Dr. Tanja Buch & Jigar Patel
Biofactory Competence Center, Fribourg, Switzerland & Repligen Corporation, Bridgewater, NJ, USA

# In-Line Slope Monitoring at 600 nm of the Fermentation Process for the Optimal Cultivation of *E. Coli* Cells

## Abstract

The increasing demand of cell and gene therapeutics (CGT) have created a need to optimize manufacturing process steps using Process Analytical Technologies (PAT) to lower costs and increase yields. Plasmid DNA (pDNA) is a precursor to the development of CGTs and is met with many challenges throughout the production process due its size, viscosity, and similarities to other impurities which can impact their yields. The generation of these plasmids in high density *E. coli* cultures can be difficult to monitor using traditional off-line OD600 methods due to the careful sample preparation, fast growth rate, and dilutions required to be in the linear range of a standard spectrophotometer.

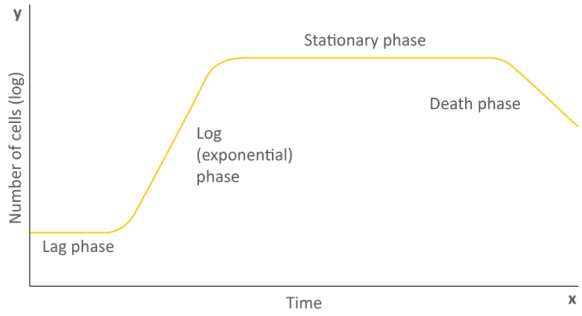


Figure 1. *E. coli* growth curve.

This study proposes utilizing an in-line variable pathlength spectrophotometer in a recirculation loop during fermentation to monitor and optimize the growth of *E. coli* cells in real time.

This provides distinct advantages over traditional methods as there can be constant modification of pathlengths to create a slope-based solution that is always within the linear range of the equipment with no buffer correction required. The growth curve is tracked in real time using the slope (Absorbance/mm) at 600 nm to measure cell density and to optimize growth performance of *E. coli* cells. This serves as a proof of concept for the optimal cultivation of *E. coli* and for the production of pDNA.

## Methods & Materials

The experiment was conducted using three different methods to detect the bacterial growth curve of *E. coli* at OD600 nm. The first method utilized a standard UV-Vis spectrophotometer that off-line measured the turbidity and optical density of the bacterial suspension sample, in a plastic cuvette using a single, fixed pathlength of 1 cm. The second method utilized an at-line variable pathlength technology (VPT) UV-Vis spectrophotometer that takes absorbance values at varying pathlengths to obtain a slope (Au/mm) value—the sample was placed into a small silica vessel and Fibrette® Optical Components (solid fiber optic pieces) were used for measurements at 600 nm. The third method utilized an in-line VPT UV-Vis spectrophotometer and performed the measurements directly in the process by creating a recirculation loop with the Roundbottom Autoclavable Lab Fermenter (RALF) bioreactor (see Figure 2). The cell broth was pumped through the spectrophotometer's 3 mm Flow Cell at 14 ml/min using a peristaltic pump. Absorbance values were taken at variable pathlengths that ranged from 3 mm to 1.5 mm in step changes of 0.15 mm, with a wavelength of 600 nm and an extinction coefficient of 1.0 ml (mg*cm) for both VPT instruments.

*E. coli* W3110 K12 cells with two liters of YPG medium (reduced glucose concentration to 5 g/l added prior to autoclaving) was used for cultivation. The lab scale 3.5L RALF bioreactor was operated at 37°C. The dipping tube inside the bioreactor was positioned pointing up to avoid introduction of air bubbles. Samples were taken at multiple timepoints throughout the process and measured in triplicate for the standard and VPT at-line spectrophotometers while continuously measuring on the in-line VPT instrument. The at-line and in-line VPT spectrophotometer data was then plotted with the standard spectrophotometer data to determine the correlation factor. This was performed at various OD ranges from 0 to 20 and was averaged together to determine the final correlation factor. The final correlation factor was then applied to the results of the at-line and in-line VPT spectrophotometers and compared to the standard spectrophotometer data at OD600. A total of three (3) RALF bioreactor experiments were performed in this study.

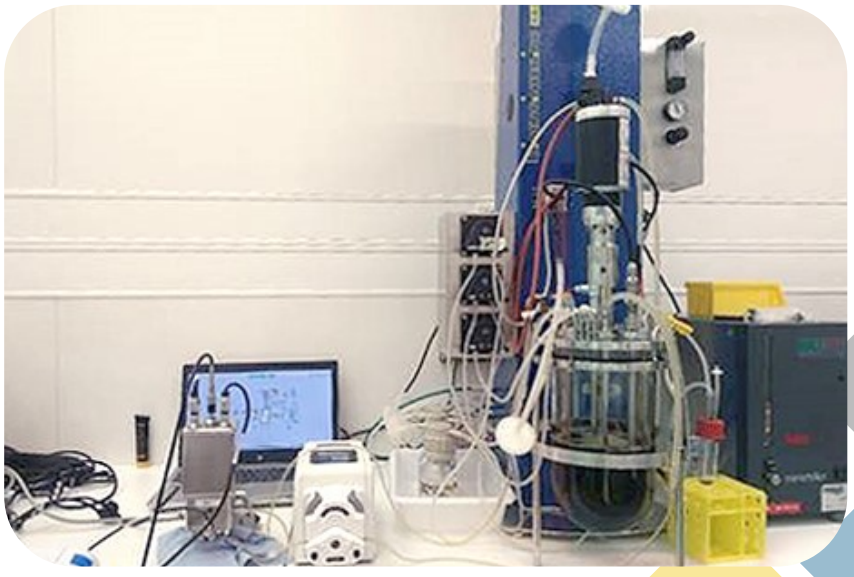

Figure 2. In-line VPT UV-Vis spectrophotometer with RALF.

## Data

Table 1 includes the data used in correlation factor determination for the at-line and in-line VPT instruments (Au/mm) to a standard spectrophotometer at various optical densities during the three RALF bioreactor experiments. *E. coli* suspensions do not follow Beer-Lambert law due to the large size and scattering properties of *E. coli* suspensions. A correlation factor determination is required, as OD600 is a scatter measurement and scatter can be affected by several factors such as diameter and number of cells.

**Table 1. Data generated from RALF bioreactor experiments**

| | *y = mx + b* | Slope (m) | *y-intercept (b)* |
|---|---|---|---|
| OD: 0-15 | RALF 1—At-line VPT | 0.0114 | 0.01070 |
| OD: 0-15 | RALF 1—In-line VPT | 0.0106 | 0.01440 |
| OD: 0-17 | RALF 2—At-line VPT | 0.0149 | 0.02420 |
| OD: 0-17 | RALF 2—In-line VPT | 0.0138 | 0.02970 |
| OD: 0-16 | RALF 3—At-line VPT | 0.0158 | 0.00480 |
| OD: 0-16 | RALF 3—In-line VPT | 0.0145 | 0.00950 |
| | **Average** | **0.0135** | **0.0156** |
| | **Standard** | **0.0021** | **0.0095** |

This can be observed in Figure 3 where the slope changes noticeably after the 7-hour mark. This noticeable change can be due to an increase in the size of the cells and/or a change in the shape of the cells.

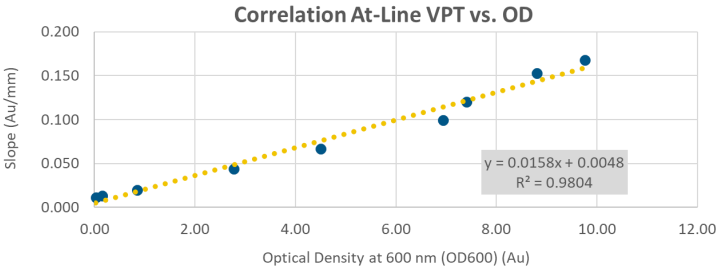


Figure 3. Correlation factor of at-line VPT vs. standard spectrophotometer data for RALF Experiment #3.

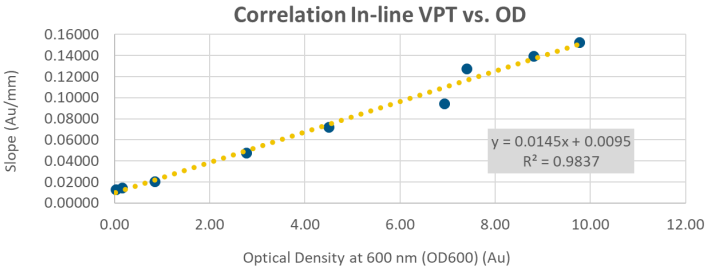


Figure 4. Correlation factor of in-line VPT vs. standard spectrophotometer data for RALF Experiment #3

## Experiment

Starting at sample 2, dilutions were required for the standard spectrophotometer, as it was outside the typical absorbance range of <0.3 Au for linear approximation of *E. coli* biomass. Three RALF bioreactor experiments were performed, and off-line samples were taken at the timepoints outlined in Table 2 in triplicate from the third RALF bioreactor experiment. The data in Table 3 was collected from the at-line and in-line VPT instruments. Pathlength vs. absorbance data was collected and a linear regression was applied to acquire a slope (Au/mm) value.

**Table 2. Data from RALF bioreactor experiment #3**

| Sample | Time (min) | Standard Spectrophotometer $OD_{600}$ | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | Blank | Dilution factor | Measured | | | Calculated | | | |
| | | | | OD-1 | OD-2 | OD-3 | Average | Std. | Final | %error |
| 1 | 0.00 | 0.174 | 1 | 0.199 | 0.198 | 0.197 | **0.198** | **0.001** | **0.02** | 0.51 |
| 2 | 55.00 | 0.068 | 10 | 0.084 | 0.084 | 0.083 | **0.084** | **0.001** | **0.16** | 0.69 |
| 3 | 110.00 | 0.068 | 10 | 0.151 | 0.151 | 0.156 | **0.153** | **0.003** | **0.85** | 1.89 |
| 4 | 165.00 | 0.059 | 50 | 0.114 | 0.115 | 0.114 | **0.114** | **0.001** | **2.77** | 0.50 |
| 5 | 195.00 | 0.058 | 100 | 0.104 | 0.1 | 0.105 | **0.103** | **0.003** | **4.50** | 2.57 |
| 6 | 225.00 | 0.058 | 100 | 0.121 | 0.128 | 0.133 | **0.127** | **0.006** | **6.93** | 4.73 |
| 7 | 270.00 | 0.058 | 100 | 0.13 | 0.13 | 0.136 | **0.132** | **0.003** | **7.40** | 2.62 |
| 8 | 325.00 | 0.058 | 100 | 0.144 | 0.146 | 0.148 | **0.146** | **0.002** | **8.80** | 1.37 |
| 9 | 385.00 | 0.058 | 100 | 0.152 | 0.154 | 0.161 | **0.156** | **0.005** | **9.77** | 3.04 |

**Table 3. Data from RALF bioreactor experiment #3 for at-line and in-line VPT spectrophotometers**

| Sample | Time (min) | Dilution Factor | Measured with At-line VPT | | | | | | In-line VPT | | Final Calculation | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | 1-P Slope (Au/mm) | 2-Q Slope (Au/mm) | 3-Q Slope (Au/mm) | Avg | Std | % error | Slope (Au/mm) | RALF #3 Corr Factor Recalculation | At-line VPT Corr Factor from all 3 RALFs (Au) | In-line VPT Corr Factor from all 3 RALFs (Au) |
| 1 | 0.00 | 1 | 0.0111 | 0.0104 | 0.01116 | 0.011 | 0.000 | 3.881 | 0.385 | 0.01300 | -0.345 | -0.189 |
| 2 | 55.00 | 1 | 0.01293 | 0.01341 | 0.01206 | 0.013 | 0.001 | 5.346 | 0.506 | 0.01434 | -0.204 | -0.090 |
| 3 | 110.00 | 1 | 0.01949 | 0.01961 | 0.01959 | 0.020 | 0.000 | 0.329 | 0.934 | 0.02026 | 0.297 | 0.349 |
| 4 | 165.00 | 1 | 0.04373 | 0.04421 | 0.045 | 0.044 | 0.000 | 0.772 | 2.479 | 0.04747 | 2.105 | 2.364 |
| 5 | 195.00 | 1 | 0.06802 | 0.0657 | 0.06538 | 0.066 | 0.001 | 2.171 | 3.897 | 0.07190 | 3.764 | 4.174 |
| 6 | 225.00 | 1 | 0.10235 | 0.099925 | 0.09509 | 0.099 | 0.004 | 3.729 | 5.970 | 0.09444 | 6.190 | 5.844 |
| 7 | 270.00 | 1 | 0.12155 | 0.11956 | 0.11929 | 0.120 | 0.001 | 1.027 | 7.300 | 0.12774 | 7.747 | 8.310 |
| 8 | 325.00 | 1 | 0.15369 | 0.15274 | 0.15307 | 0.153 | 0.000 | 0.315 | 9.390 | 0.13958 | 10.194 | 9.187 |
| 9 | 385.00 | 1 | 0.16948 | 0.16786 | 0.16674 | 0.168 | 0.001 | 0.820 | 10.331 | 0.15233 | 11.295 | 10.132 |

## Results

The standard spectrophotometer measurements after the first sample were diluted by a factor of 10. Then, after 110 minutes, they were diluted by a factor of 50, and after 165 minutes, were diluted by a factor of 100. Each sample measurement for a standard spectrophotometer must be blanked with the YPG medium at the same dilution factor as the sample. The results were then recalculated back to the actual OD600 and compared to undiluted at-line and in-line VPT data at the respective timepoints. The correlation factor average was taken across all the experiments performed in this study and applied to the at-line and in-line VPT data collected during all three RALF runs.

Figures 5 and 6 show that the at-line and in-line VPT instruments were able to monitor the growth curve of *E. coli* without the need for dilutions or buffer corrections. The data shows reliable repeatability of ~5% or less for the triplicate measurements on VPT spectrophotometers and are comparable to the standard spectrophotometer method. The percent error was calculated for the RALF fermentation experiments by using the following formula: (standard deviation/ average)*100. The %error was found to be 15.24%. It can be observed that the in-line VPT spectrophotometer data overlaps the off -line standard spectrophotometer data quite well and follows the *E. coli* growth curve in real time.

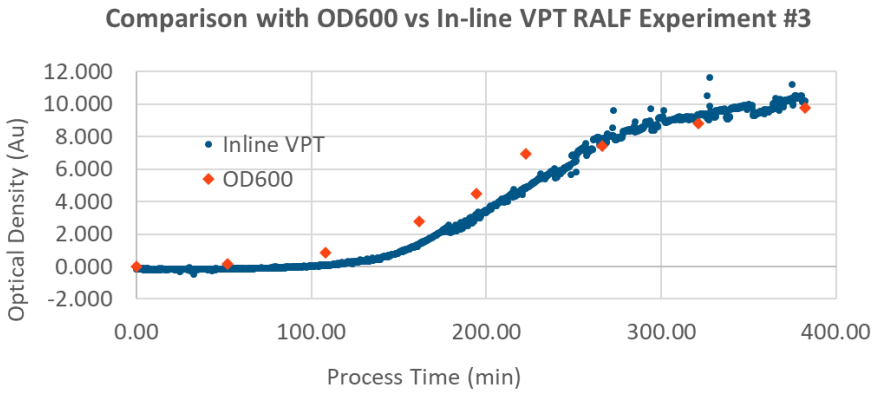


Figure 5. Growth curve of *E. coli* measured using standard and in-line VPT spectrophotometer at OD600.

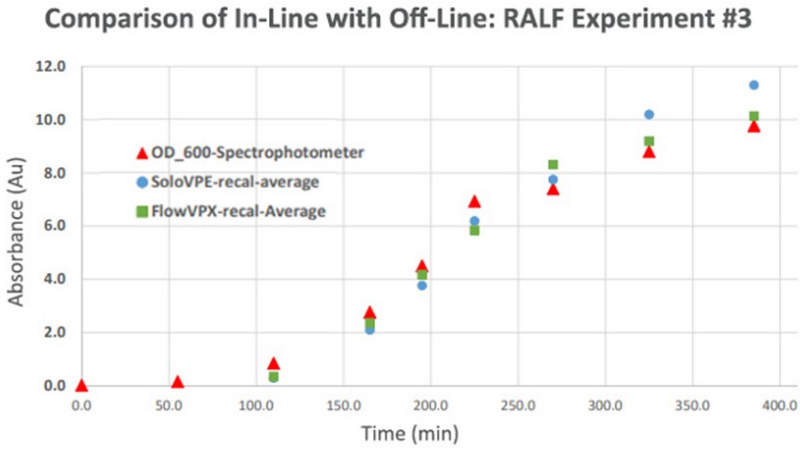


Figure 6. Comparison data for RALF Experiment #3 using all three methods.

## Conclusion

The experiments support the statement that *E. coli* cell growth curves can be monitored using at-line and in-line VPT systems with high accuracy and repeatability and without the need for dilution or baseline correction. The results show that the data is comparable to the standard spectrophotometer OD600 measurements. This study can be used as proof of concept for future experiments to determine the correct timing for the induction of pDNA into *E. coli* cells during the transformation process. This is critical, as an extended wait time can lead to metabolic changes in *E. coli* due to the consumption of all the glucose and dissolved oxygen in the medium. These metabolic changes lead to an increase in unwanted byproducts, such as acetate, which has a negative impact on cell growth and pDNA production. The ability to monitor the fermentation process in-line using process analytical technologies allows for the optimization of the process and possible yield increases for pDNA, which stands to further the development of CGTs.

## References


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