

Yeast-- practical tips for propagation & fermentation

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Outline

A. Yeast propagation & storage best practices

1. Yeast starters
2. Freezing yeast for storage
3. Storing yeast on agar slants
3. Wort oxygenation

B. Yeast fermentation byproducts

1. diacetyl
2. higher alcohols (fusels)
3. esters
4. acetaldehyde

Yeast starters, overview

- Goal is to produce, healthy, unstressed yeast cells in sufficient quantity to near ideal pitching rate

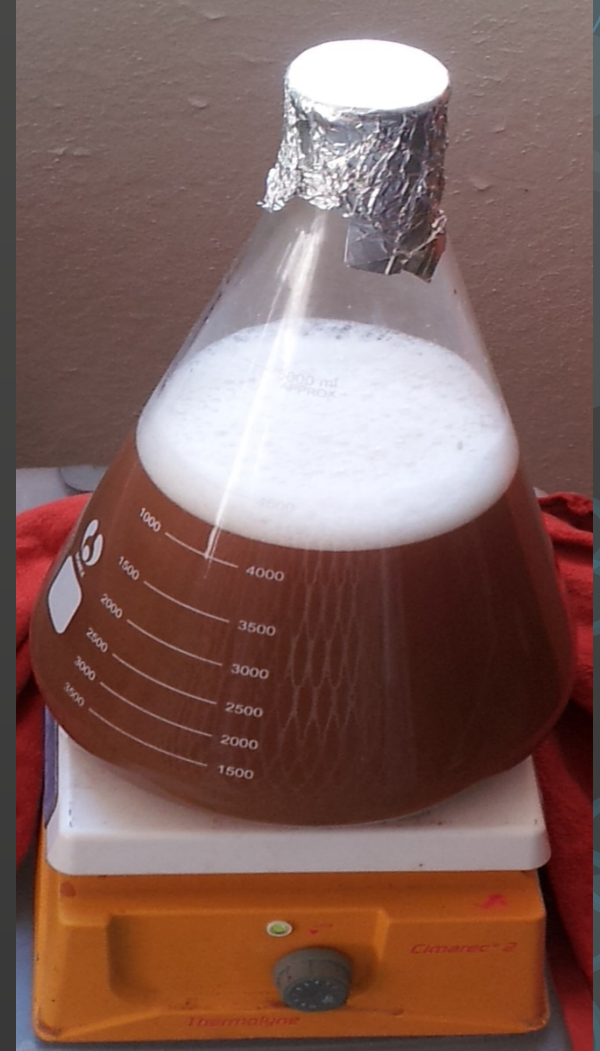
Ideal yeast pitching rate:

Ales = 1×10^6 cells / mL per ° plato

Lagers = $1.5\text{-}2 \times 10^6$ cells / mL per ° plato

- Erlenmeyer flask + stir plate has highest cell yield
- Intermittent shaking second best
- Poorest cell yield in simple starter (no shaking or aeration)

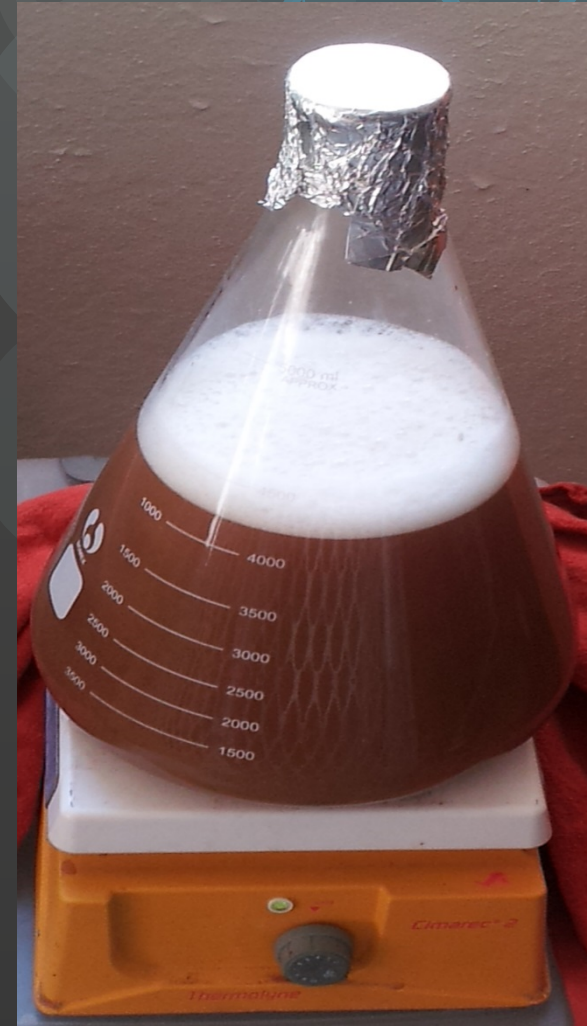
Stir Plate with a 5L flask.



Don't skimp on yeast!
Go big or go home.

Yeast starter, basic protocol

- A 10% DME solution is optimal (100g DME per 1L H₂O) OG=1.040. Boil for 10 min (Fermcap S highly recommended!) & cool.
- Grow at room temp with gentle vortex (if using stir plate) for 24-48 hours
- Crash cool to drop yeast, pour off most of spent starter wort, swirl settled yeast into a slurry, pitch

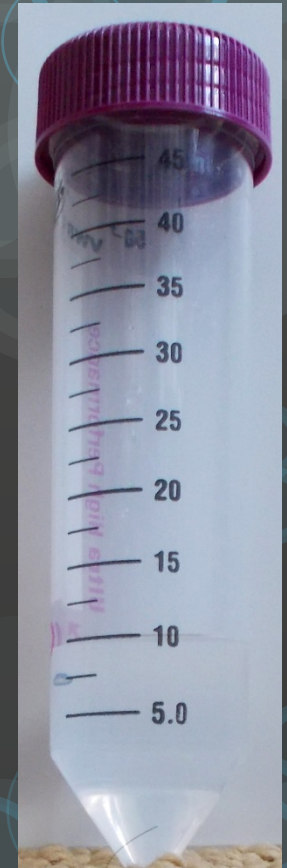


Freezing yeast for storage

- Why? -- Saves money & can have yeast bank of seasonal or discontinued strains indefinitely
- Requires ~25% glycerol (AKA glycerin) as a cryoprotectant-- Can buy glycerin in health food stores
- Freezing yeast in plastic conical tubes works very well– Can buy conical tubes online. Amazon has 50pk for \$10.



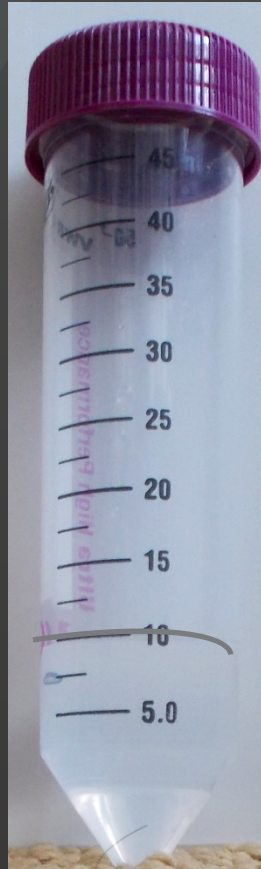
Food grade
vegetable
glycerine
bought from
organic market



50 mL conical
tube

Freezing yeast, brief protocol

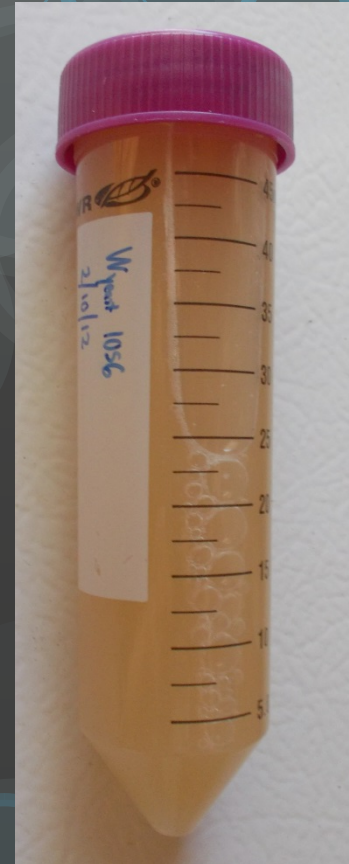
- 1) Add 10mL glycerol to 50 mL conical tubes.
- 2) Decant starter supernatant
- 3) Swirl to resuspend yeast into a slurry (want very high cell density)
- 4) Carefully pour 30 mL of slurry into conical tube to 40 mL mark, mix very well.
- 5) Place tube in freezer in small styrofoam cooler– this prevents freeze-thaw damage (if frost free freezer)



Conical with 10 mL glycerol



Settled yeast starter.
Decant most liquid



Yeast slurry with
25% glycerol
ready to be frozen

Reviving frozen yeast, brief protocol

- 1) Thaw out frozen yeast completely in fridge.
- 2) Mix gently to dislodge any suspended yeast
- 3) Add entire contents of conical to 200-250 mL prepped starter wort.
- 4) Place onto stirplate for 24-48 hours.
- 5) Add additional wort to step up to desired volume, no more than 10x dilution.

Storing yeast on agar slants

- Why? -- Saves money & can have yeast bank of seasonal or discontinued strains indefinitely
- Pros vs freezing yeast–
 - no freezing required, less space
 - can visually determine purity
- Cons vs freezing yeast
 - requires good aseptic technique!**
 - requires periodic subculturing to maintain viability
 - more prone to mutation
 - agar can be messy and is not cheap
 - can be a PITA

Materials required:



5-6in glass vial w/screw cap



Steel inoculating loop

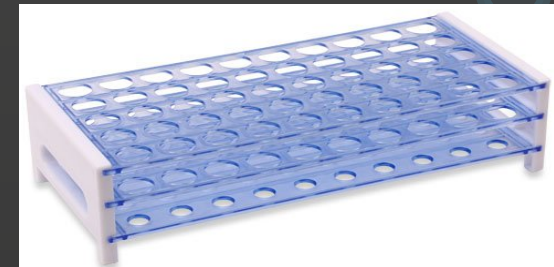


Plastic inoculating loop

OR



agar



Test tube rack

Materials recommended:

Small Erlenmeyer flask or beaker (100mL – 500mL)
Butane torch or flame source
Pressure cooker

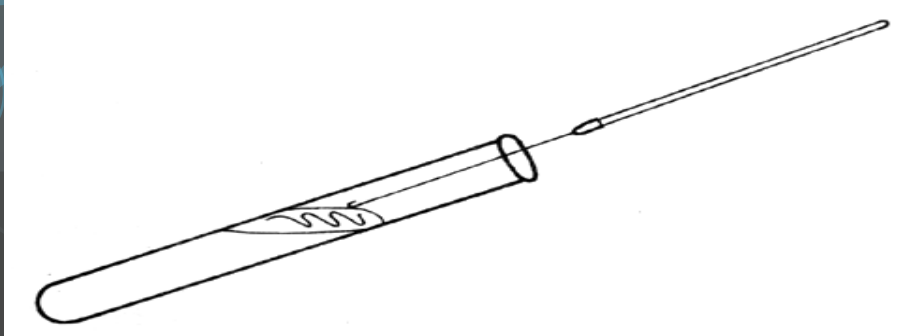
Pouring agar slants, brief protocol

- 1) Make sure glassware is clean.
- 2) Prep starter wort at usual concentration but add 1.5% agar (e.g 3.75g agar per 250mL wort)
- 3) Boil for 15 min. Agar will dissolve in a few. Better yet, place tubes in pressure cooker to sterilize.
- 4) If using flask or beaker, using mitts, carefully pour $\sim 1/2$ vol of melted agar + wort into glass tubes in rack. If boiling in pot, slowly draw out liquid with metal turkey baster and squirt gently into tubes
- 4) Cap tubes and invert on raised surface (e.g thin book)
- 5) Let solidify for a few hours. Cap and store at RT or in fridge.



Inoculating & reviving agar slants, brief protocol

- 1) From WL vial or Wyeast packet, insert sterile loop. If metal loop, flame til red hot to sterilize.
- 2) Remove loop, and working quickly, gently cross the agar slant surface using as much area as possible.



- 3) Apply cap loosely, and leave at RT for a few days.
- 4) When opaque, white colonies are visible, tighten cap and place in fridge.

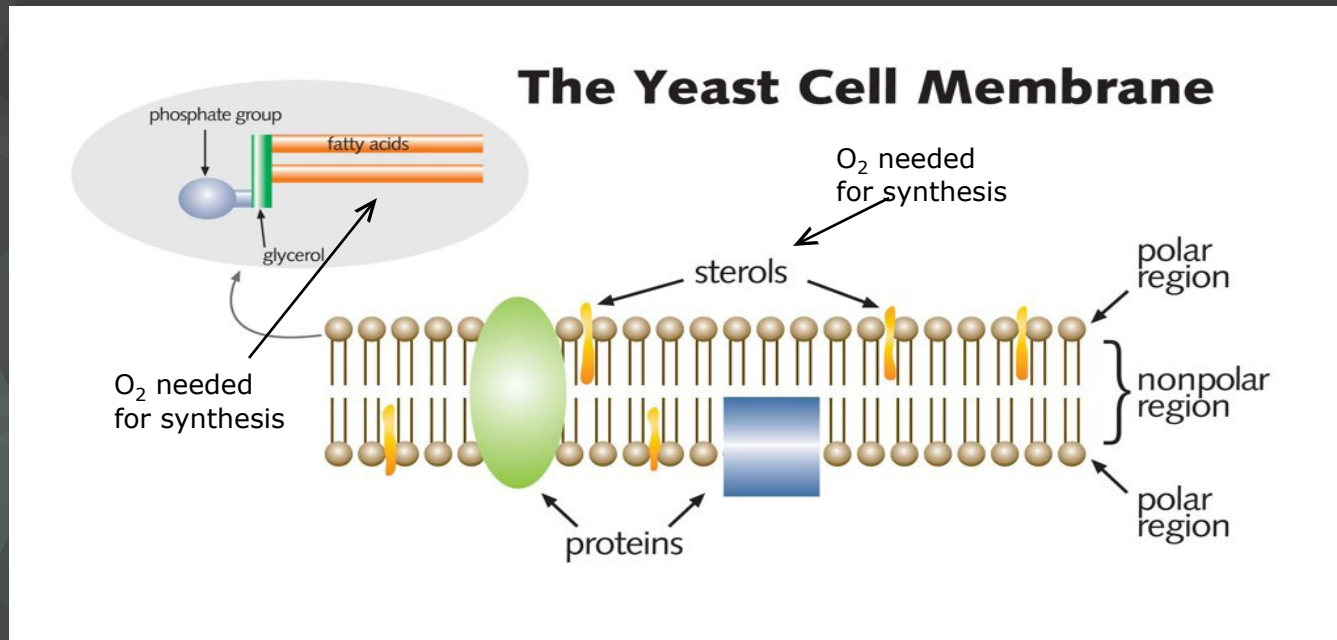


Agar slant with yeast colonies

- 5) To restart, take a hearty loopful of growth in 25-100mL fresh wort. Mix very well.
- 6) When fermentation is evident, transfer to larger volume, no more than a 10x dilution.

Wort oxygen

- O_2 needed to synthesize cell membrane sterols and unsaturated fatty acids
- Yeast growth is sterol limited
- Wort oxygenation at onset is crucial. 8-10ppm O_2 desirable for typical wort gravities. O_2 requirement is strain dependent.
- Poor oxygenation results in poor yeast vitality and poor fermentation performance



Using pure O₂ is easiest & most effective way to oxygenate

- Using an oxygen canister with a sintered 0.5 µm stone is the fastest, most sanitary method. 1 min is more than enough.
- Shaking the carboy (~5 min) works fine, if you love a workout
- Pumping filtered air (e.g. with an aquarium pump) is fine, just takes a while (~25 min)



O₂ stone costs ~45\$. O₂ tank is \$8-\$9 and lasts ~15 batches.

Yeast fermentation byproducts

- Crucial sensory components of beer

diacetyl



Higher alcohols (fusels)



esters

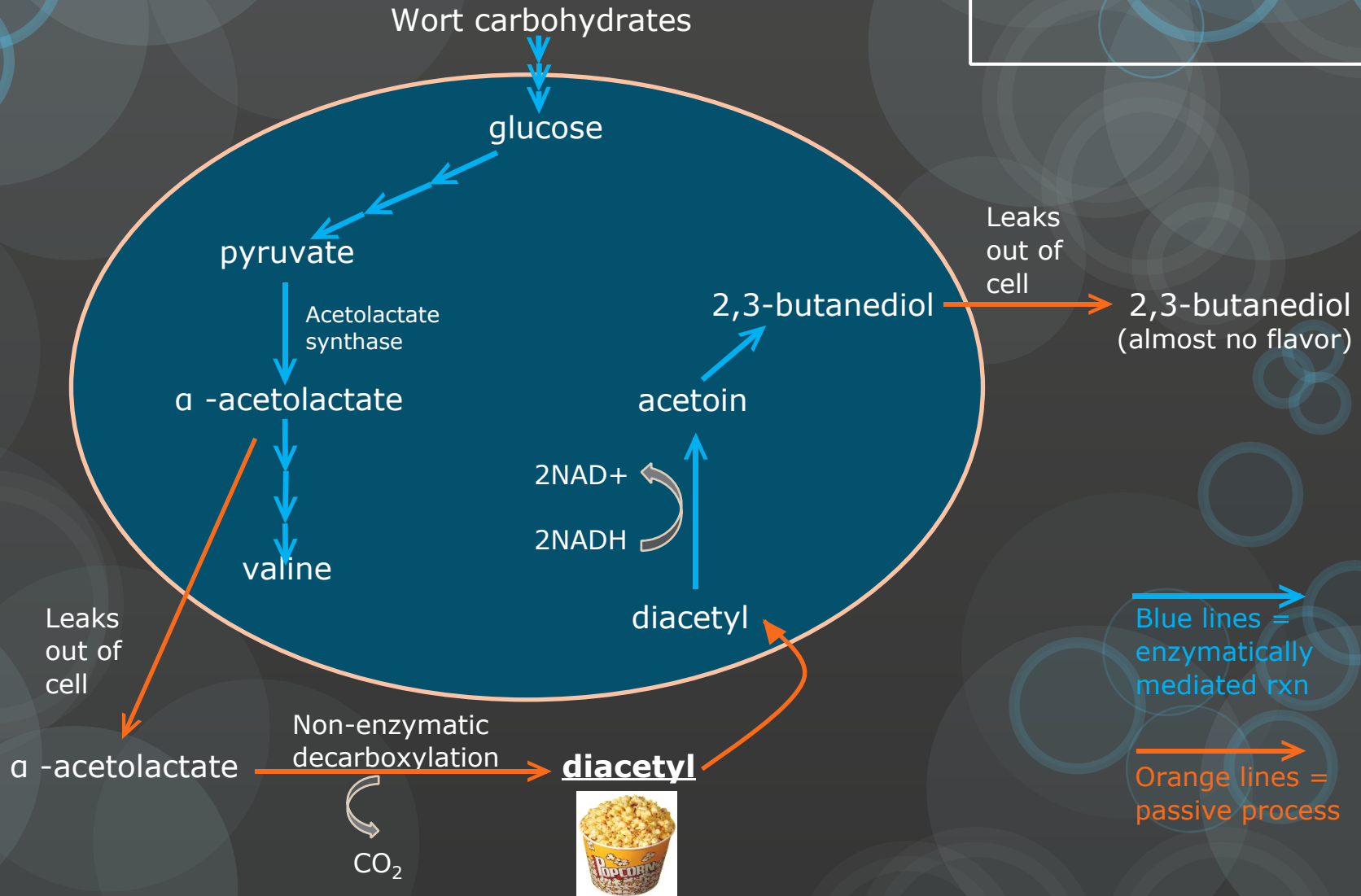
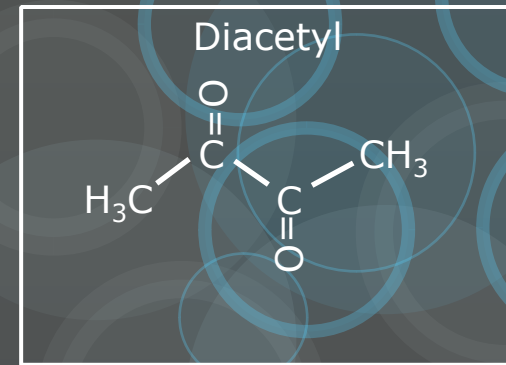


acetaldehyde



Diacetyl (2,3 butanedione)

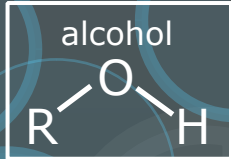
- Is highly flavor active (buttery) VDK, along with 2,3 pentanedione.
- Low flavor threshold of 0.02 mg / L
- Produced as an intermediate in valine synthesis



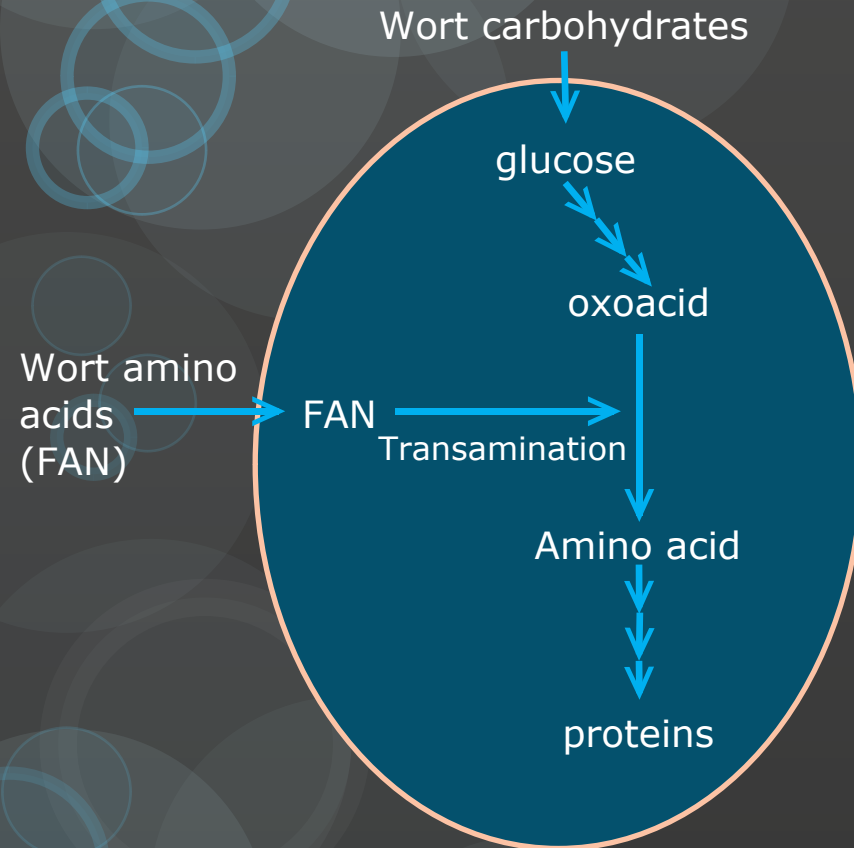
Factors affecting diacetyl formation & removal

- Provide wort with adequate FAN (not a problem with all malt grist)
- Healthy pitching yeast in proper quantity will facilitate diacetyl reduction
- Increase fermentation temp 3/4 the way to final gravity to speed up rate of diacetyl reduction– not always necessary with ales
- Maturex™? -- converts α -acetolactate \rightarrow acetoin directly
- *Pediococcus* infection results in high amounts of diacetyl.

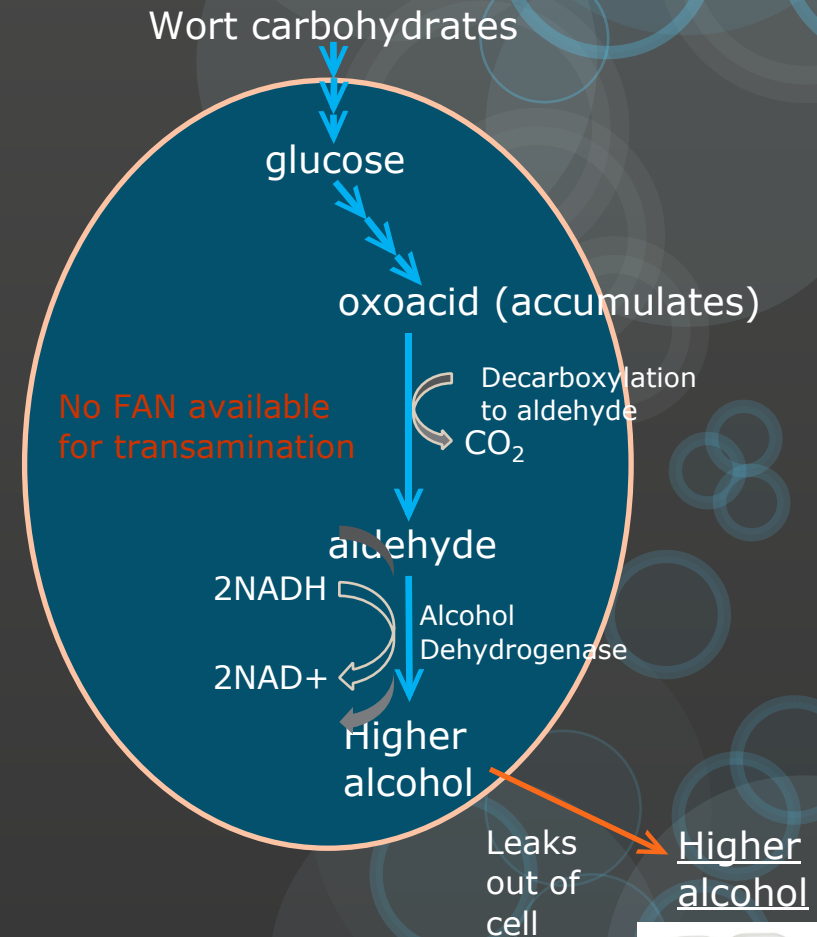
Higher alcohols (fusels)– “solvent” like



In worts with ample FAN:



In worts with insufficient FAN:



Blue lines =
enzymatically
mediated rxn

Orange lines =
passive process



Factors affecting higher alcohol formation

- Provide wort with adequate FAN (not a problem with all malt grist)
- Healthy pitching yeast in proper quantity will lower production of higher alcohols
- Keep fermentation (not ambient!) temperatures within optimum range for yeast strain
 - higher temps = rapid growth = excess oxo acid pool = higher alcohols

Johnson A419 digital external thermostat



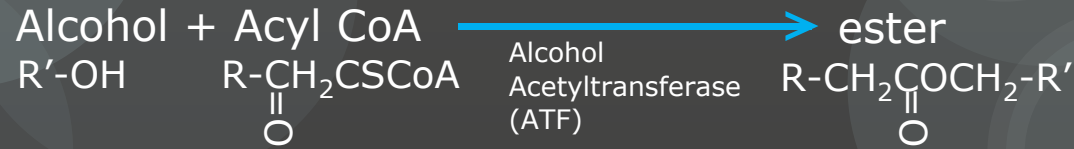
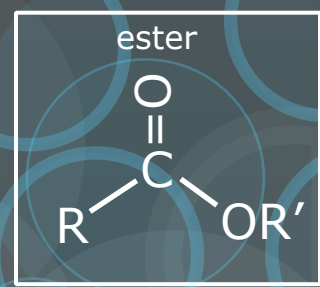
Johnson analog external thermostat



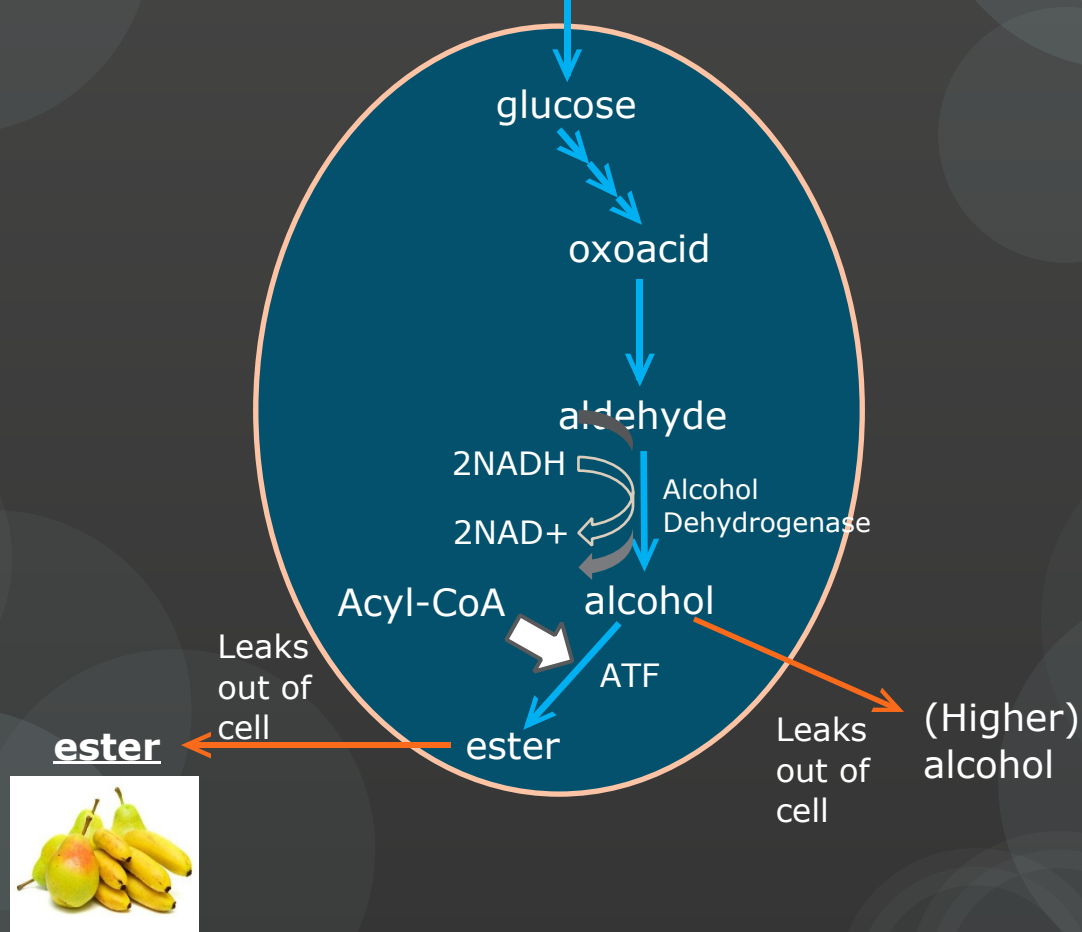
Buy a used fridge or freezer, Plug it in, set and forget!

Esters

- Highly flavor active (fruity) compounds– banana, pear, brandylike
- Produced when an alcohol reacts with an acyl CoA



Wort carbohydrates



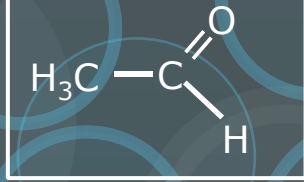
Examples of esters

Name	Formula	Characteristic	Concentration (mg / L)	Flavor threshold (mg / L)
Ethyl acetate	$\text{CH}_3\text{CH}_2\text{OC}\overset{\text{O}}{\underset{\text{O}}{\parallel}}\text{CH}_3$	Pear-like	8-70	33
Isoamyl acetate	$\begin{array}{c} \text{CH}_3 \diagdown \\ \text{CH} \\ \text{CH}_3 \diagup \end{array} \text{CH}_2\text{CH}_2\text{OC}\overset{\text{O}}{\underset{\text{O}}{\parallel}}\text{CH}_3$	banana	0.4-6	1.6
Ethyl octanoate	$\text{CH}_3\text{CH}_2\text{OC}\overset{\text{O}}{\underset{\text{O}}{\parallel}}(\text{CH}_2)_6\text{CH}_3$	Floral, brandylike	1.5	0.9
Ethyl caproate	$\text{CH}_3\text{CH}_2\text{OC}\overset{\text{O}}{\underset{\text{O}}{\parallel}}(\text{CH}_2)_8\text{CH}_3$	Goaty, barnyard	0.2-?	1.5

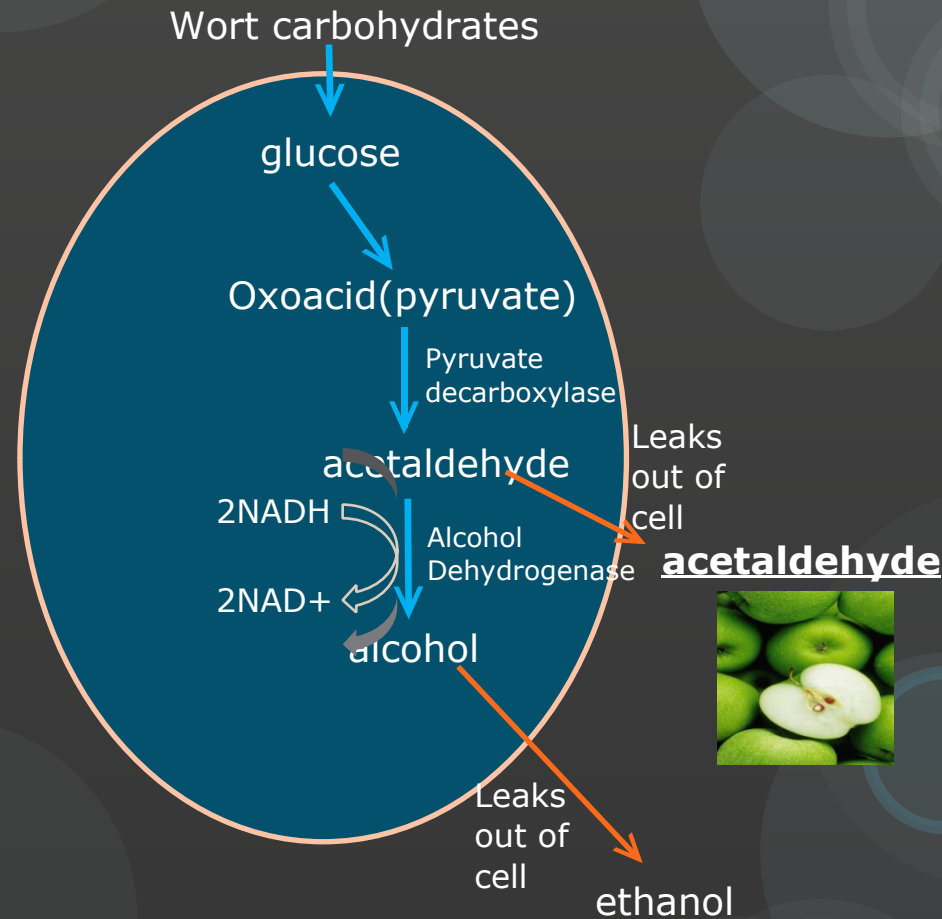
Factors affecting ester formation

- Increase in wort gravity increases esters
- Provide wort with adequate FAN (not a problem with all malt grist)
- Healthy pitching yeast in proper quantity will lower production of higher alcohols, thus lowering ester formation potential
- Ensure wort oxygenation is adequate
- Keep fermentation (not ambient!) temperatures within optimum range for yeast strain
 - higher temps -> rapid growth -> excess oxo acid pool -> higher alcohols -> esters

Acetaldehyde



- Green apple like in aroma and flavor
- Produced as an intermediate in alcohol pathway– Stressed yeast produce more
- Alcohol dehydrogenase requires Zn^{+2} as cofactor. Worts low in Zn^{+2} have increased acetaldehyde



Factors affecting acetaldehyde levels

- Low zinc levels– usually not a problem with all malt. Can supplement to 0.1-0.2mg/L
- Provide wort with adequate FAN (not a problem with all malt grist)
- Healthy pitching yeast in proper quantity will allow yeast to reabsorb acetaldehyde
- Ensure wort oxygenation is adequate– some evidence that excessive aeration will increase acetaldehyde
- Don't transfer / keg too soon.

Summary

Grow lots of yeast and treat it well.

Happy, healthy yeast = better beer (duh)

