

Application Note

Comparison of the efficacy of various yeast viability stains

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INTRODUCTION

Yeast are single cell microorganisms that reproduce by budding. A well known property of yeast is that they are responsible for the conversion of fermentable sugars into alcohol and other by products. Consistent yeast performance during the fermentation process requires both accurate cell counts plus assessment of cellular viability. The principal markets utilizing yeast fermentation are the brewery and ethanol production industries.

An important parameter for the consistent production of high quality beer is the presence of viable yeast. A common method used to assess the degree of yeast viability is the use of a vital dye and the hemacytometer. Viable yeast do not stain; whereas non-viable, non-metabolizing cells stain the color of the selected dye. An operator, using a light microscope, enumerates several hundred cells and calculates the ratio of stained cells to the total number counted. Thus, the results are expressed as percent viability.

In the brewery industry, the most common yeast viability stain is methylene blue. In fact, methylene blue is accepted as the industry standard.¹ However, in recent years, there have been reports that methylene blue stain may overestimate yeast viability.² This stain has been reported to be inaccurate when yeast viabilities fall below 95%,³ hence, the use of an alternative stain, methylene violet, has been tested by several standard committees as a suitable alternative stain for yeast viability determination.⁴

Ethanol blended fuels represent more than 12% of the US motor gasoline sales,⁵ ethanol production thereby reduces the United States dependency for energy imports

reducing overall gasoline prices and benefiting US consumers. For an efficient process, the viability of the yeast must be accurately measured. The ethanol production process measures a much wider range of cellular viabilities than does the brewing industry. Also, a vital yeast stain other than methylene blue is routinely used. Methylene violet is most often the stain of choice in the ethanol production process.⁶

Trypan blue, as a 0.4% concentrated solution, is the "gold standard" of non-fluorescent vital dyes for mammalian cells. Since, as documented above, standard committees in both the brewing and ethanol production industries have been testing vital stains, our laboratory investigated the agreement among these three most utilized stains for cellular viability measurement.

Saccharomyces cerevisiae, the species mostly commonly used in both the brewing and ethanol production industries, was used as the test organism.

MATERIALS AND METHODS

Methylene blue stain was obtained from Sigma Chemical Co. Methylene violet stain came from ICN Biomedicals. Trypan blue was prepared by EM Sciences.

The yeast species, Saccharomyces cerevisiae was ordered from Sigma Chemical. Yeast was cultured using Trypticase Soy Broth. A representative range of yeast viabilities, approximately 40-90%, were tested.



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RESULTS

Figure 1 shows the comparison of Trypan Blue with Methylene Blue vital stain over the range of yeast viabilities assayed.

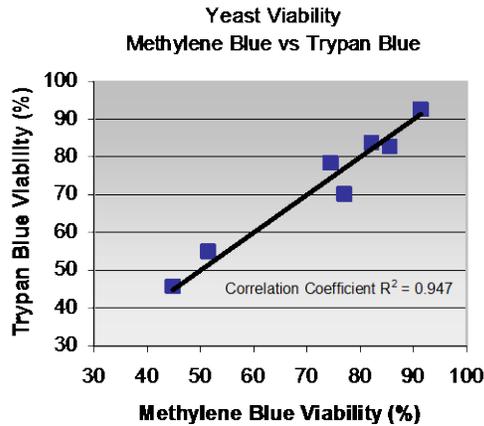


Fig. 1

Figure 2 shows the correlation of Methylene Blue and Methylene Violet stains

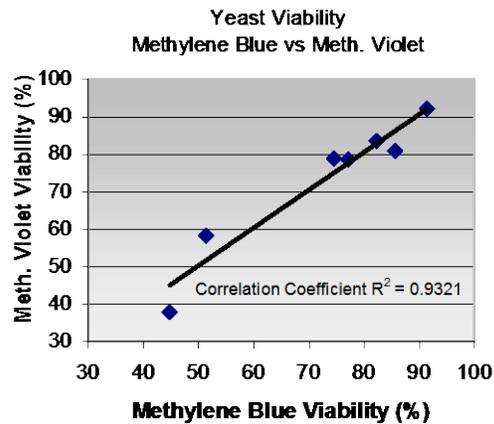


Fig. 2

Figure 3 illustrates the comparison of all three vital stains over the range of yeast viabilities tested.

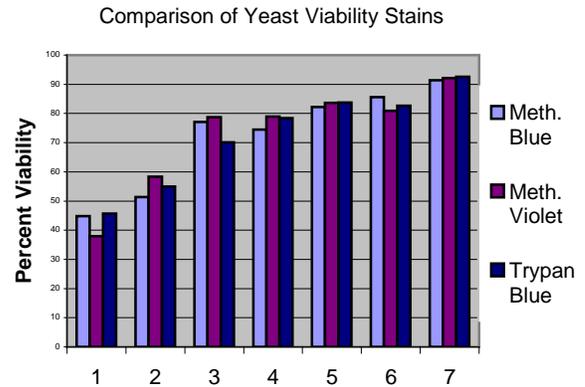


Fig. 3

CONCLUSIONS

Trypan blue stain demonstrated excellent overall correlation with both Methylene Blue and Methylene Violet stains.

The agreement extended over a wide range of yeast viabilities.

Trypan blue vital dye is suitable for the viability measurement of the yeast species, Saccharomyces cerevisiae.

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