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DIFFERENCES IN STATIONARY PHASE CELLS OF *SACCHAROMYCES CEREVISIAE* GROWN IN AEROBIC AND HYPOXIC BATCH CULTURES ASSESSED BY ELECTRIC PARTICLE ANALYSIS, LIGHT DIFFRACTION AND FLOW CYTOMETRY

X.PORTELL[1], M. GINOVART[2], R. CARBO[1], J. VIVES-REGO[3]

[1] Department of Agri-Food Engineering and Biotechnology, Polytechnic University of Catalonia, C. Esteve Terradas 8, 08860-Castelldefels (Barcelona), Spain
[2] Department of Applied Mathematics III, Polytechnic University of Catalonia, C. Esteve Terradas 8, 08860-Castelldefels (Barcelona), Spain
[3] Department of Microbiology, Faculty of Biology, University of Barcelona, Av. Diagonal 645, 08028-Barcelona, Spain

INTRODUCTION

Saccharomyces cerevisiae is a yeast of widely recognized biotechnological interest and is also used as a model to understand the cell cycle progression of eukaryotic cells. Because of the tight coupling between cell growth and division, the study of cell size distributions of yeast populations under different oxygen concentrations can reveal a wealth of information on the cell cycle regulatory mechanisms and adaptation to the environment.

From different principles, electric particle analysis and light diffraction are two current techniques that permit the obtaining of cell size distributions. Another current technique used to measure individual parameters, to identify subpopulations and to count microorganisms is flow cytometry. By this technique two parameters are collected: Forward Scatter and Side Scatter. Forward Scatter is a complex parameter as this function varies not only with cell size but also with cell shape, refractive index and number of intracellular dielectric interfaces. Side Scatter is also an intricate parameter. This signal is thought to indicate variations in cell surface or internal structure, usually referred to as "cellular granularity".

Here, we report cell population analyses of *S. cerevisiae* in stationary phase, grown in aerobic and hypoxic bath cultures by three experimental techniques: electric particle analysis, laser diffraction and flow cytometry.

MATERIALS AND METHODS

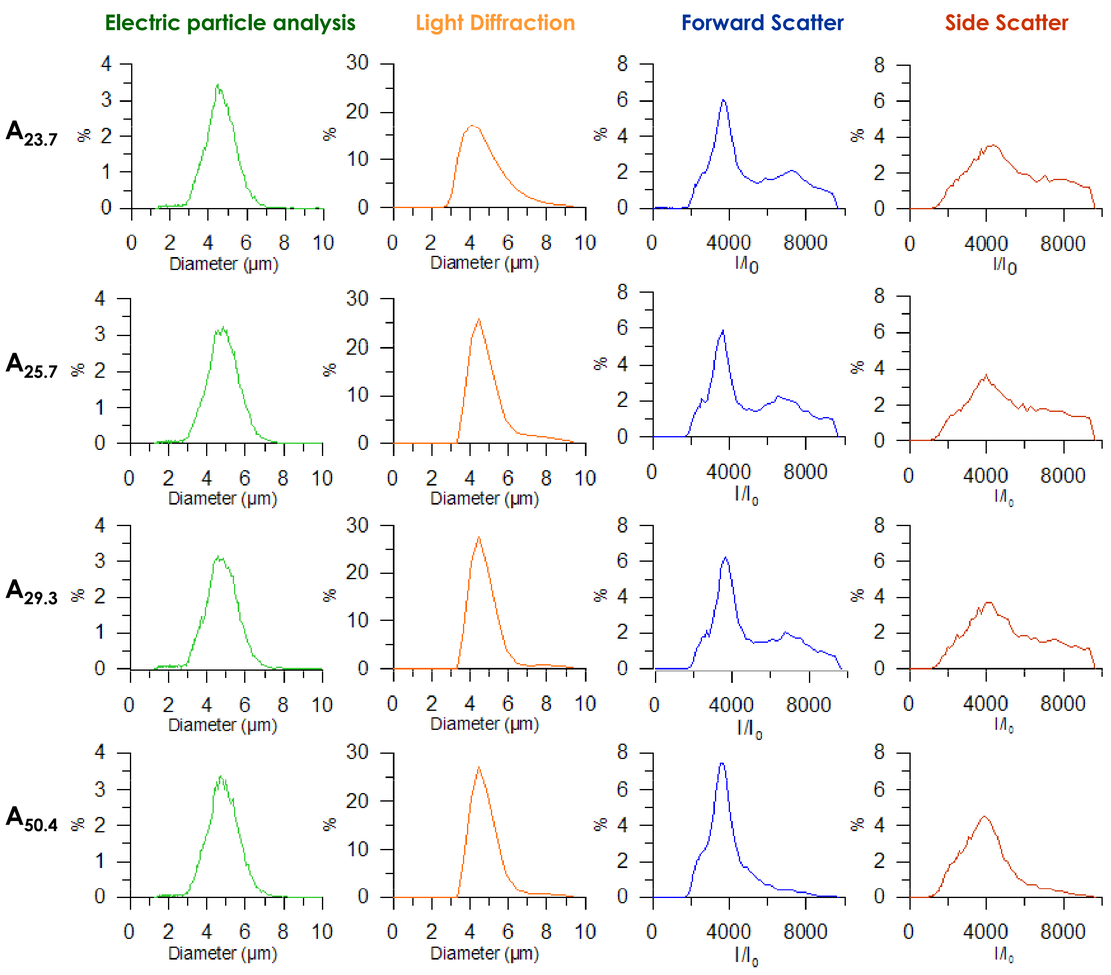
Yeast strain and culture media. The yeast strain used was *S. cerevisiae* var. *bayanus*. The medium composition was: 10 g l^{-1} glucose, 5 g l^{-1} yeast extract, and 3 g l^{-1} casein peptone. Components were dissolved in tap water. pH was initially adjusted to 3.5 with ortophosphoric acid and autoclaving for 15 min at 121ºC. To ensure hypoxic condition we added 0.5 g l^{-1} sodium thioglycolate and 0.001 g l^{-1} resazurine.

Culture conditions. The inocula, prepared and cultured in the same medium growing in aerobic and hypoxic conditions, respectively, were inoculated in 1000 ml flasks with 500 ml of the fresh medium and incubated at 27ºC, using magnetic shaking (150 r.p.m) for approximately 50 hours. Along this time, and after the steady state was confirmed, samples were removed to be analyzed.

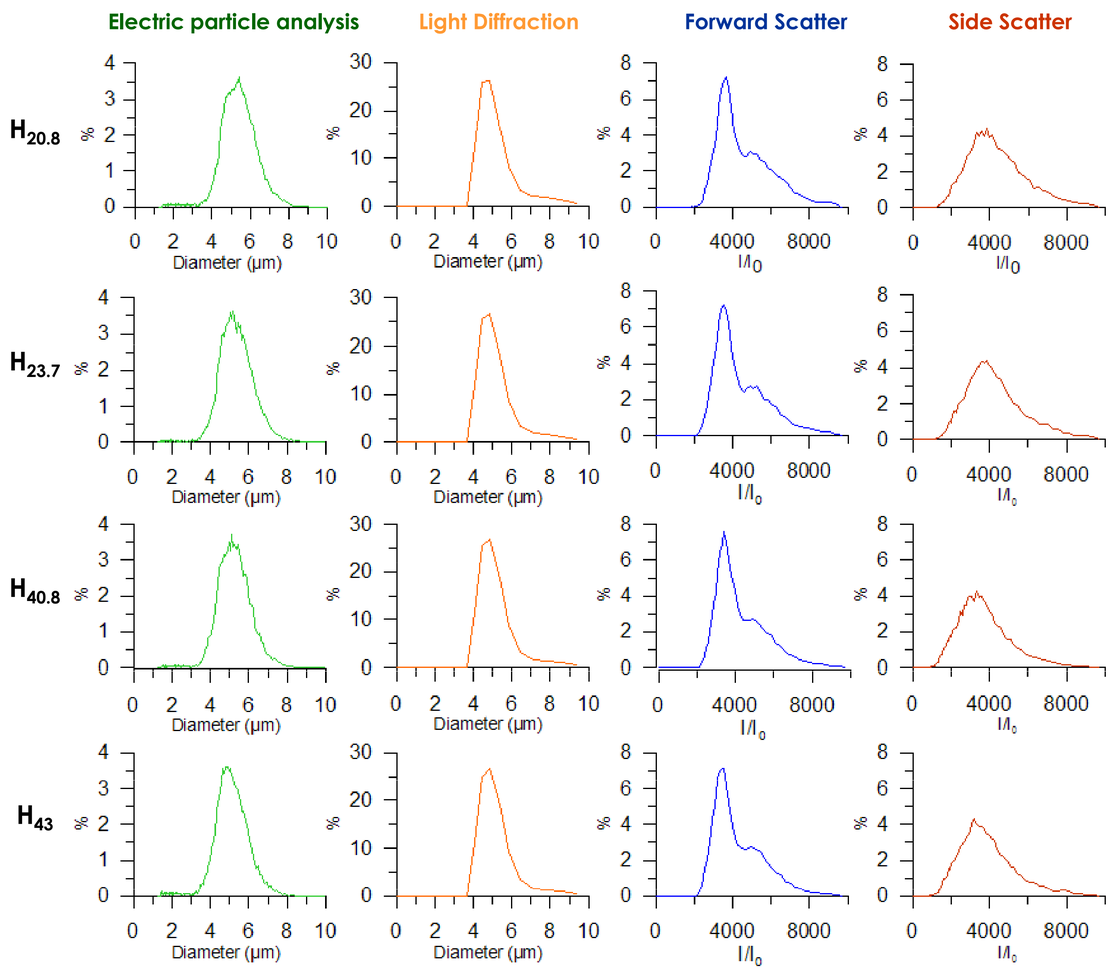
Experimental setup. The number of cells and cell size distributions were determined by electric particle analysis using a Multisizer II electronic particle analyser. Light diffraction (Beckman Coulter LS13320) was also used to determine cell size distributions. With the flow cytometry (Beckman Coulter FC cytomics 500 MPL) Forward Scatter (a measure of cell size) and Side Scatter (cell granularity) distributions were obtained.

RESULTS AND DISCUSSION

AEROBIC CULTURE



HYPOXIC CULTURE



Forward Scatter distributions show a similarly basic shape in both growth conditions, centered in a range of relative intensities between 3500-3700 with a long right tail. However, cells grown in hypoxic conditions show a subset of cells with relative frequencies between 4500 and 6000, whereas in aerobic conditions there is a higher proportion of cells with relative intensities between 6000 and 10000. This is consistent with the existence of two subpopulations, not observed with the electric particle analysis and laser diffraction technique. The two subpopulations show more differences in aerobic conditions than in hypoxia. The subpopulations in aerobic conditions join at the advanced stationary phase. **Side Scatter distributions** are essentially the same: a triangular distribution slightly positively skewed and centered at relative intensities between 4000-4200. However, there is a subpopulation of yeast cells with relative intensities between 6000 and 10000 in aerobic conditions.

	Electric particle analysis	Light diffraction
Aerobic	Mean (Coef.Var.)	M ean(Coef.Var)
$A_{23.6}$	4.46 (19.75)	4.50 (21.47)
$A_{25.7}$	4.67 (20.96)	4.66 (15.95)
$A_{29.3}$	4.60 (23.15)	4.58 (12.76)
$A_{50.8}$	4.67 (20.19)	4.61 (12.85)

	Electric particle analysis	Light diffraction
Hypoxic	M ean(Coef.Var)	Mean (Coef.Var)
$H_{20.8}$	5.23 (19.00)	4.99 (16.34)
$H_{23.7}$	5.19 (18.53)	4.98 (15.76)
$H_{40.8}$	5.09 (18.02)	4.98 (15.76)
H_{43}	4.93 (19.33)	5.00 (15.70)

During the stationary phase of culture in hypoxic conditions, the population is more homogeneous than under the aerobic cultivation.

The electric particle analysis and light diffraction show cells under hypoxic conditions that are greater than aerobic ones.

With electric particle analysis, the size distributions move to the left along the stationary phase but with laser diffraction they are stable.

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