

# Controlling Yeast and Priming Parameters for Bottle Conditioning

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# Overview

- Why Bottle Condition At All
- Problem Definition
- Carbonation Issues
  - Where to Look First / Important Parameters
- What you need to measure these parameters
- Case Study Analysis
- Calculations/Fixing Issues
- Future Work

# Why Bottle Condition At All

- Increased shelf-life
  - oxygen scavenging
- Possible Reduction of Off-Flavors
- Mouthfeel
  - Smaller Bubbles
- Improved micro stability
  - Yeast out competes low level flora
- **MARKETING**

# Problem Definition: Why Experiments Were Needed

- Complaints of some “exploding” casks at our pub
- Random bottles reaching carbonation levels of 4.0+ volumes/vol

# Where Do We Start?

- Micro Analysis: Assure your beer is free of wild yeast and/or bacteria.
- Sugar Amount/Choice: Are we using too much? Is it specifically maple syrup causing a problem vs. dextrose, etc...?
- Calculation Check: When was this spreadsheet made anyways?
- Consistency: Is mixing a problem? Is the priming source evenly distributed in the tank?
- Intensive Carbohydrate Analysis (if necessary):  
HPLC

# Micro Analysis – Bacteria

- Both pediococcus and lactic acid bacteria can form CO<sub>2</sub>. These can be detected by off flavor and appearance (turbidity, rope forming colonies).
- HLP media is best for detection of both contaminants. HLP = Hsu Lacto Pedio media
  - Facultative anaerobe, used correctly colonies form in three to five days.
- Membrane filter or plating method. Media choice is SDA in anaerobic chamber. SDA = Schwartz Differential Agar

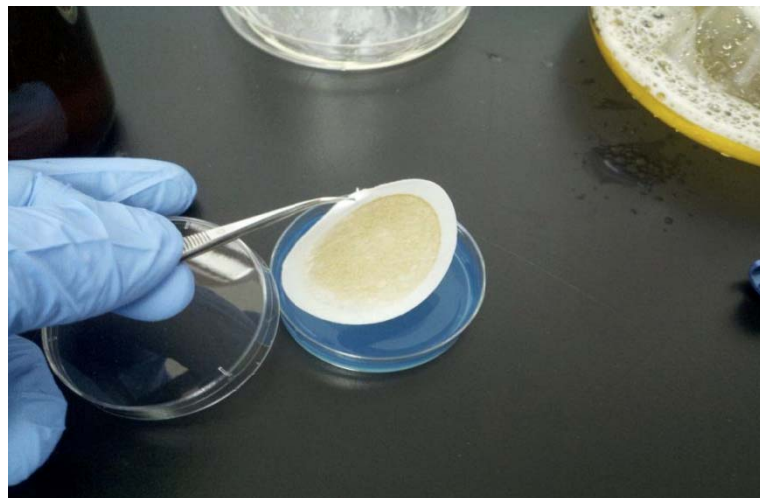
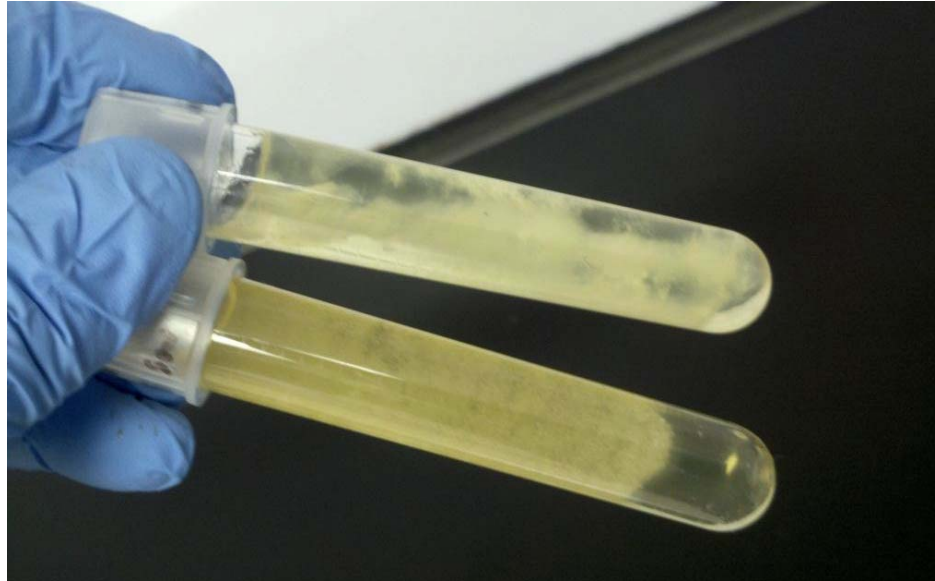
# Micro Analysis - Bacteria

- HLP Media

- ✓ Boil 7 grams HLP media in 100mL distilled water for 2-3 minutes
- ✓ Cool to less than 80 F
- ✓ Inoculate with 1 ml beer, 20 ml media, using sterile capped glass test tubes
- ✓ Incubate at 28-30 C for 3-5 days

- Contamination will show anywhere from a spot to an entire tube full of colonies/turbidity/haze.

# Micro Analysis - Bacteria





# Micro Analysis – Wild Yeast

- Heat Stress Test – Yeast thrive at different temps.
  - Ale like it warm
  - Lager like it cool
- Selective Sugar Analysis
  - Maltotriose fermentation = wild yeast
- Selective Media/Stains
  - Crystal Violet, Saccharomyces yeasts grow
  - Actidione, Wild yeast grow
  - Lysine, Non-Saccharomyces wild yeast grow
- No Issue with bacteria or wild yeast...

# Sugar Source: Maple Syrup Vs. Dextrose

- According to sources, maple syrup can contain enzymes for carbohydrate breakdown:  
unfermentable → fermentable
- We outsourced testing and found our choice of maple did not contain active enzymes,
  - this was NOT the issue
- Designed an experiment
  - Primed same beer with various sugar sources to verify fermentability

# Experiment

- Dogfish Head 60 Minute IPA
  - Hand filled sterile bottles
  - Primed with known amounts of sugar
  - Pitched with known amount of viable yeast
- Primed with
  - Brown Sugar
  - Sucrose
  - Maple Syrup
  - Dextrose
  - DME
- Target CO<sub>2</sub> 2.70 Vol/Vol

# Results: No Significant Difference In Priming Sugar

Sugar	ABV	Ea	RDF	pH	Calories
Brown Sugar	6.7	3.51	64.78	4.57	212.3
Sucrose	6.63	3.63	64.17	4.61	212.85
Maple Syrup	6.69	3.56	64.6	4.61	213.19
Dextrose	6.62	3.56	64.45	4.67	211.52
DME	6.57	3.68	63.82	4.49	211.93

# Calculation Time: Is Something Wrong?

- After digging into our old calculation spreadsheet, we found it was based on a textbook value of 2.0 P drop giving 1 gram CO<sub>2</sub>.
- Assumed we had end-fermented beer...
- Time for a new spreadsheet...

# Calculation Time

- Step 1 – Make a list of all parameters needed for this calculation
  - Current carbonation level
  - Desired carbonation level
  - Current gravity
  - Forced fermentation gravity
  - Current viable yeast cells
  - Needed viable yeast cells
  - Needed amount of priming sugar

# Calculation Time: Current Carbonation

- Haffman Gehaltemeter
  - ~\$8,000 – 25,000 depending on Capability
- Hach Orbisphere
  - Similar range
- Zahm & Nagel Shaker Method
  - ~\$1500



# Calculation Time: Desired Carbonation

- Do want the same spec for all brands?
  - 2.68 – 2.78 at Dogfish Head
- Different Carb Brand To Brand?
  - 2.3 – 2.9 depending on style



# Calculation Time: Current Gravity

- Refractometer (\$250)
- Hydrometer (\$50-300)
- Anton-Paar handheld DMA (\$1500)
- Anton-Paar AlcoLyzer (~\$60,000)

# Calculation Time: Forced Fermentation

- Make “yeast cake” out of spun-down yeast or vacuum filtered yeast.
- Put a very large amount of yeast into a sample and ferment while shaking or stirring for 24 hours to obtain its theoretical fermentation limit
- Example – 15 g yeast cake for 300 mL sample
- Measure gravity again using your method of choice (preferable same instrument as what you measure the current gravity with

# Calculation Time: Current Viability

- Hemocytometer – Counting cell with microscope. Methylene blue needed (\$100-\$1,000)
- Cellometer – Calibration required. Propidium Iodide needed. Quite expensive for purpose (\$15,000-20,000 plus disposable slides).
- Aber Meter / Capacitance Readings – Calibration Required. (\$80,000 - \$100,000 installed)



# Calculation Time: Hemocytometer Use

- Perform 1:10 dilutions of sample until there are >50 cells in the counting area at 40:1 magnification using a 1:1 dilution with Methylene Blue (usually 100:1 for a yeast slurry sample)
- $\text{Cells/mL} = (\text{Total Cells counted}/\# \text{ of Squares Counted}) \times \text{dilution factor} \times 10^4.$
- Easier way to Remember – 1:10 dilution plus 1:1 Methylene Blue = millions of cells/mL

# Calculation Time: Needed Viability

- Accepted range for bottle conditioning  
~0.75 – 1.2 million viable cells/mL
- Often times there is enough viable yeast still in your tank if it has not been centrifuged or filtered.

# Calculation Time: Sugar Needed

- This is where things get complicated...
- This number comes from a long calculation involving all of the other numbers that you have gathered.

# Calculation Time: Sugar Needed

[Conditioning Calculator.xlsx](#)



# Consistency: How Is It Measured

- Compare your tank to what you have in the bottle
  - Carbonation
  - Viable yeast count
  - Gravity
    - Pre-prime in tank
    - Post-prime in tank
    - Post-prime in bottle

# Consistency: How Is It Measured?

- Critical Factors for Bottle Conditioning
  - Storage Temperature
  - Consistent cell count and priming throughout packaging run
  - No micro contaminates
  - Low package oxygen
  - Baseline carbonation is consistent
  - **Most important consistency measurement is FLAVOR**

# Our Latest Results

- Cell counts 1.0-1.2 million cells/mL
- CO2 readings 2.71-2.88
- Taste Panel 100% Pass

# Future Work




- Determine which tanks are the best candidates for bottle conditioning using not only forced fermentations (current), but also HPLC.
- Work on mixing procedure vs Total Package Oxygen balance: Are we pumping too much?

# Take Home Thoughts

- Most important measurements for determining prime:
  - **CURRENT GRAVITY vs. FORCED GRAVITY**
- Most important factors for consistency
  - Effective and even recirculation/tank mixing
  - Lowest Dissolved Oxygen
  - Viable yeast in bottle
  - Gravity jump; pre prime to post
- **QUESTIONS???**



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