



Yeast Settling: Shutting down the party

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MBAA Conference
Jacksonville, FL



GREAT
WHITE
NORTH

Bob

LIZIE'S

Hoosier Head
BEER

Bob



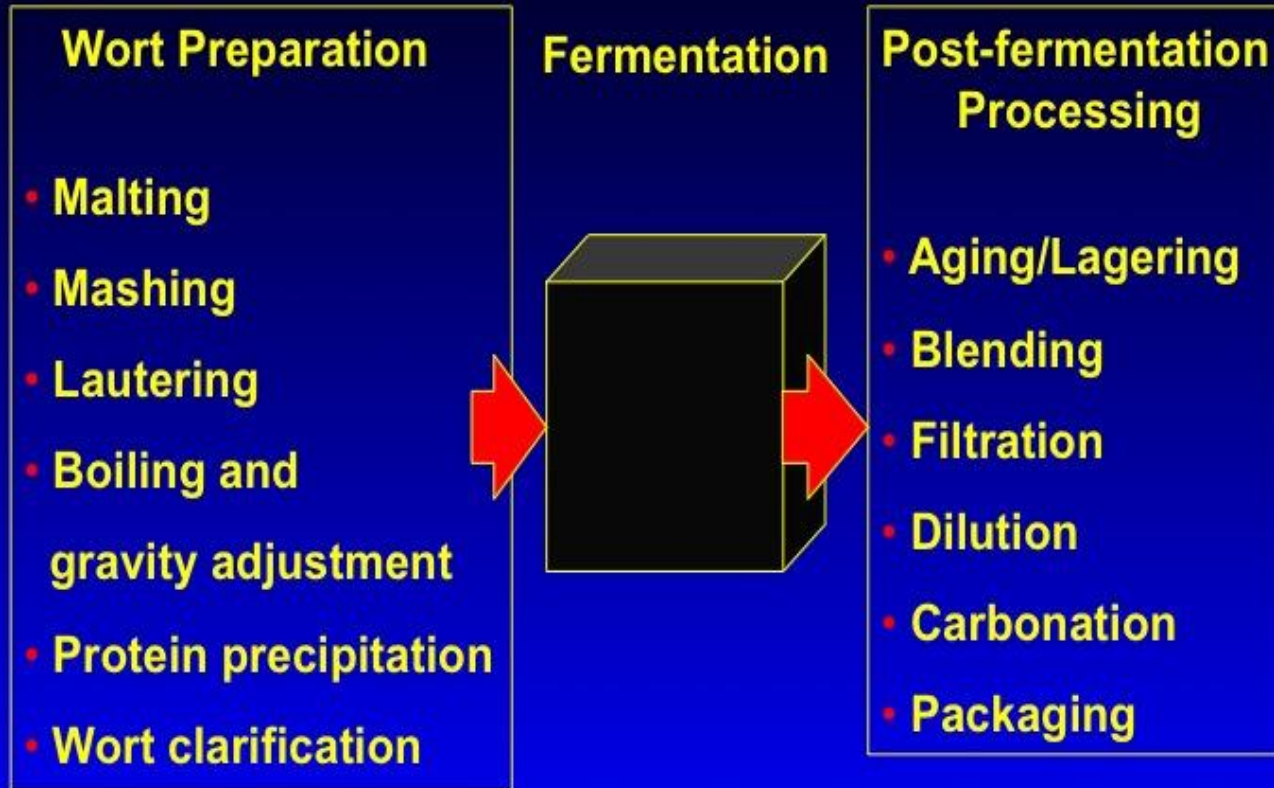
Outline

- Introduction
 - Fermentation Overview
- Flocculation
 - Why?Too much/Too little
 - Theories
 - PYF
 - Measurement

Other notes

- Summary

THE THREE STAGES OF THE BREWING PROCESS



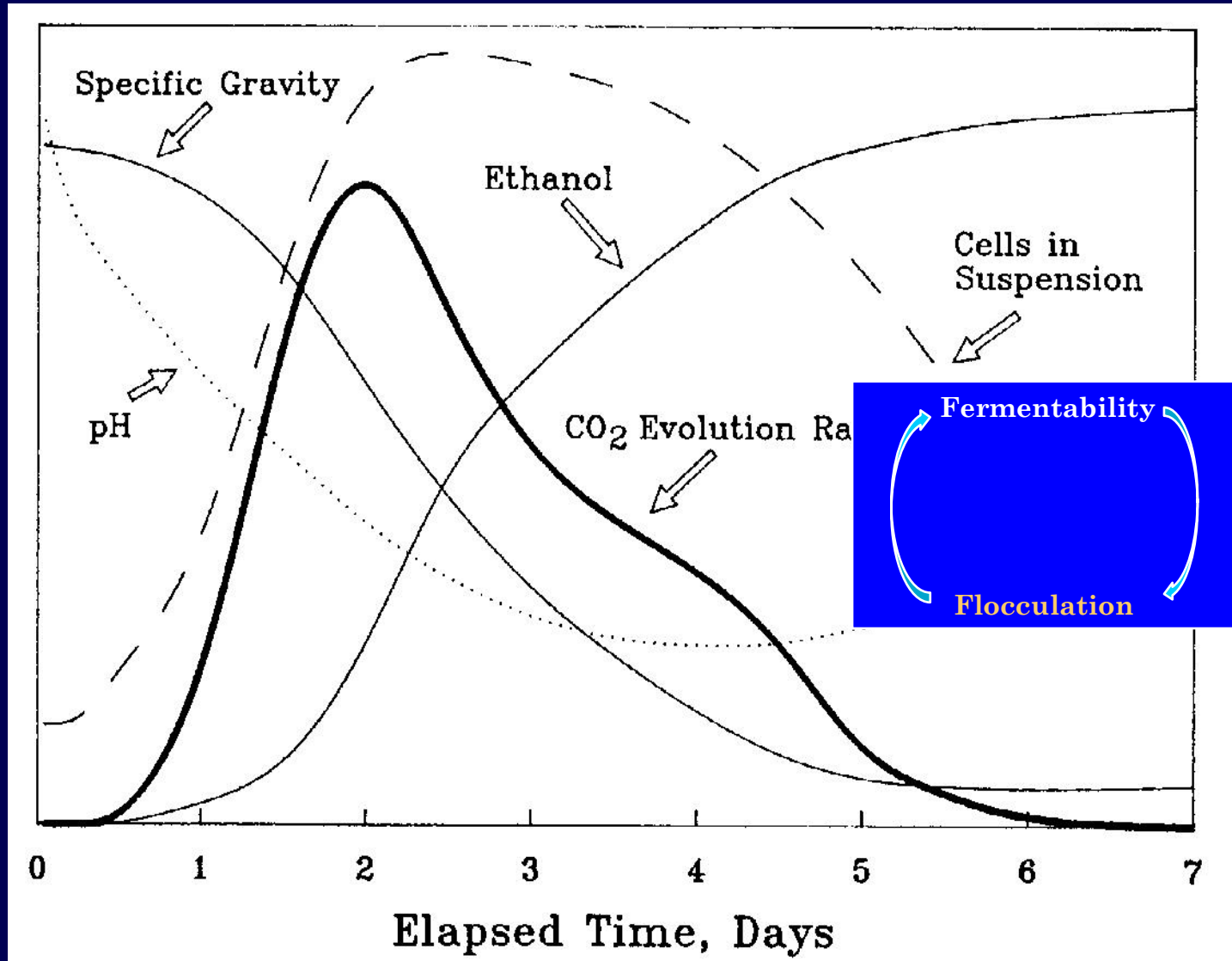
Stewart, G.G. and Russell, I. 1993

Fermentation - the "black box" of the brewing process.

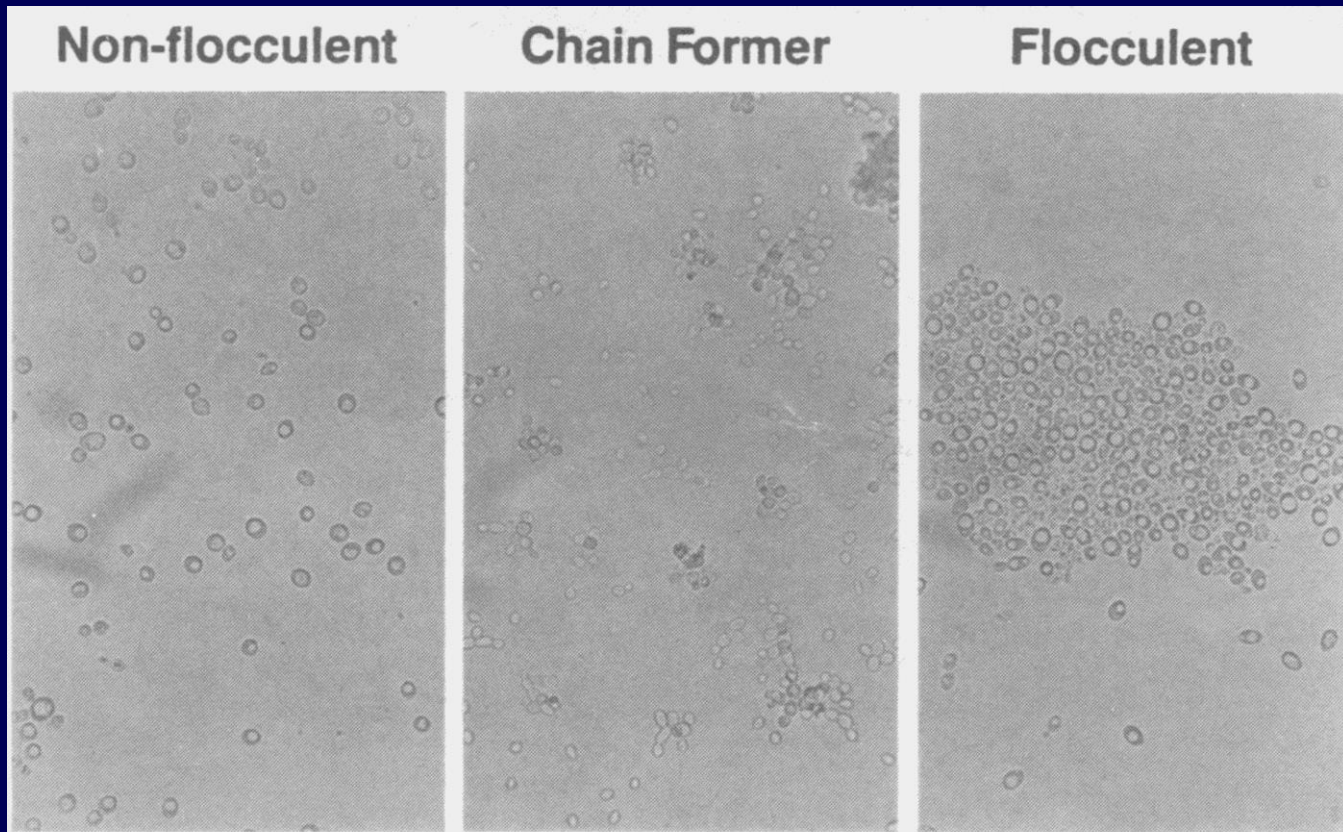
MBAA TQ. 30, 159-168.

Typical Fermentation

(Munroe, Handbook of Brewing)



Flocculation



25 μm

From G.G. Stewart, 1999 Factors influencing yeast performance during beer and industrial ethanol production. *Ferment.* 2:59-65.

Flocculation Definition

- "... the phenomenon wherein yeast cells adhere in clumps and either sediment rapidly from the medium in which they are suspended or rise to the medium's surface †".
- The process needs a micro-amount of Ca and is reversible with addition of sugars or EDTA

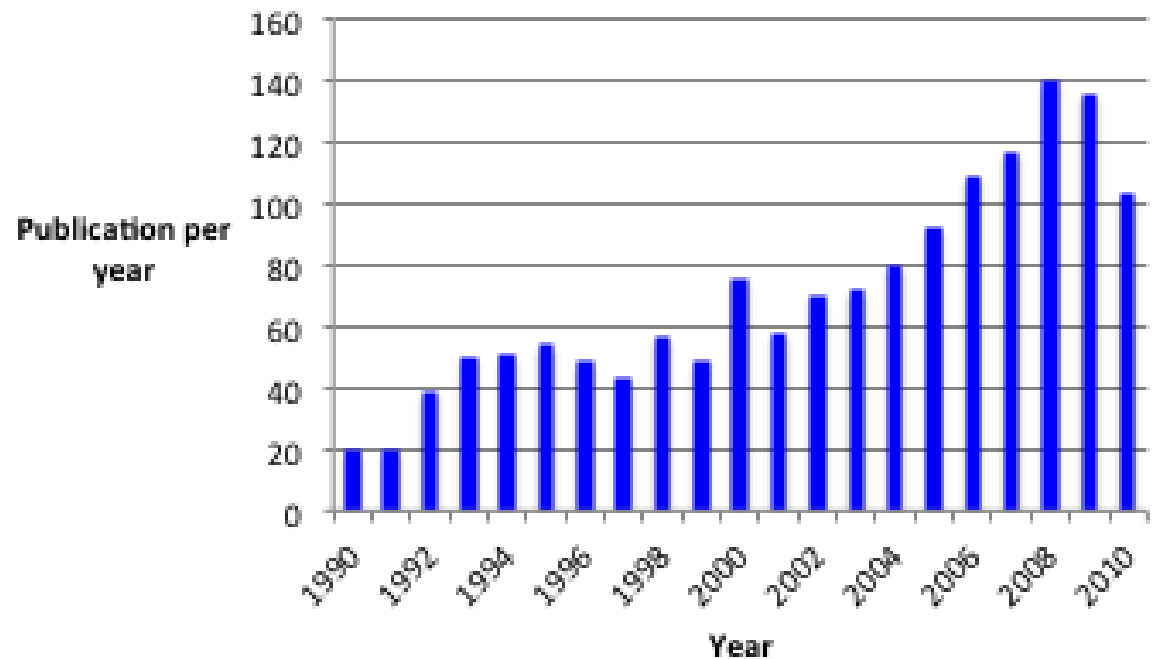
†Stewart, G. G. & Russell, 1981. Chap. 2. Brewing Science. Academic Press.

Flocculation Literature



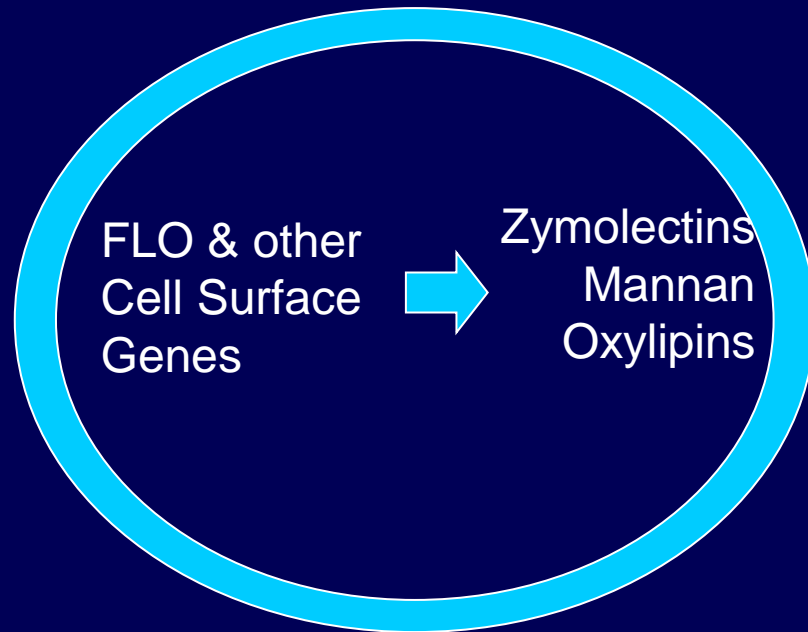
Flocculation Publication rates

- 1970-1990 ~5/y
- Google Scholar search for 'brewing yeast flocculation'



Flocculation Factors

Oxygen,
Carbon,
Nitrogen
sources,
Temperature,
Ethanol &
other
end products.



Sugars,
CO₂/Turbulence,
cell size,
Cell age??
Fimbriae
Temperature
CSH,
pH,
Ethanol,
Ca²⁺

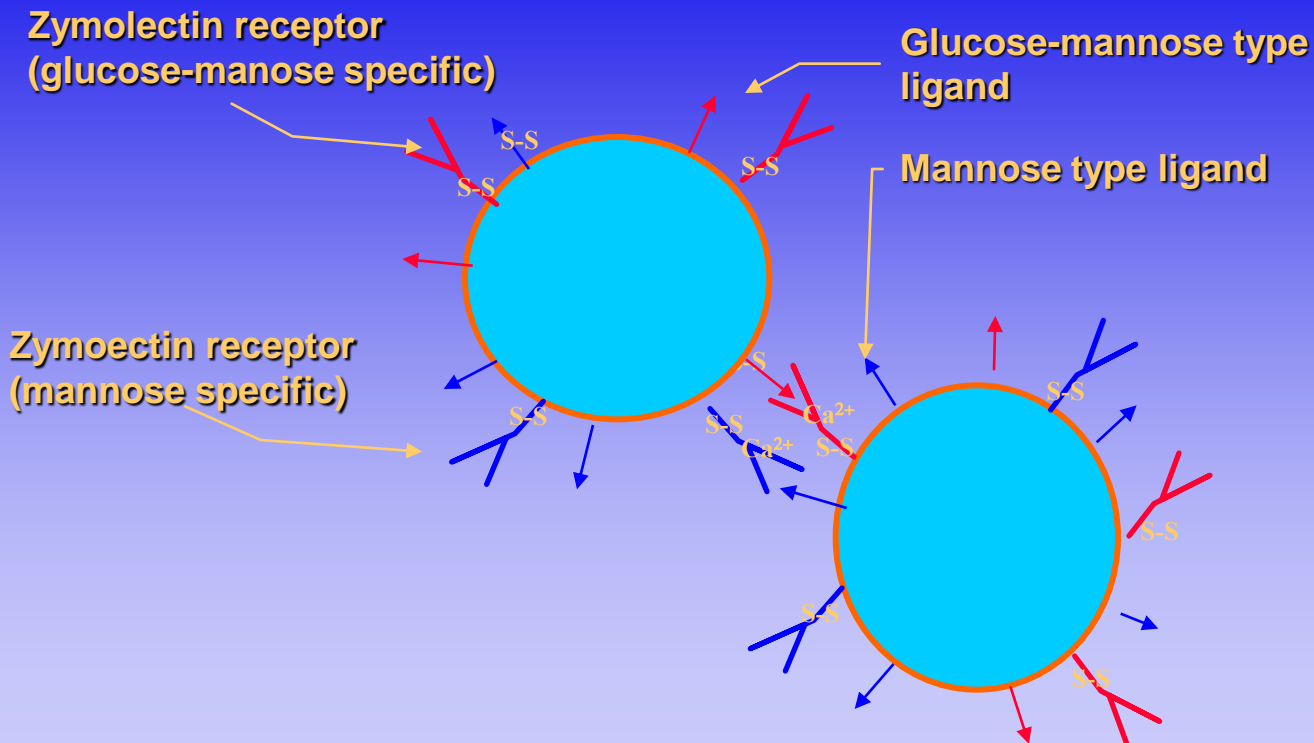
**Factors affecting
FLO/other gene activity**

**Factors affecting
cell to cell
interaction**

Theories Explaining Flocculation

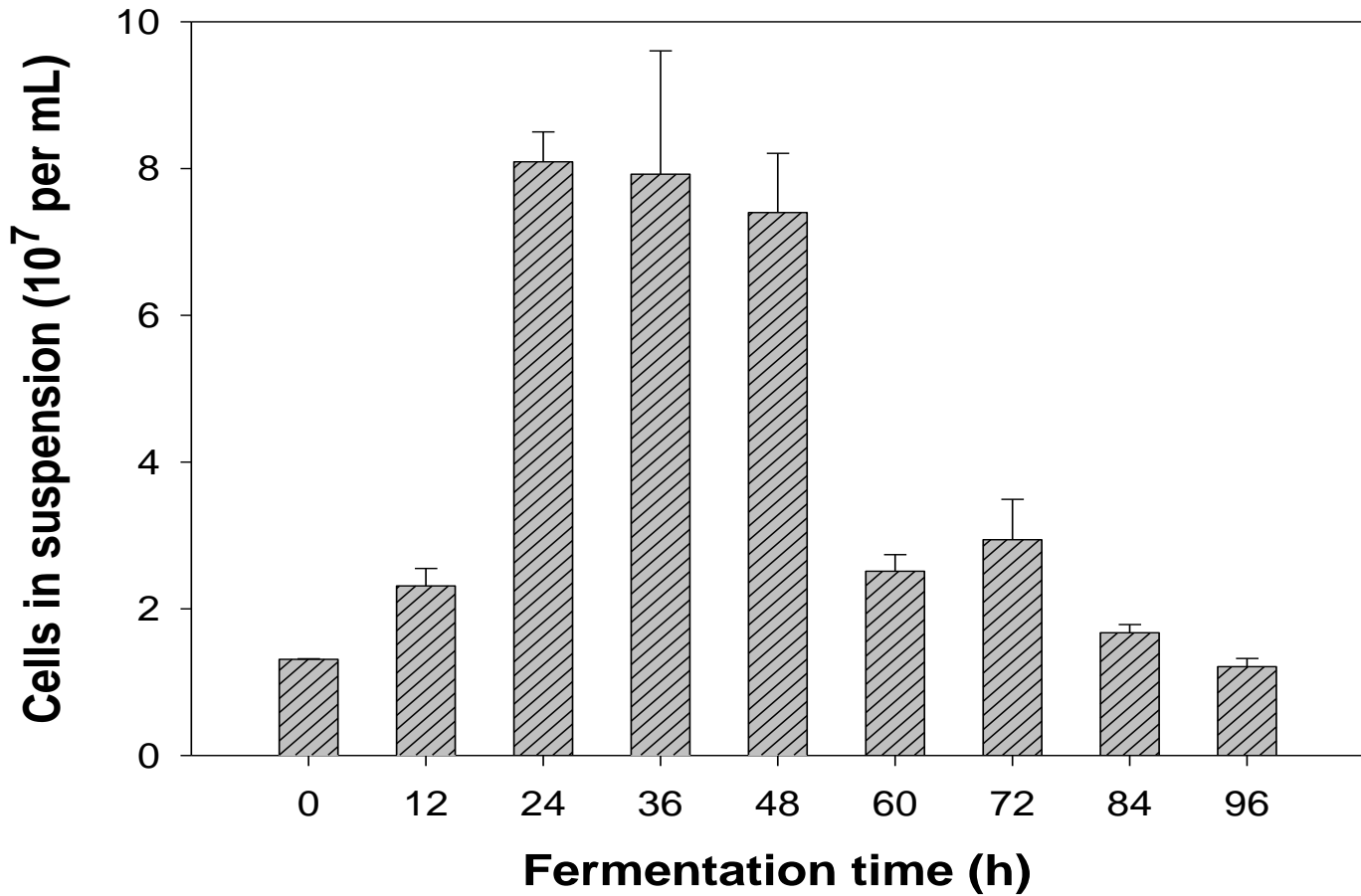
- Biochemical
 - Zymolectin binding
 - Hydrophobic interactions
 - CO₂ Shearing flow
 - Surface charge
 - Bridging factors PYF

Zymolectin Flocculation

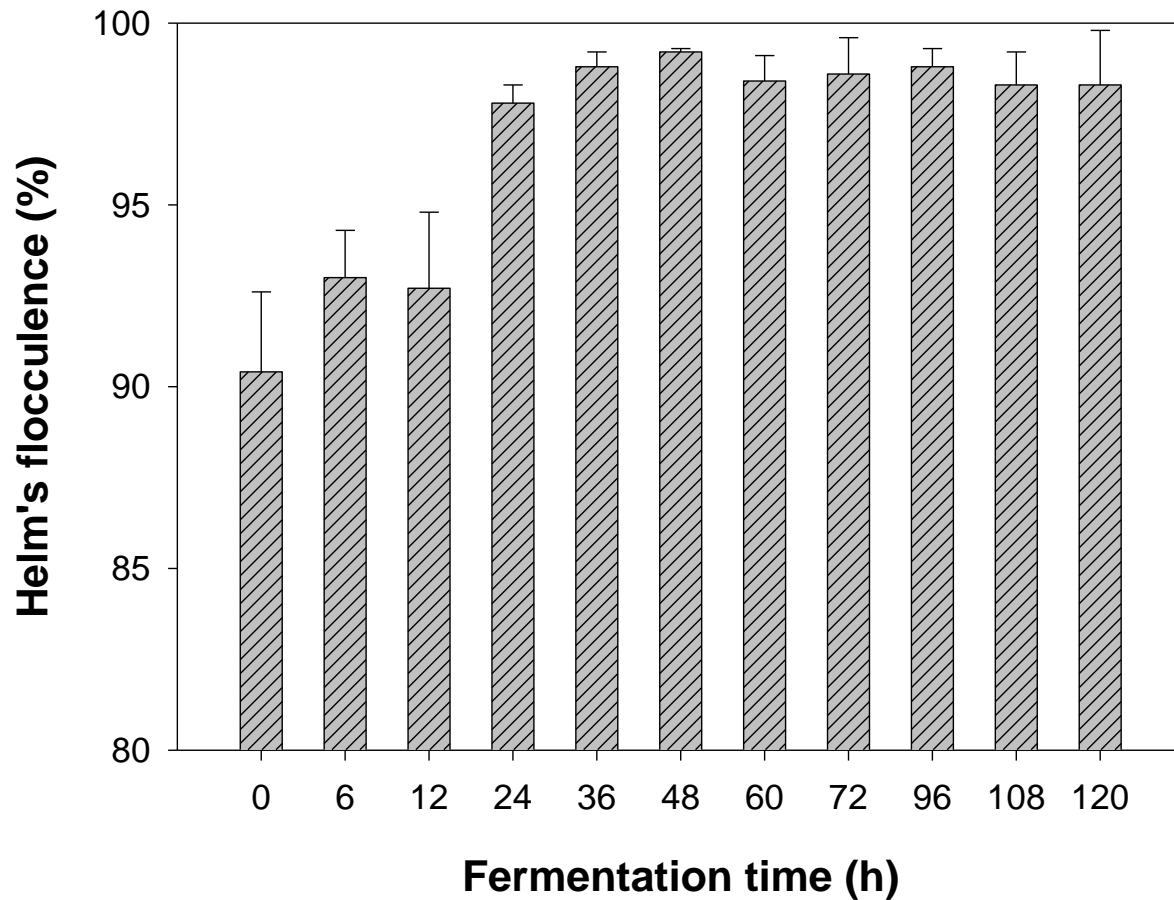


Note: Complex e.g., FLO 11 hydrophobic & self-binding

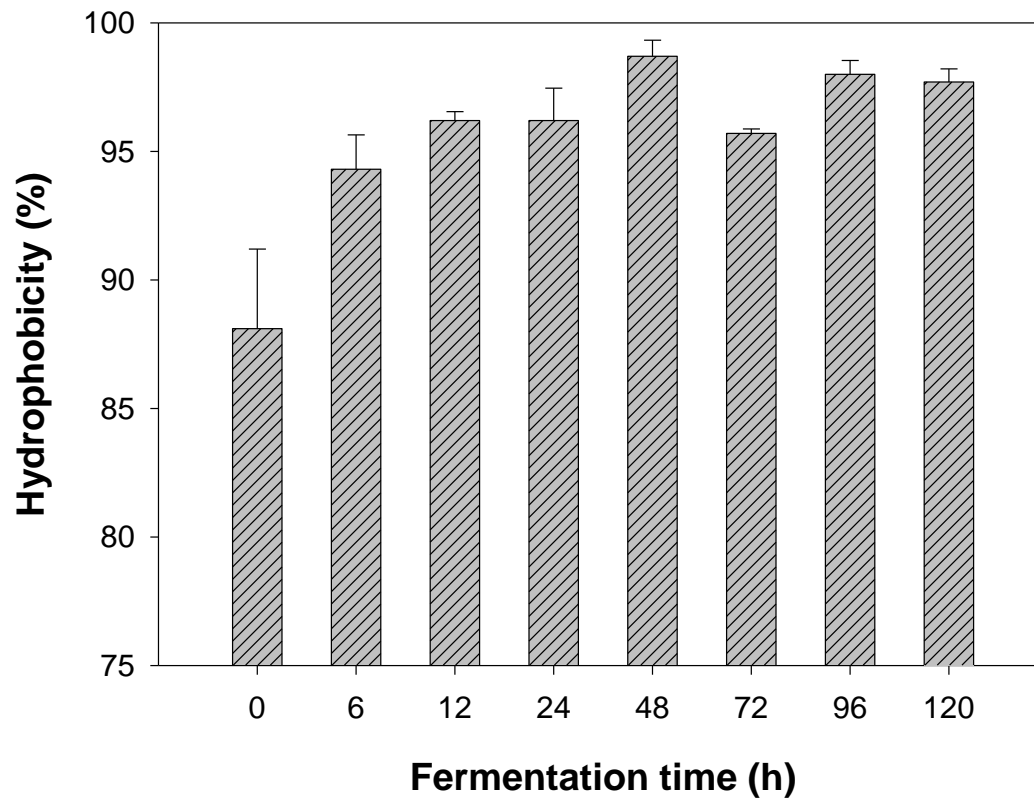
Fermenter Flocculence



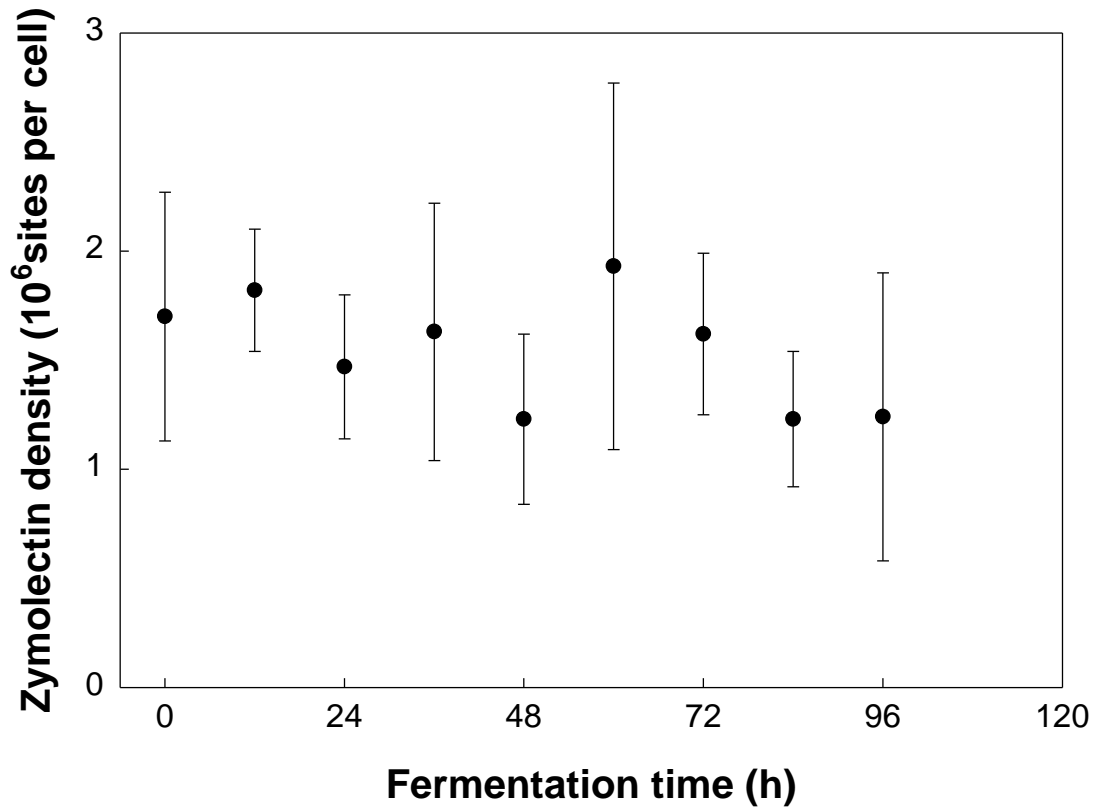
Cell Flocculence



Change of Cell Surface Hydrophobicity during Fermentation



Variation of Zymolectin Density during Fermentation



CO₂ Shearing/turbulence

- The majority of work has focused on the biochemistry and genetics of cell binding.
- However, for cells to floc, cells must first collide and then bind.

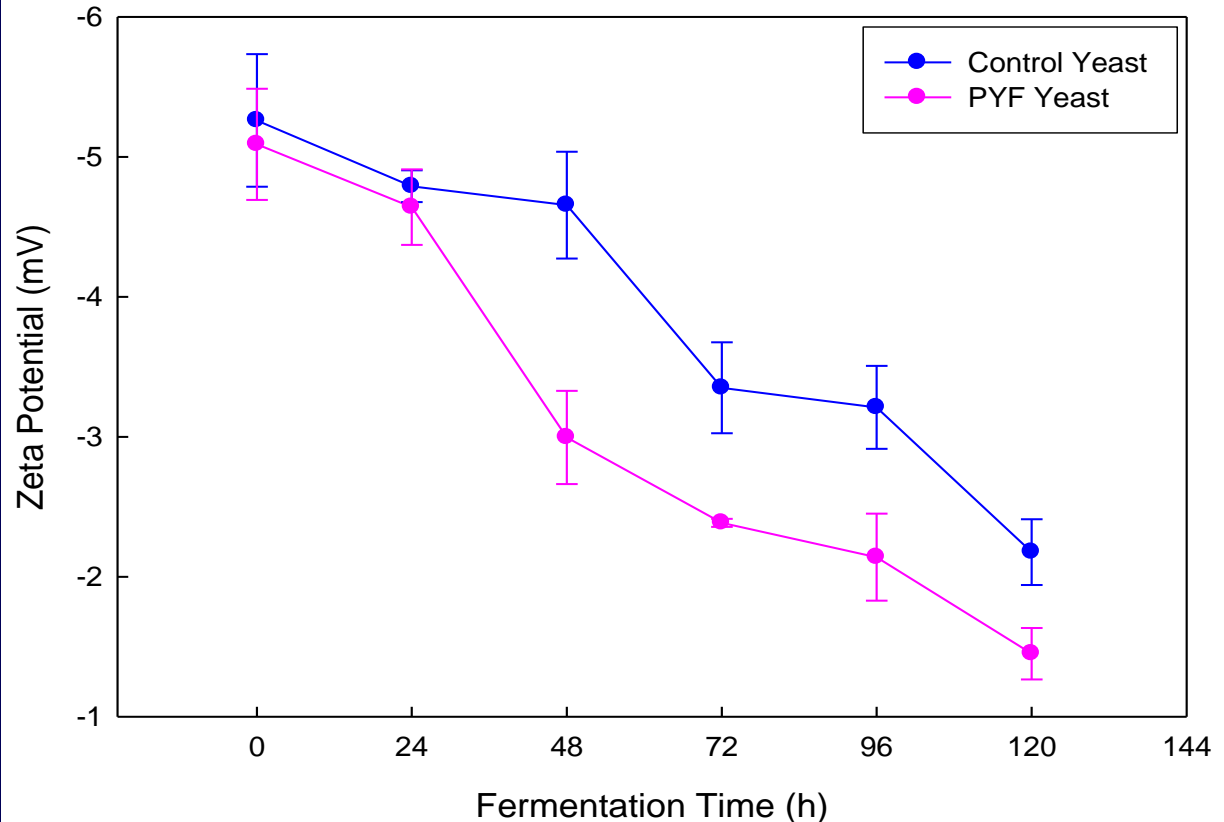


- Brownian motion is not important in flocculation - cells must collide in CO₂ driven shearing flow drives collisions and suspends flocs.
- CO₂ is also important is suspending cells & flocs

Surface Charge During Fermentation

Just not that important!

- Small decline related to pH
- Might? be important in PYF

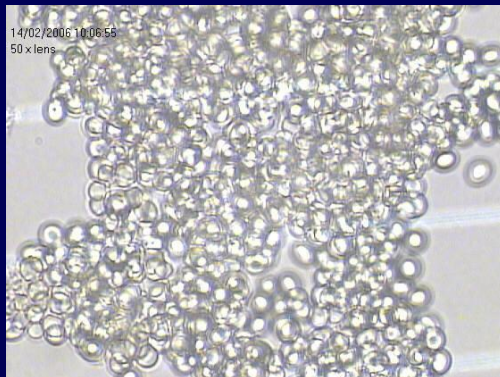


Normal Flocculation Summary

- Cells ready to floc in 24h (once hydrophobicity up ? Oxylipins?) as zymolectins always present.
- Prevented from doing so from
 - suspending shear (CO_2)
 - sugar

PYF Definition

- Premature Yeast Flocculation (**PYF**)
 - The early flocculation of fermenting yeast from the fermenting medium before all available fermentable sugars are consumed.



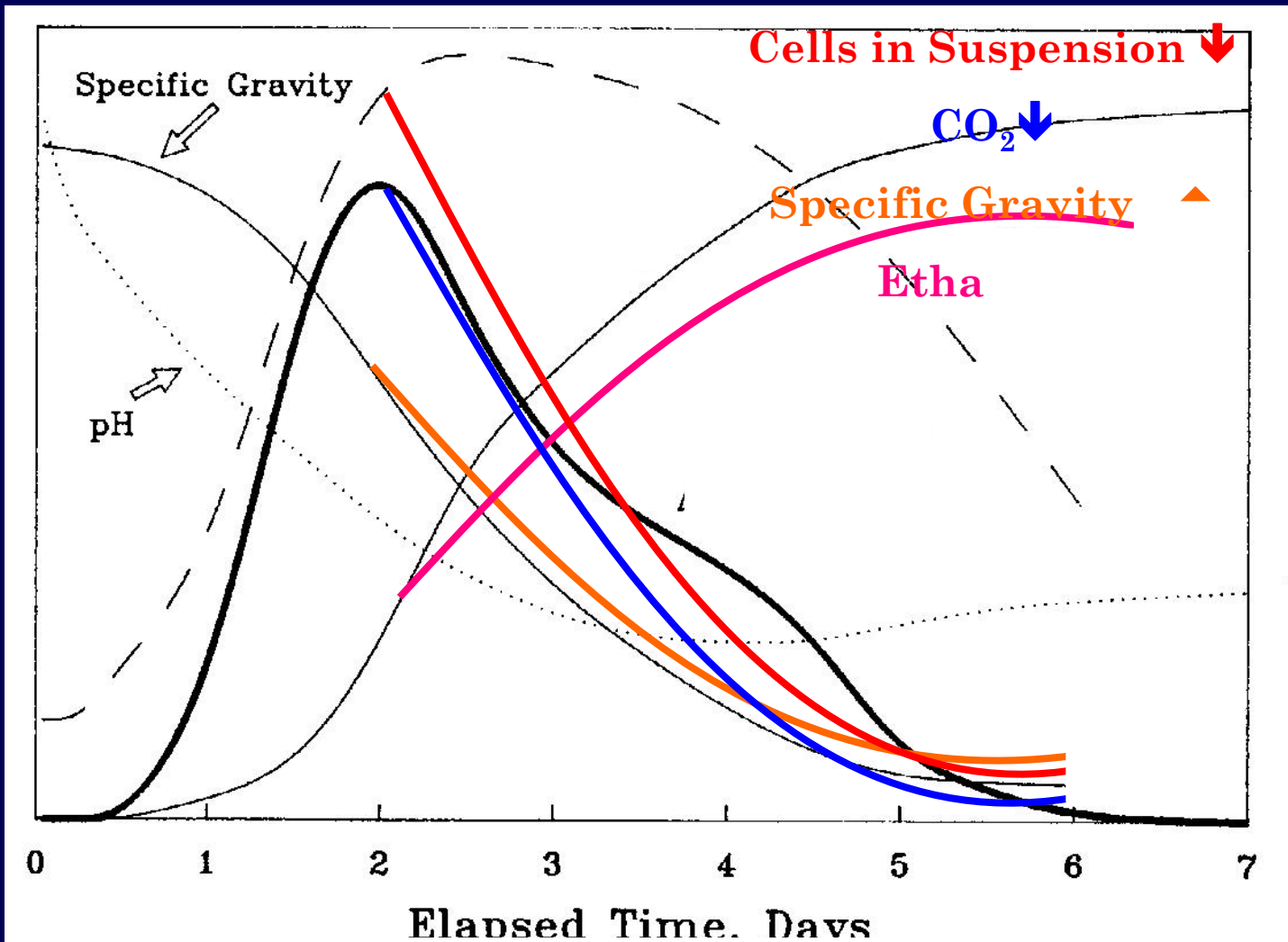
PYF

144
hr.



Control

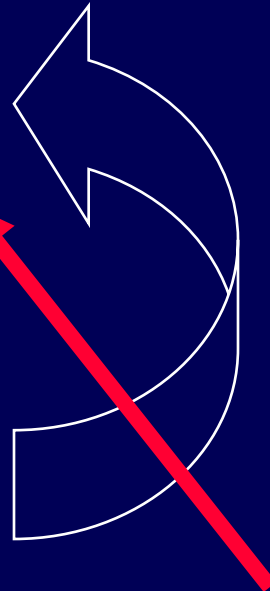
PYF Fermentation



From : J. Munroe. The Practical Brewer

PYF or Attenuation?

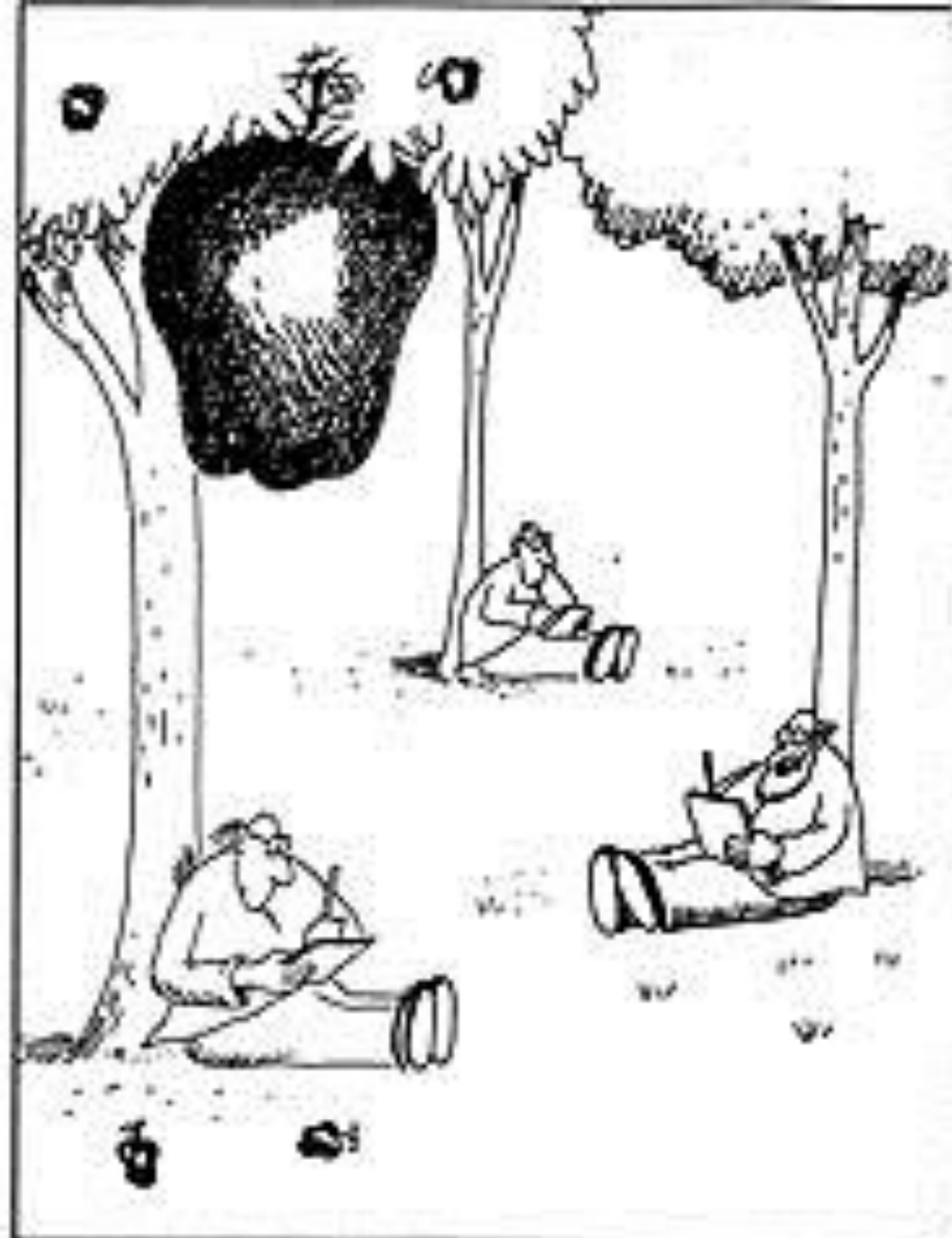
- PYF early flocculation of yeast preventing complete attenuation
- NOT poor attenuation causing early flocculation



- Define your terms and there will be no argument!
(*some Greek?*)

- Define your *assay* and there will be no argument?

Defined as a chicken!



"Nothing yet. ...How about you, Newton?"



PYF Assay

- ~~Currently~~ Formerly there was **no** way to determine PYF malt by physical or chemical means.
- Industry 'standard' ~~is~~ was to conduct ~ seven day small scale fermentation.
 - 1-2 liter cylindrical glass tube fermentors down to test tube sized fermentors.
 - Fermenting volumes ranging from 200mL-2L



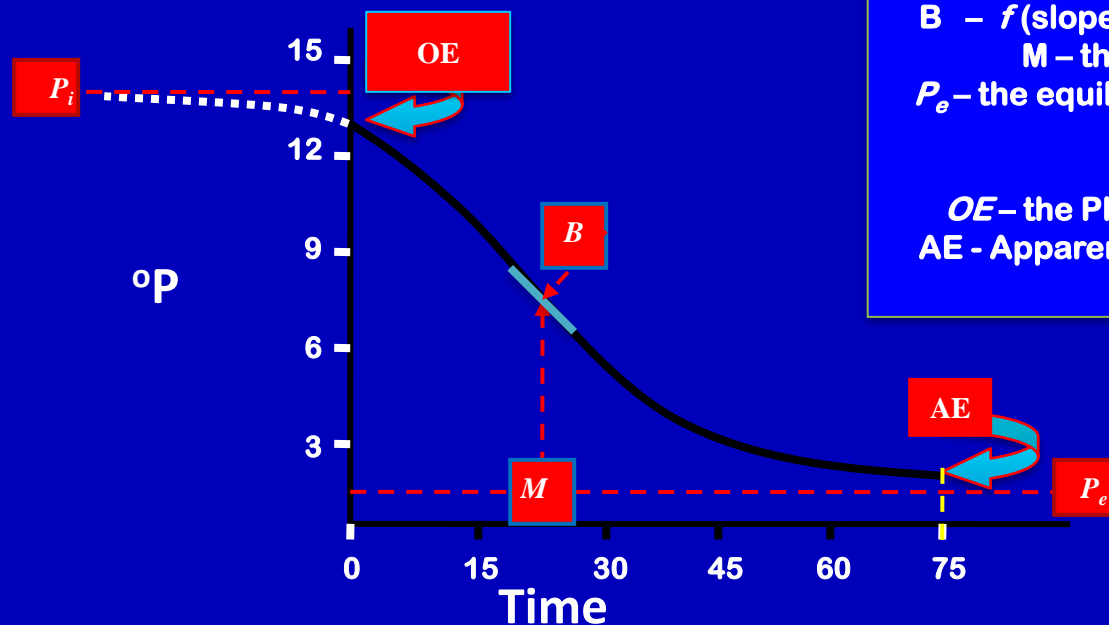
Mini-fermentation Method

- Use 15 mL of wort & 3 reps,
- Turbidity/density measurements taken 10 x' s @ 21°C over 75 h,
- Subject of ASBC collaborative. First year successful.
- Use entire dataset to get OE and AE->ADF



Modelling Fermentation

$$P_t = \left\{ \frac{(P_i - P_e)}{(1 + e^{-B \cdot (t - M)})} \right\} + P_e$$

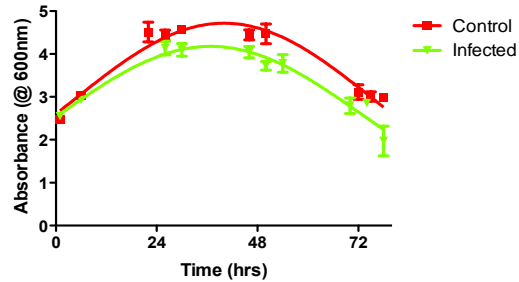


P_i – the initial asymptotic Plato value,
 B – f (slope) at the inflection point,
 M – the time at point B,
 P_e – the equilibrium asymptotic Plato value.

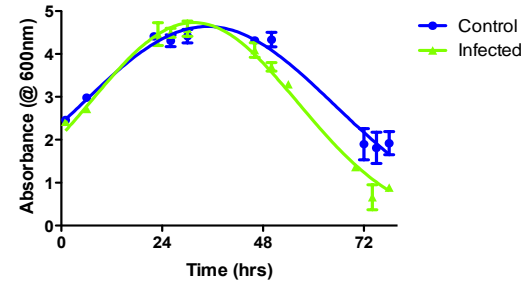
↓
 OE – the Plato value at time $t = 0$,
 AE – Apparent Extract-Plato value at time $t = 75$.

Effect of Fungus on Barley -> Fermentation

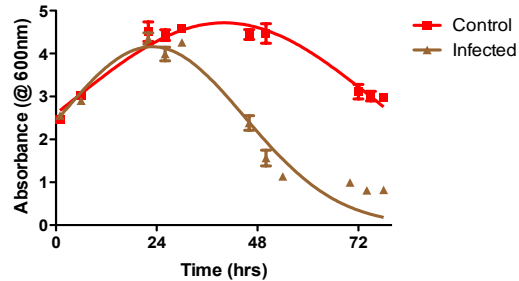
CDC Bold: net blotch (*P. teres*)



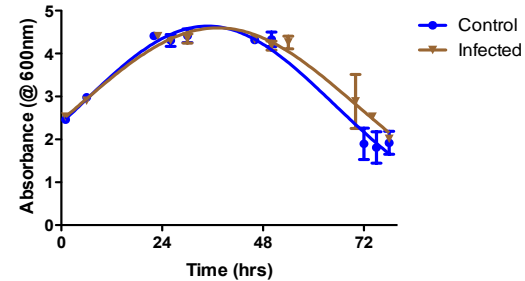
AC Metcalf: net blotch (*P. teres*)



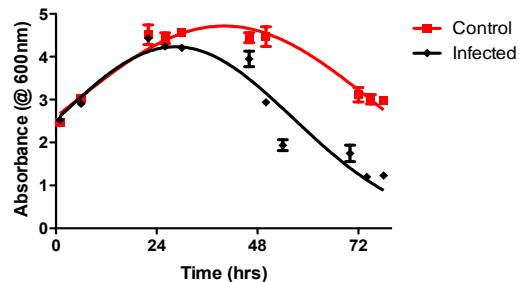
CDC Bold: spot blotch (*C. sativus*)



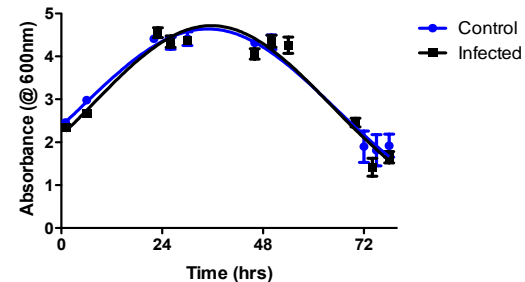
AC Metcalf: spot blotch (*C. sativus*)



CDC Bold: *F. graminearum*



AC Metcalf: *F. graminearum*



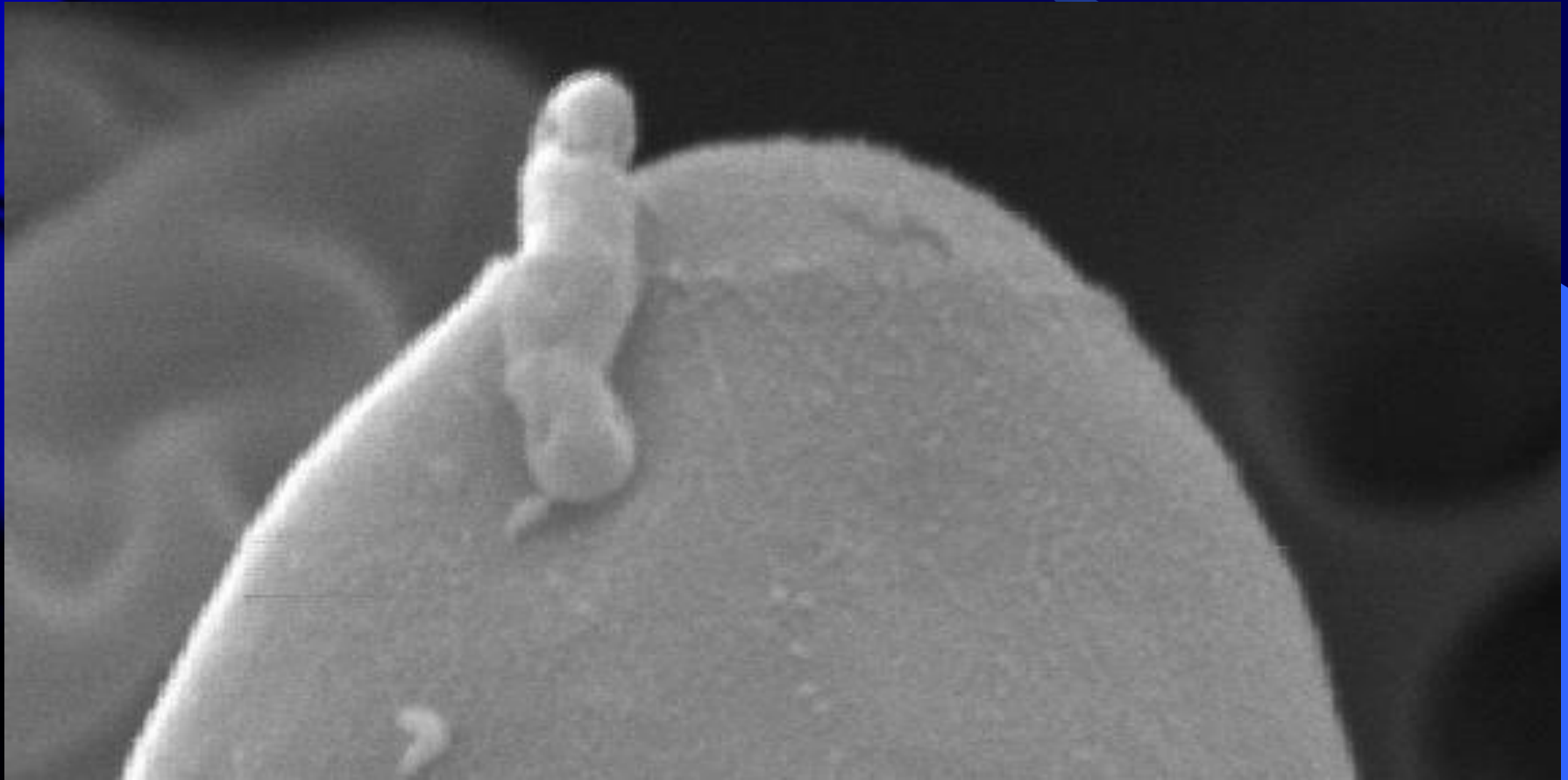
Effect of Fungi on Barley -> Fermentation

- Used two varieties – CDC Bold and AC Metcalf
- Treated with Net Bloch, Spot Bloch and *F. graminearum* in nurseries (CDC & AC)
- Malted @ CGC/Mashed Fermented @ Dalhousie

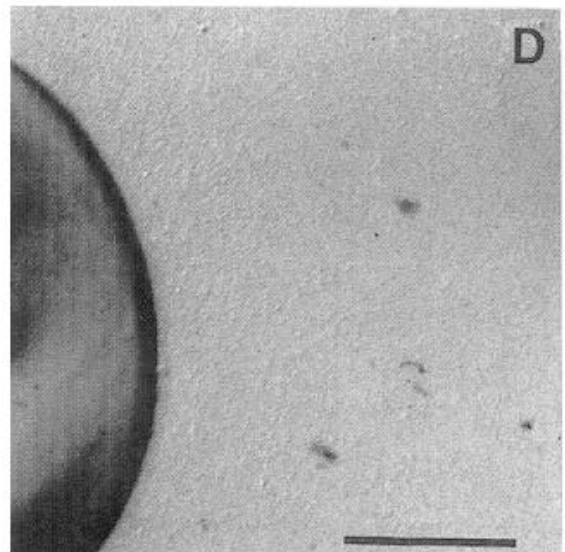
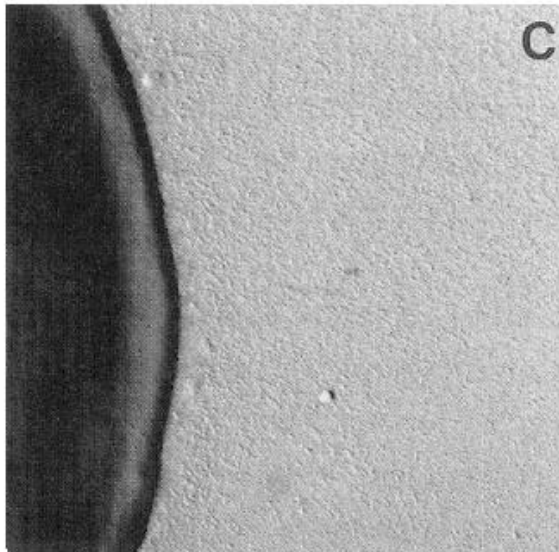
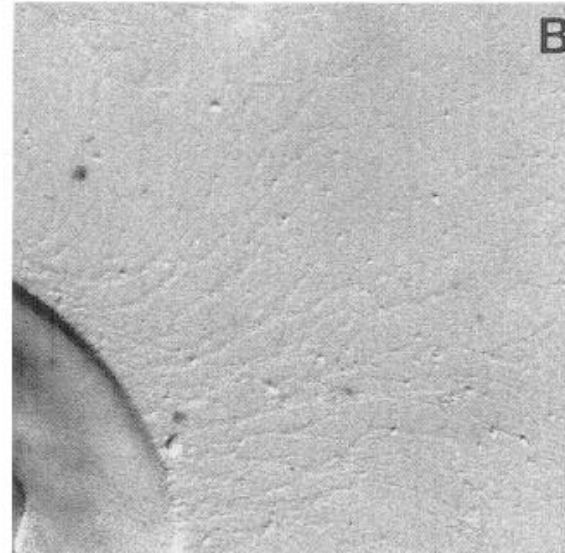
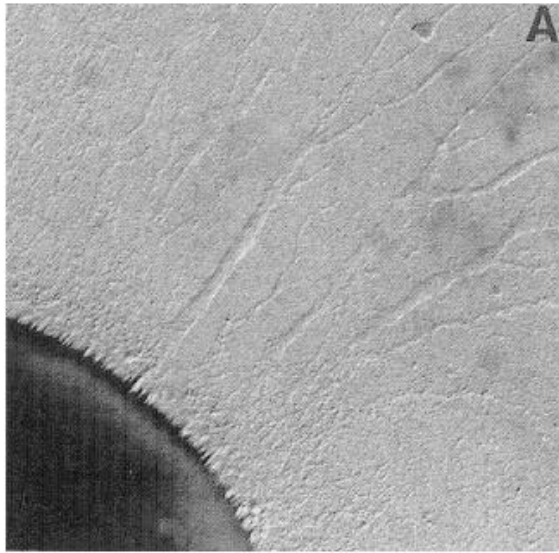
Summary:

- PYF caused by compound 100 kDa or greater isolated through filtration,
- Not associated with peptide,
- Some surface charge and binding strength differences (other work)
- Pure standards or arabinoxylan and beta-glucan did not produce PYF,
- Arabinoxylan-peptide moiety?
- ASBC Std. Assay exists.

Other notes!

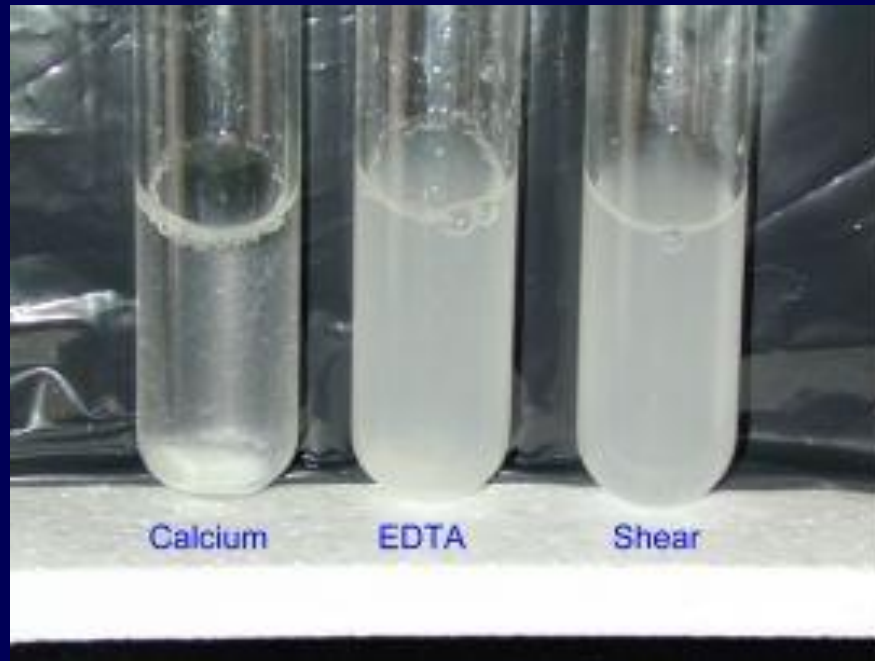


Yeast Cell Fimbriae



High shear effects

– <http://www.alumni.ca/~bebbinm/>



Acknowledgements

- NSERC, Scottish Enterprise
- MBAA, IBD, ICBD
- Canadian malting companies
- Australian, Canadian and Scottish brewing companies
- A. Macintosh and S. Murray

Questions?



And the Canadian and Scottish Taxpayers!



Don't worry it is all written down!

...so happy
...back in our arms," her
...people recognized her Smart, said yesterday. "I ha

Chip may one day fix your ailing memory

Ga-
ecided
eived
g others
al."
Angola,
and

BY CAROLYN ABRAHAM
MEDICAL REPORTER

Pa
bro
res
tic

...gap between mind and machine,
...could one day be used to boost the
...damaged brains of people who
...suffer strokes, injuries, epilepsy

A.Speers@HW.AC.UK>