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OD600 Measurements Using Different Photometers

Why does the absorbance value of turbidity measurements vary using different photometers?

Executive Summary

The turbidity measurement of microbial cultures is a widely used method to determine the cell number of growing microorganisms in a culture. This method is performed by measuring the absorbance value of a liquid microbial culture in a photometer at 600 nm. This value can vary when photometers of different manufacturers are used, since every device has a different optical set up.

Also the type of photometer, the width of the aperture and the distance between the aperture and the detector influence the results of turbidity measurements. It is important to use the same photometer for an OD600 determination when repeating experiments to avoid device specific variations in the absorbance value.

Introduction

The measurement of the turbidity of bacterial or yeast cells in liquid cultures is stated as "optical density" in microbiology (OD600). Methods like microbial cell induction for the production of recombinant proteins or harvesting microbial cells for the preparation of competent cells for cloning studies require the determination of the microbial cell number per mL. The common OD600 method estimates the total number of microbial cells at 600 nm in an easy, fast and very economical way with minimal requirements regarding technical equipment. Specialized instruments for these measurements exist, but often universal photometers are used since they offer the technique to measure samples at 600 nm. Standard photometers are designed to measure the intensity of light that is passing through a sample in clear solutions (Fig. 1). This principle relies on the ability of molecules to interact with light: The sample molecules are excited with light and partially absorb the light depending on the concentration of the sample and the wavelength of the light.

When it comes to optical density measurements the sample does not absorb the light, but the light is emitted in a random direction by each particle in the sample. That means, the microbial cells do not absorb the light, but scatter the light depending mostly on their size and shape [1].
Not all of the scattered light can reach the detector then (Fig. 3). Dead microbial cells and cell debris also scatter the light, meaning that the absorbance value does not correlate directly to viable microbial cells. The amount of light that reaches the detector depends on two parameters: 1. The number of scattering microbial cells (density) and 2. the dimension of the microbial cells (size and shape). Regarding the determination of the number of microbial cells in the solution, the decrease in the intensity of light that reaches the detector is used to determine the turbidity and with this the density and number of microbial cells in the solution [2]. It is not a real absorbance measurement in the physical sense, but more a virtual absorbance value to calculate the microbial density.

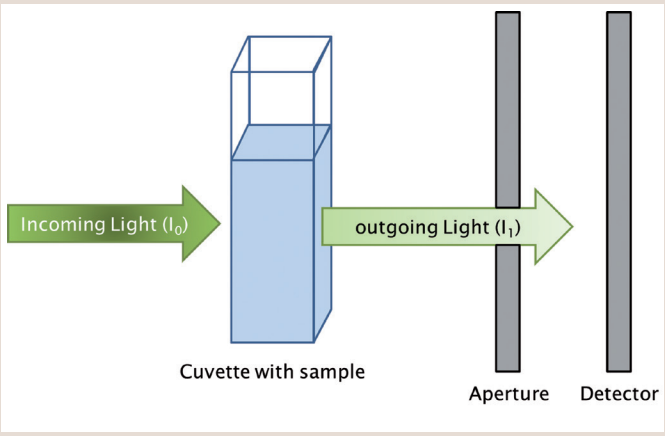

Figure 1: Typical set up of an absorbance measurement in a photometer. The incoming light intensity is reduced by absorption of sample molecules and this reduced light intensity is measured at the detector. By comparing the incoming and outgoing amount of light the concentration of a sample is calculated.

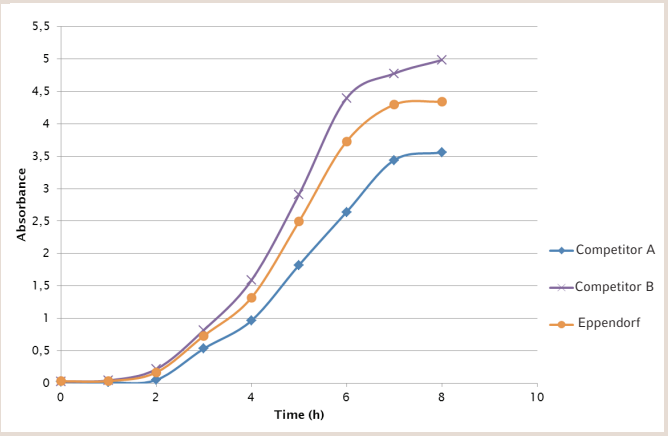

Figure 2: Growth curves of *E. coli* DH5α grown in LB medium for 8 h shaking at 180 rpm at 37 °C. Every 60 min the OD600 of the same sample was measured in three different photometers showing that the results differ when comparing different instruments. The samples were diluted using LB medium when an absorbance value of 0.8 was reached to guarantee accurate measuring at 600 nm. The dilution factor was used to multiply with the measured result for absorbance value determination.

Estimation of the optical density using McFarland turbidity standards without a photometer

The method used to estimate the turbidity of a microbial sample if no photometer is present is the McFarland turbidity standard. The standard sets are liquid samples in a glass or plastic tube that refer to an optical density (mostly 0.5, 1.0, 2.0, 3.0 and 4.0). The turbidity is reached either by polystyrene microparticles in a buffer, or barium chloride with sulfuric acid. There are many different manufacturers of standards in the market.

The method of estimation relies on visual rating. Each of the liquid standard solutions is held next to the microbial culture to assess the turbidity of the microbial culture. This method is not very precise and depends strongly on the researcher doing it.

Determination of measurement differences in different photometers

To identify the variations of optical density measurements at 600 nm in different photometers, an experiment was performed. The most common laboratory microorganism *Escherichia coli* was used to measure a growth curve. It is referred to as $1.5x10^8$ viable cells per mL at an absorbance of 0.5, measured at a wavelength of 600 nm in a photometer. The factor $1.5x10^8$ is preprogrammed in some photometers so that these devices display the cell number directly after measuring the absorbance. This factor was determined with *E. coli* DH5α. Therefore the microorganism was grown in LB medium overnight shaking at 180 rpm at 37 °C. 100 µL of this culture were used to inoculate 10 mL of fresh LB-medium in an Erlen-meyer flask. The culture was incubated shaking at 180 rpm and 37 °C for 8 h. A standard curve for *E. coli* DH5α has been established measuring the OD600 every 60 min for 8 h in 3 different photometers.

Dilutions of the microbial culture using LB medium had to be performed as soon as the absorbance value measured in the undiluted culture reached 0.8. The dilution factor was adapted according to the increasing growth of bacteria over time from 1:2 to 1:8. The measured absorbance value was multiplied with the dilution factor (e.g. OD600 0.61 * dilution factor 4 = OD600 cal. 2.44). All data points were entered in a graph to visualize the growth curve of *E. coli* DH5α (Fig. 2). Each sample was additionally plated out on agar plates to count the viable colony forming units (CFU). Thereby the reference value of $1.5x10^8$ CFU per mL at an absorbance value of 0.5 could be confirmed (data not shown).

The experimental data shows that each photometer gives a different absorbance value for the measured sample, even though the sample was exactly the same. The varying results become obvious after 2 h of bacterial growth and deviation increases over time when more bacterial cells are present in the sample. These results show that exactly the same sample can lead to different optical density results in different photometers. Even in low absorbance value ranges between 0.2 to 1.0 differences are already visible.

Generally, in spectral photometers the aperture has to be small to allow for a high spectral resolution. This in turn has the consequence for OD600 measurements that only a low amount of scattered light can reach the detector. If you are aware of this fact, you can overcome the situation of differing OD600 measurement values by always using the same photometer for OD600 applications and programming it according to the microorganism used. In some photometers one can key in the viable cell number corresponding to an OD600 value. This way one can program specific cell numbers to every microorganism used in the laboratory. The fact that OD600 values depend on the instrument used for their determination is also of relevance when one compares results to reference or literature values. With turbid samples, an absorbance value of 0.5 in one photometer can be displayed as 1.0 in another photometer without being a false result.

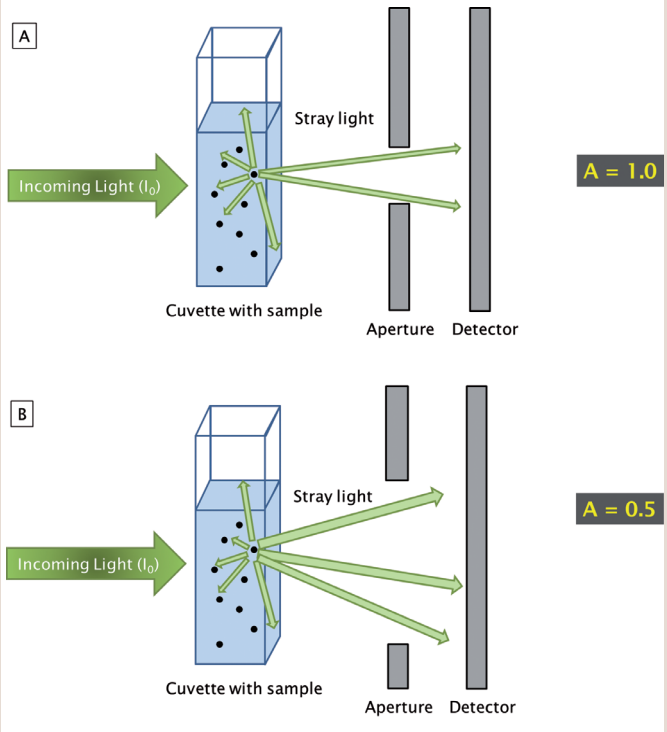

Figure 3: Photometer set up A and B show that different absorbance values are displayed on the photometer even if the sample concentration is the same, depending on the arrangement of the optical parts of the photometer.

Conclusion

Turbidity measurements using photometers are not thoroughly defined. So on balance one should always consider turbidity measurements as an approximation not an actual value of viable microbial cells and use the absorbance value measured carefully. Measuring turbidity is influenced by many different factors and is mentioned to be a reference value of when to e.g. induce/harvest the cells. And it is strongly recommended to produce an individual growth curve for every microorganism used in the lab each time a different photometer is used, since each photometer has a different optical set up and the results can differ.

The reproducibility of experiments is also dependent on the devices used and therefore each repetition of an experiment using microbial cells and measuring the OD600 should be performed with the same photometer [3].
Eppendorf BioPhotometer D30 and all members of the Eppendorf BioSpectrometer® family are suited for OD600 measurements. The optical set up of the instruments with a small aperture leads to high resolution and reliable, stable measurements.

References

[1] Janke S A, Fortnagel P, Bergmann R. Microbiological turbidimetry using standard photometers. Biospektrum, 1999, Vol. 6, 501-502.
[2] Harnack K, Spolaczyk R, Janke S A. Turbidity measurements (OD600) with absorption spectrometers. Biospektrum, 1999, Vol. 6, 503-504.
[3] White Paper; "Factors Influencing OD600 Measurements – Which factors influence microbial growth and with this varying absorbance values of turbidity measurements using the same photometer?", Eppendorf AG, 2015

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