



Monitoring Yeast Cultures with the BioLector and Multisizer 4e instruments

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Introduction

Yeasts are unicellular fungi that share cellular structures and processes that are highly conserved amongst eukaryotes (e.g., membrane-bound organelles, a cytoskeleton, nuclear DNA, secretory proteins and transcription mechanisms). In addition, they are relatively easy and cheap to culture under laboratory conditions, display rapid growth, can be easily genetically manipulated and are able to achieve most of the post-translational modifications required for a biologically active recombinant protein.¹ These characteristics make yeast cultures a popular choice for basic research (e.g., studying the function of specific genes or proteins) and protein production for various applications (e.g., chemicals, fuels, food and pharmaceuticals).^{2,3} This application note will demonstrate how the BioLector microbioreactor and the Multisizer 4e Coulter Counter can be used to optimize yeast cell culture conditions and characterize cell growth (Figure 1).



FIGURE 1: The BioLector microbioreactor and the Multisizer 4e Coulter Counter can be used to monitor yeast cultures.

Monitoring Optimal Cell Culture Conditions

The BioLector microbioreactor is a small-scale, automated system that enables high-throughput screening, cultivation parameter monitoring (e.g., pH, biomass, oxygen saturation, shaking speed, and fluorescence intensity) and feeding strategy optimization. Importantly, all these parameters are monitored online without the necessity to stop shaking or take samples. In this example, the BioLector device was used to determine the optimal pH range for efficient protein production in the yeast model *Hansenula polymorpha*.

Methods

Cultivations of *H. polymorpha* expressing green fluorescent protein (GFP) were carried out in the microfluidic 32-well FlowerPlate microtiter plate of the BioLector microbioreactor with optodes suitable for a low pH range

(M2P-MTP-MF32-BOH3). The plate was shaken at 1200 rpm and the temperature was set at 30° C. The culture medium was composed of 1.34 g/L Yeast Nitrogen Base, 5 g/L (NH₄)₂SO₄ and 20 g/L glucose. Each cultivation well was filled up with 800 µL of culture broth. A 25 mM phosphate buffer (pH 6.0) was used. The microfluidic chip was used to set different pH points between 4 and 6.5, and 3 M NaOH and 3 M HCl were used as pH adjusting agents. During the cultivation, online measurements of GFP and pH were performed. The pH was measured optically in the infrared spectrum, to reduce interference by background fluorescence in the culture medium. The GFP signal was used as a reporter of the yeast proteins synthesis.

Results and Discussion

The GFP signal and pH values of three cultivation examples are shown in Figure 2. The results showed that the lower the pH, the earlier the GFP expression starts. In addition, the rate of GFP production also was higher when the pH value was set at 4. However, as already reported, GFP is not stable in the lower pH range,⁴ thus only a qualitative comparison is possible here.

The subsequent calculation of the space-time yield (STY) of the GFP expression for each pH value confirmed that more GFP was expressed at low pH values (Figure 3).

With the help of the microfluidic option of the BioLector microbioreactor up to 32 parallel cultivation experiments at different pH setpoints were performed simultaneously on one MTP. This application note demonstrates that the BioLector system can be used to screen the best pH conditions for optimal protein expression in *H. polymorpha*. Likewise, the instrument can be used to optimize other cultivation parameters (e.g., phosphate buffer concentrations in the medium, temperature, oxygen concentration, carbon substrates, etc.). Previous studies have shown the efficacy of the BioLector microbioreactor to screen optimal culture conditions and engineered yeast strains prior to scale-up.^{5,6} In summary, the system enables the evaluation and discrimination of different cultures and therefore improves screening conditions, process development and scale-up procedures.

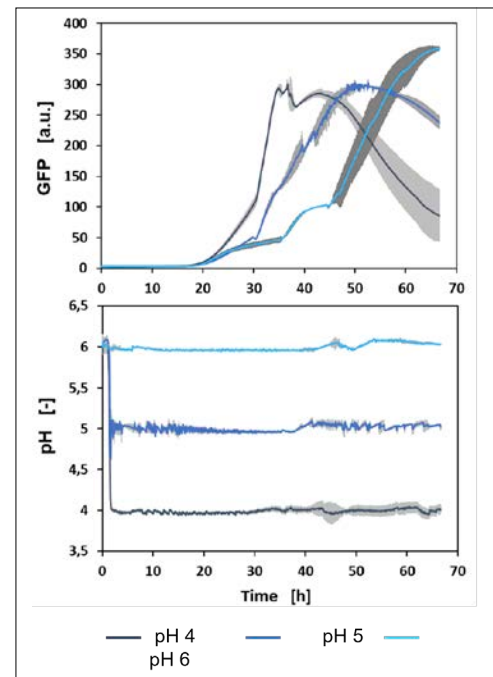


FIGURE 2: Microfluidic batch cultivation of *H. polymorpha* with online measurement of GFP and online pH regulation/measurement.

Monitoring Cell Growth

Cell size is a key parameter to gain insights into various cellular mechanisms (e.g., cell cycle, osmotic regulation, cell death, pathogenesis, phagocytosis, species diversity, etc.). The Multisizer 4e instrument uses the [Coulter method](#) to detect particles from 200 nm to 1,600 µm regardless of the particle's nature or optical properties. Particles suspended in a 0.9% electrolyte solution are drawn through a small cylindrical aperture. Two submerged electrodes located on each side of the aperture create an electric current. As each particle passes through

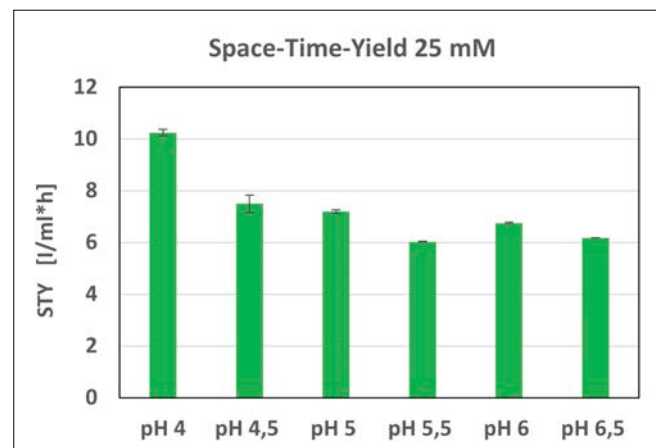


FIGURE 3: Calculation of the space-time-yield (STY) of GFP reached in *H. polymorpha* cultivations at different pH values.

the aperture, it displaces its own volume of conducting liquid, momentarily increasing the impedance of the aperture. This produces a voltage pulse that is proportional to the volume of the particle. The number of electrical pulses indicates particle count, while the amplitude of the electrical pulse produced depends on the particle's volume.² This allows researchers to accurately determine the volume, number, and cell concentration in a sample and detect real-time size changes. In the following example, the Multisizer 4e instrument was used to evaluate the range of cell sizes in a yeast culture.

Methods

A sample of the yeast culture was pre-diluted 1:200 with Isoton 2 in a 10 mL Accuvette ST. From this dilution, 500 μL was used for each measurement. The aperture used was 100 μm and each measurement lasted 120 seconds. The complete list of settings is shown in Table 1. The generated data were processed using Digital Pulse Processing (DPP) technology, which enables the acquisition, storage and display of each individual pulse. This means that individual areas of the pulse spectrum can be evaluated separately at a later date.

TABLE 1: Settings used in the experiment

Parameter	Multisizer 4e Instrument Setting
Aperture	100 μm
Current/Gain	3200 $\mu\text{A}/1$
SOM	Time 120 sec
Vessel	400 mL Beaker
Threshold	33,4 μm
Measuring Time	120 sec
Size bins	400
Dilution	1:200
Counts	92875

Results and Discussion

The DPP data of the experiment shown in Figure 4 indicates that the size of the yeast in this culture ranged between 2 and 13 μm .

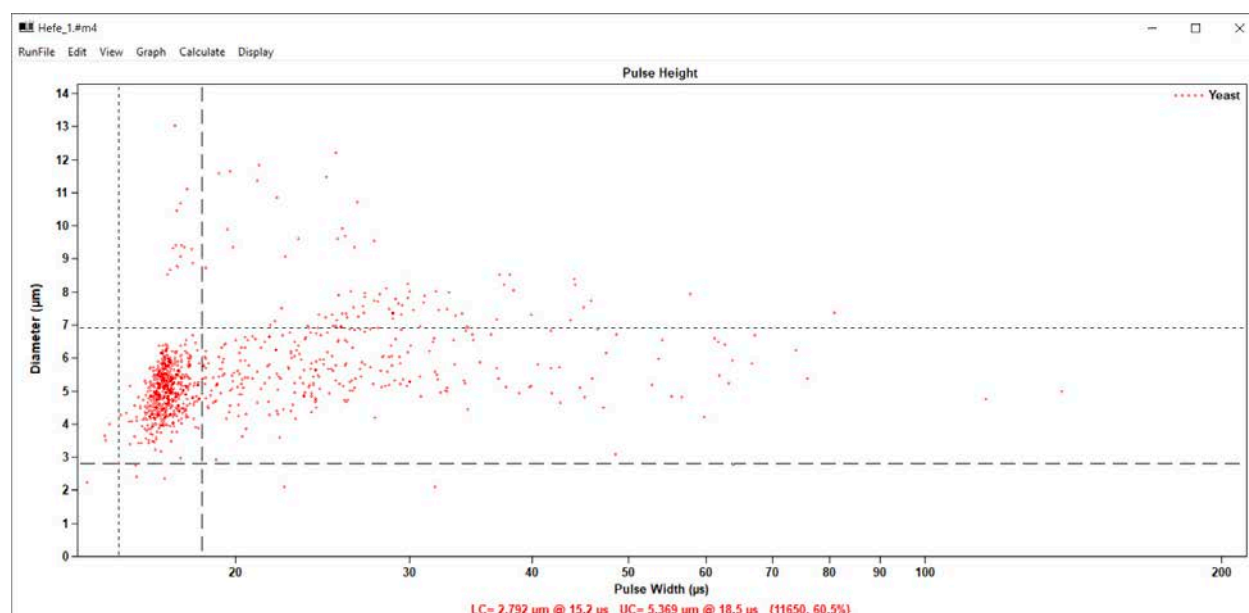


FIGURE 4: Digital Pulse data. Impulse width vs. impulse height. The dotted box indicates the data points selected for further analysis.

From this raw data it is possible to select a range of interest (dotted box in Figure 4) and create different cell size distributions (i.e., frequency histograms showing the number of cells for each volume). For example, Figure 5 showed the resulting histogram if a range between 2.8 and 7 μm is selected.

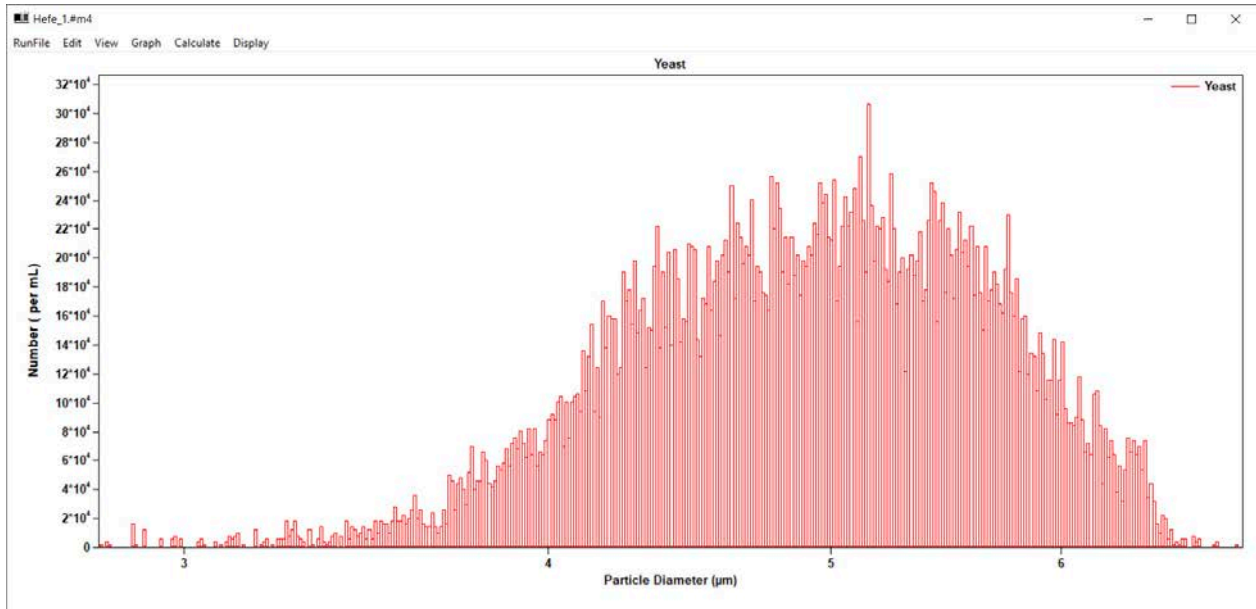


FIGURE 5: Distribution of yeast sizes across the sample (diameter, in μm).

The DPP technology provides ultra-high resolution and accuracy (i.e. detection of 1 particle in 1 mL of a sample), which is unattainable using other current technologies. Thus, it can be used for a broad range of applications. For example, the comparison of cell size obtained using different environmental conditions can help to optimize cell culture conditions while the evaluation of samples at different time points can be used to study how cell size changes over time. Moreover, it is possible to enhance the time resolution of the Multisizer 4e instrument and perform long-term measurements by creating cell culture conditions within the sample compartment.⁸ This method was used to describe yeast cell growth as a function of size and cell cycle position.

Conclusion

The BioLector microbioreactor and the Multisizer 4e Coulter Counter are benchtop devices with an intuitive user interface that can be integrated to any laboratory to optimize and monitor the growth of yeast cultures.

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*The data shown in this Application Note was generated on BioLector II and BioLector Pro microbioreactors. The BioLector model shown in this Application Note is the latest model of the series, a BioLector XT microbioreactor.

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