Concentration and Viability Measurement of Yeast in Corn Mash using the Cellometer® Vision

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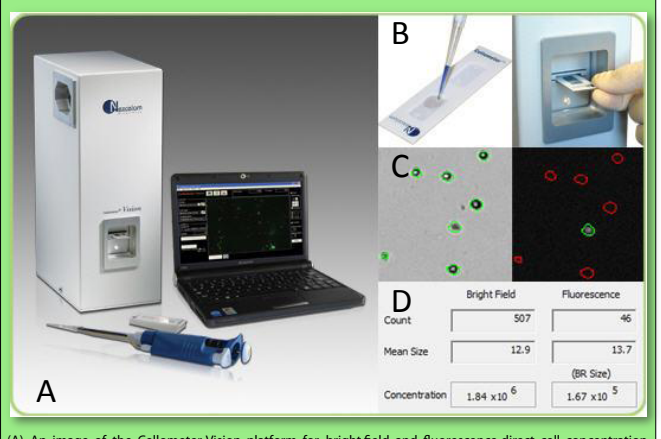
1. ABSTRACT (149)

We demonstrate a novel imaging cytometry method for concentration and viability measurement of yeasts in corn mash directly from operating fermenters. It employs an automated cell counter, a proprietary dilution buffer and staining solution from Nexcelom Bioscience to enumerate yeasts in corn mash. This novel method provides an essential tool for biofuel industries in United States to efficiently monitor yeast viability during fermentation process to ensure consistent bioethanol output.

2. CELLOMETER® VISION & CELL COUNTING METHOD



(A) An image of the Cellometer Vision platform for bright-field and fluorescence direct cell concentration measurement The counting protocol simply (B) pipette 20 l of cell sample into an inexpensive disposable cell counting chamber and place in the Cellometer Vision slide holder (C) Next, the software automatically outlines and counts the cells by bright-field or fluorescence (D) The resulting concentrations and cell sizes are calculated depending on the counted cells.

Some Application Examples

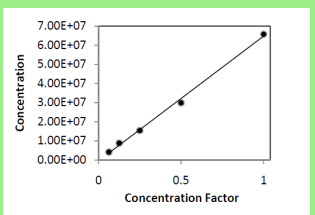
- Total cell concentration for highly viable cell population
- Live/Dead cell concentration using dual dye fluorescence
- Total and live nucleated cells without lysing
- Transfection efficiency by GFP, YFP, and RFP
- Identify and count cells base on cell size
- Capture and store cell images

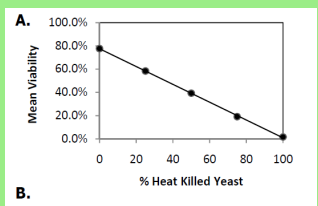
Some Cell Type Examples

- Cancer cell lines
- Stem cells
- Hepatocytes
- Splenocytes
- WBCs in whole blood
- Platelets
- Insect cells
- Yeasts and Algae

3. CHARACTERIZATION OF YEAST MEASUREMENT

- Previously, Cellometer® Vision has been shown to measure consistent concentration and viability measurement of pure yeast samples
- Concentration measurement utilizes automated bright-field counting
- Viability measurement utilizes Oxonol or Propidium Iodide (PI) to count nonviable yeasts





Dilution	Mean Concentration	CV
2	6.58E+07	6%
3	2.99E+07	4%
4	1.55E+07	7%
5	8.84E+06	10%
6	4.17E+06	13%

Live/Dead	Predicted	Measured	St Dev
100%/0%	-	78%	0.4%
75%/25%	59%	58%	1.2%
50%/50%	39%	39%	0.6%
25%/75%	20%	19%	1.3%
0%/100%	0%	0%	0.4%

Concentration Measurement

- 10 mg of Munton brewing yeasts are dissolved in 500 µl of cell culture H_2O
- The samples are diluted in 6 steps of half dilution
- The CV remains approximately 10% after 6 dilution steps

Viability Measurement

- "Live" cell samples are mixed with heat-killed yeasts at various percentages
- Using PI to specifically stain the dead yeast cells
- The predicted and measured viabilities of yeasts are consistent

4. ISSUES OF CURRENT YEAST DETECTION METHODS

Methods for Pure Yeast

- Colony-based optical density
- Manual hemacytometer using microscopy
- Flow cytometry
- Fluorescence microscopy

Disadvantages

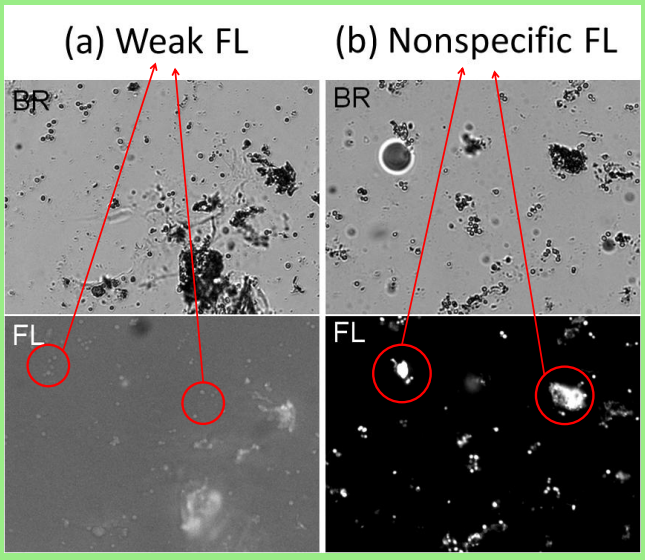
- Long assay time and tedious
- Tedious and prone to human error
- Expensive and high maintenance
- Weak and nonspecific fluorescence

Cellometer® Vision

- Pure yeast
  - Bright-field Total cell count
  - Fluorescence Dead cell count
- Yeast in corn mash
  - Fluorescence Live/Dead cell count

Fluorescence Microscopy

- Pure yeast
  - Bright-field Total and Methylene Blue
  - Fluorescence Live/Dead cell count
- Yeast in corn mash
  - Bright-field manual hemacytometer

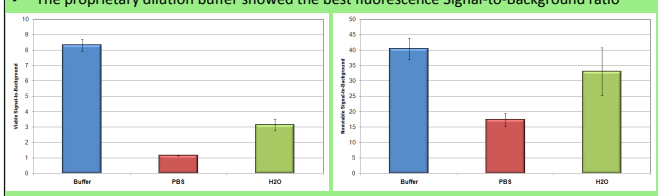


(a) An example of weak fluorescence signal of yeasts in corn mash using fluorescence microscopy (B) An example of nonspecific fluorescence signal of corn mash. Both examples show the difficulty in accurately measuring concentration and viability of yeasts in corn mash.

5. OPTIMIZATION OF YEAST FLUORESCENCE DETECTION

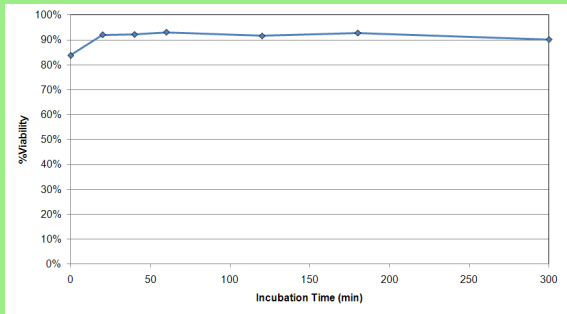
Fluorescence Signal Optimization

- Using a proprietary staining solution and dilution buffer to enhance yeast fluorescence signal
- Comparison of fluorescence Signal-to-Background ratio between H_2O, Phosphate Buffered Saline (PBS), and the proprietary buffer
- The proprietary dilution buffer showed the best fluorescence Signal-to-Background ratio

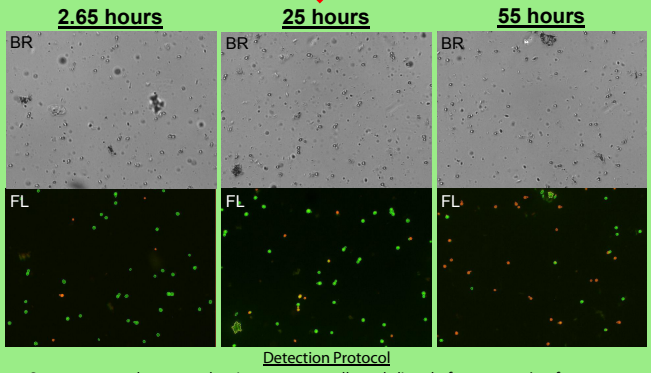


Buffer Stability Assessment

- The viability showed consistent results for 0, 20, 40, 60, 120, 180 and 300 min of incubation
- The yeast viability is stable for 5 hours of incubation in the proprietary buffer



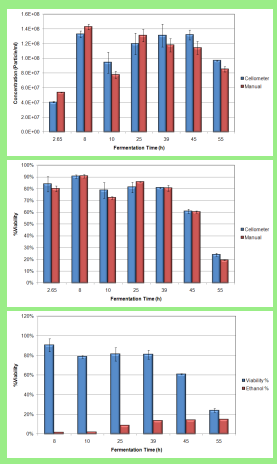
6. CONCENTRATION AND VIABILITY MEASUREMENT



Detection Protocol

- Seven yeast and corn mash mixtures were collected directly from operating fermenters at 2.65h, 8h, 10h, 25h, 39h, 45h, and 55h of fermentation
- Samples were diluted in Yeast Dilution Buffer and stained with Yeast Staining Solution
- Concentration and viability were measured directly from samples
- As fermentation period increases, more nonviable yeasts are observed

7. RESULTS AND DISCUSSION



Concentration

- Concentration of yeast at each fermentation stage was measured using Cellometer® and manual counting
- The results between the two methods are extremely consistent

Viability

- The viability of yeast at each fermentation stage was measured and compared to manual counting method
- It showed a trend in decreasing viability as fermentation period increased

Ethanol %

- Ethanol content of each fermentation stage was measured using a Hamamatsu HPLC system
- As Ethanol content in the sample increases the viability of yeast decrease, which is agreeable with physiological response of yeast

8. CONCLUSION & ACKNOWLEDGEMENT

We have demonstrated the capability of determining yeast concentration and viability in corn mash, where strong fluorescence signals from detected and counted from viable and nonviable yeast cells, while nonspecific staining of corn mash is minimized to facilitate effective automated cell counting algorithm. The development of the counting protocol employing Cellometer® Vision provides a simple tool for the biofuel industries to rapidly monitor yeast viability throughout a fermentation process to ensure optimal bioethanol output.

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