Quick Yeast Counting and Viability Testing using Flow Cytometry

Optimizing Flow Cytometry for Microbial Monitoring in Beer Fermentations

Introduction: Flow Cytometry (FCM) is a specialized tool used for monitoring and sizing particles in a liquid sample. In the case of alcoholic fermentations, flow cytometry is best suited for monitoring microbial populations for fermentation quality, consistency, and contamination control. Traditional methods, such as plate counting and microscopy, are time-intensive, variable between operator, and may underestimate viable but non-culturable (VBNC) cells. FCM enables rapid, single-cell analysis of microbial populations quickly and accurately. In contrast to other alcoholic beverages, beer presents a complex analytical matrix due to proteins, β-glucans, hop compounds, and suspended solids, which can interfere with the optical measurements of the flow cytometer.

Hypothesis: By developing a repeatable sample preparation workflow for beer fermentation samples, we aimed to reduce the interference from complex matrix components to improve accuracy and reproducibility of flow cytometry measurements. This should also decrease variability in readings between beer styles and yeast strains.

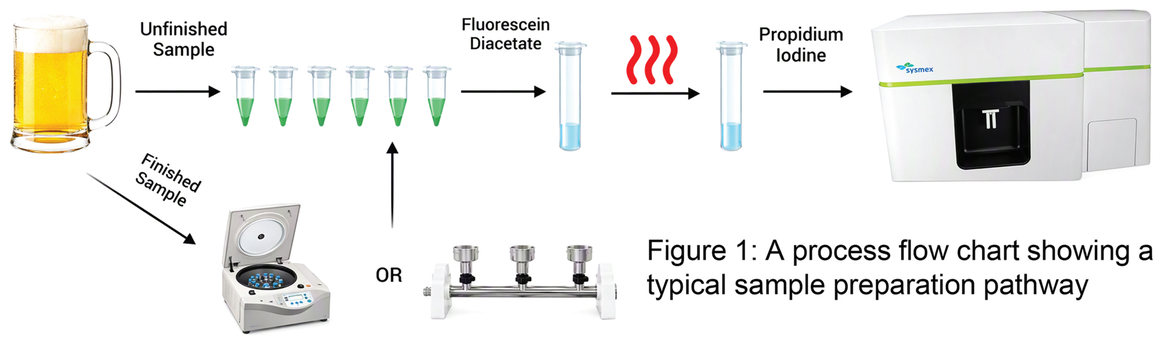


Figure 1: A process flow chart showing a typical sample preparation pathway

Method:

- A Sysmex CyFlow RI-300 Benchtop Flow Cytometer outfitted with an AL-20 Autoloader was used to analyze all samples
- Viability staining was done using Fluorescein Diacetate (FDA, a membrane permeable dye) and Propidium Iodide (PI, a non-membrane permeable dye) to differentiate between live cells with their membranes intact and dead cells
- Forward (FSC) and Side (SSC) scatter detectors were utilized to differentiate yeast from other particulate
- Green and Red fluorescent detectors were utilized to detect FDA and PI, respectively
- Samples were diluted using PBS to bring concentrations down within machine capacities, with a goal of events-per-second between 100 and 2000
- Diluted samples were incubated for 15 minutes with FDA before PI was added for analysis

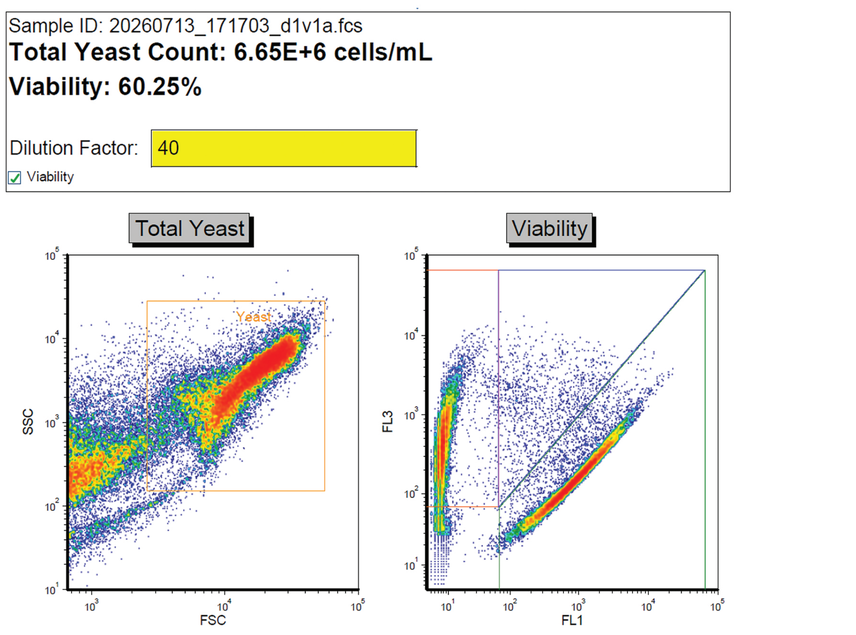


Figure 2: Example results showing a total yeast count along with yeast viability. The left plot shows the yeast differentiated by size from other particulate, and then the right plot further distinguishes live and dead cells based on their red and green fluorescent properties.

Results: We tracked yeast viability and populations throughout beer fermentations from two different commercial breweries. The yeast strains tracked were different, not following the exact same path, but a general fermentation curve. The yeast did follow a predicted trend, which helped cement that the machine is useful when tracking yeast health and cell counts. Any errors present were most likely due to the sample prep being misdiluted. This was the thing we found most difficult; understanding at what dilution to test.

Conclusion: Overall, we found that using flow cytometry to count and find the viability of the sample to be relatively accurate. The repeatability between users and within triplicates showed low coefficient variables, especially when compared to more manual hemocytometer counting. Further removing the background noise from both the samples as well as the dyes would clear up the results and increase the accuracy of the machine. Sterile filtration of reagents and consumables could help lower the background noise seen especially during viability staining. Dilution factor was vitally important, as with the background noise it was difficult to differentiate noise from cells on smaller populations.

Discussion: The beer matrix is more complex than wine, for which flow cytometry has already been proven to work. Using flow cytometry for cell counts throughout beer fermentation allows labs to obtain counts and viability faster than other methods. Since we had relative success with using the flow cytometer throughout beer fermentations, we feel this process could be expanded to other fermented products, such as cider, kombucha, and sour beers. While ciders could be analyzed similarly to wine, kombucha and sour beers would be more complex because they do not contain just yeast, but a combination of yeasts (both domestic and wild) and bacteria. Bacteria and wild yeast show clumping properties that could also make them difficult to reliably count as single particles.

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