Optical densities of suspensions

I. Analysing optical densities of suspensions

Beer-Lambert law vs. optical density of suspensions

Measuring the optical density (OD) is a common method to quantify the concentration of substances, since the absorbance is proportional to the concentration of the absorbing species in the sample (Beer-Lambert law). But the Beer-Lambert law is only true for homogenous substances and not for suspension! Cells or particles can be shadowed by another or can reflect light many times. This effect plays a minor role at low particle concentrations and when thin optical cuvettes are used. But when cell suspension are analysed at higher concentrations (OD > 0.25) or in cuvettes with larger thickness (>2.5 mm) the relation between OD and concentration is in a non-linear way dependent on more or less unpredictable factors*. One mean effect is, that the OD value saturates as the biomass concentration is getting higher. For example, suspension with $OD_{primary}$ = 2 will not have $OD_{primary}$ = 1 after 1:1 dilution.

To circumvent this multifactorial error cell concentrations measurements are frequently made off-line on high diluted samples (best results are obtained, when the optical density is adjusted below 0.25). Because the dilution factor can be relative high, this method often leads to other errors in the resulting values. In addition, such practice is laborious and risky. Non closed cuvettes do not prevent from toxic or hygienic disasters, contaminations, infections and so on.

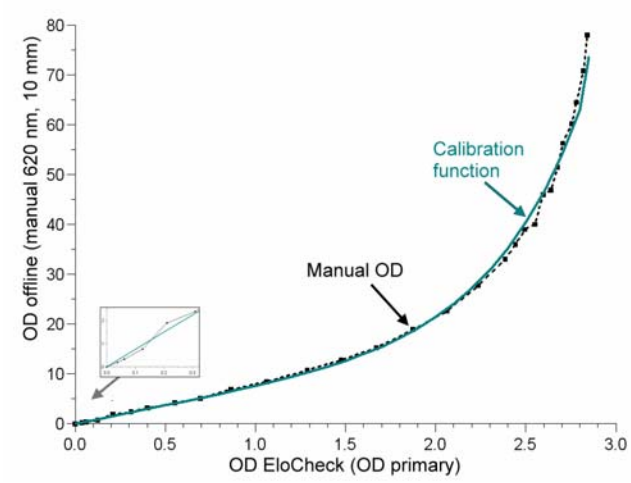

Fig. 1: *L. plantarum* were diluted and analysed optically with a standard spectralphotometer at wave length 620 nm in 10 mm cuvettes. The diagram shows the relationship between primary EloCheck data ($OD_{primary}$) and the optical density as determined via high dilution ($OD_{off\text{-}line}$).
With a polynomial function editor or with the EloCheck calibration function individual calibrations of optical densities up to 50 or more can generated for dilution free measurements of high OD in real time.

Correct measurement of OD without any dilution.

For further understanding of the term "optical density" or OD, it is important to distinguish two different definitions. '$OD_{primary}$' defines the optical density of samples within an optical cuvette. This is the basic parameter of EloCheck and most other photometers. EloCheck calculates $OD_{primary}$ by the formula $OD = -\log (I/I_o)$ where I_o is the intensity of the light before it enters the sample and I is the intensity of light that has passed through (transmitted light). $OD_{primary}$ is not proportional to the particle concentration at higher concentrations. Also it depends on various factors as light scattering and cell size.

*Cells or particles can be shadowed by another or can reflect light many times. The physical interpretation of data from this measurements is complex and dependent on many factors such as wavelength, cell size etc. For more information see http://en.wikipedia.org/wiki/Rayleigh_scattering or http://en.wikipedia.org/wiki/Mie_scattering

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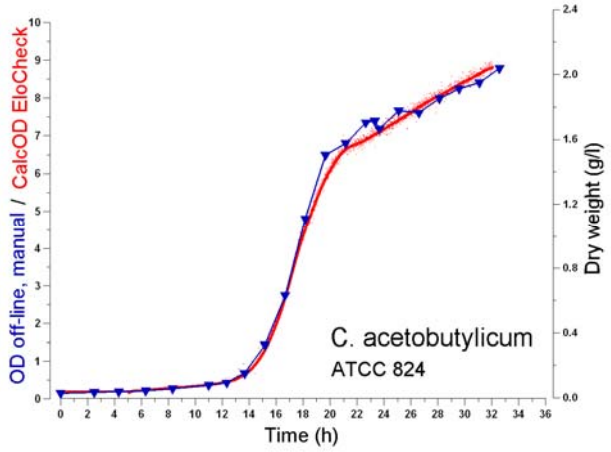

Fig. 2: Comparison between EloCheck data (CalcOD, red) and manually obtained data (blue). During 36 hours of a fermentation of *Clostridium acetobutylicum* EloCheck collected automatically continuous actual data. The manual analysis allowed the quantification of about one samples per hour.
As also seen in the diagram on the right, EloCheck data can be calibrated to units as dry weight / liter and other.

'$OD_{off\text{-}line}$' is often called 'off-line laborious optical density'. Here it represents the optical density as quantified via high diluted samples. In such highly diluted samples scattering errors are negligible and the resulting $OD_{off\text{-}line}$ is proportional to the particle concentration.

EloCheck allows the automatic calculation of $OD_{off\text{-}line}$ values from $OD_{primary}$. Corrected values of OD can be calculated in real time without any dilution. Only one empiric calibration experiment is necessary to take into account all cell and medium type specific parameters. As the result all laborious work as pipetting and dilution is not necessary any more.

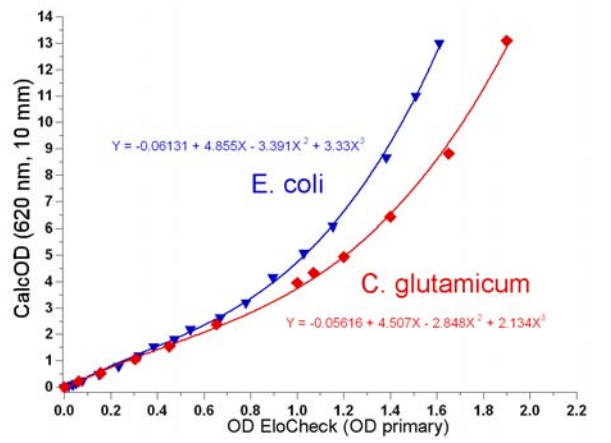

Fig. 3: Relationship between OD primary and OD offline of two different strains. The values of OD offline are shown as manual determined via high dilution (▼and ♦) and as calculated in real time with a polynomial function (CalcOD, ▬).

Individual $OD_{off\text{-}line}$ calibration

If you want to see automatically the correct value of optical density in real time, you have to make an individual calibration first.

The calculation to $OD_{off\text{-}line}$ is dependent on the medium type specific parameters and especially the cell strain. Differences in particle or cell shape influence the calibration formula more or less. The example on the left shows the correlation between $OD_{primary}$ and $OD_{off\text{-}line}$ of two different cell strains grown in clear medium. Obviously, best results are obtained, when calibrations are done individually for each cell type and medium (if the cultivation medium contains particles).

Evaluation of individual calibration functions

a) Implemented calibration function (recommended for dilution free measurement of higher optical densities)

Software Version 2.1 or higher

The EloCheck software version 2.1 and higher has a integrated calibration function for direct quantification of higher optical densities without prior dilution.

Let give an example to show how an individual calibration function can be evaluated.

① Get three to five samples of your suspension with typical optical densities of your fermentations (higher OD recommended).

② Determine $OD_{primary}$ of each undiluted suspension with EloCheck.

③ Make suitable dilutions of these suspensions, so that the final OD is the range between 0.1 and 0.25

④ Determine the OD of these diluted samples (with EloCheck or any other photometer). Because many references in the literature are based on optical thicknesses of 10 mm, it is recommended to us 10 mm cuvettes.

⑤ Recalculate the $OD_{off\text{-}line}$ of undiluted samples by multiplying these values with the used dilution factors. If you have used a 10 mm cuvette, the $OD_{off\text{-}line}$ values represents of course values accordant 10 mm cuvettes.

⑥ Enter the specified pairs of $OD_{primary}$ and $OD_{off\text{-}line}$ values within the EloCheck calibration window. Enter other necessary values into the form (as thickness of the EloCheck cuvette and of the cuvette you have used for analyzing the diluted samples). Press the compile button to update the calibration function.

⑦ ODcalc in the chart represents now the user-defined $OD_{off\text{-}line}$ value

b) Polynomial function (recommended for other correlations)

Software Version 1.5 or higher

① Get three to five samples of your suspension with typical optical densities of your fermentations.

② Determine $OD_{primary}$ of each undiluted suspension with EloCheck.

③ Make suitable dilutions of these suspensions, so that the final OD is the range between 0.1 and 0.25

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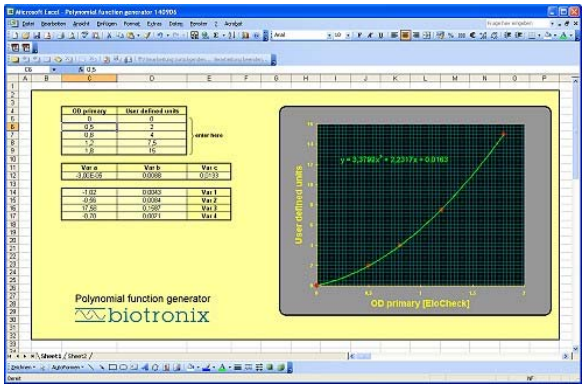

Fig. 4: The Excel spread sheet "Polynomial function generator" allows the generating of a polynomial function for automatic calibration of primary OD to other user defined units ($OD_{offline}$). EloCheck can quantify high OD values (up to 35 or higher) in this way in real time without any manual dilution prior to measurement.

⑥ The specified pairs of $OD_{primary}$ and $OD_{off\text{-}line}$ values have to be transferred to a polynomial correlation function. The final function contains all necessary parameter for real-time calculating the exact optical densities. The user can find a helpful Excel file "Polynomial function generator" free to download via www.biotronix.de*. The spread sheet calculates suitable polynomial functions based on up to 5 pairs of parameters. Of course other programs can be used to get similar polynomial functions. EloCheck allows the use of such polynomial functions up to the third order.

* "Polynomial function generator" = biotronix freeware: http://www.biotronix.de/Polynomial_function_generator.xls

⑦ Enter the polynomial function in the OPTION menu within the EloCheck software. If you want, you can also specify an own name for the defined $OD_{off\text{-}line}$ units. That's it. EloCheck shows now in real time an additional optical parameter, which corresponds to the manual measured values. All calculated user defined parameters can be of course stored to hard disk.

Fig. 5: Polynomial function menu

⑧ ODcalc in the chart represents now the user-defined $OD_{off\text{-}line}$ value

Calibration to other user defined units

Similar to the definition of $OD_{off\text{-}line}$ representing OD values measured via high dilution, the user can define other correlations and units. For example the $OD_{primary}$ can be correlated to corresponding values of dry weight, CFU / ml, NTU and other. As seen in Fig. 6, with the help of such calibration EloCheck can show in real time the actual dry weight concentration on the screen.

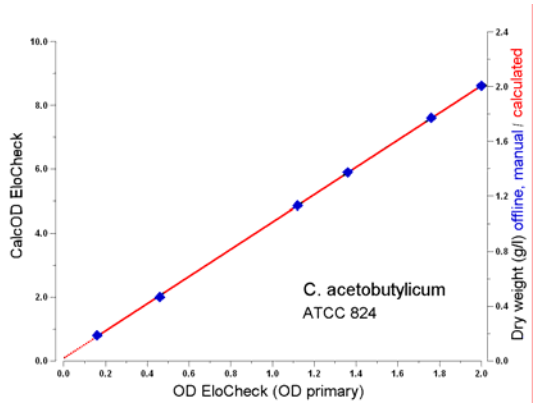

Fig. 6: $OD_{primary}$ data can be calibrated to free definable units. After calibration the calculated can be shown in real time without any further manual work or calculation.

This application note should provide the use with helpful information for optimal use of the EloCheck photometer. If you need additional technical information, do not hesitate to e-mail biotronix Technical Service Department (info@biotronix.de)

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