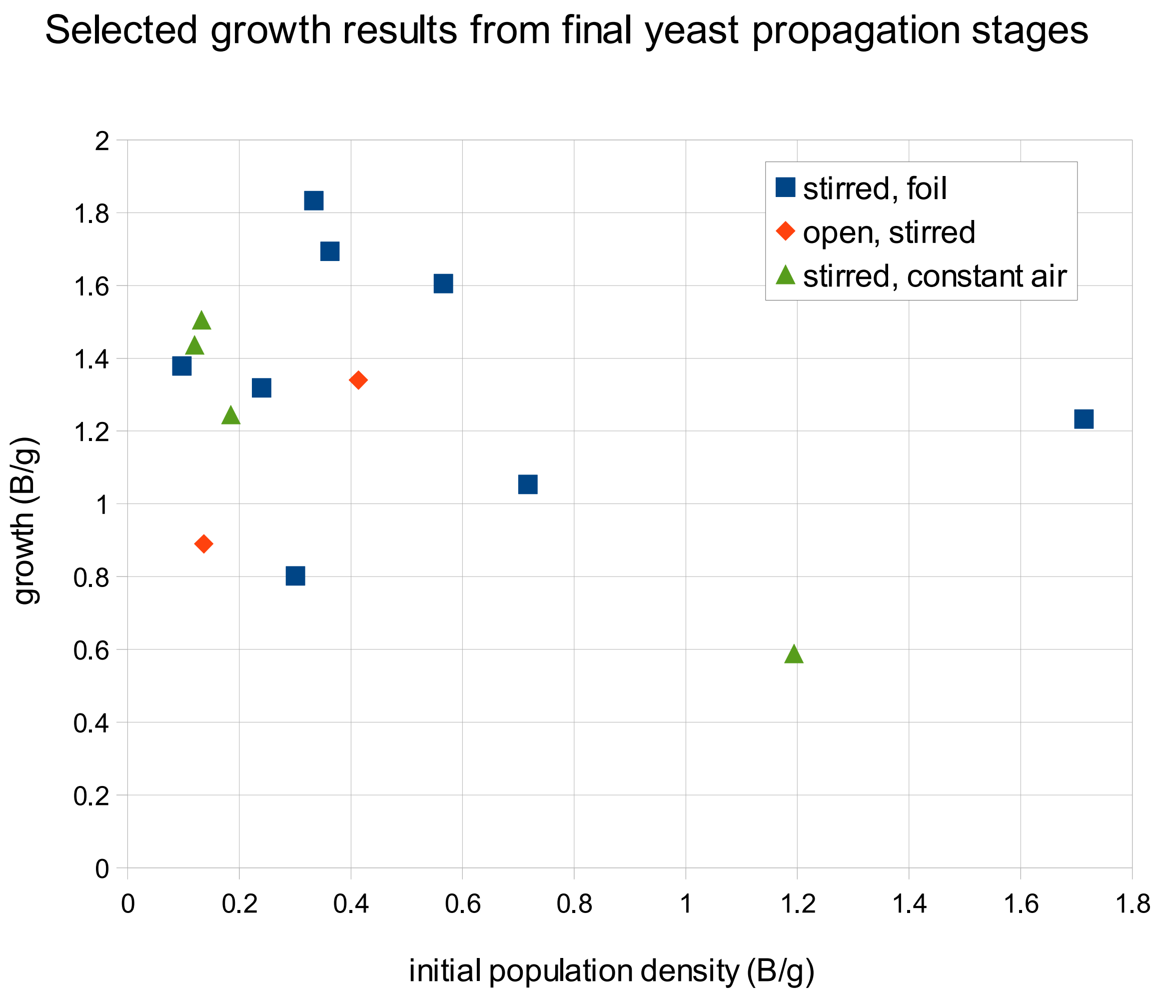
- Two different yeasts (WY 2042 and WLP 036)
- Notably different growth factors (2x difference)
- But WY 2042 is older series and used lightly hopped brewer's wort. WLP 036 series used DME only wort and was done in sets of 4 side-by-side





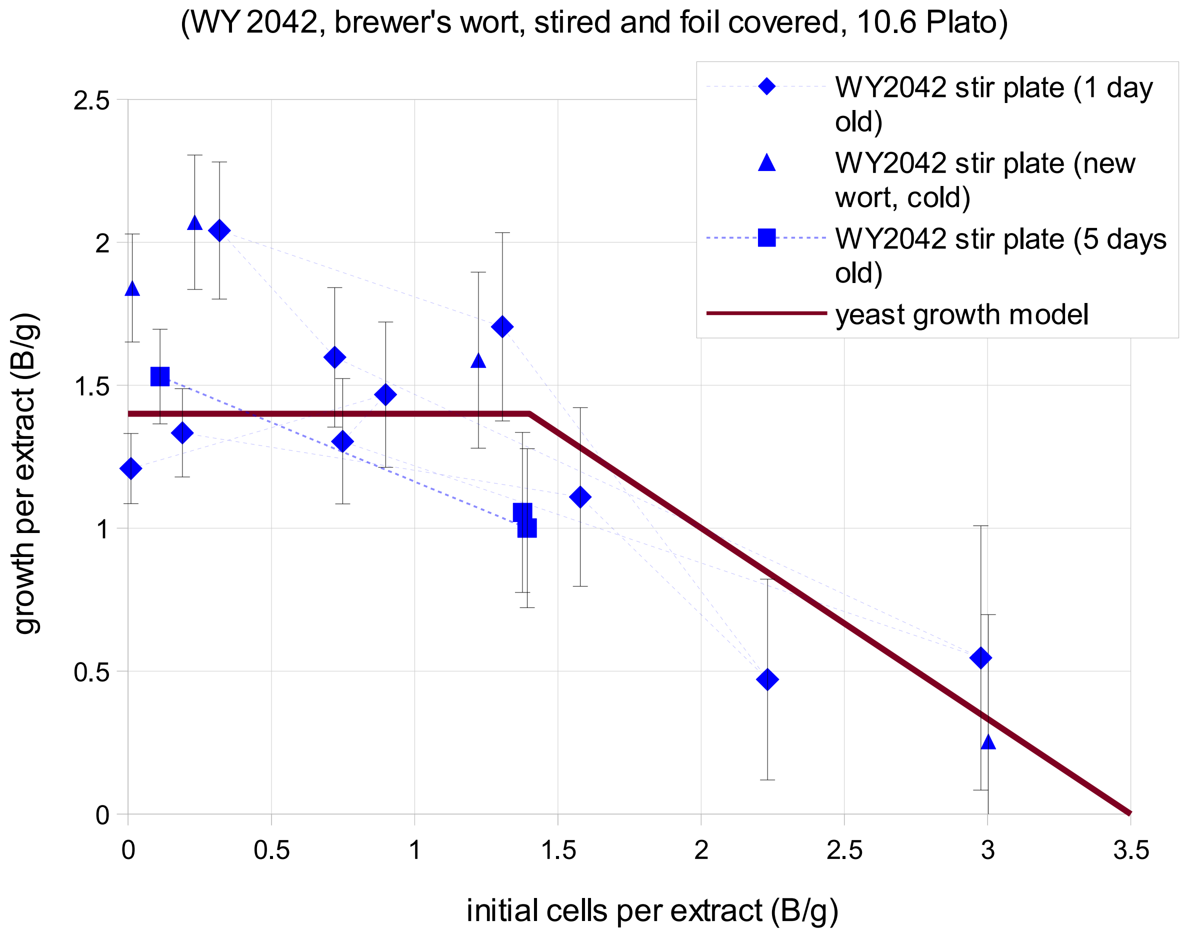
Practical yeast propagation observations

- Data from a few final yeast propagation stages
- No clear pattern even between similar types of starters
- This makes practical yeast growth prediction difficult
- Propagation medium was always leftover wort, not pure DME based wort



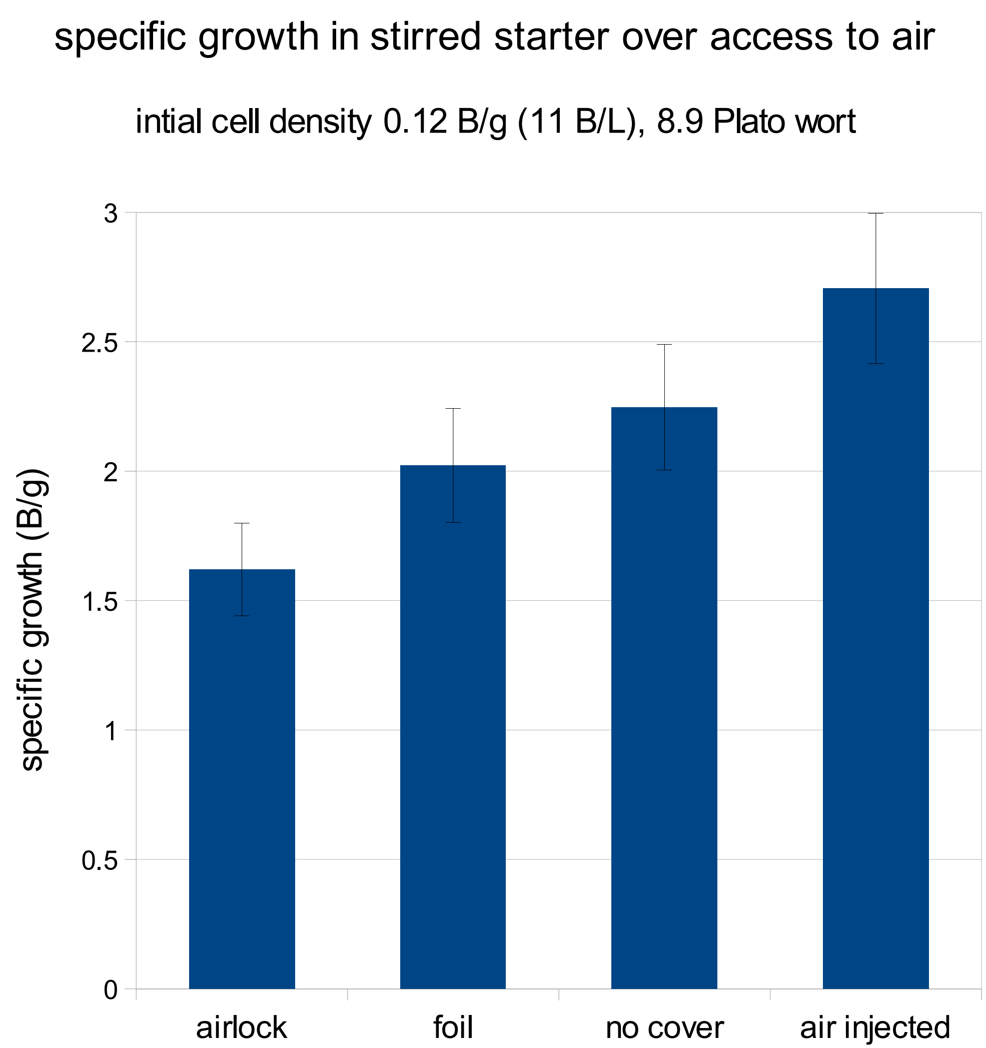
Yeast growth model?

- Based on data I had last year I proposed a growth model for stirred starters
- Implemented by Brewer's Friend and Yeast Calc
- For foil capped stirred starters only.
- Good enough for general use
- Does work well for my general brewing
- New data suggests that for a better model a lot of characterization is needed



Yeast growth and Access to Air

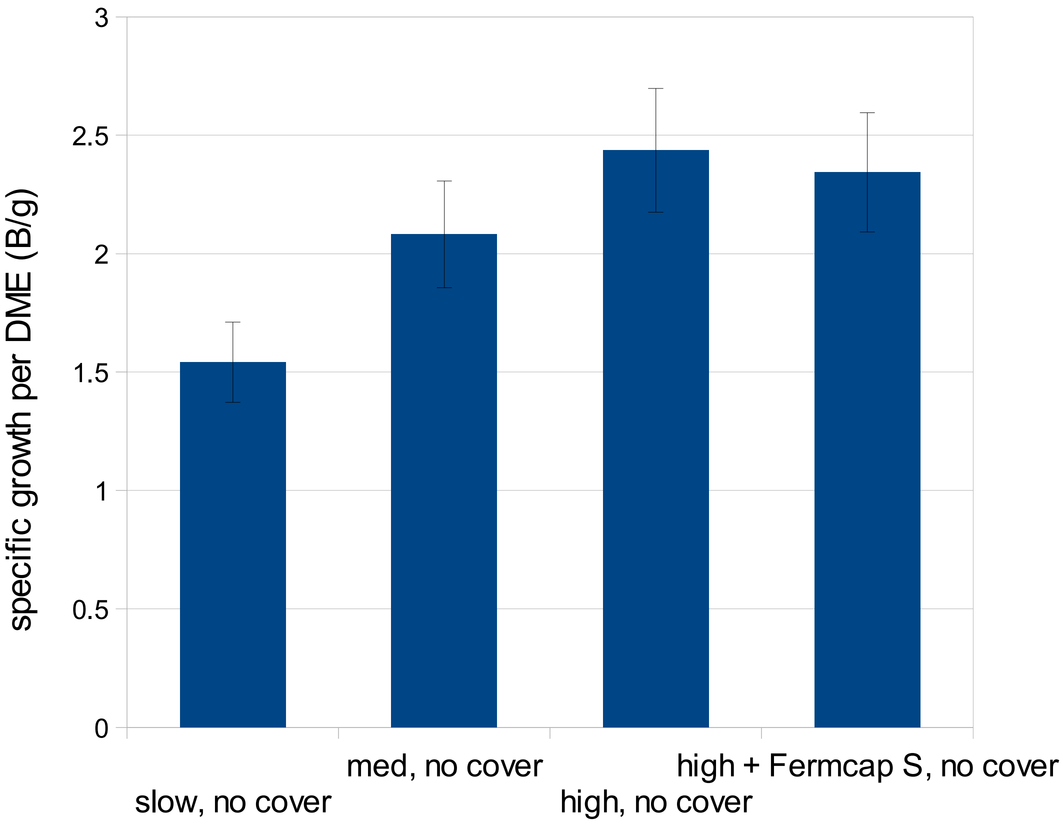
- Access to air ranged from airlock to active air injection
- The better access to fresh air → the more yeast growth
- Likely the result of increased amounts of aerobic respiration and increased sterol production



Yeast growth and Stir Speed

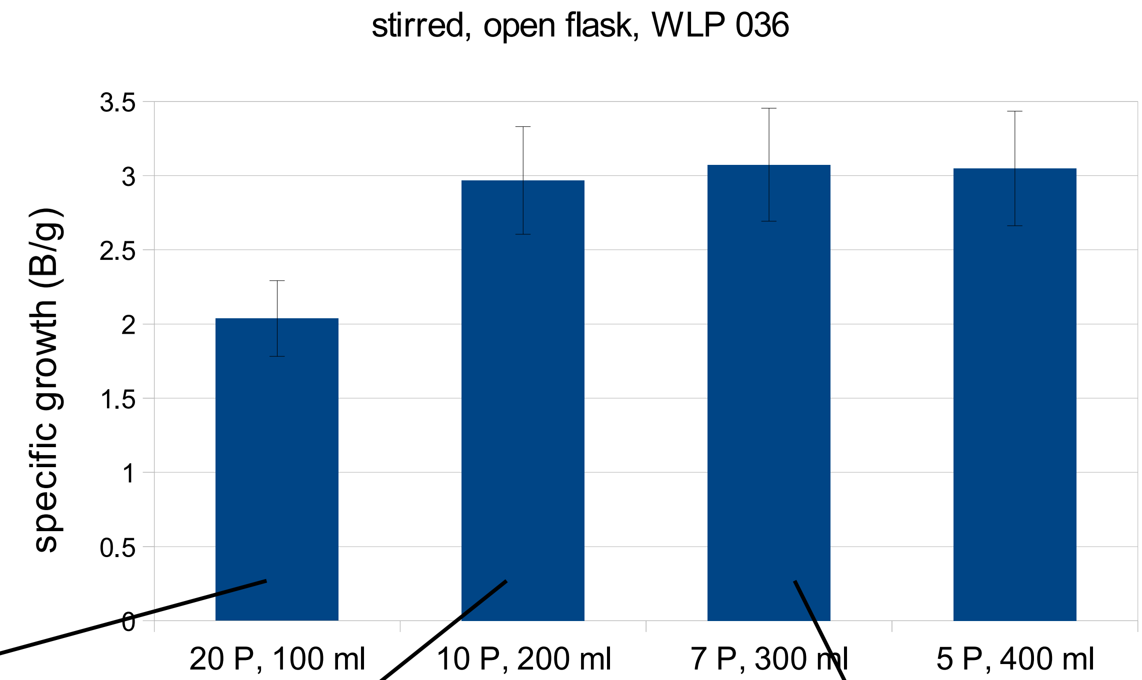
- Stir speed has a similar effect as access to air
- The faster, the more yeast growth
- Fermcap S was only tested to see if reduction of foam would make a difference
- Neither starter foamed a lot, though

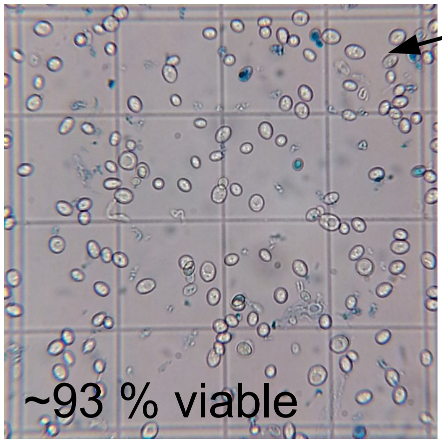
(8.8 Plato unhopped wort, initial cell density 0.11 B/g, 260-320 ml wort, 500 ml flask, WY2042)

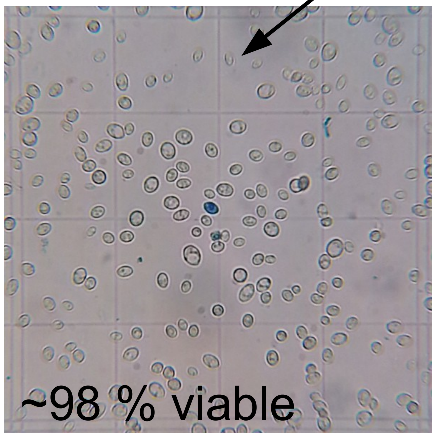


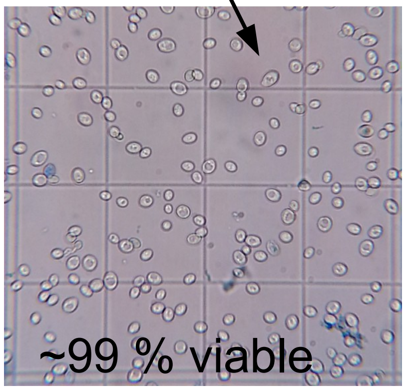
Original Extract
(Starting Gravity)

- If stir speed and access to air make a difference what about changes in starter volume while the extract is kept constant
- Result: high gravity starters don't work as well
- Also impacts yeast health









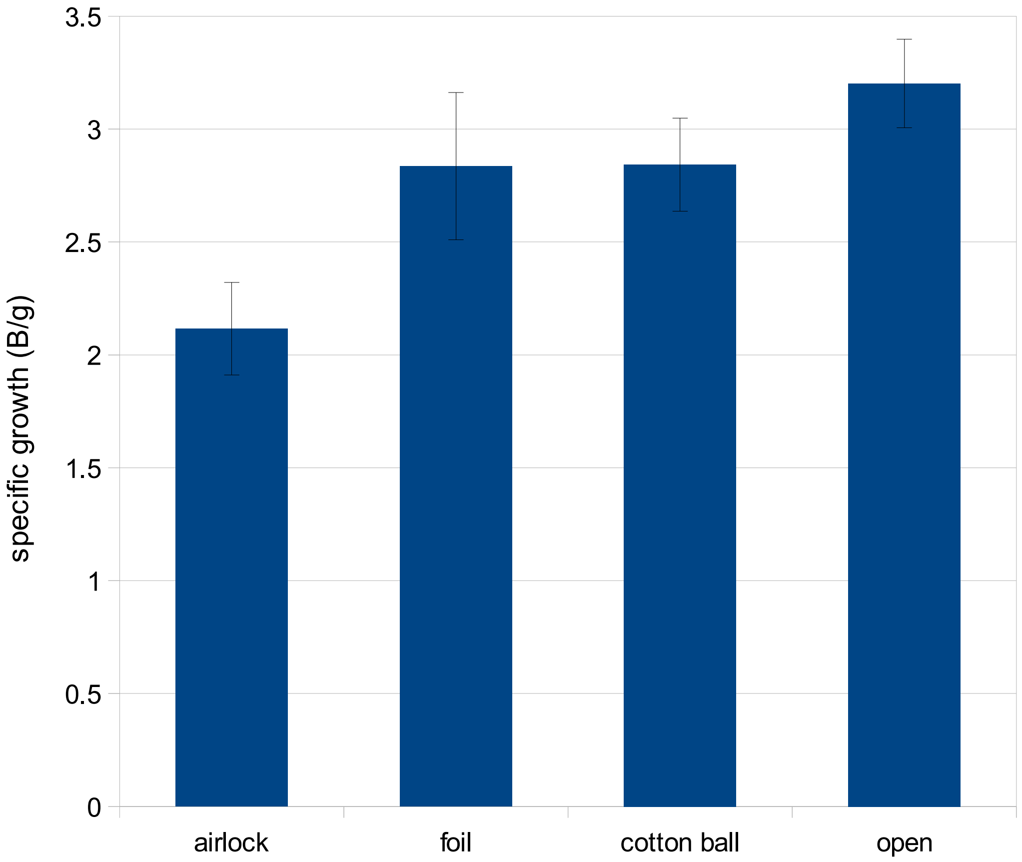
Quality vs. Quantity

- So far the experiments only considered cell growth as a metric
- But brewers care more about how well yeast ferments their wort
- To answer that question yeast propagated under different conditions was also used for fermentation tests
- Fermentation test used VHGW (Very High Gravity Wort) to stress the yeast
- Starting extract was about 30 Plato (~1.130 sg)
- Fermentation progress was monitored via weight loss (CO_2 escape) during fermentation

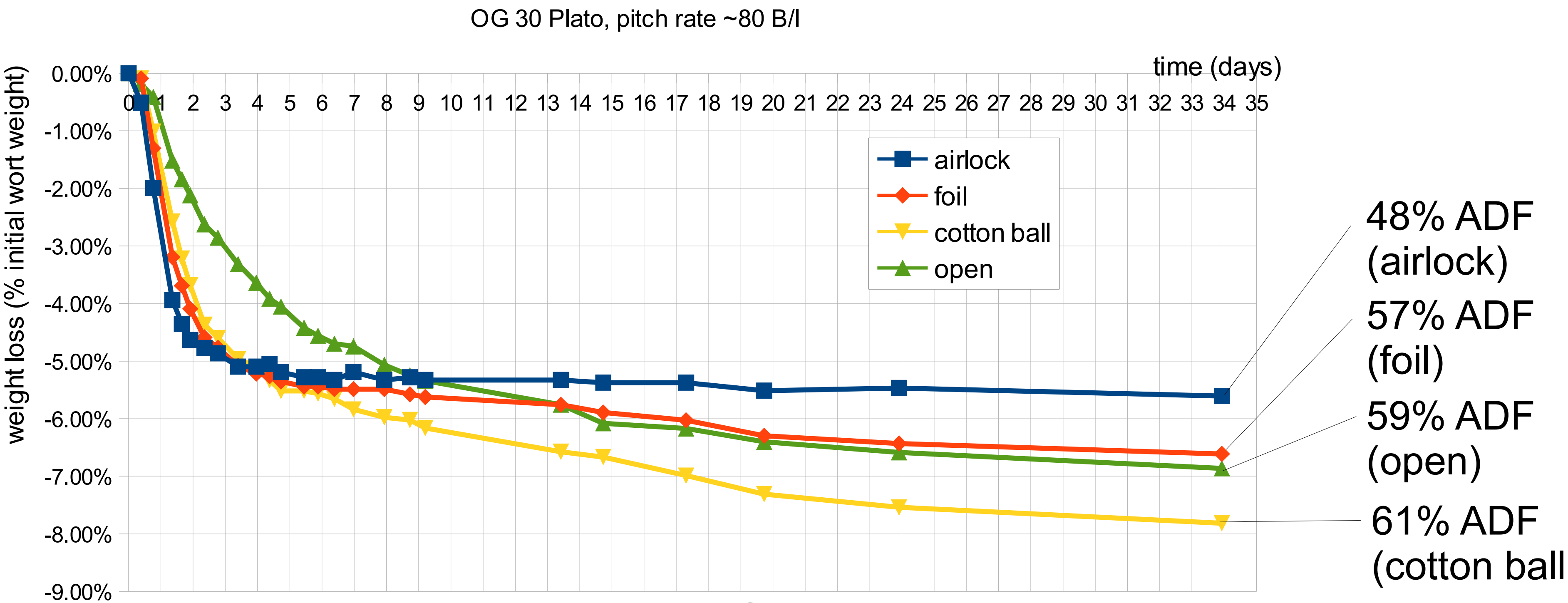
Access to air and fermentation performance

- This experiment was done with WLP 036
- The yeast growth was as expected: better access to air → better growth
- But test fermentation was not as expected:
  - Airlock covered yeast took off the fastest
  - Cotton ball covered yeast had best attenuation
- Airlock covered yeast showed notably less viability after 6 days

(WLP 036, 500 ml flask, ~220 ml wort, 8.5 Plato)

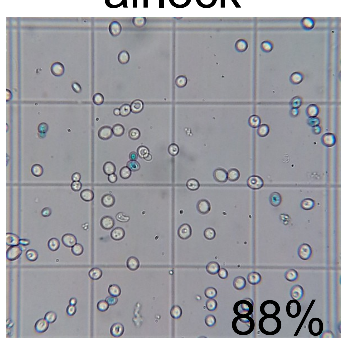


Access to air and fermentation performance

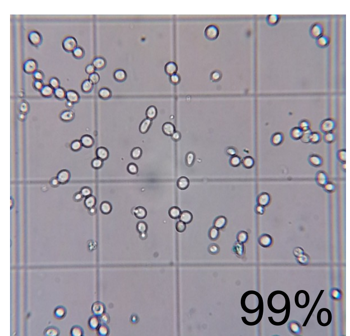


Viability on day 6

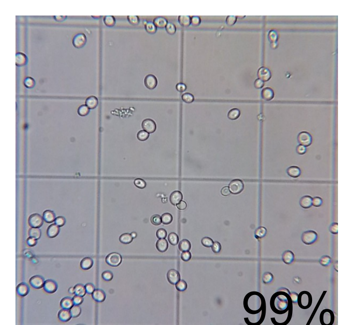
airlock



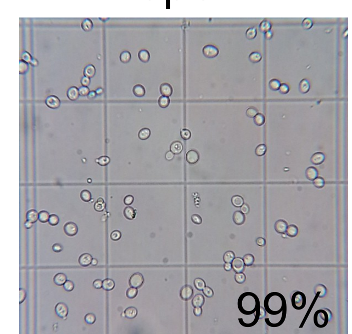
foil



cotton ball



open

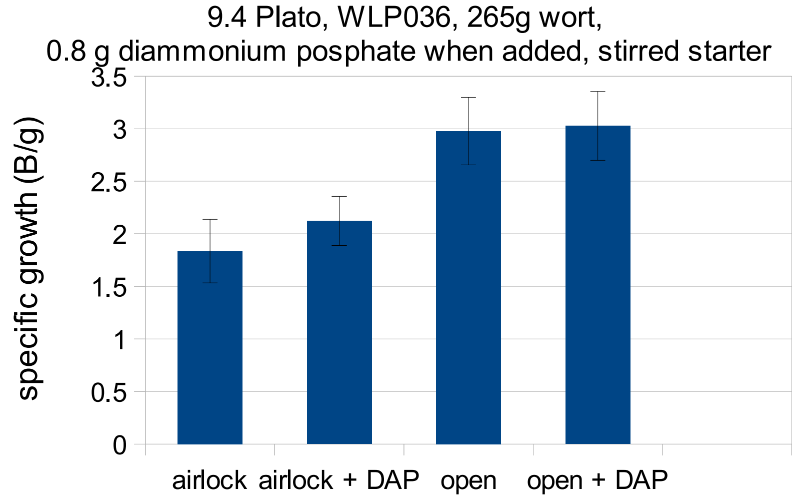


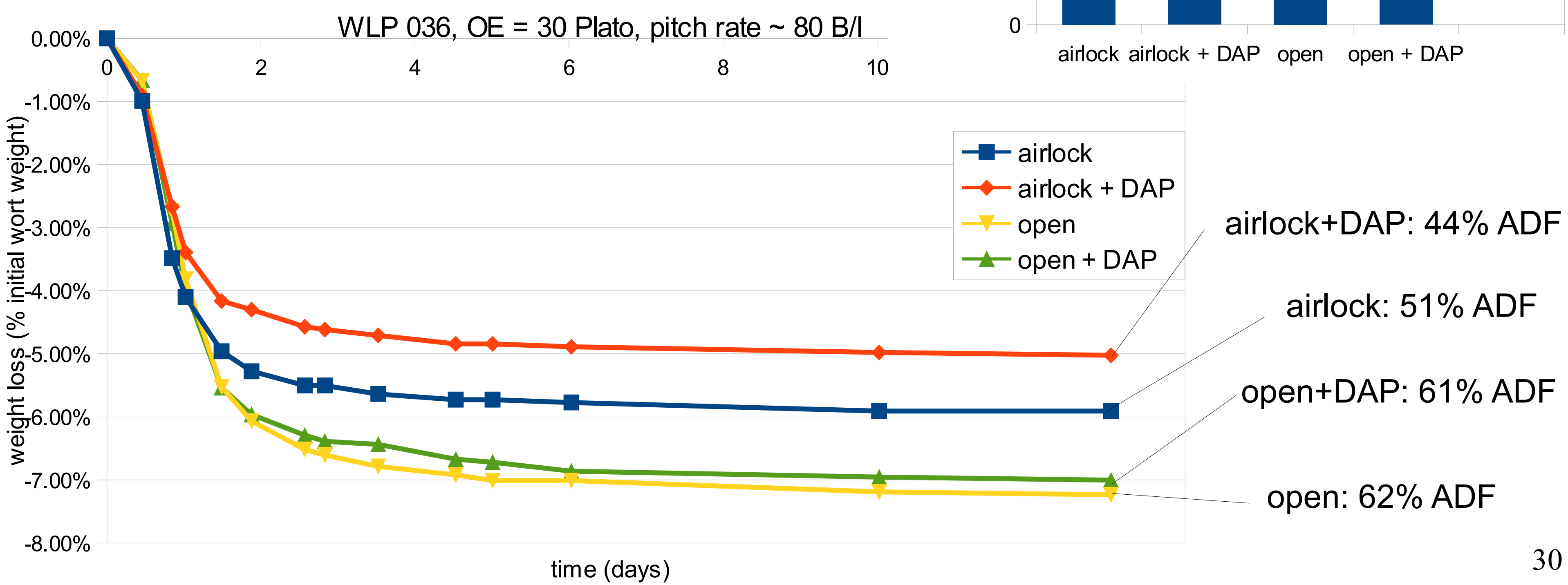
The problem of enhanced yeast growth

- Starter wort provides limited amount of nutrients
- Yeast needs more than just the energy it gets from metabolism
- The more yeast is grown the more yeast have to share the limited pool of nutrients
- Resulting yeast population may be larger but each individual cell might be low on certain nutrients e.g nitrogen or zinc
- Yeast nutrient additions may be beneficial
- The yeast growth and fermentation performance experiment was rerun with the addition of DAP (diammonium phosphate) but results were not as expected

Added nitrogen

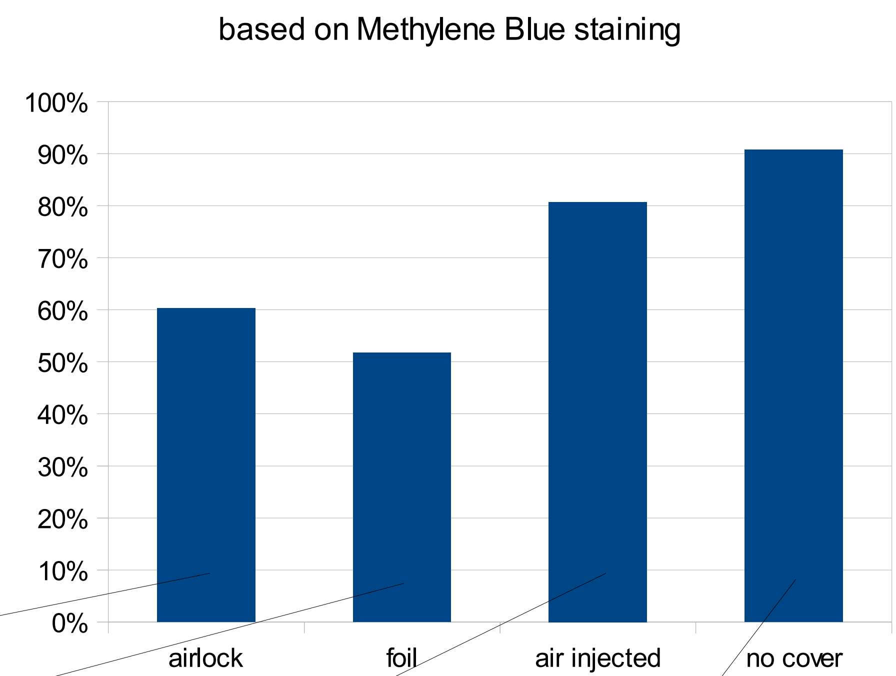
- Yeast with added nitrogen resulted in less attenuation
- The open propagated yeast did not have a slow start. This is different from the previous experiment
- More work needed to make sense of these results !!

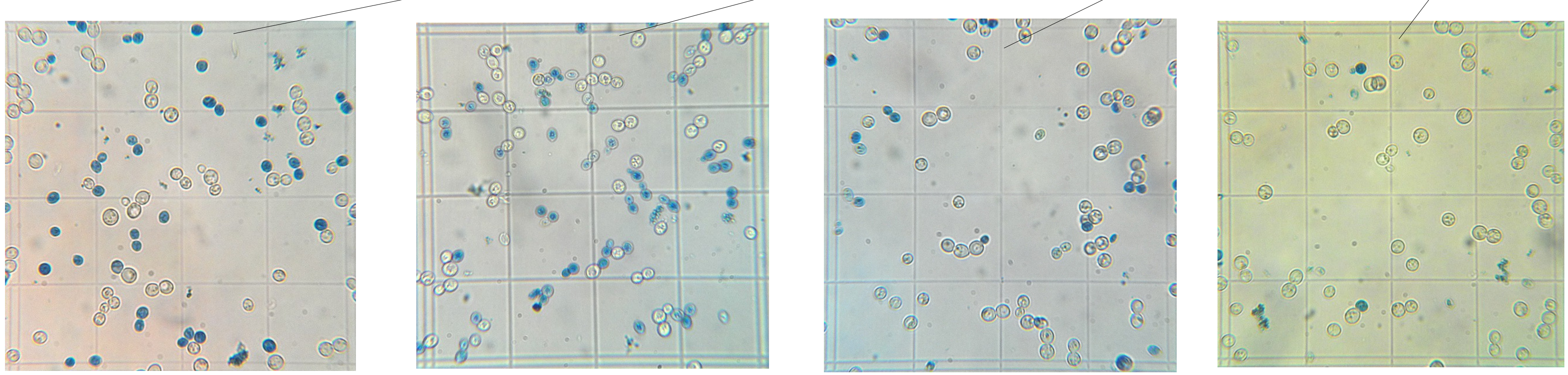




Final Viability

- Results from a fermentation test with yeast propagated with airlock, foil, open and air injected.
- Yeast sediment was stained with methylene blue after 15 days of fermentation
- shows tendency that more air access leads to more alcohol resistant yeast





Conclusions

- Access to air during yeast propagation helps growth and (more importantly) improves alcohol resistance
- Different strains may show different growth per available extract. This makes predictions difficult.
- Starter pitch rate does not have a significant effect on growth in stirred starters → stepping up may not be needed
- More work is needed on
  - Yeast propagation for best fermentation performance
  - Growth differences between strains

Links

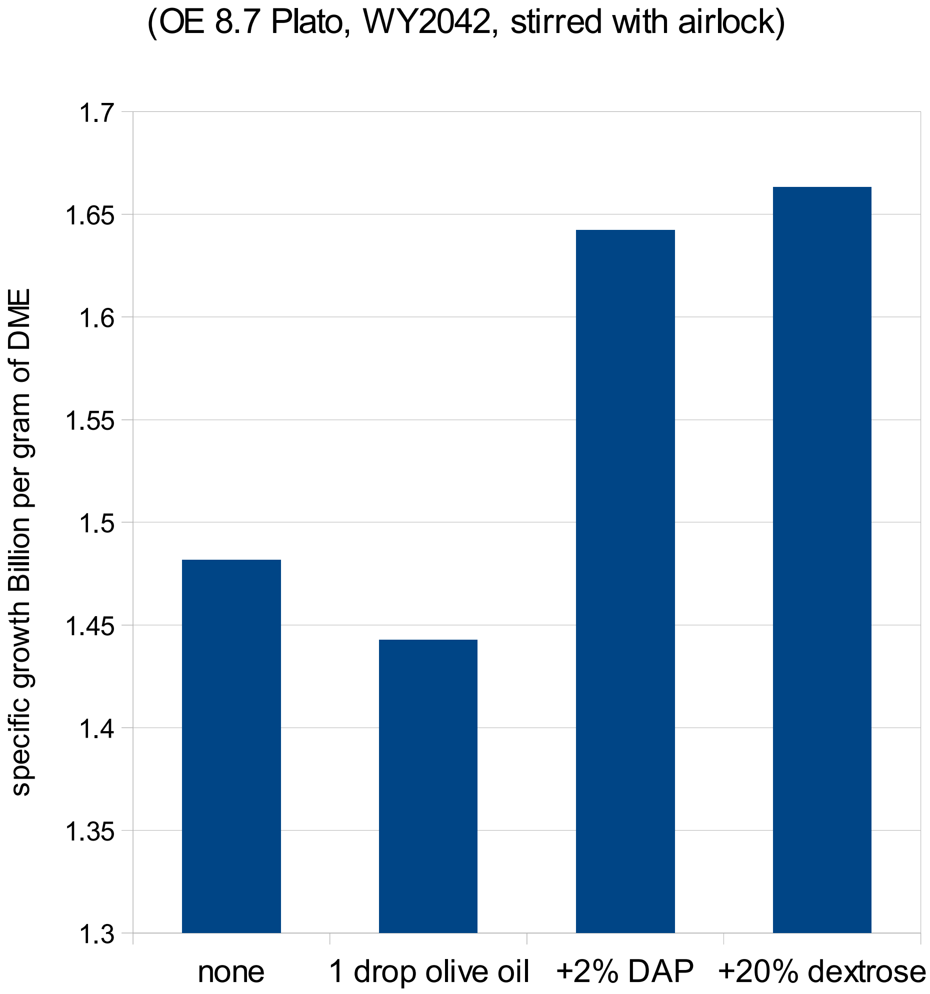
- Detailed publication of the experiments:
    - http://braukaiser.com/blog/brewing-science/
- Thomas Kurz' dissertation:
    - http://tumb1.biblio.tu-muenchen.de/publ/diss/ww/2002/kurz.pdf
- An Examination of the Crabtree Effect **in** Saccharomyces cerevisiae (Barford and Hall, 1978):
    - http://mic.sgmjournals.org/content/114/2/267.full.pdf+html

Backup

Even more data

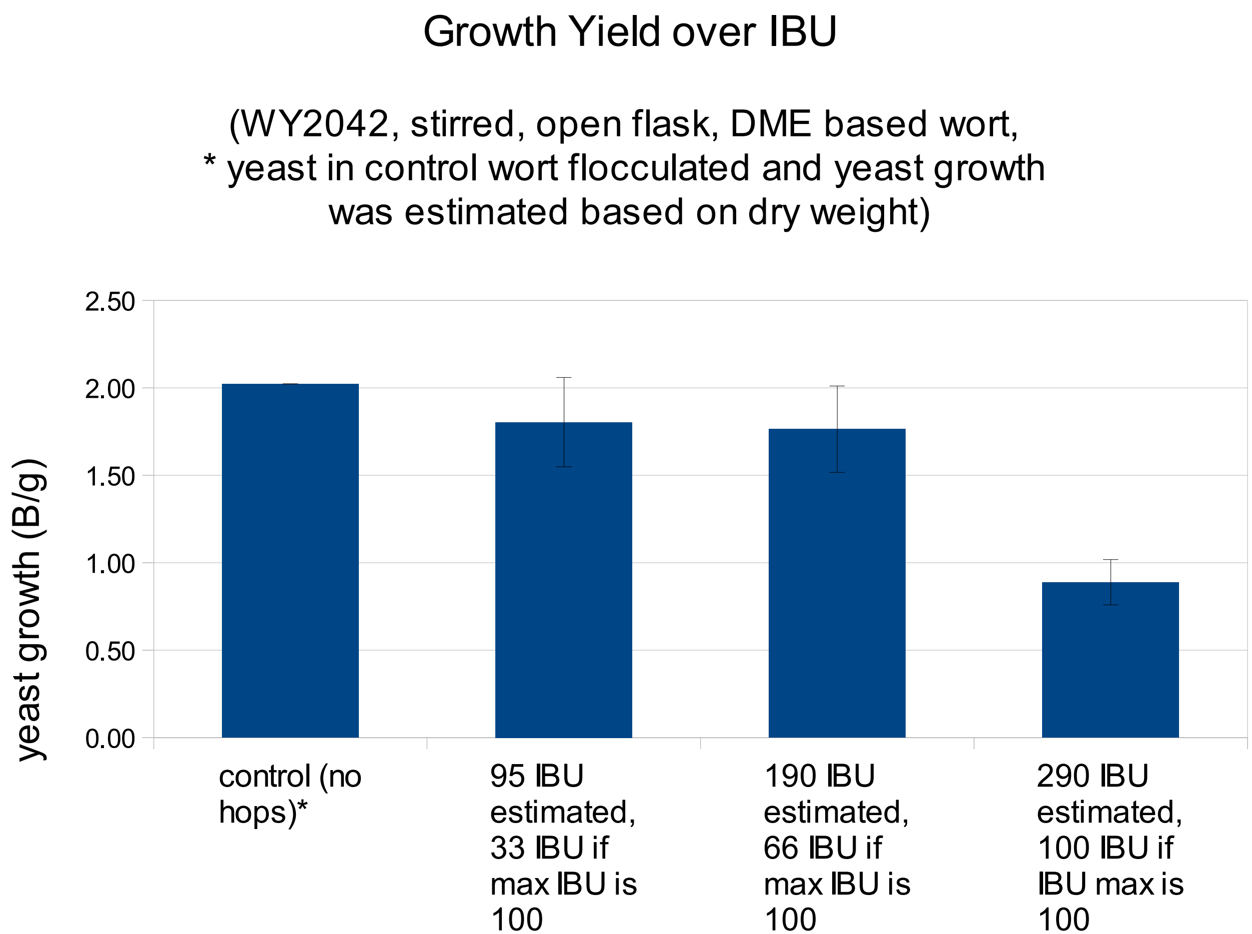
Additional nutrients

- Yeast growth is limited by nutrients
- Depending on the nutrient additional supply may or may not boost yeast growth
    - Olive oil was added to supply sterols since the starter had limited access to air
    - DAP (diammonium phosphate) adds nitrogen for protein synthesis
    - Dextrose (corn sugar) adds more energy for yeast growth



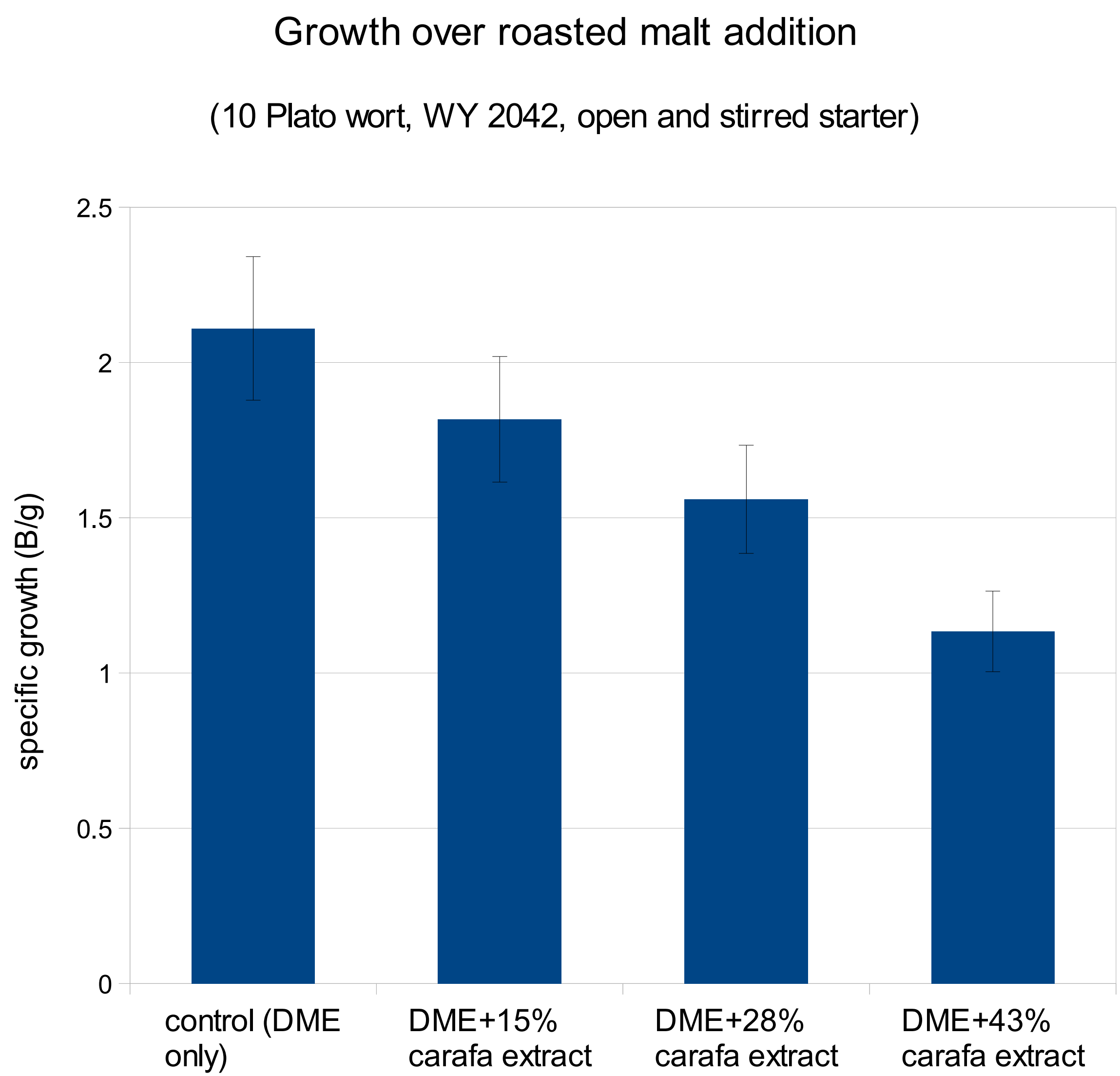
Hops (iso alpha acids)

- Iso alpha acids do affect yeast growth negatively
- Impact is not significant until very high bitterness (> 0.6 oz/gal, 13 % AA)
- Likely not a problem for practical yeast propagation even if wort from previous batches is used.



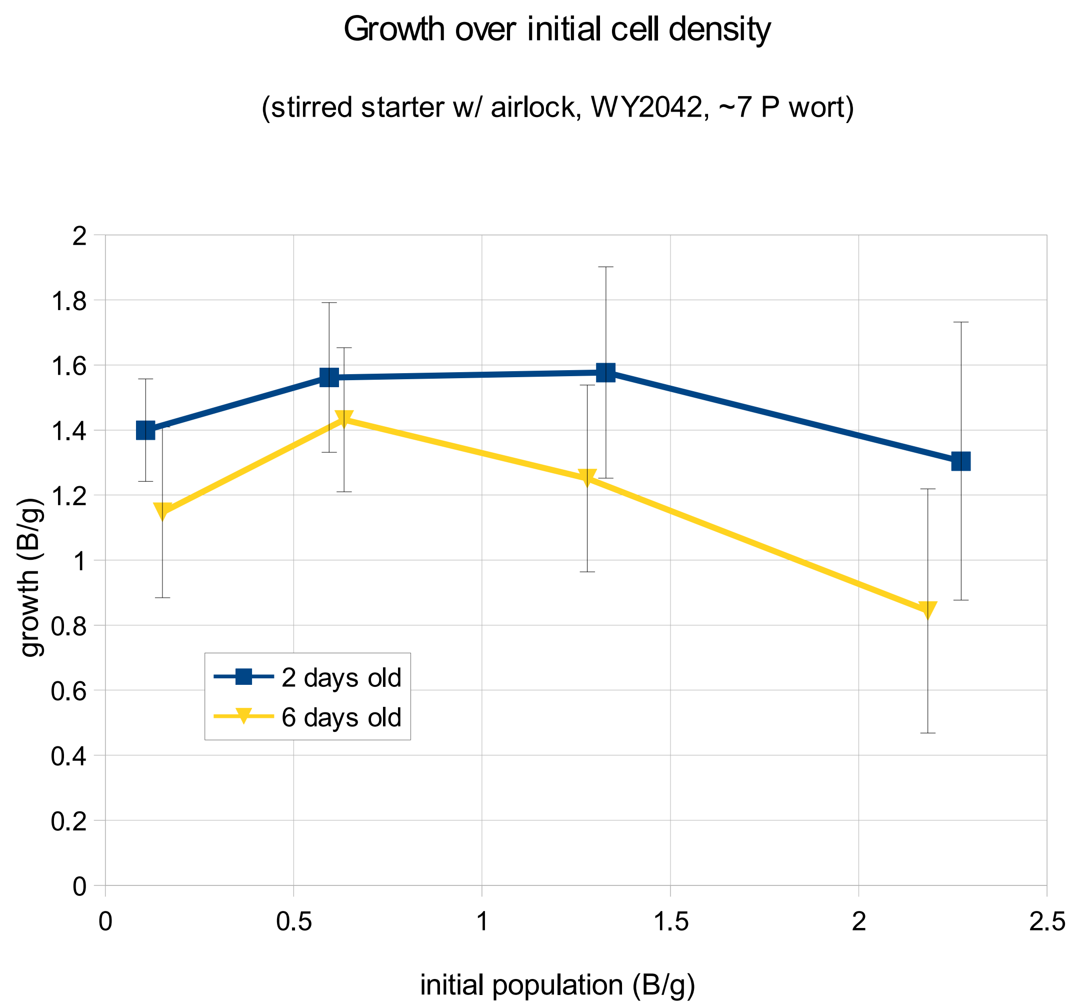
Dark Malts

- Melanoidens effect yeast growth negatively
- Significant effects only observed for 15 % dark malt extract
- The experiment did not evaluate how much growth reduction came from less fermentable wort



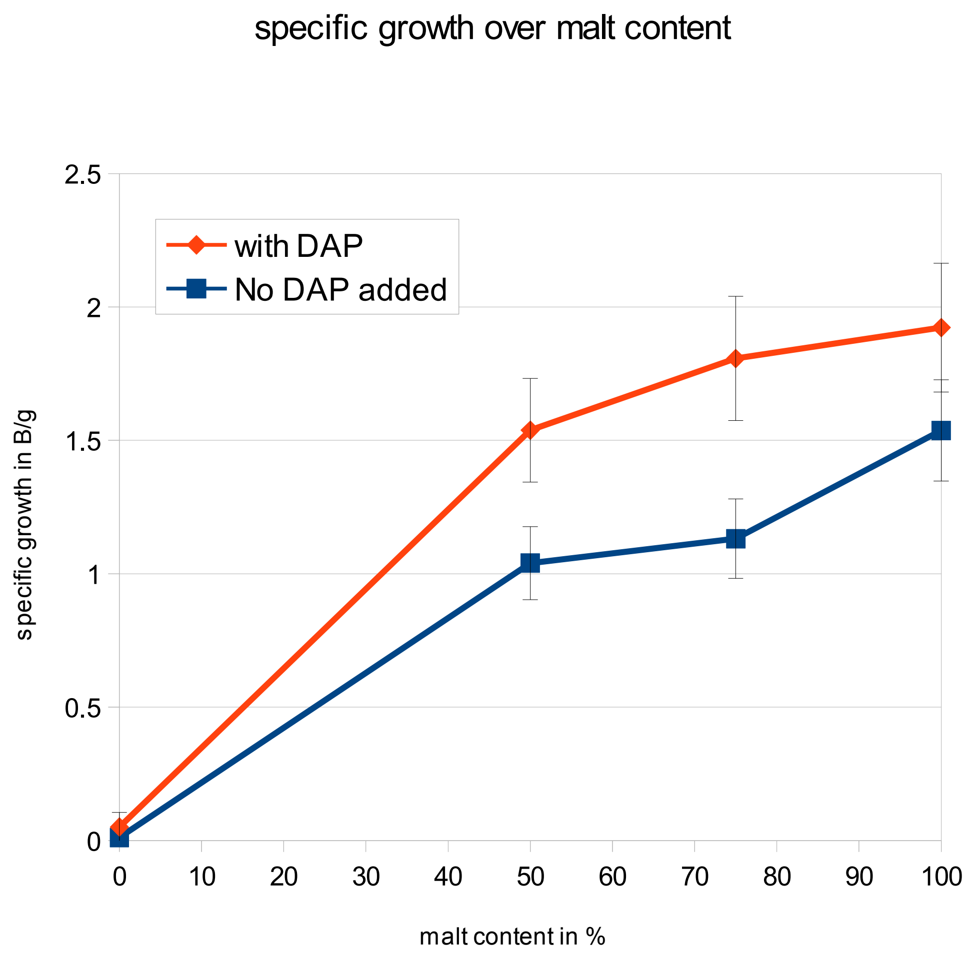
Growth in airlocked starter

- Fresh data
- Sterol reserves may be limiting growth at low initial population sizes



DME and glucose mix

- Brewer's know that all malt starters are best
- The less malt the less growth
- Even with added nitrogen



BUT

- A fermentation test showed interesting results
- More corn sugar in starter → better fermentation performance ?
- More work is needed