TITLE: USE OF MCFARLAND STANDARDS AND SPECTROPHOTOMETRY FOR *YARROWIA LIPOLITICA* QU69 CELL COUNTING

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ABSTRACT:

Yarrowia lipolytica is an aerobic dimorphic fungus considered a non-pathogenic yeast with the ability to grow in hydrophobic environments. It is also used to produce citric acid, proteins, and lipids in processes of bioconversion. McFarland standard and Neubauer hemocytometer are used for cell counting in bioconversion processes. McFarland standards, which range from 0.5 to 10, are used as a visual reference to adjust the turbidity of a suspension and it is designed to be used for estimating concentrations of Gram negative bacteria. Neubauer hemocytometer is a device employed for blood cell counting under a microscope. In order to count *Yarrowia lipolytica* cells according to McFarland scale, the ideal wavelength for determining absorbance was established, the absorbance of the standards was measured, and the cell counting in Neubauer hemocytometer was performed. The yeast was maintained in GYP agar at 28C for 48h. Colonies were added to pre-sterilized saline solutions as to make a homogeneous suspensions until the turbidity was similar to McFarland scale. The samples were read in a microtiter spectrophotometer (Thermo Multiskan GO). The highest absorbance was recorded at a wavelength of 500 nm. The McFarland scale absorbance ranged from 0.114 to 1.340 and the *Y. lipolytica* samples from 0.104 to 0.904. The yeast cell counting in Neubauer hemocytometer varied from 2.1-23 x 10^6 CFU/mL. We can conclude that using the McFarland scale for yeasts could lead to an overestimate result due to the difference in weight, size and mass in relation to Gram negative bacteria since the 0.5 standard reflects a 10^8 CFU/mL cell counting and our results reflect a 10^6 CFU/mL cell counting. It's possible to achieve faster and more reliable results when allying absorbance and cell counting, and as a consequence, decrease possible mistakes due to visual analysis.

Keywords: Neubauer hemocytometer, turbidity, bioconversion, absorbance