WORLD BREWING CONGRESS
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#ElevateBeer

Terminal acidic shock inhibits sour beer bottle conditioning by *Saccharomyces cerevisiae*

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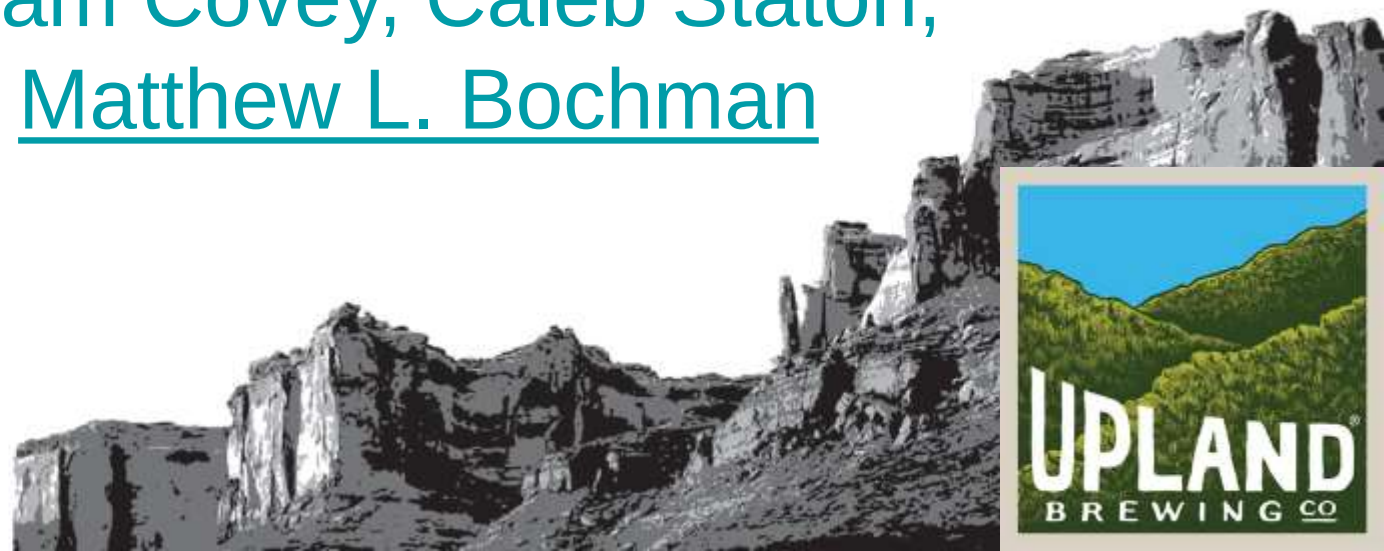
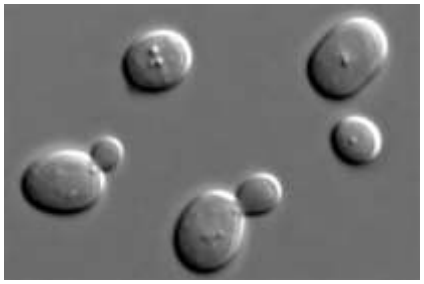
INDIANA UNIVERSITY

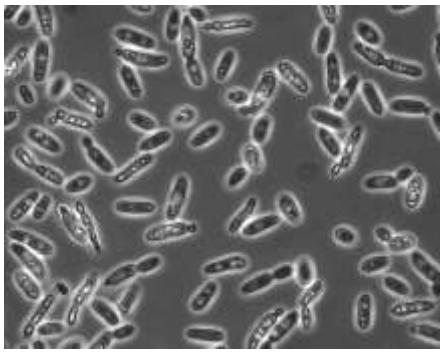


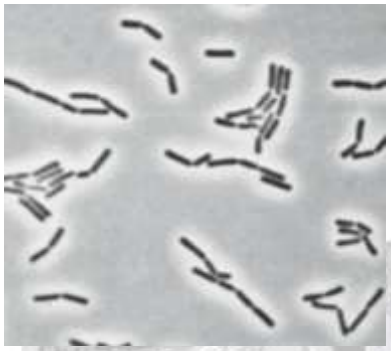
UPLAND BREWING CO

Sour beers are an ancient beer style

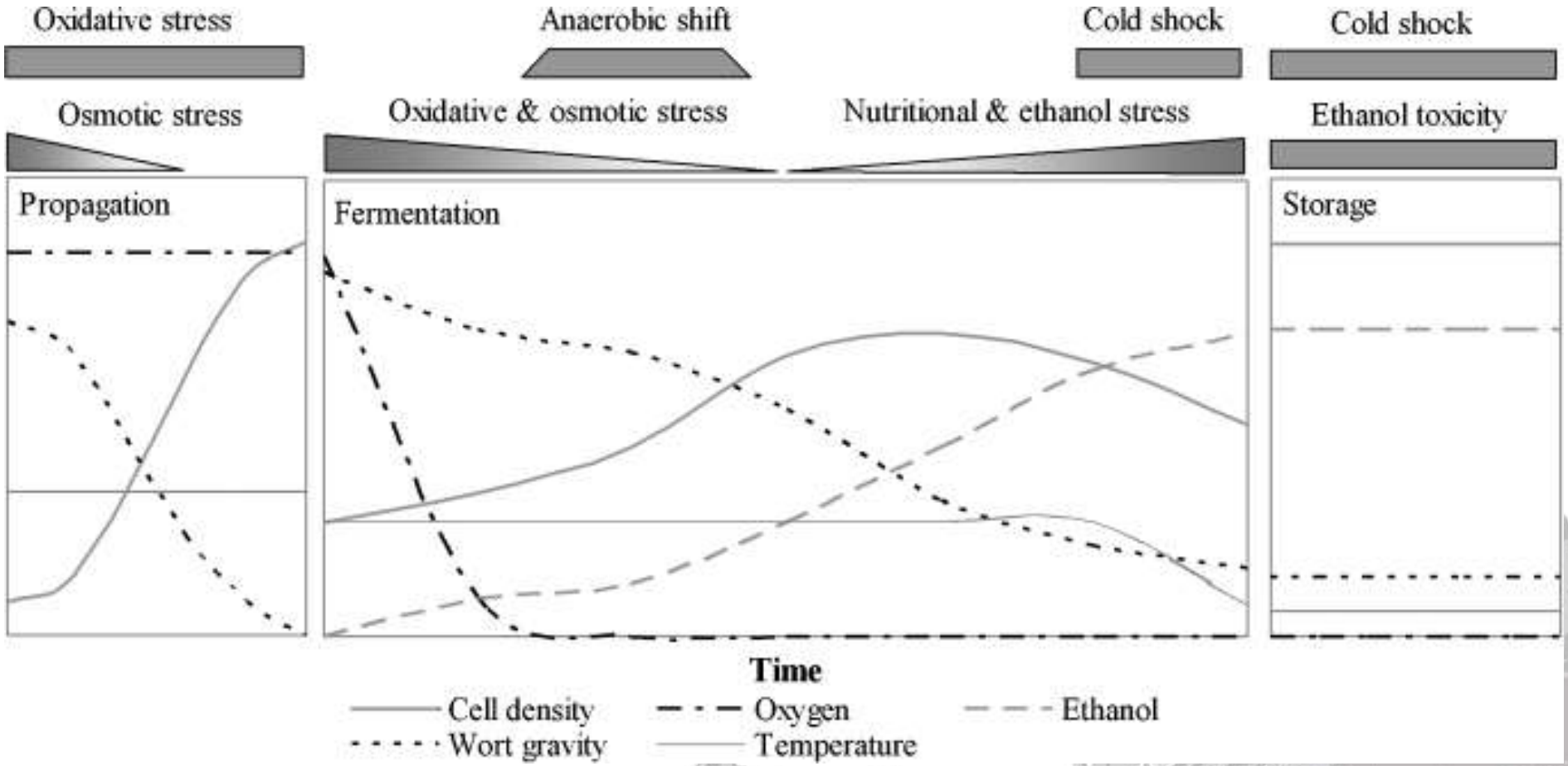
- Lambics
- Oud bruins
- Flander’s red ales
- Goses
- Berliner weiss
- American wild ales







Beer is a stressful environment



Gibson *et al*. (2007) FEMS Microbiol Rev

Sour beers are even more stressful

- Lower pH (lactic and acetic acid)
  - >0.8% lactic acid
  - >0.05% acetic acid
  - pH = 3-4

  } Decrease fermentation efficiency
- Barrel aging
  - Tannins
- Fruit, herb/spice, and other additions
  - Phytochemicals

Cauldron by Upland Brewing Co.

https://twitter.com/uplandbrewco/status/537652344750485504

- Blend of American wild ale and Flander's red ale
- Aged on Montmorency cherries in oak
- **Won't bottle condition**
- **Why?**

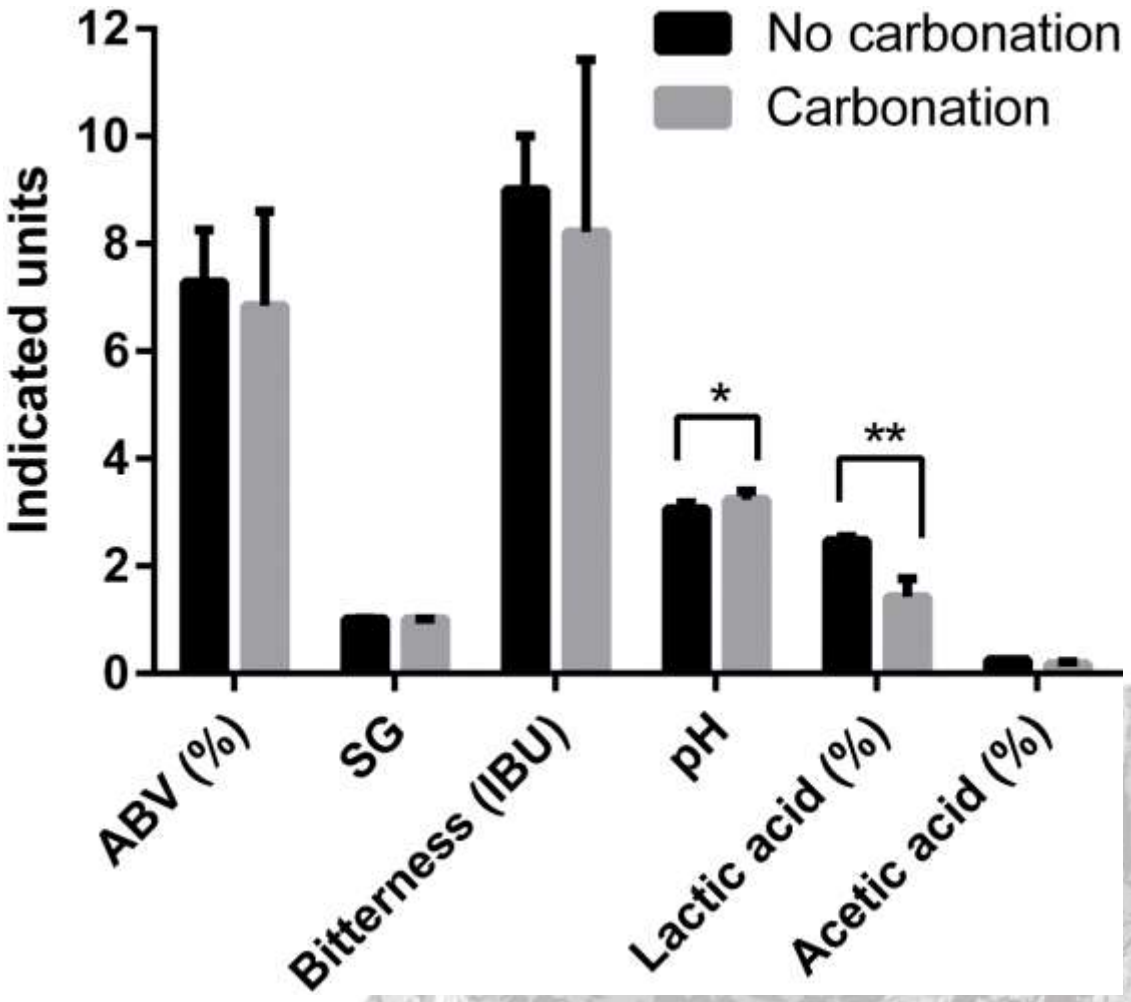
Comparison of carbonated *vs.* uncarbonated sours

Beer	ABV (% at 20°C)	Bitterness (IBU)	pH	Total acidity (% lactic acid)	Volatile acidity (% acetic acid)
Blueberry Lambic 1	6.52	8.0	2.95	2.54	N.D.
Blueberry Lambic 2	6.97	9.0	3.08	2.38	N.D.
Cauldron	8.37	10.0	3.17	2.50	0.25
Carbonated sours	6.84 (4.81-9.78)	8.22 (5-13)	3.23 (3.02-3.47)	1.43 (0.99-1.63)	0.17 (0.15-0.21)

Average (Range)

Chemical analyses performed by Brewing & Distilling Analytical Services, LLC (KY, USA)

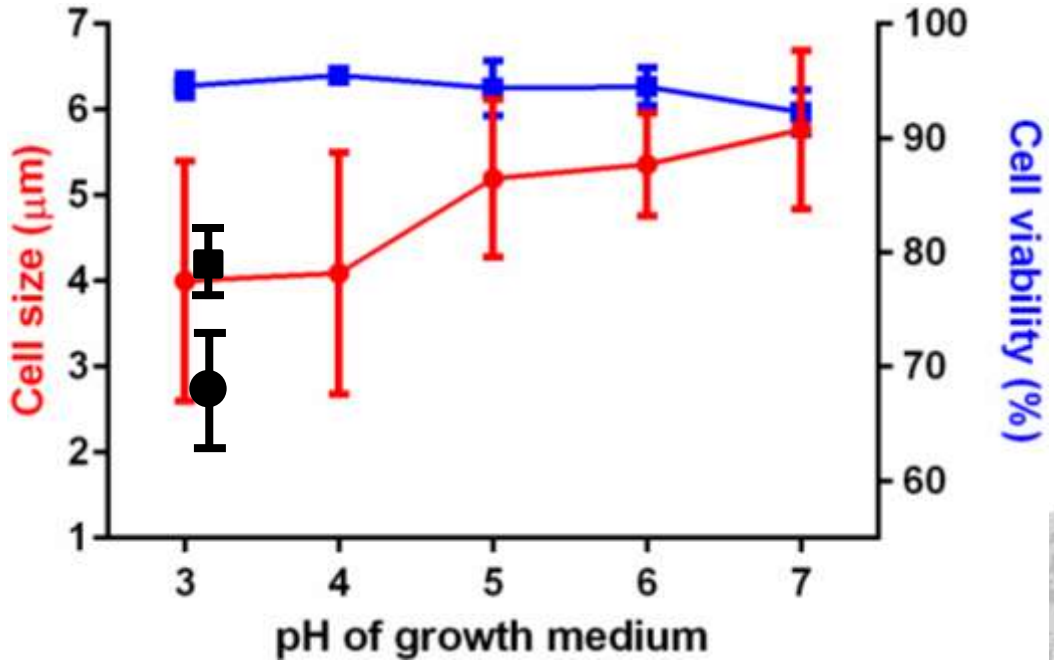
Failure to bottle condition correlated with low pH



* $p < 0.05$; ** $p < 0.01$

The yeast did not die during bottle conditioning

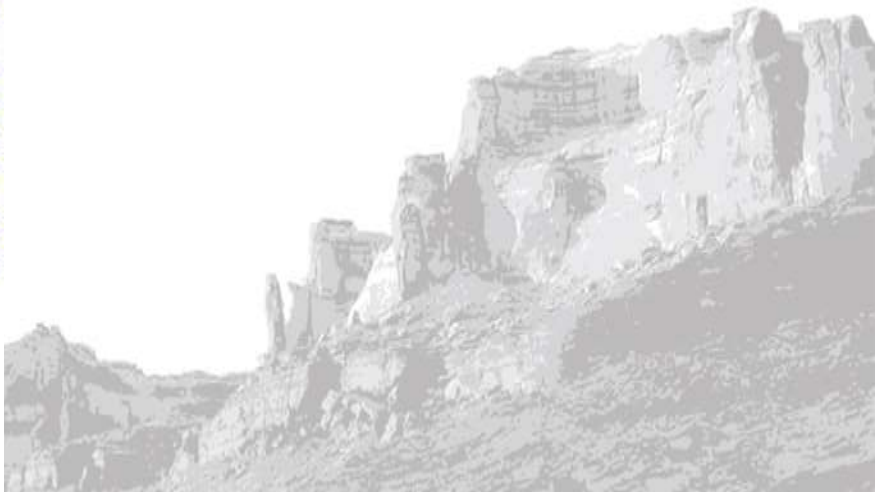
Growth medium	pH	Cell size (μm)	Viability (%)
Water	7.2	2.68 ± 0.68	68.8 ± 10.4



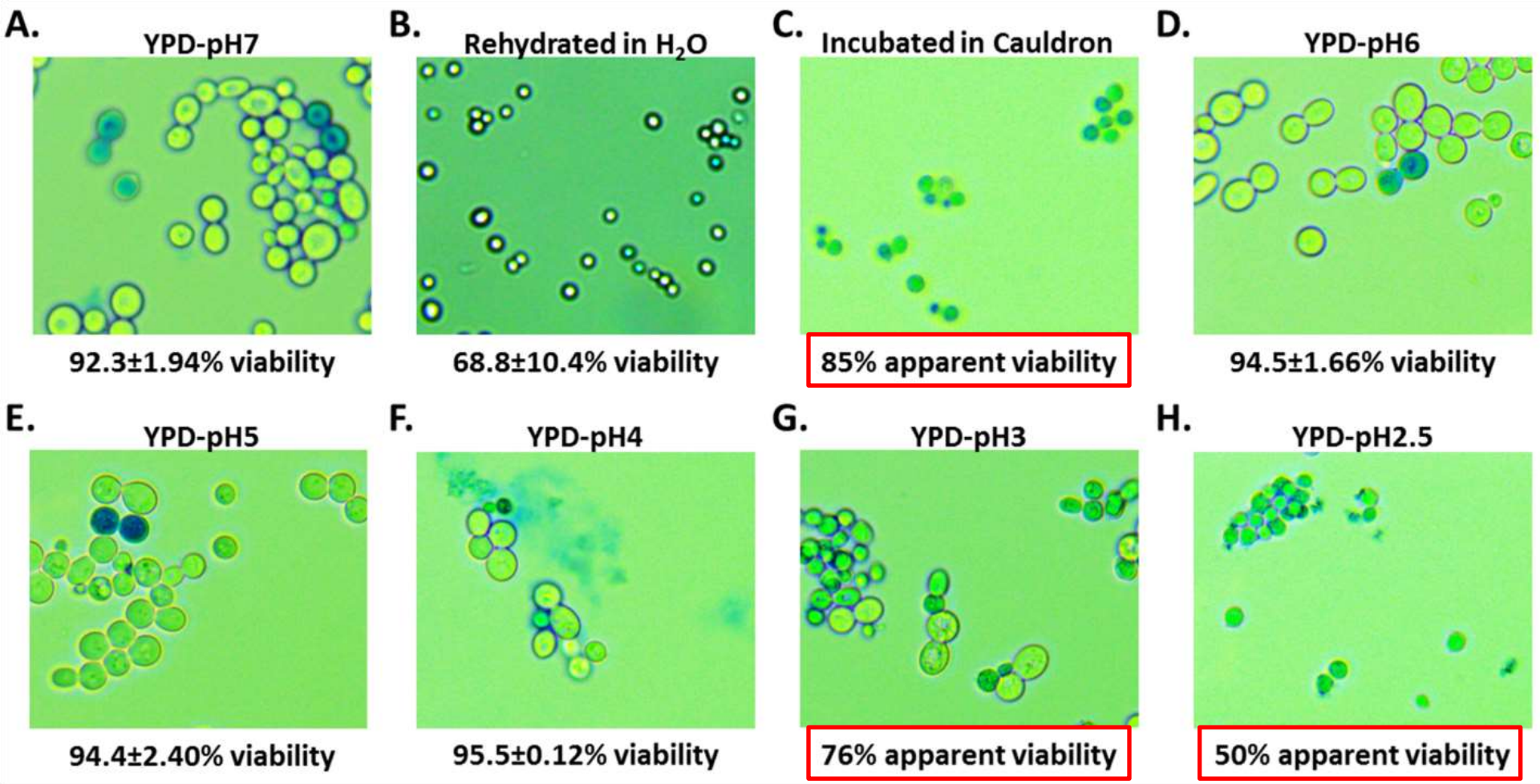
Cell size decreases with decreasing pH

Viability is stable

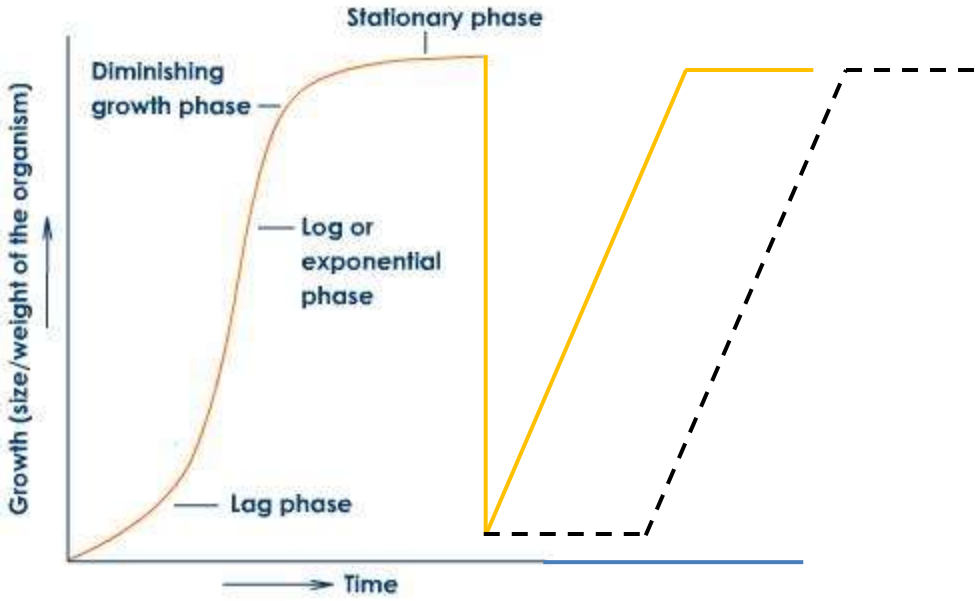
Cauldron is an outlier…

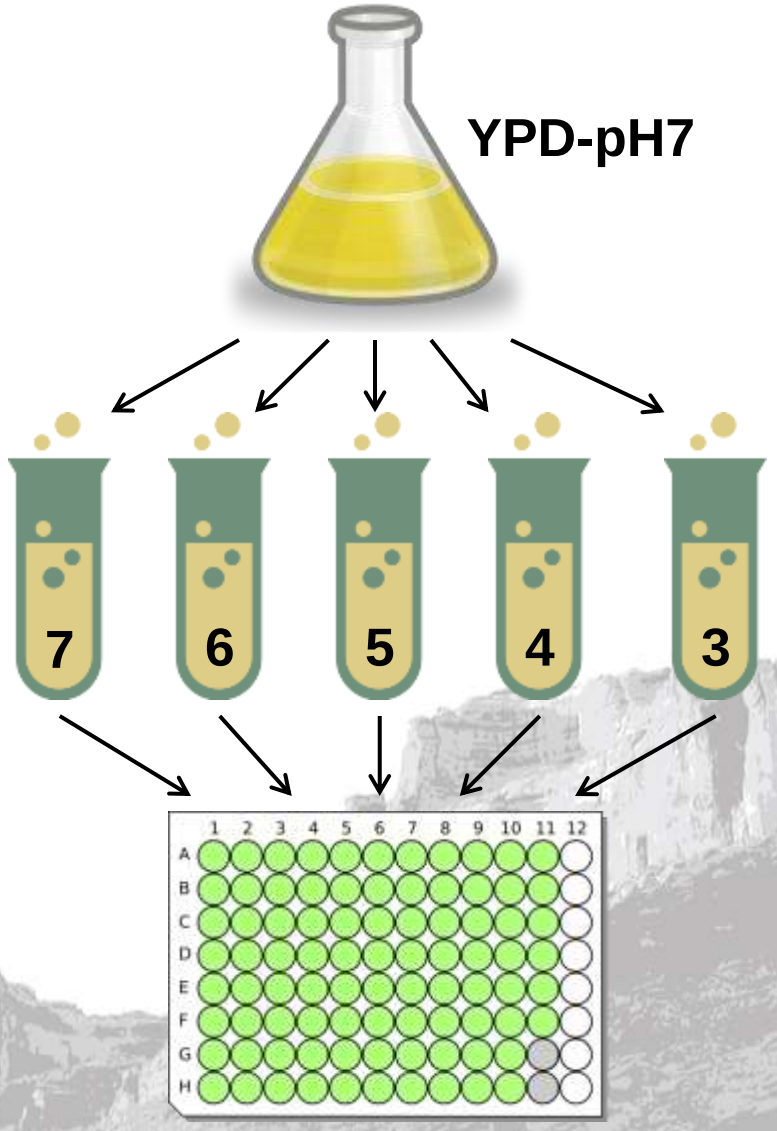


Methylene blue staining is unreliable at low pH

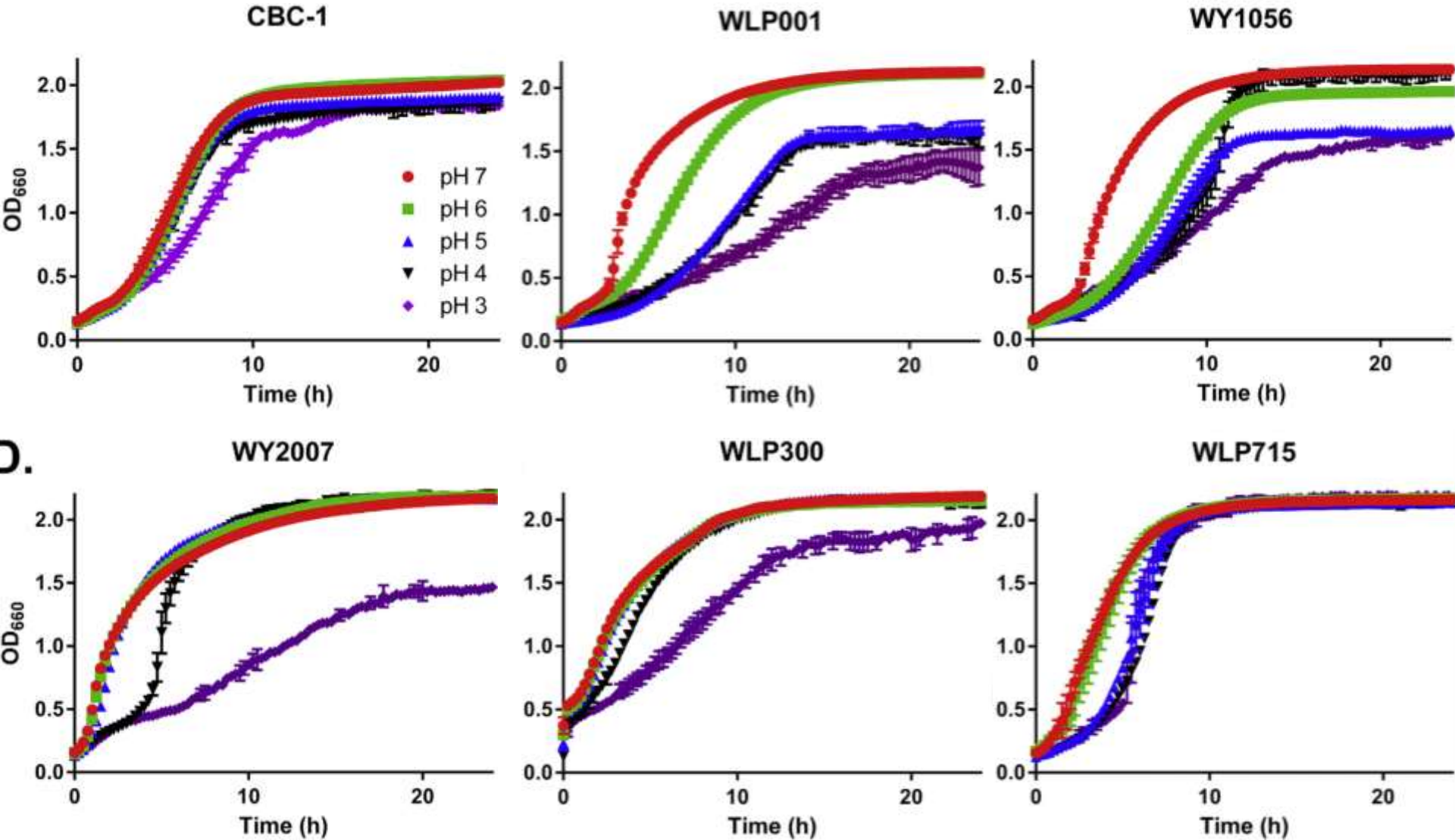


Does the high [lactic acid] in Cauldron terminally shock CBC-1 cells?



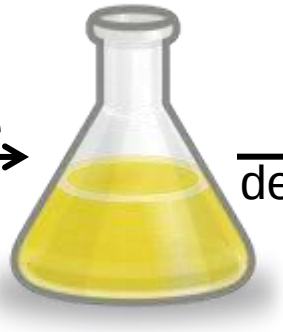


Lactic acid differentially affects brewing strains



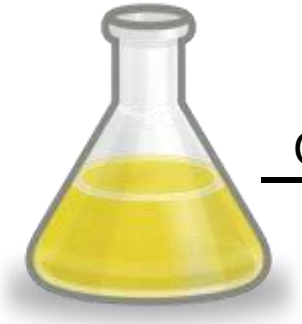
Does recovery in rich medium fix the problem?

Rehydrate in H_2O



Recirculate with dextrose in Cauldron

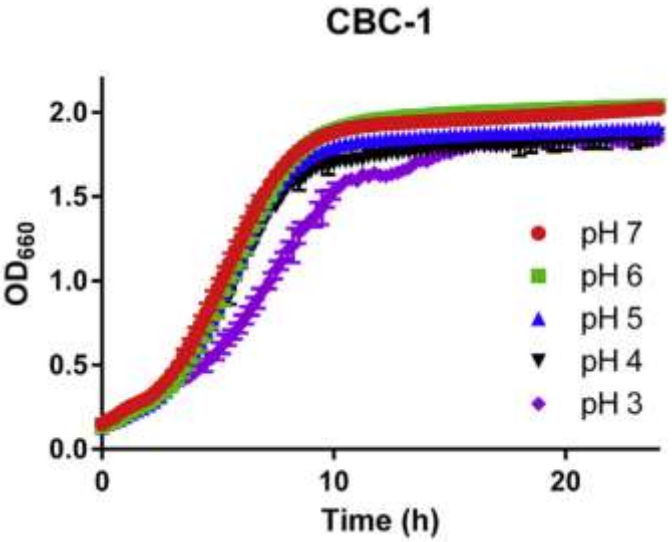
Rehydrate in rich medium

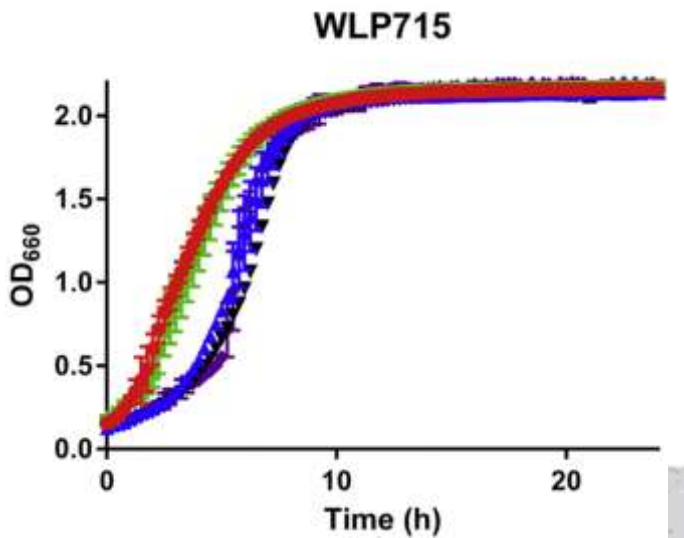


Grow in rich medium

Recirculate with dextrose in Cauldron

Does recovery in rich medium fix the problem?





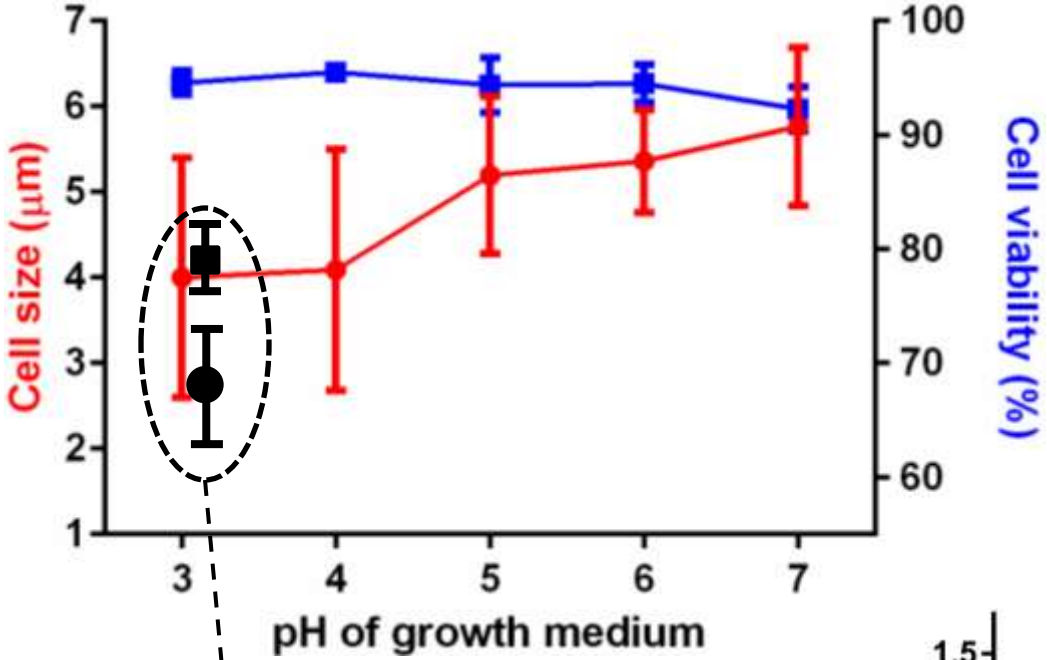
CBC-1

Uncarbonated

WLP-715

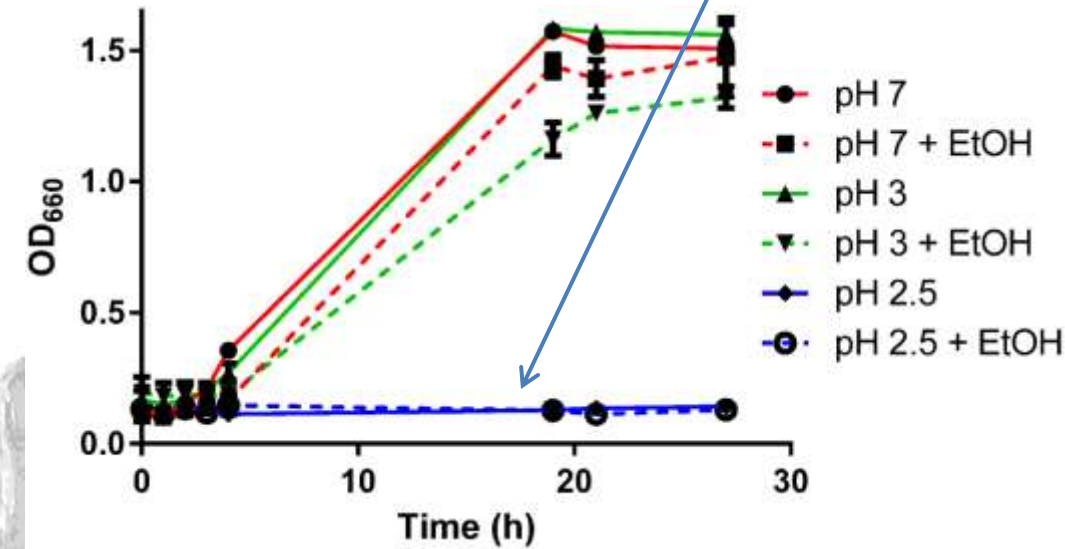
Uncarbonated

Can we better recapitulate Cauldron in the lab?



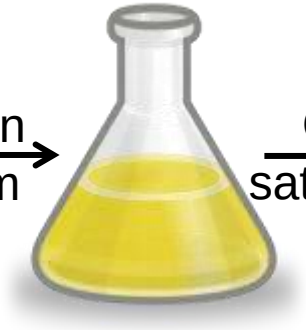
We're not mimicking Cauldron well enough

Terminal acidic shock



Can we pH-adapt yeast to carbonate Cauldron?

Rehydrate in rich medium →



→ Grow to saturation in:

- YPD-pH7
- YPD-pH7 + EtOH
- YPD-pH3
- YPD-pH3 + EtOH
- 1:1 YPD + Cauldron

pH adaptation overcomes terminal acidic shock

CBC-1
Uncarbonated

WLP-715
Uncarbonated

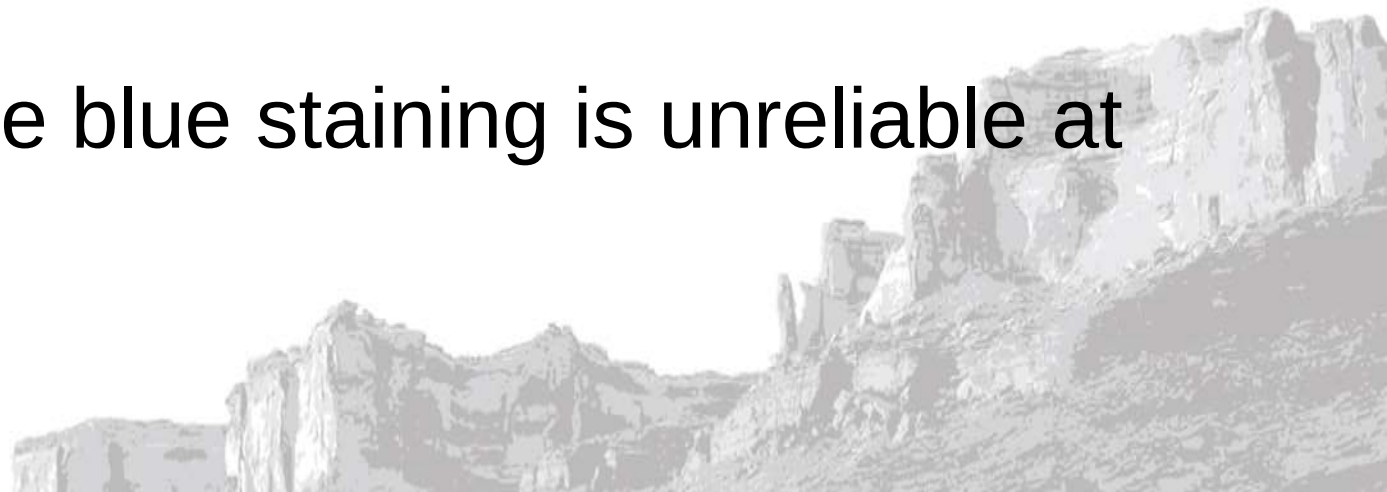
YPD-pH7
YPD-pH7 + EtOH

1:1 YPD + Cauldron (2 weeks)

YPD-pH3 (4 weeks)
YPD-pH3 + EtOH (4 weeks)

Take home messages:

- Pre-adapt your yeast to the conditions they'll face for best fermentation efficiency
  - There are stressors other than pH
- Dry yeast should be used with caution for "extreme" beers
- Methylene blue staining is unreliable at low pH



Acknowledgments

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