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Use of McFarland Standards and Spectrophotometry for Yarrowia Lipolytica QU69 cell counting

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Abstract*—New researches on microorganisms capable of synthesizing different carbon sources have been made to fulfill the growing use of biotechnology to obtain products with economic value and the search for reducing the environmental impact caused by inadequate waste disposal. The yeast Yarrowia lipolytica has stood out for its ability to grow in hydrophobic environments and has been used in bioconversion processes to produce various industrial products of interest. McFarland standards and Neubauer chamber are the two most common methodologies employed to count viable cells, but they were originally made to count bacterial cells and blood cells, furthermore, those methodologies can be quite subjective. In order to optimize yeast cell count for use in bioprocesses, McFarland standard associated with spectrophotometry was used to estimate the amount of strain Yarrowia lipolytica QU69 cells present in a suspension. It proved to be a reliable, accurate and reproducible method, and it could be applied in routine analysis and classroom experiments.*

***Keywords*—hemocytometer, turbidity, bioconversion, absorbance.**

I. EXPERIMENTAL AND RESULTS

Only high purity reagents and solvents without any prior purification were employed. *Yarrowia lipolytica* strain QU69 was kindly provided by Professor Patrícia Valente (Department of Microbiology, Immunology and Parasitology - UFRGS - Brasil - RS). Cell counting was previously performed in a Neubauer chamber, Kasvi, Ref. OG200 with an Olympus CX21FS1 optical microscope. The yeast maximum absorption spectrum was determined using a Thermo Multiskan GO microplate spectrophotometer. McFarland scale was prepared in a Fume Hood and the yeast inoculum in a Bioseg 12 Class II type A1 Biosafety cabinet.

II. PREPARATION OF MCFARLAND STANDARDS

First, solutions of 1% (w/v) Barium Chloride ($BaCl_2$) ($BaCl_2 \cdot 2H_2O$) and 1% (v/v) Sulfuric Acid (H_2SO_4) with constant stirring were prepared.

Then, 11 assay tubes of the same size were sequentially numbered, and 1% barium chloride solution and 1% sulfuric acid solution were added according to Table 1. The final scale is shown in Figure 2.

Yarrowia lipolytica QU69 was isolated in GYP [5]. Agar after incubation at 28°C for 48 h. After that, some cells were transferred to test tubes containing 10 mL of sterile saline until turbidity was adjusted to meet McFarland standards.

Yeast cell counting was performed in a Neubauer chamber, prior to being transferred to a 96-well plate for optical density readings at 500 nm using a microplate reader, as well as the McFarland standards. Considering that the precipitate could easily sediment, it was standardized that before the reading the microplate would be agitated for thirty seconds so that there was no sedimentation and possible alteration in the results.

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Table 1. Correlation between turbidity, bacteria, and yeast cell counting

Tube	$BaCl_2$ 1% V (mL)	H_2SO_4 1% V (mL)	[Bacteria] ($x10^8$)	Abs (500 nm)	[Yeast] ($x10^6$)
0.5	0.05	9.95	1,5	0.104	1.2
1	0.1	9.9	3	0.159	2.6
2	0.2	9.8	6	0.242	5.0
3	0.3	9.7	9	0.375	6.7
4	0.4	9.6	12	0.401	8.5
5	0.5	9.5	15	0.499	9.4
6	0.6	9.4	18	0.580	11.2
7	0.7	9.3	21	0.661	13.2
8	0.8	9.2	24	0.742	14.9
9	0.9	9.1	27	0.823	16.1
10	1,0	9.0	30	0.904	16.4

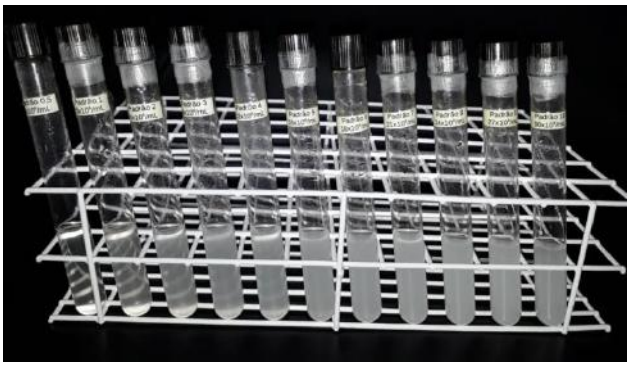

Fig.1: McFarland standard.

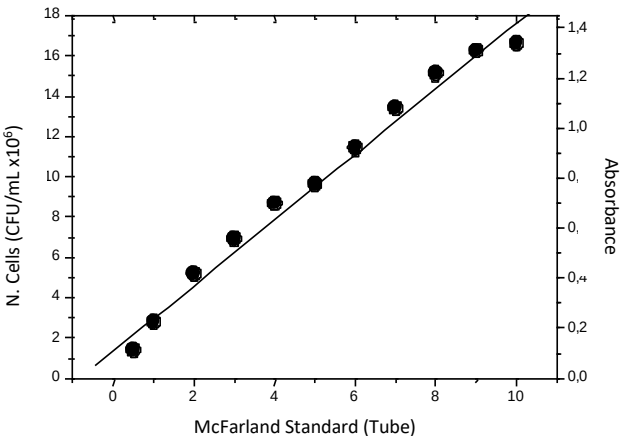

Fig.2: Correlation between Yarrowia lipolytica QU69 cell counting and the absorbance achieved by the McFarland suspensions

Figure 2 shows that the cell count reached a standard of 10^6 CFU/mL, differently from the original McFarland standard corresponding to 10^8 CFU/mL. This is due to the yeast cell size, much bigger (20 to 50 µm) than bacteria, from 1 and 10 µm [6]. Therefore, fewer yeast cells are required to reach the intended turbidity of McFarland scale.

The tested methodology presents advantages due to its speed, simplicity and greater accuracy in comparison to the colony counting or the naked eye comparing technique.

The absorbance reading can be used to estimate the number of *Y. lipolytica* QU 69 cells present in suspensions that will be used in bioprocesses. Hence, the quantification of cells becomes more precise, optimizing the time spent in Neubauer chambers and reducing the error that may occur when employing visual comparison with the McFarland standard alone.

III. CONCLUSION

The present methodology allows two jobs, presents speed and efficiency, making the results more precise.

Confirms that the scale of use of the method in question for yeasts is around 10^6.

It presents greater reliability of the results, considering the absorbance reading and the visual interplay of the turbidity of the medium, presents potential to be used in research routines

IV. DECLARATION OF INTERESTS

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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