Getting The Most Out Of Your Hemocytometer

Peter Hoey
BSG CraftBrewing
March 28, 2013
Introduction

- Brewers have access to the same set of ingredients.
- How you combine them and control the process is what makes your beer unique.
- Monitoring yeast health and pitching rates is one more point of control to fine tune your process.
Getting Started

• The technology is simple and relatively inexpensive
• A hemocytometer: $15-30
• A microscope: $50-200
  – 400x objective
• 1 ml transfer pipet
• A scale that can measure grams accurately or a 100 ml volumetric flask
• A Pasteur pipette or a fine tipped glass pipet
• A hand held counter
• Pipette Pump
• Methyene Blue Stain
What is a Hemocytometer?

- A slide that you can look at under a microscope
- Chamber has a known volume of 0.0001 ml³

Photo by Jeffery M. Vinocur
What is a Hemocytometer?

- Laser etched in the surface are gridlines to aid in counting cells.
- Cell counting occurs in the matrix where the triple lines converge.
• If the equipment is not accessible and ready to use you and your brewers won’t count cells regularly.
  – Not everyone has a lab.
  – Or an office.
  – Or a desk.
• Build and maintain a kit.
  – Use a tray, tool box to store everything.
• Develop or borrow a spreadsheet to calculate pitching quantities.
Cell Counting Procedures

• Sampling of yeast slurry
  – Yeast should be stored in a vessel that make mixing and sampling easy.
  – Sampling needs to be done in a sanitary manner.

• Sample Size
  – Will you pitch by weight or by volume?
  – 1 ml if pitching by volume or 1 g if pitching by weight.
• Create a dilution
  – 1:100 is target using 1 part yeast slurry and 99 parts distilled water
  – 1 gram of slurry to 99 grams of water
  – 1 ml of slurry to 99 ml of water
  – Include the methylene blue as part of the dilution to determine viability
• A word about Viability
  – Viabilities below 95% may not be accurate
  – The test only demonstrates the yeasts ability to metabolize the methylene blue stain
  – We are interested in the yeasts ability to ferment wort
  – The test measures viability but what we are really interested in is vitality
Cell Counting Procedures

• Load the hemocytometer
  – Make sure the hemocytometer is clean and dry
  – Place a lens on the hemocytometer
  – Take the Pasteur pipette and fill with dilution
  – Create a droplet at the tip of the pipette
  – Touch the droplet to the edge of the slide and the sample will wick into the chamber

http://commons.wikimedia.org/wiki/File:Hemocytometer.jpg
Cell Counting Procedures

• Counting Cells
  – No need to count every cell in the chamber
  – The grid is 5 x 5 of the large squares
  – Use a hand held counter.
  – Budding cells are counted as **one** cell unless the bud is at least one half the size of the mother.
• **Counting Cells**
  – Count 5 of the 25 squares in the 5 x 5 grid.
• Move methodically through the grid
• If the cell touches to top or right line of the grid do not count.
• If the cell touches the bottom or left line of the grid count it.
Determining Pitching Rate

• Traditional pitching rates
  – 1 million cells per ml per degree plato for ale
  – 2 million cells per ml per degree plato for lager

\[
\text{\(\circ\text{Plato wort} \times \frac{1 \times 10^6 \text{ viable cells/ml_{wort}}}{1 \text{ \(\circ\text{Plato}\)}} = \text{viable cells/ml_{wort}}\)}
\]

\[
\text{\(bbl_{wort} \times \frac{117.35 \text{ L_{wort}}}{1 \text{ bbl_{wort}}} \times \frac{1,000 \text{ ml_{wort}}}{1 \text{ L_{wort}}} \times \frac{\text{viable cells}}{\text{ml_{wort}}} = Total \text{ cells needed}\)}
\]
Cell Counting Procedures

- The Calculation:

\[
\frac{(\text{viable cell count})(5)(\text{dilution})}{(\text{chamber volume})} = \frac{\text{yeast cells}}{\text{ml or g}_{\text{slurry}}}
\]

- *Viable* cells are what we are interested in.
- The number 5 in the equation takes our sample of 5 squares up to 25 squares to represent the whole grid.
- The dilution factor in this example would be 100 because we did a 100:1 dilution.
Determining Pitching Rate

\[
\frac{\text{Total Cells Needed}}{\text{yeast cells/ml}_{\text{slurry}}} = \text{Volume of yeast slurry required (in ml)}
\]

\[
\frac{\text{Total Cells Needed}}{\text{yeast cells/g}_{\text{slurry}}} = \text{Volume of yeast slurry required (in g)}
\]

- Convert to gallons or pounds for easier use:

\[
\text{ml}_{\text{slurry}} \times \frac{1 \text{ gal}_{\text{slurry}}}{3785 \text{ ml}_{\text{slurry}}} = \text{gal}_{\text{slurry}}
\]

\[
\text{g}_{\text{slurry}} \times \frac{1 \text{ lb}_{\text{slurry}}}{453.592 \text{ g}_{\text{slurry}}} = \text{lbs}_{\text{slurry}}
\]
Yeast requirements for brewing 15 barrels of 13 plato wort of ale
13 million cells per ml would be target pitching rate

\[
15 \text{ bbl}_{\text{wort}} \times \frac{117.35 \text{ L}_{\text{wort}}}{1 \text{ bbl}_{\text{wort}}} \times \frac{1,000 \text{ ml}_{\text{wort}}}{1 \text{ L}_{\text{wort}}} \times \frac{1.3 \times 10^7}{\text{ml}_{\text{wort}}} = 2.288 \times 10^{13} \text{ cells needed}
\]

\[
\frac{(\text{viable cell count})(5)(\text{dilution})}{(\text{chamber volume})} = \frac{(98 \text{ cells})(5)(100)}{(0.0001 \text{ ml})} = 4.9 \times 10^8 \text{cells/ml}_{\text{slurry}}
\]

\[
\text{Total Cells Needed} = \frac{2.288 \times 10^{13} \text{ cells}}{4.9 \times 10^8 \text{ yeast cells/ml}_{\text{slurry}}} = 4.67 \times 10^4 \text{ ml}_{\text{slurry}}
\]
• Convert to gallons or pounds for easier use:

\[
4.67 \times 10^4 \text{ ml}_{\text{slurry}} \times \frac{1 \text{ gal}_{\text{slurry}}}{3785 \text{ ml}_{\text{slurry}}} = 12.34 \text{ gal}_{\text{slurry}}
\]

\[
4.67 \times 10^4 \text{ g}_{\text{slurry}} \times \frac{1 \text{ lb}_{\text{slurry}}}{453.592 \text{ g}_{\text{slurry}}} = 102.96 \text{ lbs}_{\text{slurry}}
\]
Determining Pitching Rate

• Non traditional pitching rates
  – Some fermentation flavors from expressive yeasts can be manipulated by intentional under or over pitching
  – Not all strains can perform well with under pitching, you still need to achieve attenuation
  – Rates of 750,000 cells per ml per degree plato in German Style Hefeweizen and Belgian styles have worked well balancing flavor and performance
• High Gravity Beers
  – Over pitching high gravity beers typically leads to better attenuation rates
  – Can lead to lower flocculation and an abundance of yeast in the fermenter

• Beyond fermentation flavors
  – Yeast cells absorb isomerized alpha acids
  – Pitch rate effects finished IBU levels in beer
  – Consistent pitching rates will result in more consistent bitterness.
Beyond Primary Fermentation

• Determining the best time to filter beer
  – Establish a *measureable* target for pre-filtration clarity

• Establishing consistent yeast levels in unfiltered beer.
  – Consistent haze and yeast count in beer package with yeast across several operators
• Determining bottle or can conditioning pitching rates
  – Evaluate unfiltered beer prior to package
  – Determine fresh yeast dosing rates
    • Too much yeast in the bottle leads to off flavors and shorter shelf life
    • Too little viable yeast can result in a failure to re-ferment in the bottle.
  – 500,000 cells/ml is sufficient if only a portion of final co2 is gained from bottle conditioning.
  – Over 1 million cells/ml can result in excessive yeast in the bottle.
Conclusion

• You monitor your mash temp, calculate IBUs and weigh out your grain already
• All are points of control that impact the final flavor of your beer
• Using a hemocytometer gives the brewer one more point of control to fine tune flavor and ultimately achieve a more consistent product.
Thank You

Steve Parkes – American Brewers Guild and Drop In Brewing Company
Ray Romero – Brewers Supply Group
The Brewers Association

QUESTIONS?
PHoey@BSGCraftBrewing.com