EN_58767570 Method Report

Materials and Methods

The following steps were taken to establish whether a plate count and a counting chamber procedure yield the same results when quantifying live Saccharomyces cerevisiae cells per gram in a solution.

Plate count

A glass container of 5 g/L yeast suspension was gently vortexed into a homogenous suspension, after which 50 L of this suspension was added to 50 mL of tap water to create a 103-dilution.

A 104-dilution was made by transferring 0.1 mL of the 5 g/L yeast suspension into a sterile Eppendorf tube containing 0.9 mL of physiological saline solution. This procedure was repeated twice to create 105- and 106-dilutions.

0.2 mL of each dilution was spread on three malt agar plates. The plates were then incubated for 48 hours at 37°C. After the incubation period, the number of colonies were counted on the plates containing a number of colonies within a range of 5 to 300.

Counting chamber procedure

5 L of the homogenized 5 g/L yeast suspension was transferred to a counting chamber, after which the counting chamber was microscopically observed with a magnification of 400x. The number of cells were counted within ten individual squares containing 10-50 squares each.

Determining the live/dead-ratio with methylene blue

1 mL of the homogenized 5 g/L yeast suspension was transferred into a nonsterile Eppendorf tube. The tube was then placed in a heating block at 100°C for three minutes. Once the suspension was properly cooled, 0.1 mL of 0.5% methylene blue was added. A wet mount of the suspension was microscopically observed with a magnification of 400x to determine whether all yeast cells were dead.

1 ml of nonboiled yeast was stained and observed following the same procedure, to determine the live/dead-ratio of the cells in the yeast suspension.

Calculating the number of living cells per gram of yeast

Counting the number of colonies on the 106- and 105-dilution plates resulted in the number of colonies per 0.2 mL of either dilution. Subsequently, the following consecutive formulas were applied to establish the cell density in the original 5 g/L yeast suspension.

colony count × 1/(0.2 mL)=cells/mL of dilution

cells/mL of dilution × dilution factor =cells/mL of undiluted suspension

1000/5=200 mL of suspension per gram of yeast

cells/mL of original suspension × 200=cells/g of yeast

Combining the results from the counting chamber and the viability determination, the cell density in the original 5 g/L yeast suspension calculated again, by application of the following consecutive formulas.

Counting chamber volume (large square) = 0.04 mm2 × 0.100 mm = 0.004 mm3 = 0.004 L

cells per square (average)× 1/(square volume (mL))=cells/mL of suspension

cells/mL of suspension × 200=total cells/g of yeast

cells/g of yeast× viability factor=living cells/g of yeast

A two-tailed Welch's t-test for heteroskedastic data was applied with significance level $\alpha=0.05$ was applied to assess whether the two different quantification methods had yielded significantly different results, by comparing the two different outcomes of the viable plate counts and their average to the outcome of the counting chamber procedure with and without viability-correction. The effect size was displayed with Cohen's d to show the standardized difference between the two quantification procedures in relation to their pooled standard deviation.