53

Image-Based Cytometric Analysis of Fluorescent Viability and Vitality Staining Methods for Saccharomyces Cerevisiae

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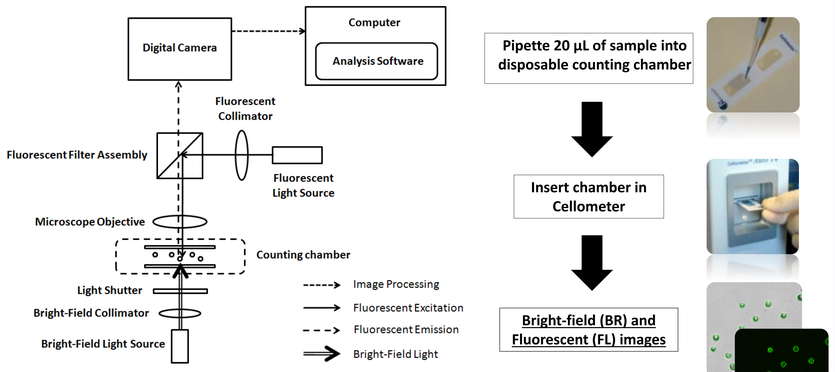
1. ABSTRACT

Saccharomyces cerevisiae has been an essential component for the production of beer for centuries. The viability and vitality of yeast during standard brewing process is especially important for proper cell growth, consistent production of flavor, and optimal yield for fermentation. Viability refers to the ability of the yeast to live and continue dividing, while the vitality refers to the metabolic activity of the yeast. Yeast may be viable and dividing, while not vital and allowing for fermentation. Traditional method for yeast viability measurement depended on mainly manual counting of methylene blue stained yeast cells in a hemacytometer. However, this method can be time-consuming and has user-dependent variations. In the recent years, fluorescent viability and vitality stains have become widely used for flow and image-based cytometry methods. Specifically for image cytometry, it has been previously demonstrated for rapid yeast concentration and viability measurements. In this work, we demonstrate the capability of Cellometer Vision image cytometry for yeast viability and vitality measurement, validating the methods against methylene blue. Various fluorescent stains were employed for viability and vitality measurement, such as nucleic acid stains (PI, EB, 7-AAD and DAPI), membrane potential, intracellular, and enzymatic stains (oxonol, MgANS and CFDA-AM), and dual-fluorescent stains (AO/PI and CFDA-AM/PI). In addition, we performed a time-course study to compare viability and vitality of lager and ale yeast, in order to understand yeast physical and metabolic characteristics during a standard fermentation process.

2. CURRENT METHODS FOR MEASURING YEAST VIABILITY AND VITALITY

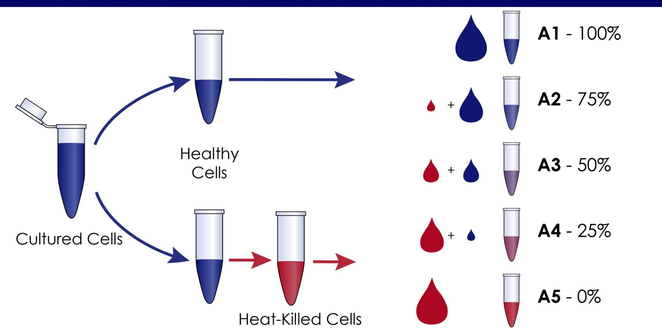
Methods	Description	Known Issues
Hemacytometer	Manually counting budding cells	•Time-consuming and tedious process •Requires experienced user for accurate counting
Fluorescence Microscopy	Visualization of fluorescently labeled yeast cells	•Qualitative observe instead of quantitative analysis •Not automated, low throughput
Flow-Based Analysis	•Quantitative analysis •Automated analysis	•Relatively expensive and high maintenance •Requires experienced user for proper operation •Cannot visually observe yeast cells

3. CELLOMETER IMAGE CYTOMETRY INSTRUMENTATION



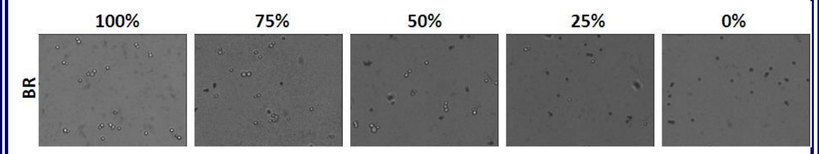
- Cellometer image cytometer utilizes an epi-fluorescence setup for fluorescent image analysis

4. YEAST "VIABILITY" AND "VITALITY" DETECTION EXPERIMENT



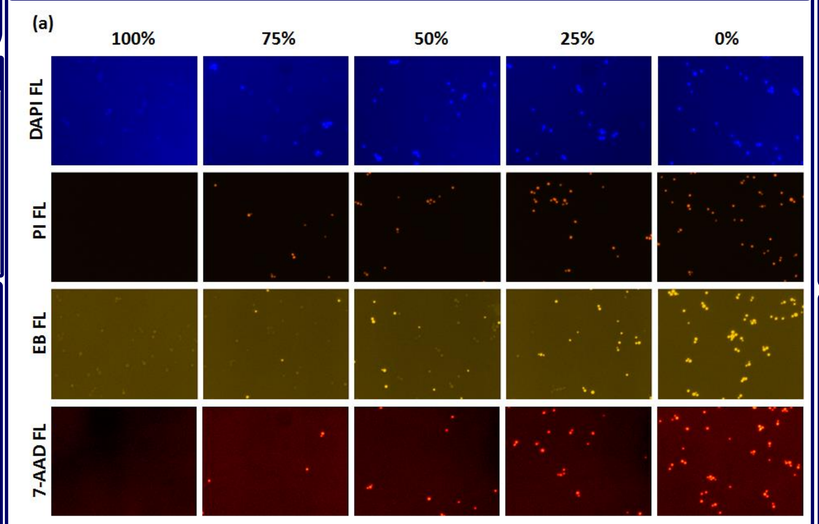
1. Yeast is cultured overnight using YPD media
2. Half of the yeast is heat-killed and mixed proportionally with fresh yeast at 100, 75, 50, 25, and 0%
3. Each mixture is stained with viability and vitality dyes DAPI, PI, EB, 7-AAD, CFDA-AM, Oxonol, and MgANS

5. YEAST "VIABILITY" AND "VITALITY" DETECTION USING METHYLENE BLUE



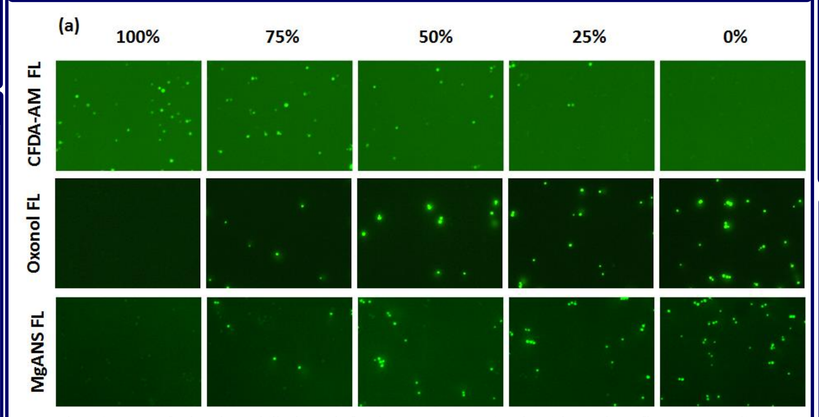
- Each mixture is stained with methylene blue and counted manually using the traditional method of hemacytometry
- Since the yeast cells are heat-killed, the viability measurement has high linearity
- Methylene blue is actually a "vitality" stain that metabolically react with dehydrogenase and convert the blue color to a colorless substance

6. YEAST VIABILITY DETECTION USING PI, EB, DAPI, AND 7-AAD



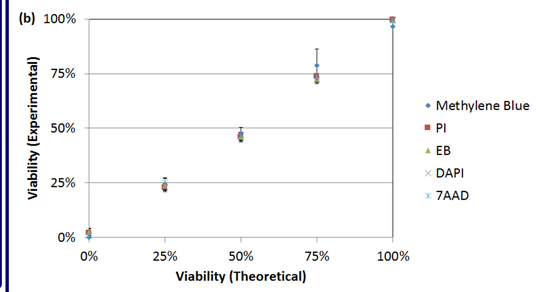
- Each mixture of the heat-killed and fresh yeast is stained with Propidium Iodide (PI), Ethidium Bromide (EB), 4',6-diamidino-2-phenylindole (DAPI), and 7-aminoactinomycin D (7-AAD)
- These viability stains can only enter membrane-compromised dead cells, binding to the DNA in the nucleus, thus only dead cells fluoresce

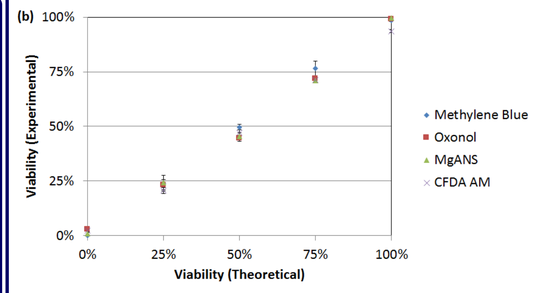
7. YEAST VITALITY DETECTION USING CFDA-AM, OXONOL, AND MGANS



- Each mixture of the heat-killed and fresh yeast is stained with carboxyfluorescein diacetate, acetoxymethyl ester (CFDA-AM), $DiBAC_4(3)$ (Oxonol), and Magnesium salt of 1-anilino-8- naphthalene sulfonic acid (MgANS)
- CFDA-AM can be cleaved by esterase to generate fluorescence to indicate vitality, Oxonol is a membrane potential dye that enters dead cells, and MgANS stains cytoplasmic proteins in dead cells

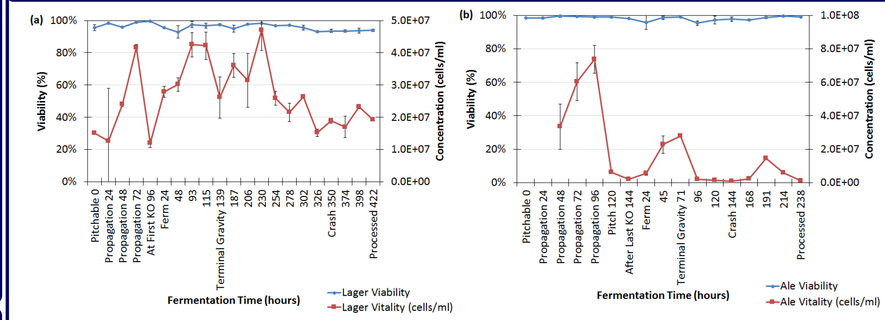
8. COMPARISON OF FL-BASED DETECTION METHOD TO METHYLENE BLUE





- Fluorescence-based nucleic acid viability stains PI, EB, DAPI, and 7-AAD showed highly comparable viability results to traditional methylene blue
- Membrane potential detection method using Oxonol was also comparable
- Cytoplasmic protein staining method using MgANS was also comparable
- CFDA-AM for vitality detection was also comparable
- Since the yeast cells were heat-killed and mixed with fresh cells, the viability measurement should be highly linear
- In addition, the heat-killed cells are easily stained with methylene blue without creating much artifact in comparison to yeast cells dying under apoptosis
- These methods allow quick and rapid measurement of viability and vitality of yeast to monitor its characteristics during fermentation process

9. VIABILITY AND VITALITY MEASUREMENT OF LAGER AND ALE FERMENTATION



- Standard lager and ale fermentation was performed at Avery Brewing Company to monitor their viability and vitality throughout the fermentation process
- Viability and vitality data was collected each day to study the yeast characteristics
- The viability was consistently high throughout the fermentation for both lager and ale yeast
- The vitality measurement was performed by observing the concentration of vital cells in suspension
- It seems like the lager is more stable during the end of the fermentation, where it showed higher concentration of vital cells in comparison to ale yeast

10. CONCLUSION

In conclusion, we have demonstrated the capability of using image cytometry to perform fluorescence-based viability and vitality measurements on yeasts. In addition, we were able to measure both viability and vitality throughout the course of fermentation in a full-scale industrial brewing fermentation. These physiological parameters are of great importance to brewers, as poor yeast health can lead to the production of many off flavors in beer. The ability to efficiently monitor yeast health is essential to both maintaining consistent product flavor, as well as minimizing financial losses from the impact of unsuccessful fermentations.

In subsequent experiments, it would be very interesting to investigate the factors unique to different beer brands, and their impact on the same yeast strain. For example, it would be important to understand the effect of osmotic stresses on lager yeast in high and low gravity beers. Additionally, it would be interesting to investigate both high and low alcohol beers, and the effect of alcohol concentration on viability and vitality of the same ale yeast. Lastly, it would also be significant to perform similar study on measuring intracellular glycogen reserves, and to understand their rate of depletion during fermentation.