# Optical densities in biotechnology

I. Use of optical densities

Measuring the optical density of growing cultures is a common method to quantify various important culture parameters like cell concentration, biomass production or changes in the cell morphology. Online photometry allows continuous real time analysis of those parameters without any laborious work. Continuous measuring of optical density is the most basic and powerful tool for providing optimal yields and controlling reproducibility in many fermentation strategies.
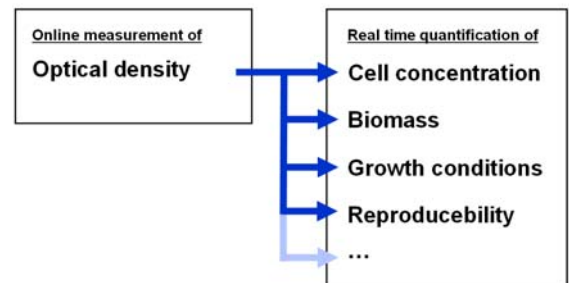

II. Quantification of optical densities

In general, measuring the optical density (OD) is a common method to quantify the concentration of substances (Beer-Lambert law), since the absorbance is proportional to the concentration of the absorbing species in the sample. Photometers quantify the optical density of liquid samples by comparing the intensity of light that has passed through (I) and the intensity of the light before it enters the sample (I_o). In spectroscopy the absorbance A is usually defined as:

$$A = -\log(I / I_o)$$

A = the absorbance
I_o = Intensity of light before it enters the sample
I = Intensity of light, that has passed a sample (transmitted light)
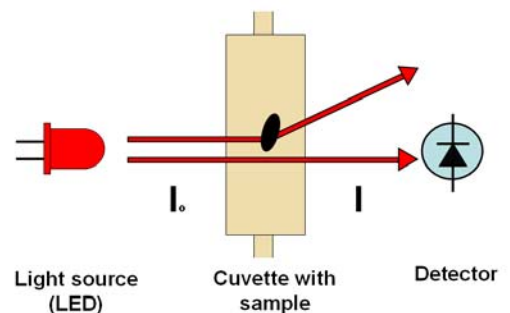
The optical density is depending on the thickness of the sample. The absorbance of an object for a given wavelength per unit distance is:

$$OD = A / L$$

OD = optical density
A = the absorbance
L = thickness of sample

III. Optical density in practice

1. Sample thickness
Although cuvettes with smaller thickness have many advantages for measurements, the optical density is most often specified in publications with sample thicknesses of 10 mm. EloCheck is an automatic photometer, which quantifies the optical density with 2.1 mm cuvettes (and 1.6 mm respectively). To get conformity the primary OD values have to be multiplied with the corresponded factor.

Examples:

Calculation of OD_{10mm}, when using a 2.1 mm cuvette:

$OD_{10mm} = OD_{primary}$ * 4.76 [4.76 = 10 mm / 2.1 mm]

Calculation of OD_{10mm}, when using a 1.6 mm cuvette:

$OD_{10mm} = OD_{primary}$ * 6.25 [6.25 = 10 mm / 1.6 mm]

EloCheck can do all necessary calculation automatically so that values as "OD_{10mm}" are directly shown directly in the chart.

2. Free calibration to other cell parameters
The automatic multiplication routine can also be used for showing other helpful parameters as "CFU / ml" or "Dry weight / l" in the chart in real-time.

Examples:

Calculation of cell concentration, when using a 2.1 mm cuvette:

CFU [10^6 / ml] = $OD_{primary}$ * 44.5 [empirical data, dependent on cell strain etc.]

Calculation of Dry weight per liter, when using a 2.1 mm cuvette:

Weight [g / l] = $OD_{primary}$ * 0.238 [empirical data, dependent on cell strain etc.]

3. Complex calibration with polynomial functions
Cell suspensions with optical densities of about 0.4 or higher require more complex (polynomial) formulas for calculation (for more information about using the powerful calibration function see *Application Note II* from biotronix).

Example for higher optical densities:

Calculation of OD_{10mm}, for higher optical densities (up to OD_{10mm} = 70):

$OD_{10mm} = 0.642*OD^3 + 0.36*OD^2 + 4.427*OD + 0$ [dependent on cell strain etc.]

Polynomial calibrations are done with EloCheck in the same way by using the same function editor within the OD calculation menu.

With the graphical output small changes in cell metabolism and morphology (lag-phases, diauxie, osmotic changes etc.) are now visible in real time. Due to the continuous measurement time points of induction (e.g. IPTG addition) or harvest can be defined much more precisely. And finally, the OD-alarm function will inform the experimenter promptly, when the optimal OD is reached.
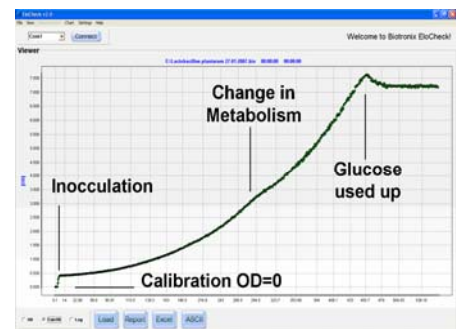
Growth curve of a *L. plantarum* fermentation monitored with EloCheck.
MRS, pH 5.6, T= 36°C