APPLICATION INFORMATION

Cellular Solutions

ENUMERATION AND SIZE DISTRIBUTION OF YEAST CELLS IN THE BREWING INDUSTRY

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Introduction

Breweries, such as those operated by Anheuser Busch, Inc., utilize the Coulter Principle for their yeast cell enumeration and sizing. This technology uses the Z™ Series instrument models and the Multisizer 3. This document will focus on the Z Series models: the Z1 Single Threshold, the Z1 Dual Threshold, and the Z2 Analyzer.

Yeast cells are analyzed during the fermentation process. Carbohydrates in the wort, which is the water containing sugars from the grains, are converted by the yeast strain into alcohol, carbon dioxide, and numerous by-products.

There are several stages in this process, during which the yeast cells are analyzed. The first, and very critical stage for cell enumeration is during the pitching process. Pitching is the introduction of the live yeast culture into cooled wort to begin the fermentation. It is important to deliver the proper cell concentration for a successful fermentation. In addition, the yeast concentration will affect beer taste. A typical pitch concentration is 30.3 x 10^6 cells/mL. Yeast counts using either a Z1 single or dual threshold can be used to determine the initial cell concentration. If size distribution is being measured, a Z2 model is necessary.

Often, samples are taken during the fermentation process. During this time, the yeast cells may be budding. The Z Series instruments will enumerate a budding cell as a single unit. The third stage for sampling is at the end of the fermentation process. A sample analyzed at this time is highly likely to have proteinaceous material present. To accurately measure these samples, a Z2 analyzer is needed. The size-distribution histogram may show a bi-modal size population. One peak is the yeast cell population; the other, flocculated protein (Figure 1). To remove the protein material, simply add several drops of 2 M sodium hydroxide (NaOH) to the yeast sample, mix, and analyze on the Z2.

Equipment

One of the following instruments

• Z1 Single Threshold	P/N 6605698
• Z1 Dual Threshold	P/N 6605699
• Z2 Analyzer	P/N 6605700
• Accuvette™ Cups	BCI P/N 8320592

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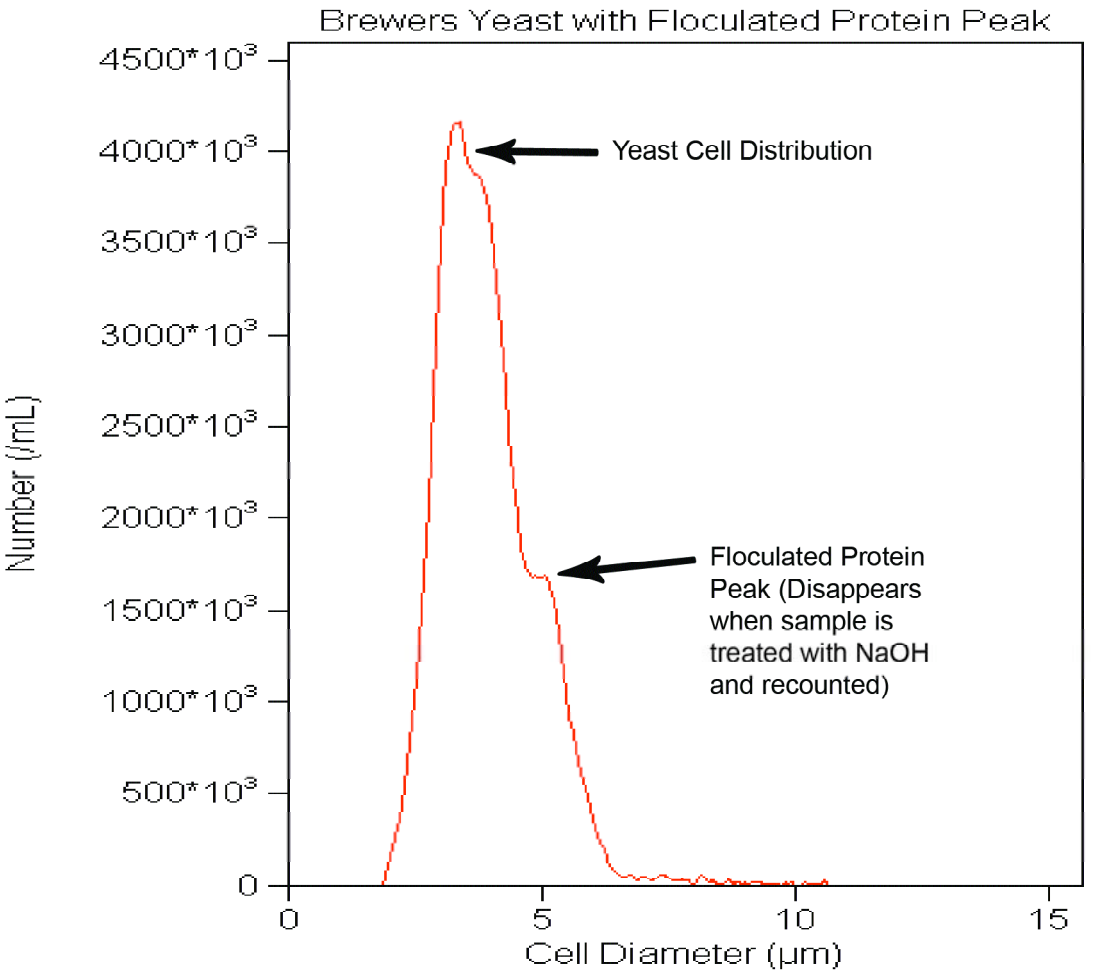

Figure 1: *A yeast sample with a bi-modal peak. The first peak is yeast, while the second peak is that of flocculated protein. The secondary peak can be larger than the yeast cell peak.*

Reagents

• 2 M NaOH Sigma	P/N S8263
• Z pak	BCI P/N 8320312
• L3 Beads	BCI P/N 6602793
• L5 Beads	BCI P/N 6602794
• L10 Beads	BCI P/N 6602796

Method

Basic Instrument Setup for Z1 Dual Threshold and Z2 Analyzer

1. Sample preparation: 10 µL of yeast sample into 20 mL Isoton II
2. 100 micron Aperture Tube.
3. Set Lower Threshold to 3.0 microns.
4. Set Upper Threshold to 10.0 microns.
5. Count Mode - between TL and TU.
6. Set Dilution Factor 2000.

Basic Instrument Setup for Z1 Single Threshold

1. Sample preparation: 10 µL of yeast sample into 20 mL Isoton II
2. 100 micron Aperture Tube.
3. Set Lower Threshold to 3.0 microns.
4. Count Mode - above TL.
5. Set Dilution Factor 2000.

Procedure

1. Calibrate Z system. Add 5 drops of L10 Beads to 20 mL of Isoton II.
   a. Mix gently by inversion.
   Note: Do not mix vigorously as air bubbles will form.
   b. Press Cal button on the keypad to select the C1 page.
   c. Set units to µm. In the calibration size, enter Number Mode.
   d. Press Start.
   e. Record calibration factor.

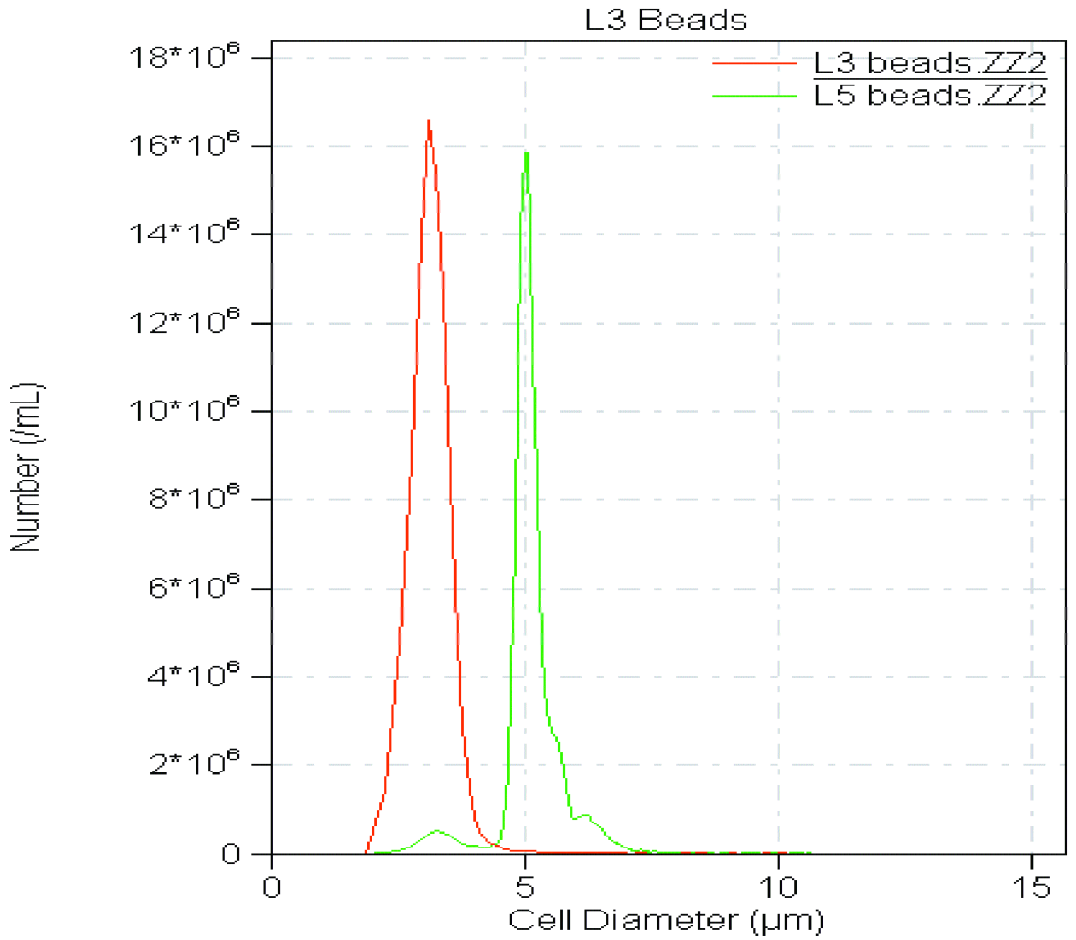

Figure 2: *L3 and L5 beads used to set size paramters.*

f. Repeat 3 times.
g. Average results and enter in new calibration factor.

2. Running size standard
   a. Select Set Up.
   b. Set lower threshold to 3.0 microns.
   c. Set Upper Threshold to 10 microns.
   d. Set count mode to between.
   e. Add 5 drops of L5 Beads to 20 mL of Isoton II.
   f. Mix by inversion.
   g. Press start.
   h. After run, acquire data with Accucomp software (Figure 2).
3. If there are sample controls, run these at this time.
4. Run Samples.
   a. Select output.
      i. Set dilution factor to 2000.
      Note: Dilution factor may vary based on sample density.
      ii. Set result type to concentration.
   b. Add 10 μL of sample to 20 mL Isoton II.
   Note: Sample may have aggregate; mix thoroughly prior to adding to Isoton II.
   c. Mix gently.
   d. Press start.
   e. After run, acquire data with Accucomp™ software (Figure 3).
5. Samples that have a bi-modal peak (Figure 1).
   a. Add 4 drops of 2 M NaOH.
   b. Mix gently.
   c. Incubate 5 min. and Press start.
   d. After run acquire data with Accucomp software.

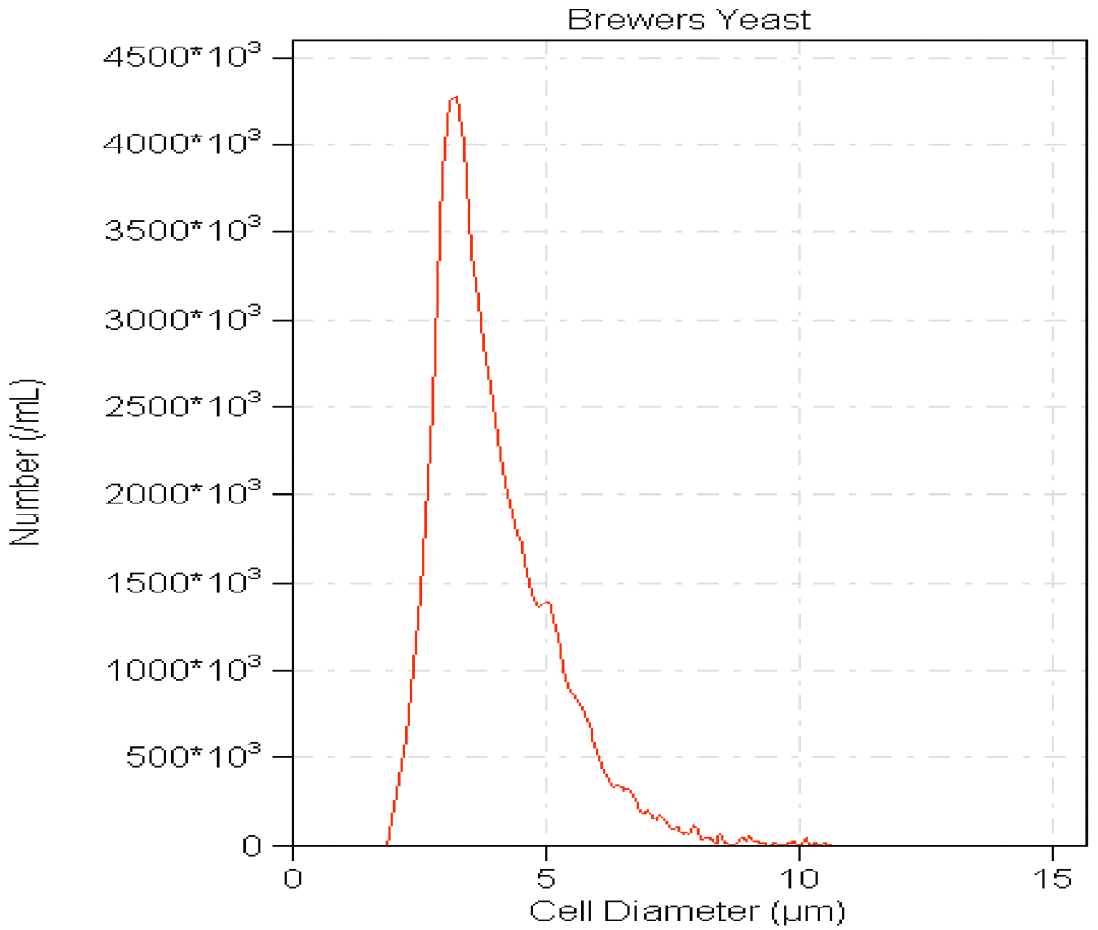

Figure 3: *Brewers Yeast, which was grown at 37ºC 2 hours in distilled water. Average yeast cell size is 3.5 microns. Aggregates show as 7 microns or higher.*

Selected References

1. ASTM D F2149-01. Standard Test Method for Automated Analyses of Cells-the Electrical Sensing Zone Method of Enumerating and Sizing Single Cell Suspensions. *ASTM International*. February, 2002.
2. H.A. Teass, Jr., J. Byrnes & A. Valentine. Correlation of Yeast Measurement Between Spin Down and Coulter Counter Laboratory Analysis and In-Line Measurements With the McNab Cell Counter. *Master Brewers Association of the Americas Volume 35, Number 2, 1998* Pages 101-103.

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