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A novel cytometry-based Lactobacillus counting method for the production of kettle sour beer

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1. ABSTRACT

Craft beer has enjoyed a tremendous growth over the last decade. As more craft breweries open, the desire for more flavors of beer also grow, which may require new methods to monitor the production and quality of the products. The majority of the craft beers are fermented using a variety of strains of yeasts, which can be counted using manual hemacytometer or automatically counted using image cytometers such as the Cellometer X2. In the recent years, craft breweries have begun to introduce bacteria for new flavors. One such beer, the kettle sour beer, has grown in popularity. This beer utilizes a combined fermentation process, where lactic acid bacteria is allowed to first ferment for some time, before pitching the yeast to complete the product.

Traditionally, bacterial particles have to be counted using colony outgrowth on an agar plate to determine the concentrations or using a microscope with high magnification to count cells in hemacytometer. However, these methods are time-consuming, highly tedious, and large operator-dependent error.

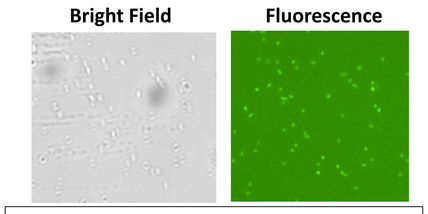
In this work, we have developed an image cytometry-based bacteria counting method to measure and monitor three lactobacilli strains in a kettle sour beer fermentation environment. We developed the counting methods using fluorescent stains SytoBC and Syto 9 to directly count L. plantarum, L. bulgaricus, and L. brevis. The three strains were grown for 24 hours in MRS broth and counted at different titrations. The concentrations of the serial dilutions were evaluated using fluorescence stains and image cytometry which were then evaluated against standard plate counts. In addition, lactobacilli were pitched in a standard kettle sour beer fermentation recipe and protocol. Samples were collected every 3 hours to monitor the growth of lactobacilli strains using image cytometry and standard plate counts. Future work will be focused on developing a method to directly measure lactobacilli viability.

2. CURRENT METHODS FOR BACTERIA ENUMERATION

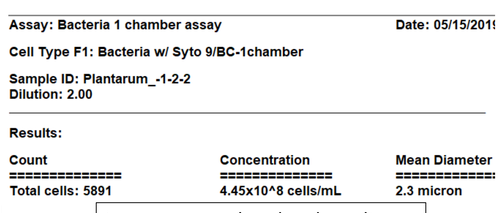
Methods	Description	Known Issues
pH Monitoring	Measure with pH meter at end of fermentation ~3.1-3.9	•Under pitching or growth of Lactobacillus may not be detected immediately
Colony Formation	Serial dilutions of Lactobacillus samples onto MRS agar dishes	•Requires at least 24-48 hours for growth •Time consuming (1-4 mins/plate) •Low number of particles •Sample counting variation and operator variation
Hemacytometer	Manual counting of Lactobacillus using bright field microscopy	•Time consuming (~10 min/sample) •Operator dependent error •Unreliable due to small size of bacteria

3. CELLOMETER IMAGE CYTOMETRY INSTRUMENTATION AND PROTOCOL

Stain sample with Syto 9/BC | Pipette 4 µL of stained cells | Tape holes with scotch tape | Insert slide in instrument | Select corresponding assay



Bright field and fluorescent images are acquired and analyzed



Images are analyzed and results are automatically displayed

- Cellometer X2 automatically counts bacteria using SYTO9, SYTOBC, and SYTOX Green fluorescent stains

4. LACTOBACILLUS COUNTING COMPARISON PROTOCOL

Plating Assay

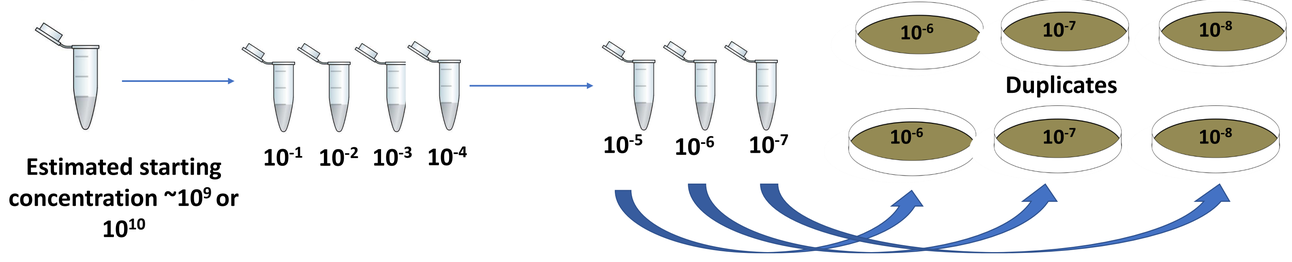
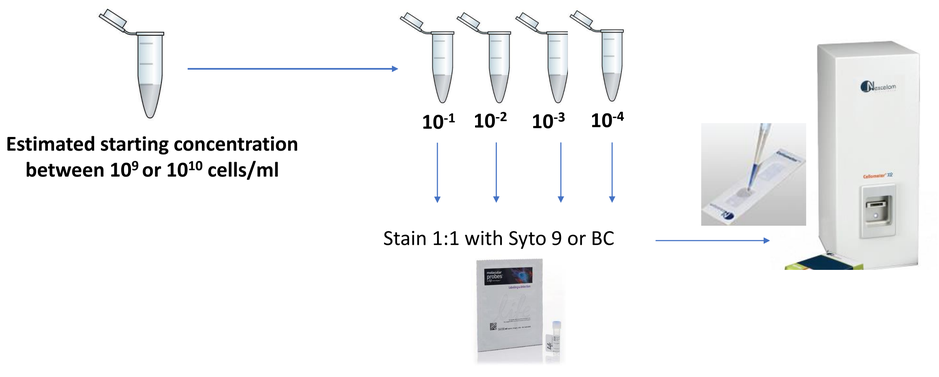
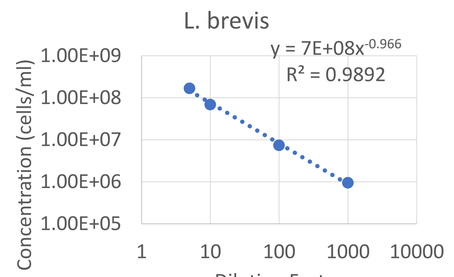


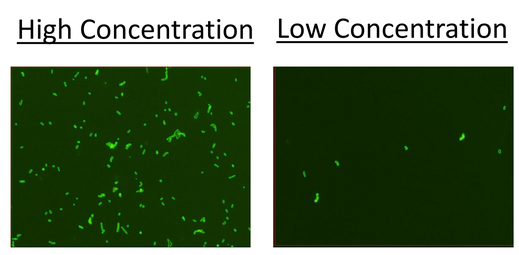
Image Cytometry

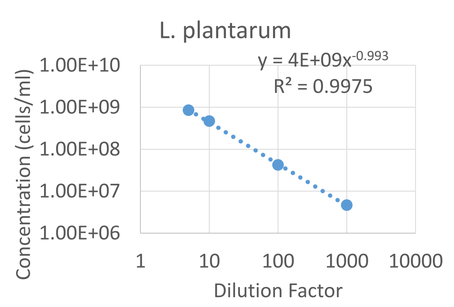


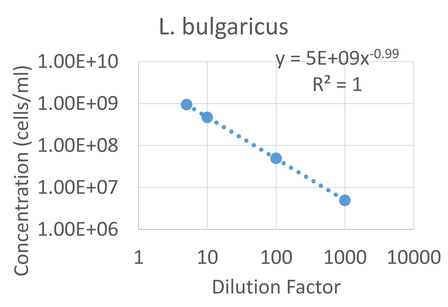
- *L. brevis – Syto BC*
- *L. plantarum – Syto BC*
- *L. bulgaricus – Syto 9*

5. LINEARITY RESULTS AND COMPARABILITY TO PLATING







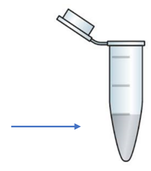


	Cellometer X2		Manual Counting		
	Cells/ml	**Log10**	**CFU/ml**	**Log10**	**Differences**
L. plantarum	4.23E+09	9.63	6.90E+09	9.84	0.21
L. bulgaricus	4.71E+09	9.67	7.10E+09	9.85	0.18
L. brevis	8.32E+08	8.92	6.20E+08	8.79	-0.13

- Results showed differences less than 0.3 Log
- Indicating comparability between Cellometer and manual counting

6. KETTLE SOUR FERMENTATION PROTOCOL

Prepared wort



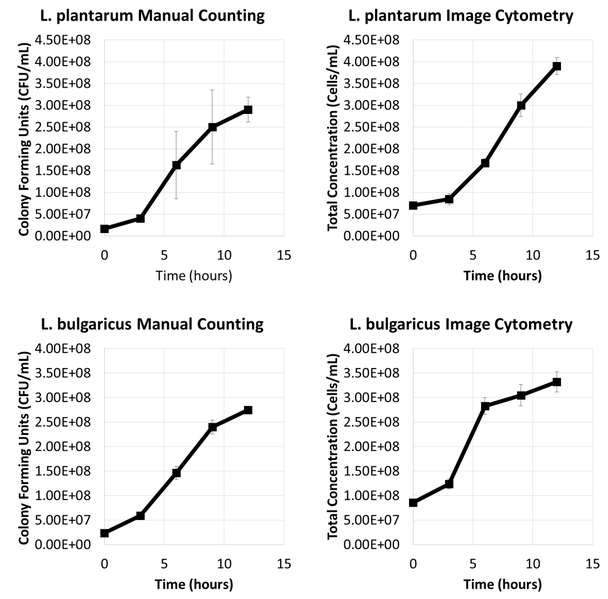
Cell concentration measured using Cellometer X2

Pitched 67 million cells into fermentation vessel

University of Maine Brewery Lab

- Two different strains of *Lactobacillus*
  - *L. plantarum*
  - *L. bulgaricus*
- Analyze at 0, 3,6, 9 and 12 hours
- Compared plating and image cytometry methods

7. LACTOBACILLUS GROWTH CURVES COMPARISON



- Image cytometry proved effective in measuring the concentration over time (n = 3)
- Plating produced inconsistent counting with high standard deviation (n = 2)
- Image cytometry performed 1 min/sample
- Plating assay required 24-48 hours and 1-4/min for counting per plate

8. CONCLUSION

- Image cytometry produces accurate, rapid and consistent counts
- Brewery operators can become more efficient and save time
- Higher accuracy may improve beverage quality