



# Measuring Yeast Stress in the Brewery

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# What is Yeast Stress?



## Environmental Stress

- Excretion of Ethanol
- Organic acids lower pH
- Osmotic/hydrostatic pressure

## Human Associated Stress

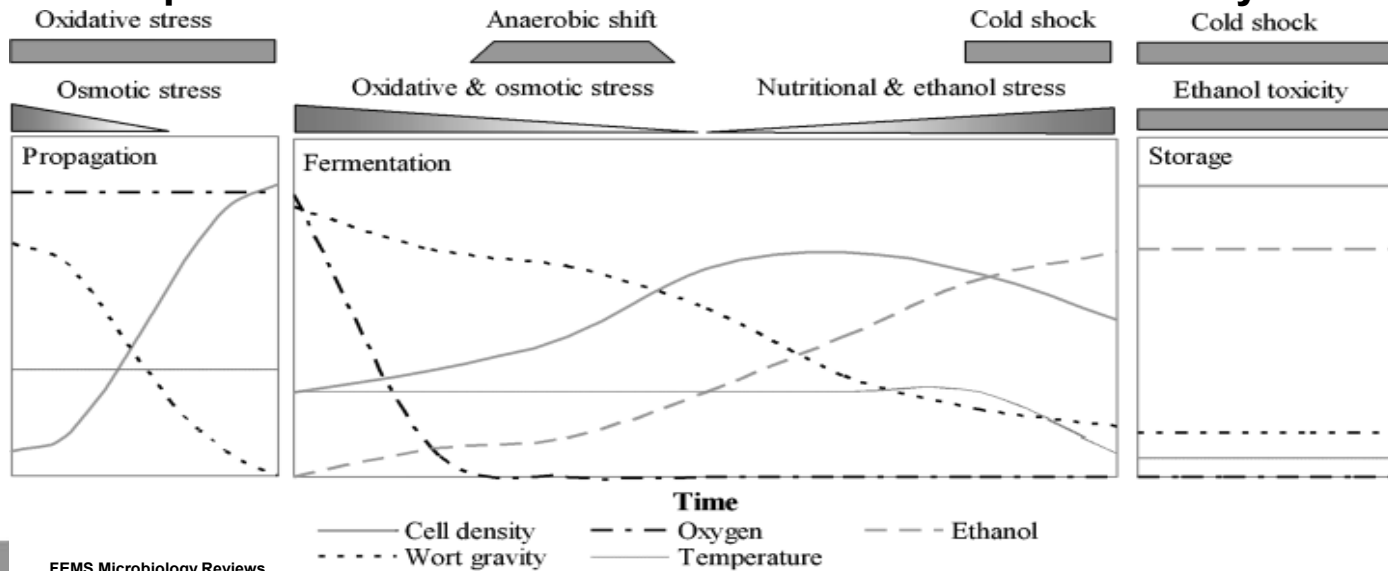
- Poor handling and storage
- Excessive agitation
- Gaseous pressure
- Oxidative

**The brewing process already creates a harsh environment for yeast.**

**Don't exacerbate it with human error.**

**If yeast is stressed out, it makes poor beer**

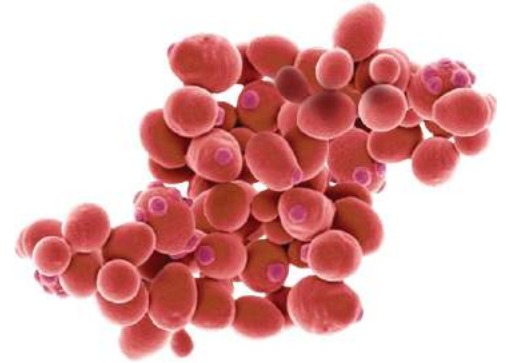
# Yeast responses to stresses associated with industrial brewery handling



# Measuring Yeast Stress

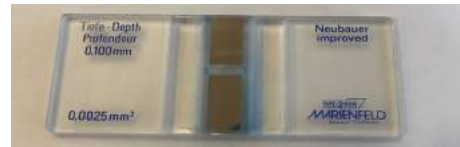
**Viability:** A measure of the proportion of living yeast cells to dead cells.

**Vitality:** A measure of the metabolic activity of yeast cells.

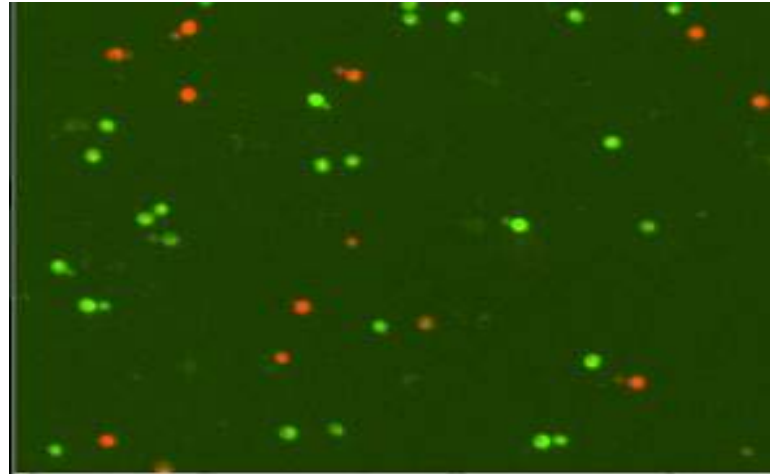
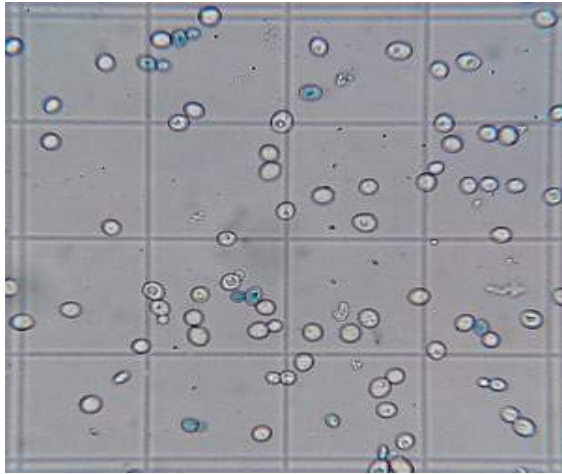


# Measuring Yeast Stress

- Hemocytometer
- Fluorescence based assays
  - Excitation of fluorescent dyes causes coloration
  - Total computational time 60-80 seconds
  - Roughly 1,000,000 cells per second



# Hemocytometer vs. Cellometer



# Yeast Stressors in Breweries

- Oxidative
- **pH**
- Osmotic
- **Temperature**
- Pressure
- **Ethanol toxicity**
- Nutritional Stress
  - Fermentable Carbohydrate
  - Assimilable Nitrogen

# Yeast Stressors in Breweries

Optimal Conditions	Sub-optimal Conditions
pH: 4.4 - 4.2	pH: <4.2
Temperature: 32 - 34°F	Temperature: >40°F
Pressure: 0 psi	Pressure: >0 psi
Time: 1-3 days	Time: >4 days
Ethanol: 0-5%	Ethanol: >5%

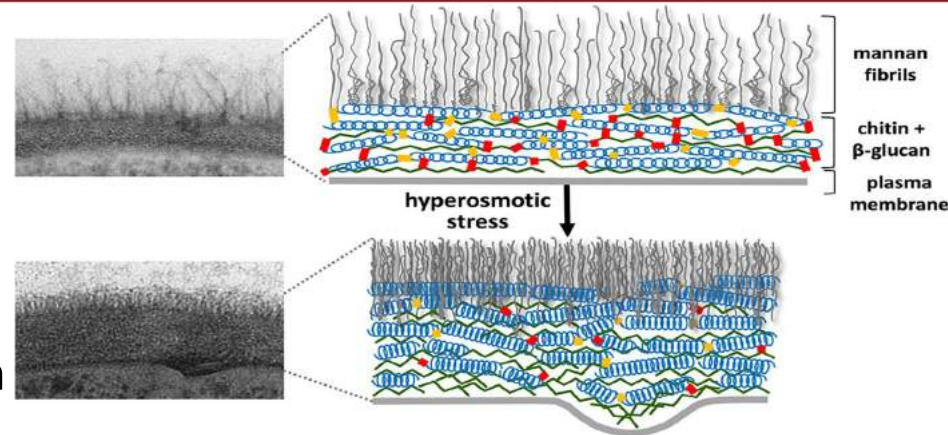


**Sub-optimal yeast storage puts you at risk for a poor fermentation**



# Cellular Responses to Stress

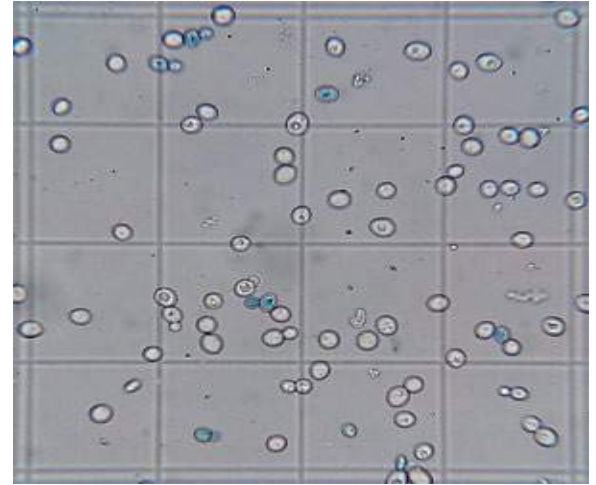
- Genes turn on
  - ESR (Environmental)
  - HSR (Heat)
  - GSR (General)
- Repression in Protein Synthesis
- Membrane fluidity/Cell wall composition
- Antioxidant production



Ene, I. V., Walker, L. A., Schiavone, M., Lee, K. K., Martin-Yken, H., Dague, E., . . . Brown, A. J. (2015). Cell Wall Remodeling Enzymes Modulate Fungal Cell Wall Elasticity and Osmotic Stress Resistance. *MBio*, 6(4). doi:10.1128/mbio.00986-15

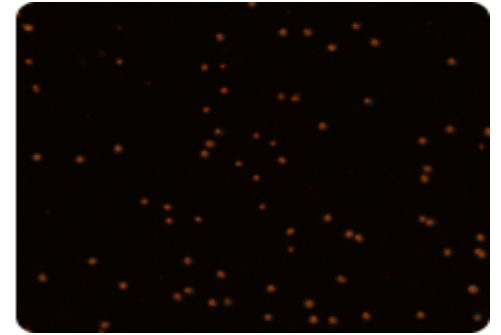
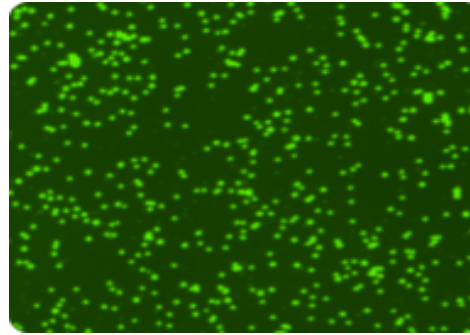
# Viability

- A measure of the proportion of living to dead cells
- Assays often based on uptake of dyes
- Methylene Blue
- Other assays
  - CFU, Spotting Test, Zone of Growth Inhibition, **AOPI**



# AOPI

- Acridine Orange/Propidium Iodide
- AO: Is cell permeable and stains live cells.
- PI: Is not cell permeable and stains dead cells



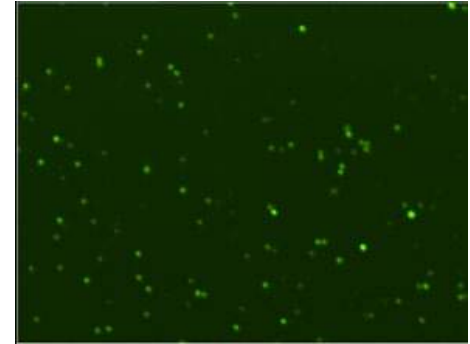
Viability using Acridine Orange/Propidium Iodide View all Viability applications →. (n.d.). Retrieved January 29, 2018, from <http://www.nexcelom.com/Applications/cell-viability-2-viability-using-aopi.php>

# Vitality

- A measure of the health of yeast in a culture
- Assays often based on enzymatic reduction of the stain resulting in fluorescence
- Assays include
  - FUN-1, WST-8, XTT, Rhodamine, Luciferase, **CFDA**

## CFDA-AM

- 5-Carboxy Fluorescein Diacetate
- Acetomethyl ester: aids in diffusion across the membrane
- Diacetate groups are cleaved by esterases resulting in the fluorescence of 5-Carboxy Fluorescein
- Carboxy group increases negative charge helping cells retain fluorescein at physiological pH



# Experimental Design

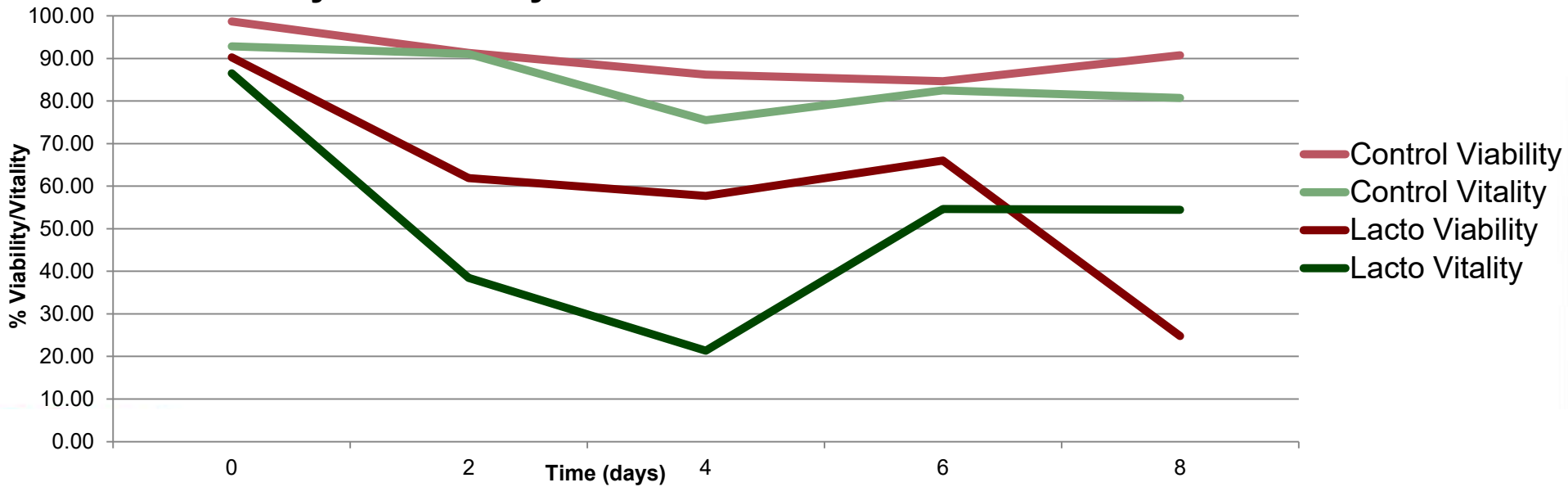
- Designed to test the AOPI and CFDA assays
- Testing the most common stress factors in the brewery
- BSI-1 California Ale chosen for popularity and efficacy for both assays



## pH Stress Practical vs. Experimental

- Typical end of boil wort pH starts around 5.1- 5.4 (depending on style) and ends around 4.2-4.4
- In kettle soured beers pH is between 3.2 - 3.5 upon pitching yeast
- Wort growth medium soured to pH 3.4 with *L. delbrueckii*
- Ferment BSI-1 to terminal gravity in both soured and controlled medium
- pH, Viability, Vitality readings every two days

## Viability and Vitality of BSI-1 in Controlled and Acidic Conditions

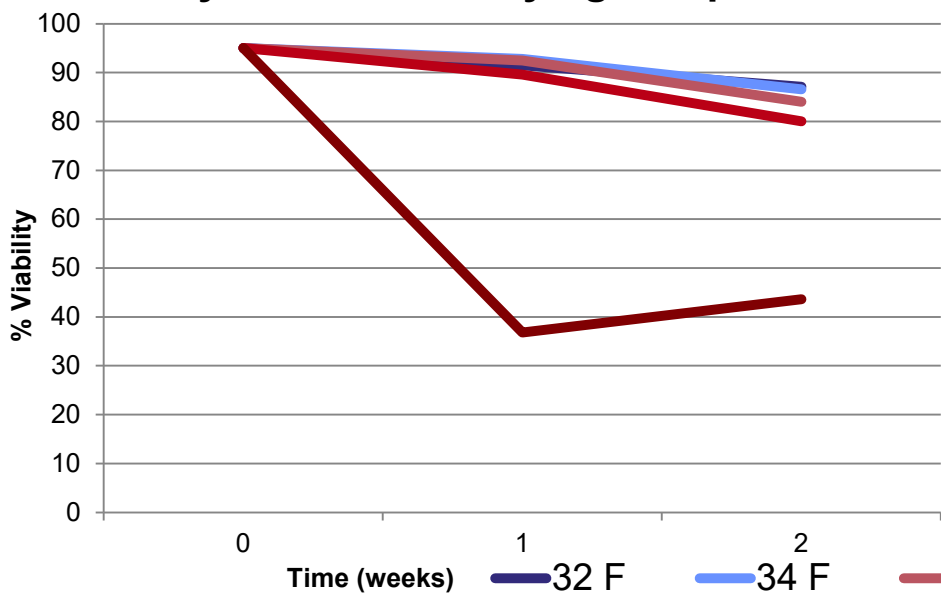




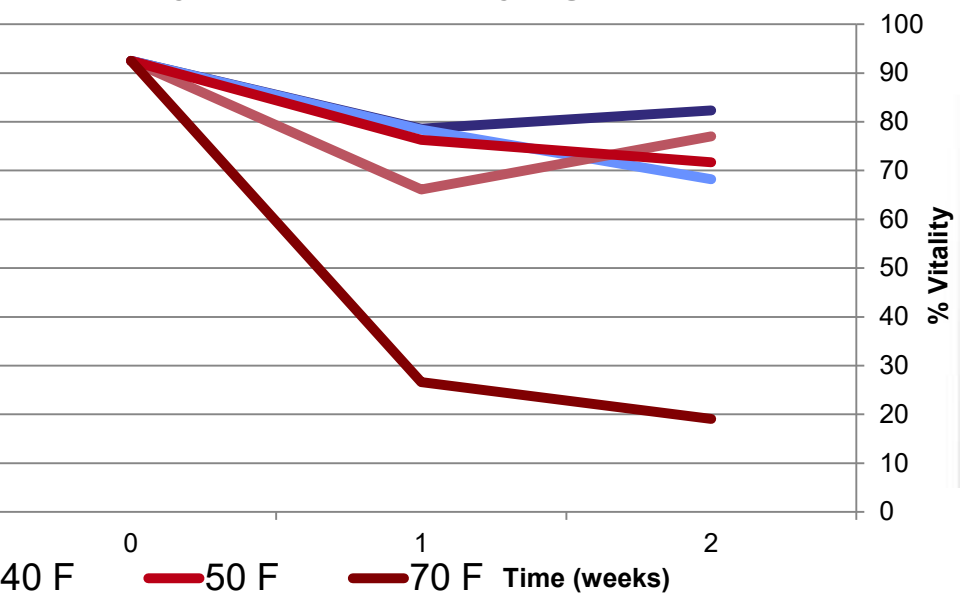
# Time/Temperature Stress Practical vs. Experimental

- Warm yeast depletes its glycogen resulting in compromised cell membrane synthesis upon repitching
- Diacetyl rests at 70°F are harmful.
- Glycol jackets cool concentrated yeast slurry to a depth about ~6 cm. Yeast at the center of a cone can heat up 10-15°F higher.
- Ferment BSI-1 to terminal gravity in wort growth medium
- Yeast was stored at 32, 34, 40, 50, and 70°F
- Viability and Vitality tested at 1, 2, and 3 weeks

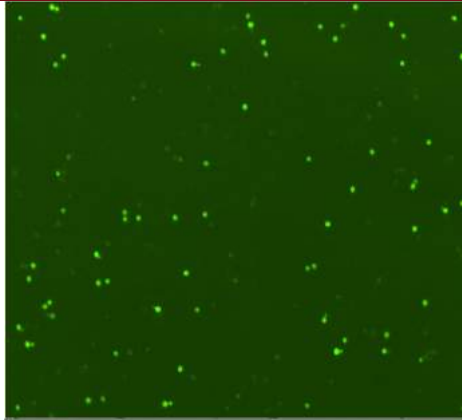
### Viability of BSI-1 at Varying Temperatures



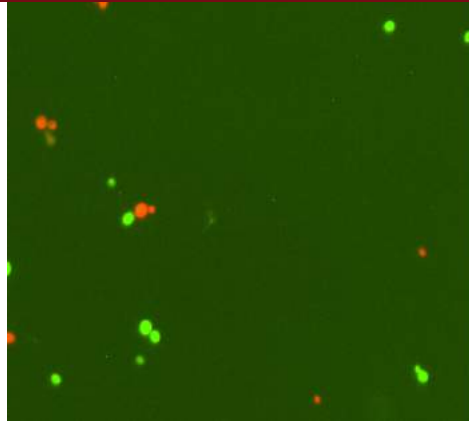
### Vitality of BSI-1 at Varying Temperatures



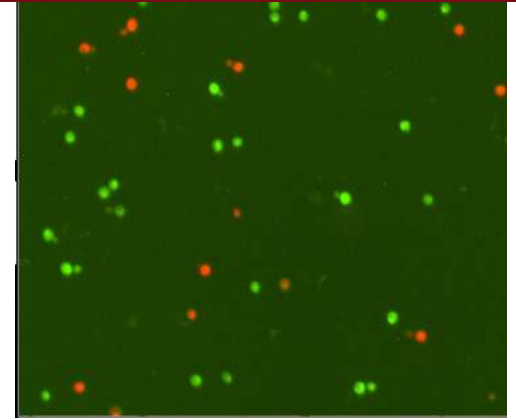
BSI-1 Fresh Sample



BSI-1, 1 month old



BSI-1, 2 month old



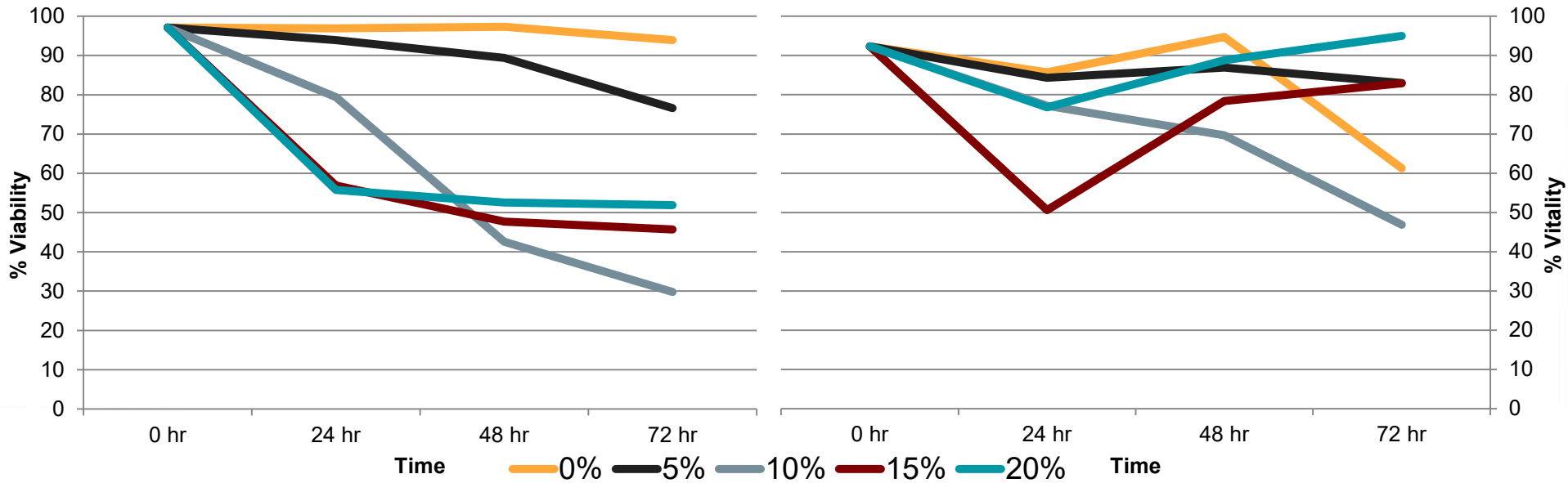
# Ethanol Stress Practical vs. Experimental

- High gravity brewing
- Elevated ethanol causes damage to cell membrane, inhibition of cell growth, loss of viability, vitality and reduced fermentation.



- Ethanol dosed at 5, 10, 15, and 20% concentrations
- Viability and vitality assays done every 24 hours for 3 days.

## Viability and Vitality of BSI-1 at Varying Ethanol Concentrations



**LIVE DEMO!**  
**WELCOME NAVA THAPA!**  
**THE YEAST WHISPERER!**



# AOPI Assay Procedure

Pipette 10  $\mu$ L diluted yeast sample



Add 10  $\mu$ L yeast dilution buffer and mix



Add 20  $\mu$ L AOPI stain and mix



Preview, Focus and Count



Select AOPI assay on Cellometer



Load 20  $\mu$ L into counting chamber

# CFDA Assay Procedure

100  $\mu$ L diluted yeast sample mixed with 100  $\mu$ L CFDA solution



Incubate for 45 mins at 30°C in the dark



Load 20  $\mu$ L sample into counting chamber



Select yeast vitality assay on Cellometer software



Preview and focus



Count



## Benefits and Limitations of AOPI

- Simple, fast, and easy procedure for cell count and viability
- Works well with samples containing debris and non specific particles
- The diameter of the yeast cell is fluorescent dependent
- The total cell count is focus/exposure dependent
- Expensive chemical and maintenance cost of Cellometer is high

## Benefits and Limitations of CFDA

- Simple and easy procedure
- Store desiccated and protected from light at -20°C
- Vital cell concentrations is focus/exposure dependent
- Stain depends upon the cell membrane permeability and flocculation characteristics
- High cost per sample

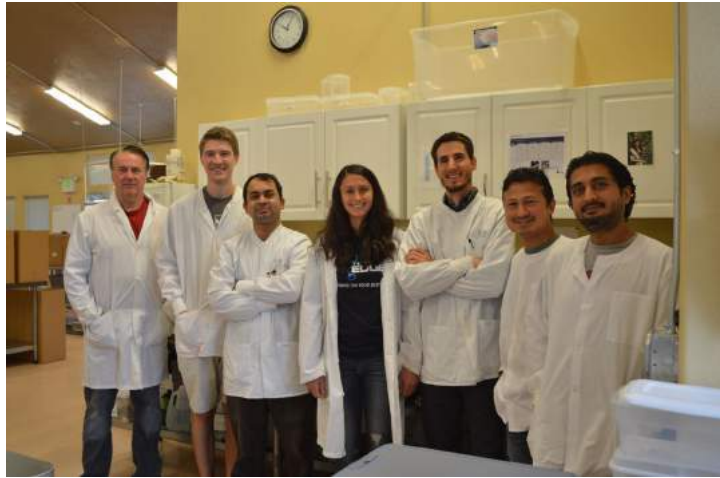
## Contributors

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# Questions



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