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METHODS FOR SIMULTANEOUSLY DETERMINING THE CONCENTRATION OF YEAST AND BACTERIAL CELLS

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ABSTRACT

In scenarios where yeast and bacteria coexist, such as the food industry and sugarcane biorefineries, it would be of interest to simultaneously quantify the concentrations of both cell types, since traditional methods used to determine these concentrations individually take more time and resources. In the present work, we conducted a comparative evaluation of different methods for simultaneously quantifying yeast and bacterial cells in microbial suspensions. The following methods were investigated: bright field microscopy; spread-plate with manual and automatic counting; drop-plate; flow cytometry; and Coulter Counter. We observed that flow cytometry, the Coulter Counter, and both spread-plate options yielded statistically similar results, while the drop-plate and microscopy-based methods gave statistically different results for yeast counts. Concerning the bacterial quantification, flow cytometry (1:1), microscopy-based method, drop-plate, and both spread-plate plating options yielded statistically similar results, whereas the Coulter counter and flow cytometry (100:1) gave statistically different results. The results from this work indicate that each method has limitations, advantages, and disadvantages, meaning that the best option will always depend on the application. We present a comparison of the methods, in terms of time-to-results, cost of analysis and equipment, range of detectable cell/particle diameters, adequacy for simultaneous enumeration, and general pros and cons.

Keywords: Cell counting. Yeast. Lactic acid bacteria. Cell viability.

1 INTRODUCTION

The enumeration of yeast and bacterial cells is a critical task in diverse scientific and industrial domains, encompassing microbiology, biotechnology, food, medicine, and environmental studies.[1,2] These microorganisms play pivotal roles in fermentation, food production, wastewater treatment, and the pathogenesis of infectious diseases.[1,3] Precise measurement of yeast and bacterial concentrations is essential for comprehending, monitoring, and controlling biotechnological processes, as well as for disease diagnosis, and water and food monitoring, among other applications. Within diverse fields employing yeast and bacterial cells, the selection of microbial enumeration methods varies, and several studies explored the comparative analysis of total enumeration and viability of yeasts and bacteria individually. However, many fermentation processes involve mixed cultures containing multiple microbial species. In the food industry, for instance, there are numerous products and processes where yeast and bacterial cells coexist, particularly lactic acid bacteria, exemplified by kefir, a fermented beverage.[4] In the context of Brazilian sugarcane biorefineries, the quantification of yeasts and bacteria is essential for monitoring both the cellular concentration of the *Saccharomyces cerevisiae* yeast and contaminating bacterial cells, mainly composed of lactic acid bacteria.[3,5] Consequently, methods requiring less training and time for obtaining results, as well as methods with lower associated costs for equipment, are commonly preferred. On the other hand, in environments such as pharmaceutical industries, where there is more investment in infrastructure, equipment, and operator training, methodologies with higher accuracy, specificity, and complexity are typically adopted.[2] A recent study demonstrated successful and consistent simultaneous enumeration of yeasts and bacteria cells in mixed cultures using a specific image-based method, emphasizing the need for further comparative studies across different quantitative methods for complex samples. [6] The present work performed a comparative assessment of currently employed techniques for quantifying yeast and bacterial cells, with a special focus on simultaneous quantification in mixed samples through a single analysis.

2 MATERIAL & METHODS

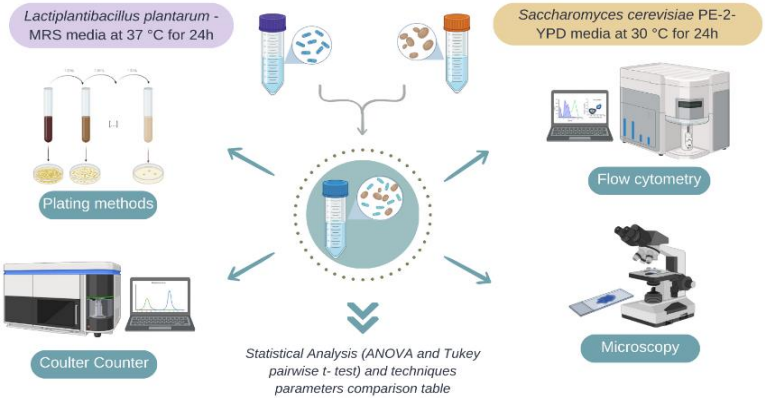

Figure 2 Methods applied in this work.

3 RESULTS & DISCUSSION

Cell suspensions were prepared by cultivating yeast and bacterial cells in YPD and MRS media, separately. For plating techniques (Spread-plate and Drop-plate) and bright-field microscopy, mixed suspensions of *S. cerevisiae* yeast and *L. plantarum* bacteria populations were prepared in a 1:1 cell concentration ratio and analyzed. As for flow cytometry and Coulter Counter techniques, using the Multisizer IV equipment, mixed suspensions were prepared in ratios of 1:1 and 100:1 and analyzed. The results for the yeast and bacterial cell simultaneous enumeration are presented in Figure 2.

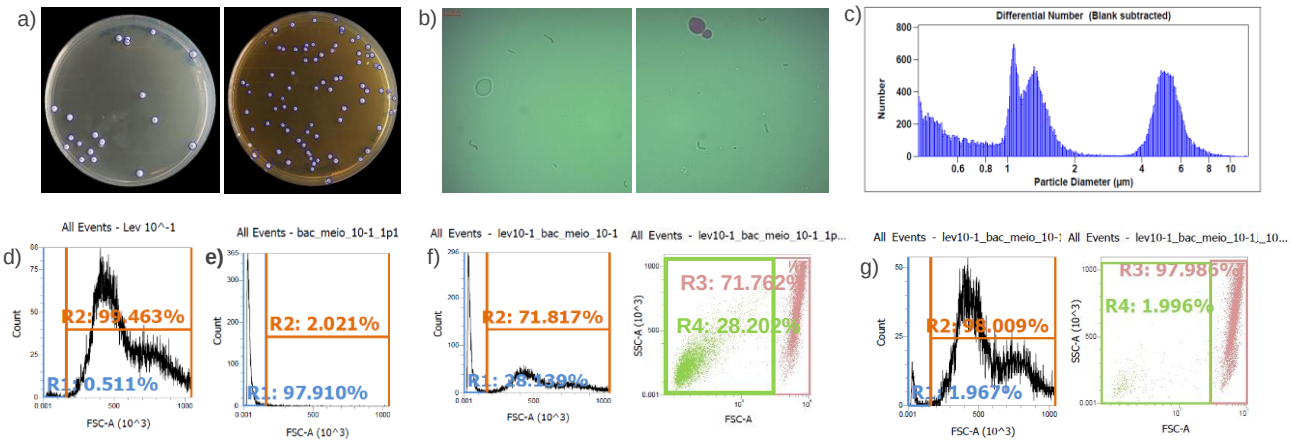

Figure 2 Results from the simultaneous enumeration of yeast and bacterial cells in the (a) plating, (b) microscopy, (c) Coulter Counter, and (d,e,f,g) flow cytometry techniques.

By subjecting the same mixed cell suspension to these different techniques in quintuplicate, statistically similar results ($p<0.05$) were observed for yeast cell counts with flow cytometry (Attune NxT, ThermoFisher), the Coulter Counter (Multisizer IV, Beckman), and both spread-plate options (manual and automatic CFU counting) (Table 1). Conversely, drop-plate and microscopy-based methods provided statistically distinct results. Regarding bacterial cell quantification, flow cytometry (1:1 yeast:bacterial cells), microscopy-based method, drop-plate, and both spread-plate options showed statistically similar results ($p<0.05$), whereas the Coulter Counter and flow cytometry (100:1 yeast:bacterial cells) produced statistically different results (Table 1).

Table 1 Cell concentration values from the simultaneous enumeration of yeast and bacterial cells in the plating, microscopy, flow cytometry, and Coulter Counter techniques.

	Technique	*Drop-plate*	*Spread-plate*	*Auto.Spread-plate*	Microscopy	*Flow cytometry (1:1)*	*Flow cytometry (100:1)*	*Multisizer IV (1:1)*	*Multisizer IV (100:1)*
S. cerevisiae (PE-2)	*Concentration unit* ($x10^7$)	CFU/mL	CFU/mL	CFU/mL	cell/mL	cell/mL	cell/mL	cell/mL	*It was not possible to distinguish between bacterial cells and yeast cell debris at the 100:1 ratio*
	mean	**7,8**	**4,2**	**4,2**	**8,1**	**3,40**	**2,99**	**3,89**	
	s.d	*0,9*	*0,9*	*0,8*	*1,3*	*0,17*	*0,20*	*0,22*	
L. plantarum	*Concentration unit* ($x10^9$)	CFU/mL	CFU/mL	CFU/mL	cell/mL	cell/mL	cell/mL	cell/mL	
	mean	**2,17**	**2,19**	**2,00**	**2,40**	**1,47**	**3,16**	**4,87**	
	s.d	*0,14*	*0,33*	*0,26*	*0,70*	*0,14*	*2,21*	*0,44*	

The results of yeast and bacterial cell concentrations from a mixed culture obtained in quintuplicate for each evaluated technique in this study, including Drop-plate plating, Spread-plate with manual counting, Spread-plate with automatic counting, microscopy, flow cytometry (at yeast-to-bacteria cell ratios of 1:1 and 100:1, respectively), and automatic counting using the Coulter Principle (Multisizer IV), were grouped in Figure 3.

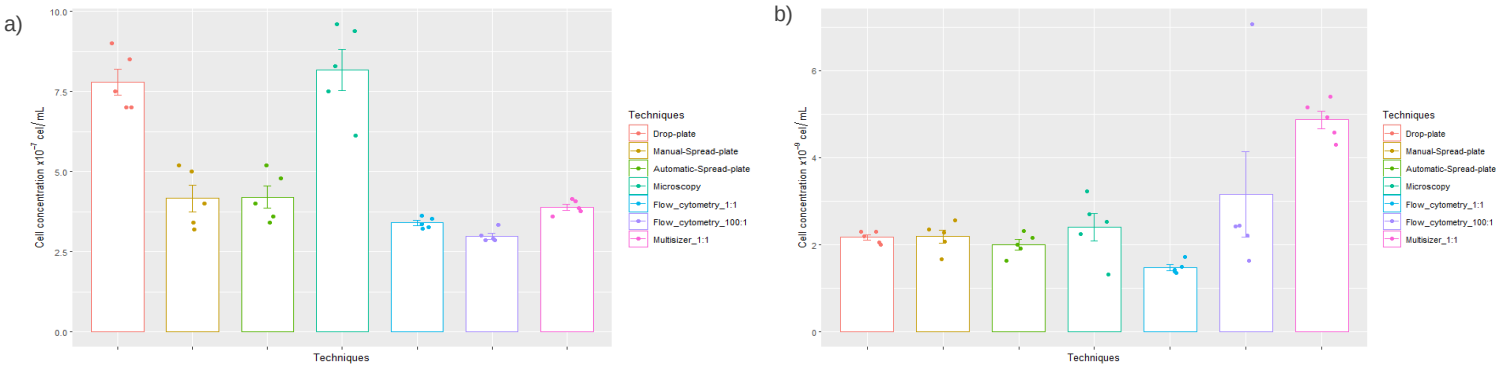

Figure 3 Compilation of cell concentration values of the mixed suspension of yeast (3a) and bacterial (3b) cells in each technique.

The results from this work indicate that each method has limitations, advantages, and disadvantages, meaning that the best option will always depend on the application. For instance, while plate counts may underestimate microbial diversity and absolute cell concentration, flow cytometry, although efficient and rapid, may require expensive equipment and specialized training to analyze results. We present a comparison of the methods, in terms of time-to-results, cost of analysis and equipment, range of detectable cell/particle diameters, adequacy for simultaneous enumeration, and general pros and cons (Table 2).

Table 2 Main characteristics of each selected technique for simultaneous enumeration of yeast and bacterial cells.

Methods		Principles	Analysis time	Cell diameter range	Simultaneous Enumeration Capacity	Advantages	Disadvantages	Estimated Equipment Cost
Culture-based methods	***Spread-plate***	Counting viable and cultivable microorganism colonies	48-72 h	N/A	No*	Low cost; ability to assess cell viability; ability to characterize colonies (contribution to identification); ease of result analysis	Long analysis time; more "laborious" method; operational difficulty - requires technical training; inability to quantify VBNC; "lower" reproducibility**	N/A
	Drop-plate		17-24 h					N/A
Microscopy		Bright-field microscopy; cell counting on hemocytometer or glass slide; viability cell stains	~10 min	0.2 µm - 1 mm***	Yes	Common equipment in laboratories; ability to identify yeast and bacteria; ability to assess cell viability;	Medium analysis time; requires technical training; resolution limitation for smaller cells; subjective viability determination; requires viability stains; choice of stain may interfere with analysis; "lower" reproducibility**	$ 4,035.00
Flow Cytometry		Light scatter and fluorescence (combination of fluidic, optical, and electronic system)	< 1 min	0.5 - 50 µm	Yes	Ease of result analysis; viability cell information; precise technique; short analysis time; ease of operation;	High cost; requires viability stains; more complex result analysis; need for sample filtration;	$ 61,500.00
Coulter Counter (Multisizer IV)		Coulter Principle; detection of electrical zone by passage through an aperture	< 1 min	0.4 - 12 µm (linearity from 2 to 60%)	Yes	Ease of result analysis; information on cell size distribution; precise technique; short analysis time; ease of operation.	High cost; does not provide cell viability information; requires sample filtration;	$ 80,200.00

*Although it is possible to start from a mixed culture, colony counting is done separately for yeast and bacteria on plates with a selective medium.**Based on the results generated in this study.***Lower limit with lower image resolution.

4 CONCLUSION

This work shows that, in the context of simultaneous quantification of yeast and bacterial cells, it is challenging to pinpoint a single technique as ideal or better for all possible situations. The choice of technique depends on various factors related to the development of academic research or research/application in industries, such as the budget available for obtaining equipment and reagents, qualified training, required result turnaround time, the accuracy of obtained results, etc. Ideally, an integrated approach, combining complementary methods, might be used to gain a comprehensive understanding of the microbial community. Additionally, it is crucial to consider the costs, ease of implementation, and specific limitations of each method in the decision-making process.

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