A comparative study of the use of an electronic particle analyser and a flow cytometer to assess the changes of cell size distributions of *Saccharomyces cerevisiae* var. *bayanus* during aerobic and anaerobic growth in batch cultures

M. Ginovart[1], X. Portell[2], R. Carbo[2], A. Gras[2], J. Vives-Rego[3]

[1] Department of Applied Mathematics III, Technical University of Catalonia, Campus Baix Llobregat, Avda. Canal Olímpic 15, 08860-Castelldefels (Barcelona), Spain

[2] Department of Agri-Food Engineering and Biotechnology, Technical University of Catalonia, Campus Baix Llobregat, Avda. Canal Olímpic 15, 08860-Castelldefels (Barcelona), Spain

[3] Departament de Microbiologia, Facultat de Biologia, Universitat de Barcelona, Av. Diagonal 645, 08028-Barcelona, Spain

INTRODUCTION

The sizes and growth rates of individual cells, as in *Saccharomyces cerevisiae* cultures, are highly heterogeneous, mainly due to both the asymmetric cell division of budding yeast and to ageing. The availability of ready-to-use commercial strains of *S. cerevisiae* var. *bayanus* has prompted their widespread use in wine production. A comprehensive understanding of the growth processes of a culture must be based on a quantitative understanding of how individual cells change through time and of how they interact with their environment. Yeast cell size is an important physical parameter since it can be related to the growth stage of yeast cells and by extension to fermentation performance.

Electric counters and flow cytometry are valuable and advanced tools that offer direct rapid assays to reveal particle size, number of particles, cell size distributions, and even biochemical and physiological characteristics of individual cells.

The aim of this contribution is to analyze and compare the data sets obtained by using these two types of measurements in order to assess the changes in cell size distributions of *S. cerevisiae* var. *bayanus* during aerobic and anaerobic growth in batch cultures.

MATERIALS AND METHODS

Yeast strain

The yeast strain used was *S. cerevisiae* var. *bayanus* Vitilevure DV10 from the Oenological Station in Champagne (Epernay, France). It was first hydrated and then spread on Sabouraud Dextrose agar to purify the strain.

Culture medium

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. We added 0.5 g l^{-1} sodium thioglycolate and 0.001 g l^{-1} resazurine for the anaerobic conditions.

Culture conditions

Two culture conditions, aerobic and anaerobic, were examined to study the different stages of their temporal evolution: lag phase, exponential growth and stationary phase.

Yeast was seeded in vessels that contained 500mL of the described media. Cultures were incubated at 27ºC for approximately 65-70 hours to guarantee that they were in the stationary phase. They were shaken at 150 rpm.

The inocula used in the experiments (previously grown in Petri dishes) were prepared and cultured: i) in the aerobic case, using active oxygenation achieved by magnetic shaking in cotton plugged flasks of 250 mL with 100 mL and, ii) in the anaerobic case, using screw capped tubes with the medium supplemented with sodium thioglycolate and resazurine for adjustment .

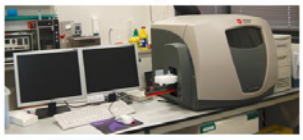
Samples were analysed at several times points with an electronic particle analyser and a flow cytometer to study changes in the size distributions of the yeast population during the evolution of the culture.

Experimental setup

The number of cells and cell size distribution were determined by electrozone counting. For this, a **Multisizer II electronic particle analyser** (Coulter® Corporation), with an aperture tube of 30 µm in diameter and a capacity to process 500 µl of the cell suspension in Isoton was used. Data were analysed with AccuComp software version 1.15. Trials were also carried out using a Beckman Coulter **FC cytomics 500 MPL flow cytometer** (ref.: 20061121, Coulter Corporation, Miami, Florida). A standard 488nm air-cooled argon-ion laser with 15mW power was used. The **forward scatter (FS)** and **side scatter (SS)** distributions were obtained for each measurement. The FS detector in the Elite flow cytometer is a photodiode that collects light between 1-5º and 19º from the laser axis and which is able to detect particles as small as 0-5 µm in diameter. The SS detector is situated at a 90º angle from the laser axis. Data were analysed with Summit ® V3.1 software (Cytomation, Inc.).

Multisizer II

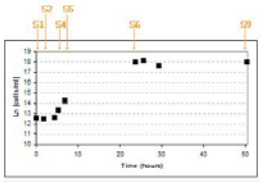
FC cytomics 500 MPL

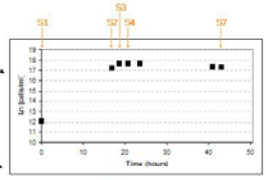


RESULTS AND DISCUSSION

AEROBIC CULTURE

ANAEROBIC CULTURE



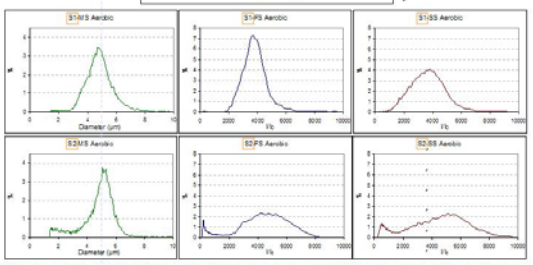


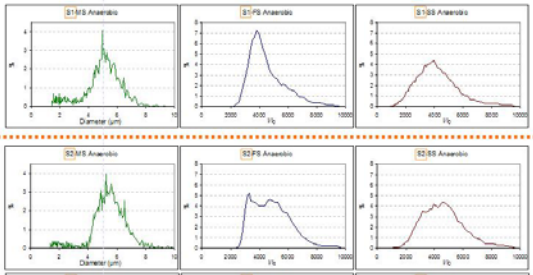
Cell concentrations and the size distributions obtained with the Multisizer II are shown. The sizes are given in terms of equivalent spheres. The output yields the diameter of a sphere that has the same volume as the yeast cell.

The flow cytometric techniques provide the distributions of forward scatter FS and side scatter SS in terms of channels. Each channel collects the scattered intensity within a certain interval. These distributions are shown in terms of intensity rather than channels. Relative scattered intensity (I/I_0) in a linear scale was used.

Light scattering is a complex function of the cell size and also depends on the shape, structure, chemical composition, refractive index, etc.

Flow cytometric cell sizing is mostly estimated from the intensity of the forward light scatter (FS), which is used in preference to the 90º scatter (SS) because of its insensitivity to subcellular structure. FS is assumed to be proportional to cell size, but a correlation between narrow-angle light scatter and yeast size remains to be investigated in depth.





Lag Phase

Exponential Phase

At the end of the Exponential Phase

Stationary Phase

Table 1: Descriptive statistics for each sample obtained from the Multisizer II, the diameter (µm) distributions.
M: Mean, SD: Standard deviation, Q1: First quartile, Q2: Second quartile or Median, Q3: Third quartile, R: Range, IQR: Interquartile range, CV: Coefficient of variation

	M (SD)	Q1 - Q2 - Q3	R - IQR	CV
AEROBIC CULTURE				
S1	4.64 (1.04)	4.08 - 4.62 - 5.16	37.63 - 1.08	22.51
S2	4.38 (1.47)	3.60 - 4.81 - 5.31	25.09 - 1.71	33.58
S3	4.72 (2.11)	3.09 - 5.05 - 5.85	18.95 - 2.76	44.73
S4	5.43 (1.37)	4.81 - 5.38 - 6.09	29.88 - 1.28	25.17
S5	5.13 (1.41)	4.55 - 5.16 - 5.77	25.09 - 1.21	27.56
S6	4.43 (0.88)	3.91 - 4.43 - 4.95	17.86 - 1.04	19.75
S7	4.64 (0.97)	4.08 - 4.62 - 5.23	23.66 - 1.16	20.96
S8	4.57 (1.06)	4.02 - 4.56 - 5.16	33.07 - 1.14	23.16
S9	4.64 (0.94)	4.08 - 5.62 - 5.23	21.66 - 1.16	20.19
ANAEROBIC CULTURE				
S1	4.59 (1.48)	3.91 - 4.88 - 5.53	11.46 - 1.62	32.19
S2	5.19 (1.57)	4.62 - 5.31 - 5.93	17.84 - 1.31	30.27
S3	4.58 (1.83)	3.09 - 4.95 - 5.77	17.05 - 2.68	40.05
S4	5.28 (0.80)	4.68 - 5.23 - 5.77	4.93 - 1.08	15.11
S5	5.16 (0.96)	4.62 - 5.16 - 5.69	13.58 - 1.07	18.53
S6	5.06 (0.91)	4.55 - 5.02 - 5.61	13.79 - 1.05	18.02
S7	4.90 (0.95)	4.43 - 4.88 - 5.45	9.49 - 1.02	19.33

Batch cultures can be used to simulate different nutrient availabilities thereby making it possible to study the fundamental mechanisms which allow microorganisms to survive in changing conditions.

The three types of distributions observed for the yeast strain *S. cerevisiae* var. *bayanus* throughout the course of the two batch cultures, aerobic and anaerobic, are shown in the figures.

The overall shape of these distributions gradually changed through the course of the diverse stages.

Further experimental data is needed in order to characterize the distributions at different phases of the yeast cultures.

Table 2: Percentages of different subpopulations of yeast cells for the samples obtained from the Multisizer II.

	AEROBIC CULTURE								ANAEROBIC CULTURE							
	S1	**S2**	**S3**	**S4**	**S5**	**S6**	**S7**	**S8**	**S9**	**S1**	**S2**	**S3**	**S4**	**S5**	**S6**	**S7**
Cell diameter < 3	3.4	19.9	24.2	5.5	7.0	4.2	3.5	3.8	3.0	17.6	10.3	24.4	3.1	2.2	2.2	3.7
3 ≤ Cell diameter ≤ 8	96.1	79.7	73.1	91.9	91.3	95.7	96.3	95.9	96.8	81.7	88.0	74.2	95.6	97.4	97.6	96.2
Cell diameter > 8	0.5	0.4	2.7	2.6	1.7	0.1	0.2	0.3	0.2	0.7	1.7	1.4	0.3	0.4	0.2	0.1

Acknowledgements
We are grateful to Clara Prats (UPC) and Jaume Comas-Riu (Scientific and Technical Services of the University of Barcelona).
We gratefully acknowledge the financial support of the Plan Nacional I+D+i of the Ministerio de Educación y Ciencia CGL2007-65142/BOS, and the European Social Fund and AGAUR - Generalitat de Catalunya 2008FIA-00307.

REFERENCES

Erlebach C.E., Illmer P., Schinner F. (2000) Changes of cell size distribution during the batch culture of *Arthrobacter* strain PI/1-95. *Antonie van Leeuwenhoek* 77, 329-335.
Ginovart M., Carbó R., Gras A., Vives-Rego J. (2007) The use of flow cytometry and particle size analysis in the individual-based model INDISIM-YEAST, a simulator of yeast populations. In: G. Nychas et al. (Eds) *Proceedings of the 5th International Conference Predictive Modelling in Foods*, p. 341-344.
Prats C., Ferrer J., Flix B., Giró A., López D., Vives-Rego J. (2007) Evolution of biomass distribution during lag phase through flow cytometry, particle analysis and individual-based Modelling. In: G. Nychas et al. (Eds.) *Proceedings of the 5th International Conference on Predictive Modelling in Foods*, p. 301-304.
Vives-Rego J., Lebaron P., Nebe-vonCaron G. (2000) Current and future applications of flow cytometry in aquatic microbiology. *FEMS Microbiology Reviews* 24, 429-448.