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A Simple, Cost-Effective Treatment for Declumping Super Yeast

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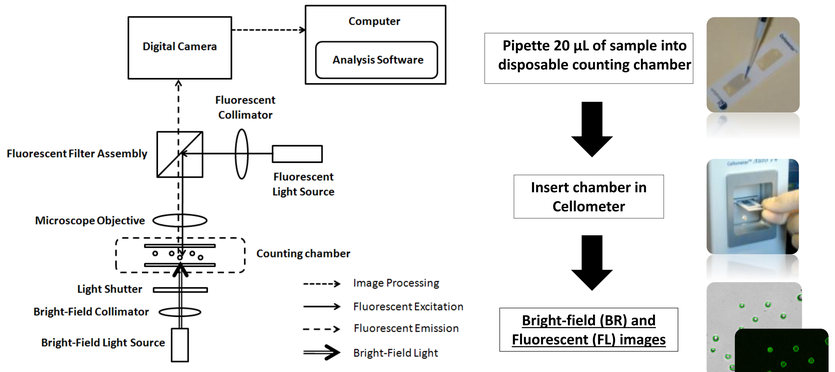
1. ABSTRACT

In the early fermentation stages, many types of yeast cells form clusters that can increase the difficulty of both manual cell counting and automated image-based cell counting. In this work, we demonstrate a simple and cost-effective method for declumping yeast cells in order to facilitate accurate cell counting and viability measurements. We examined the WLP090 San Diego Super Yeast which is characterized by high clumping during fermentation. A beer sample with Super Yeast was collected from the fermentation tank and treated with a small dose of hydrogen chloride (HCl) for 10-30 seconds. A control sample was treated with the same dose of water for the same duration. Concentrations of both samples were then measured in the Cellometer X2 image cytometer. The yeast concentration as measured by the Cellometer was higher in the sample treated with HCl due to more accurate yeast identification in the single cell suspension. The accuracy of yeast counting can be easily verified visually by the user after a count. The declumping method also improved the accuracy of measuring viability with PI using Cellometer X2. By utilizing the acid-based declumping method, clumpy yeast or yeast in the early stages of fermentation can be made into a single cell suspension, which improves the accuracy in cell counting and viability.

2. CURRENT YEAST TYPE USED AT BREW HUB

Characteristics	WLP090 San Diego Super Yeast	California Ale Yeast
Advantages	• Fast fermenting yeast • Clean aroma • High alcohol tolerance • Optimum fermentation temperature is 65 - 68°F (18 - 20°C) with attenuation of 76 - 83%.	•Similar fermentation stats as the San Diego Super yeast
Drawbacks	•Poor viability for first 24 hours •High clumping cells, flocculate and crash	•Medium to Low clumpiness

3. CELLOMETER X2 IMAGE CYTOMETRY INSTRUMENTATION

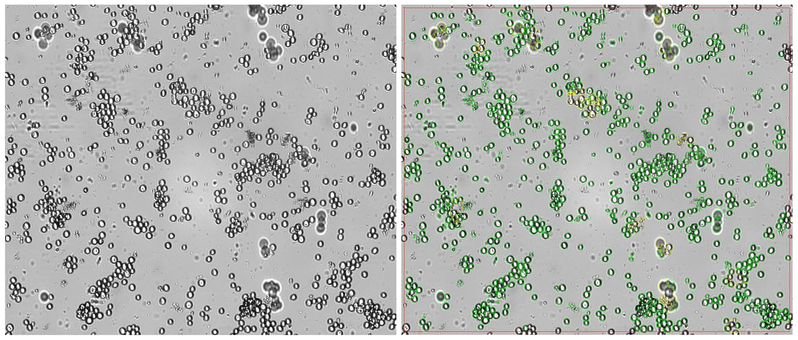


- Cellometer X2 image cytometer utilizes an epi-fluorescence setup for fluorescent image analysis

4. SUPER YEAST DECLUMPING AND DETECTION PROTOCOL

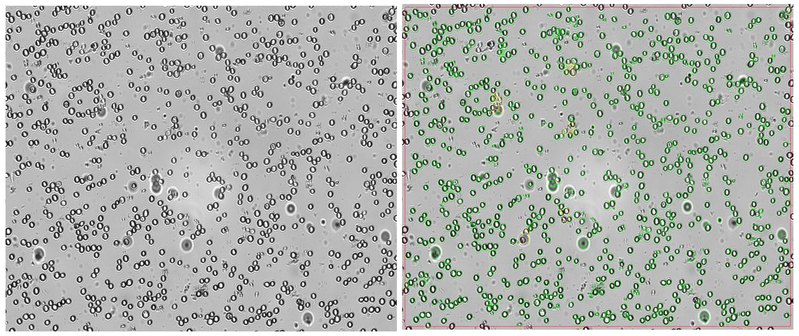
- The beer sample from the fermentation tank contains the San Diego Super yeasts, which are highly clumpy, and makes it difficult for both manual and automated counting
- One drop of 3M HCl is added into a 15 ml beer sample, which is diluted to a final concentration of 10 mM
- The HCl treatment is between 10 – 30 seconds, which is enough time to separate the yeast into single particle
- The sample is also vortexed and pipetted multiple times to mechanically declump the yeast
- The yeast concentration is determined by capturing and analyzing bright-field images to count all the yeast cells in the images
- The Revvity counting chamber has a fixed height, which is used to accurately calculate cell concentration

5. INITIAL IMAGE ANALYSIS SETUP FOR UNTREATED YEAST SAMPLE



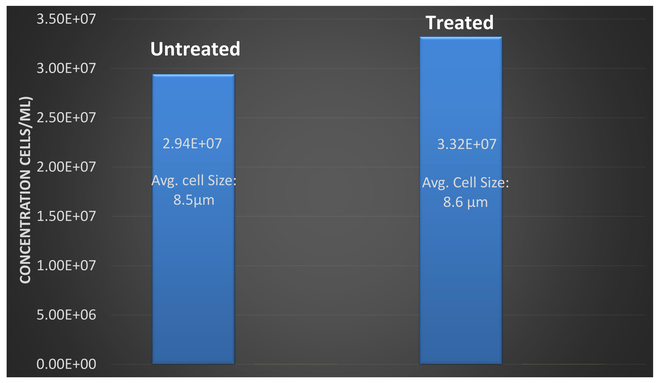
- The captured bright-field images showed clumpy yeast cells without HCl treatment
- The Cellometer X2 software is used to analyze the captured images, where all the yeasts are counted
- Even though the yeasts are clumpy, the declustering algorithm can count each yeast as long as the cell membrane is visible and well defined

6. IMAGE ANALYSIS FOR HCL DECLUMPED YEAST SAMPLE



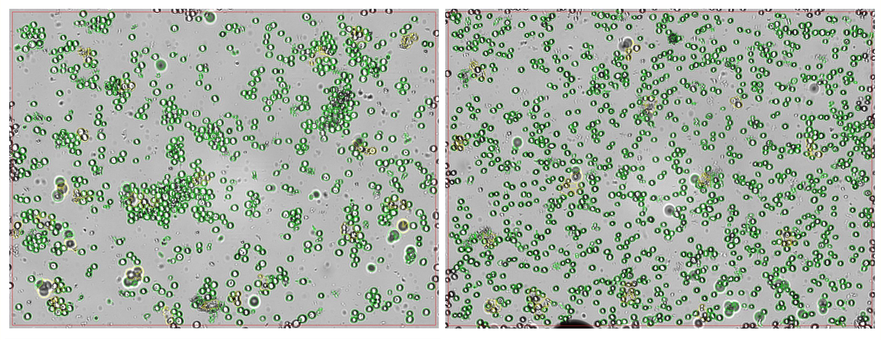
- The captured bright-field images showed declumped yeast cells with HCl treatment
- The single yeast cell is much easier to count than the clumpy cells, which can generate more accurate yeast cell concentration results
- It also showed that the HCl did not damage the yeast cell membrane

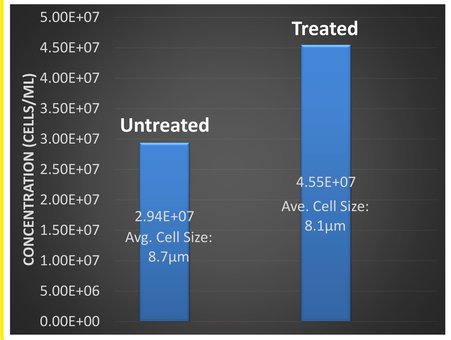
7. PRELIMINARY CONCENTRATION RESULTS COMPARISON



- The yeast sample treated with HCl showed higher concentration results by ~13%
- The counted cell size is similar between the treated and untreated samples

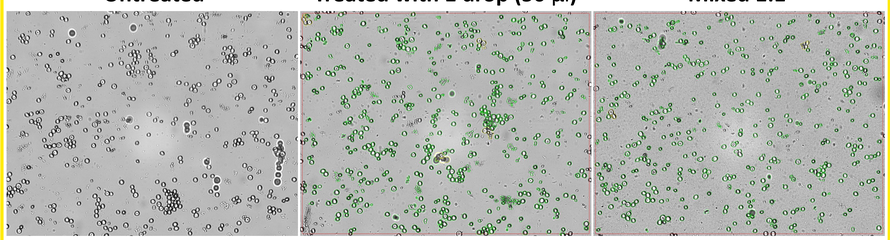
8. HCL TREATMENT OF HIGHLY CLUMPY SAN DIEGO SUPER YEAST SAMPLE





- The captured bright-field images showed highly clumpy yeast cell clusters
- The sample is more difficult to count with manual and automated counting due to the large clusters they form
- The clusters form a three-dimensional structure making the yeast cell membrane not clearly visible
- By utilizing HCl treatment, the large clusters of yeasts are separated into single cells, which allowed easy automated analysis with Cellometer X2
- The results showed that the average cell size is larger for untreated cells, and a 55% concentration increase

9. DECLUMPING YEAST CLUSTERS WITH CITRIC ACID



- Lemon juice was purchased from a grocery store at pH ~ 2.3, and it is used to treat the clumpy yeast sample
- The untreated yeast sample only showed a small portion of clustered cells, thus the increase was only ~4%
- By treating with just one drop of the lemon juice, it seemed that the clusters are not eliminated
- When the yeast sample is mixed with the lemon juice at 1:1 volume ratio, the cells seemed to decluster more than just adding one drop
- However, if highly clumpy yeast is used such as WLP002 English Ale, lemon juice may not be sufficient to decluster the cells

10. CONCLUSION

The declumping method of HCl is highly effective for physically declustering the San Diego Super yeast. Although the Cellometer X2 software can utilize a declustering algorithm to count the yeast sample, if the clusters of yeast is large and forming a three dimensional structure, the cell membrane will not be visible for the declustering algorithm to work properly.

Therefore, it is better to physically decluster the yeast by using the acidic treatment method. By using either HCl or lemon, the yeast clusters can be physically declustered into single cells, which will improve the counting ability with both manual and automated counting.

The method is cost-effective and can be easily incorporated into daily workflow, which allow brewers to accurately count yeasts. The future work includes understanding the treatment's effect on cell viability, conducting a concentration response of the HCl, and testing other acidic treatment.