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Determination of cell density in bacterial cultures using the Hellma® fiber optic probe in combination with the Eppendorf BioSpectrometer®

Sarah Kühnapfel[1], Jochen Hallmann[3], Ines Hartmann[1], Tanja Musiol[1], Wolf Wente[2], Martin Armbrecht[1]
[1] Eppendorf AG, Hamburg, Germany
[2] Eppendorf Instrumente GmbH, Hamburg, Germany
[3] Hellma GmbH & Co. KG, Müllheim, Germany

Abstract

Photometric measurement of turbidity for the purpose of determining cell density in a bacterial suspension is a basic method in every molecular biology laboratory. In addition to consumption of single use cuvettes and pipette tips, reduction of the culture volume is an effect of frequent sample removal and subsequent disposal for the purpose of measurement.

The present work will show that the alternative use of a fiber optic probe, which operates without consumables and without reduction of the bacterial suspension, in combination with the Eppendorf BioSpectrometer, yields comparable results and thus presents a good alternative to a common measurement method.

Introduction

Liquid culture of bacteria serves as a basic method for many experiments, as high cell densities may be achieved by this culture format. Expression of recombinant proteins and amplification of plasmid DNA in bacteria are only two examples. For the purpose of liquid culture, the broth is inoculated either directly with a single colony from an agar plate, or with a defined volume from a pre-culture, followed by incubation - predominanthy at 37 °C – in an incubator shaker for a specific period of time. Depending on the experiment, it is frequently required to monitor cell growth in regular intervals, allowing definition of the optimum moment for cell harvest.

Measurement of optical density of a bacterial suspension is based on the light scatter which is captured by the photometer or spectrometer. Such "loss of light" is measured as optical density or extinction in a spectral photometer at 600 nm. To this end, sample volumes of 1-2 mL are removed at different time points for measurement in plastic cuvettes. The consumption of items such as single use cuvettes and pipette tips is considerable, and removal of the sample is time consuming. In order to reduce both, the suitability of fiber optic probes for determination of optical density was evaluated as an alternative to the common method of cuvette measurements.

The areas of application for fiber optic probes range from process control in chemical plants, to monitoring of bioreactors, to simple control of syntheses on the laboratory scale. Fiber optic probes (figure 1) enable measurement directly inside the solution to be measured, thus also simplifying spectroscopic analyses of samples. Additional advantages of this method include time savings and increased yield. Connection of the light conductors of a probe is possible either directly via SMA-connection on the photometer, or via an adapter in the cuvette shaft.

For the Eppendorf BioSpectrometer, the latter configuration constitutes the easiest solution. The light is de-coupled by the adapter and guided to the probe by the light conductor. At the tip of the probe the light traverses the measurement gap of defined width, is reflected by a mirror, re-coupled to a second light conductor and guided back to the photometer (figure 2). Exchangeable measurement gaps allow quick and flexible selection of 1, 2, 5, 10 and 20 mm path lengths.

Figure 1: Fiber optic probe by Hellma

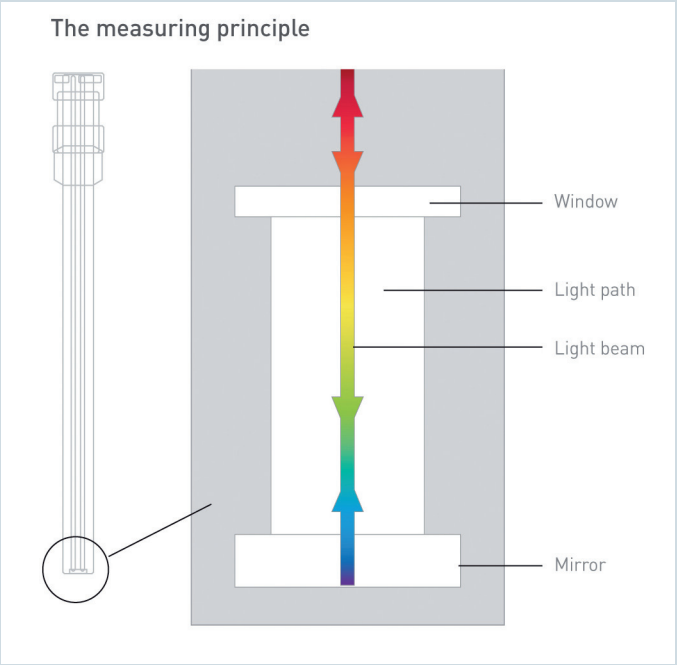

Figure 2: Measurement principle of the fiber optic probe

In order to ensure optimum growth and aeration, culture in flasks is mainly carried out in orbital incubator shakers. In addition to exact temperature control for optimum growth conditions, the use of a programmable refrigerated incubation shaker such as the Eppendorf New Brunswick™ Innova® 42R ensures timed programming of different temperatures and speeds. Hence, bacterial cultures of a known growth kinetic may be incubated under shaking conditions at 37 °C for a defined period of time, followed by growth cessation by stationary cooling at 4 °C.* Thus, "overgrowth" of the cultures past the exponential phase is prevented. Under good cooling conditions liquid cultures survive for approximately one week. The culture my therefore be kept overnight or even over the weekend without compromising quality.

Materials und Methods

Materials

> Eppendorf BioSpectrometer® kinetic
> Programmable refrigerated Incubator shaker Eppendorf New Brunswick Innova® 42R
> Hellma® Analytics 66.1668-U.V, mini probe fiber optic probe, path length: 10 mm
> PMMA Vis Cuvettes, path length: 10 mm
> Bacterial culture: *Escherichia coli* DH5α
> C. ROTH® LB-medium (Luria/Miller) for molecular biology

Methods

In order to obtain a bacterial culture suitable for a series of measurements, a pre-culture of *Escherichia coli* DH5α was first generated. The pre-culture was incubated overnight for approximately 18 h at 37 °C and 180 rpm with shaking in the incubator shaker Eppendorf New Brunswick Innova 42R. The following day, two times 2 mL pre-culture were removed and transferred to two 1 L flasks containing 200 mL LB medium. These two cultures formed the basis for the subsequent series of measurements. A third flask with 200 mL LB medium only was incubated in the same manner and served as the blank. Incubation of cultures during the measurement series was carried out in the incubator shaker at 37 °C and 180 rpm. The respective display selections are shown in figure 3.
Measurements were performed once per hour for the first three hours. With increasing turbidity of the cultures, subsequent measurements were carried out in half hour intervals, until a total of 4.5 h, at which time the incubator shaker was programmed to lower the temperature to 4 °C. The cultures were then stored in a stationary position overnight. One additional measurement of optical density was performed the following day.

* The Innova 42R provides temperature control from 20 °C below ambient (with a minimum setpoint of 4 °C) to 80 °C. Naturally, both these ranges depend on relative humidity and other ambient factors, as well as the options installed in the unit.

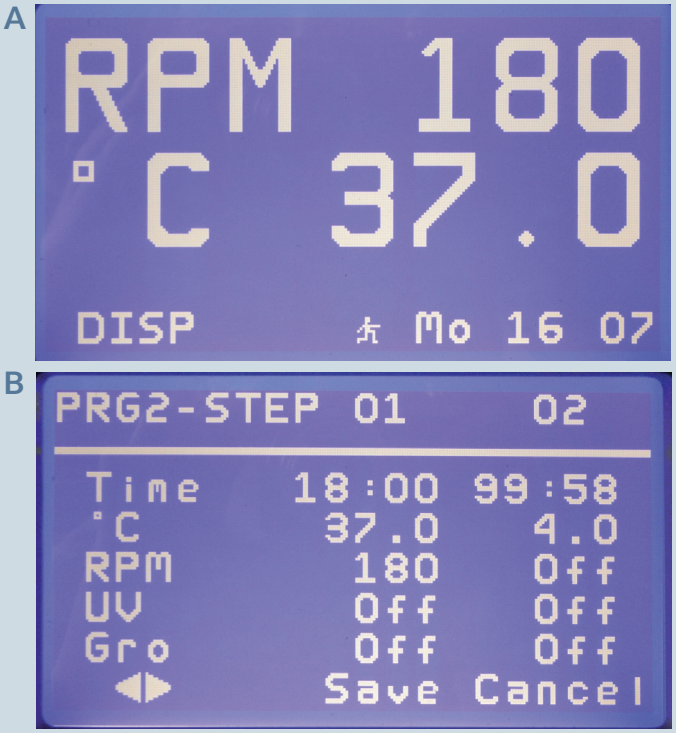

Figure 3: Settings on the incubator shaker Innova 42R.
A) Main display shows current number of revolutions per minute and temperature.
B) Programming function with selection of times, temperature and number of revolutions per minute.

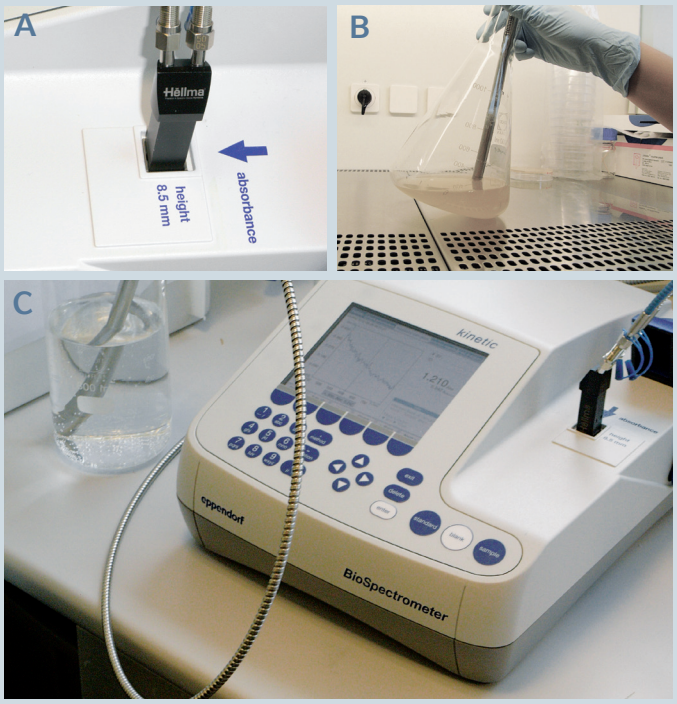

Figure 4: Use of the Hellma fiber optic probe with the Eppendorf BioSpectrometer
A) Cuvette adapter of the fiber optic probe for insertion into the cuvette shaft.
B) Probe for insertion into the bacterial culture.
C) Cuvette adapter and probe are connected by a cable to allow greater freedom of movement

The measurements were performed in an Eppendorf BioSpectrometer kinetic using the pre-programmed method OD600. Growth of the bacterial culture could be deduced from the measured optical density. Figure 5 depicts the path for selection of the method outlined above, as well as the relevant parameters.

The measurements were performed as follows: medium was measured to obtain a blank value, followed by determination of optical density of the bacterial cultures. The probe was inserted in as much of an angle as possible to ensure sufficient coverage with medium (figure 4B). In order to avoid interfering air bubbles, motion of the culture during measurement should be minimized.

For comparison, 1 mL of the bacterial culture was removed and measured PMMA macro Cuvettes. 5 replicates were measured, respectively.

In order to ensure sterile conditions, the probe was cleaned with 70 % ethanol prior to each new series of measurements. Between time points the probe was kept in VE-water (figure 4C) to avoid precipitation of contaminants on the probe.

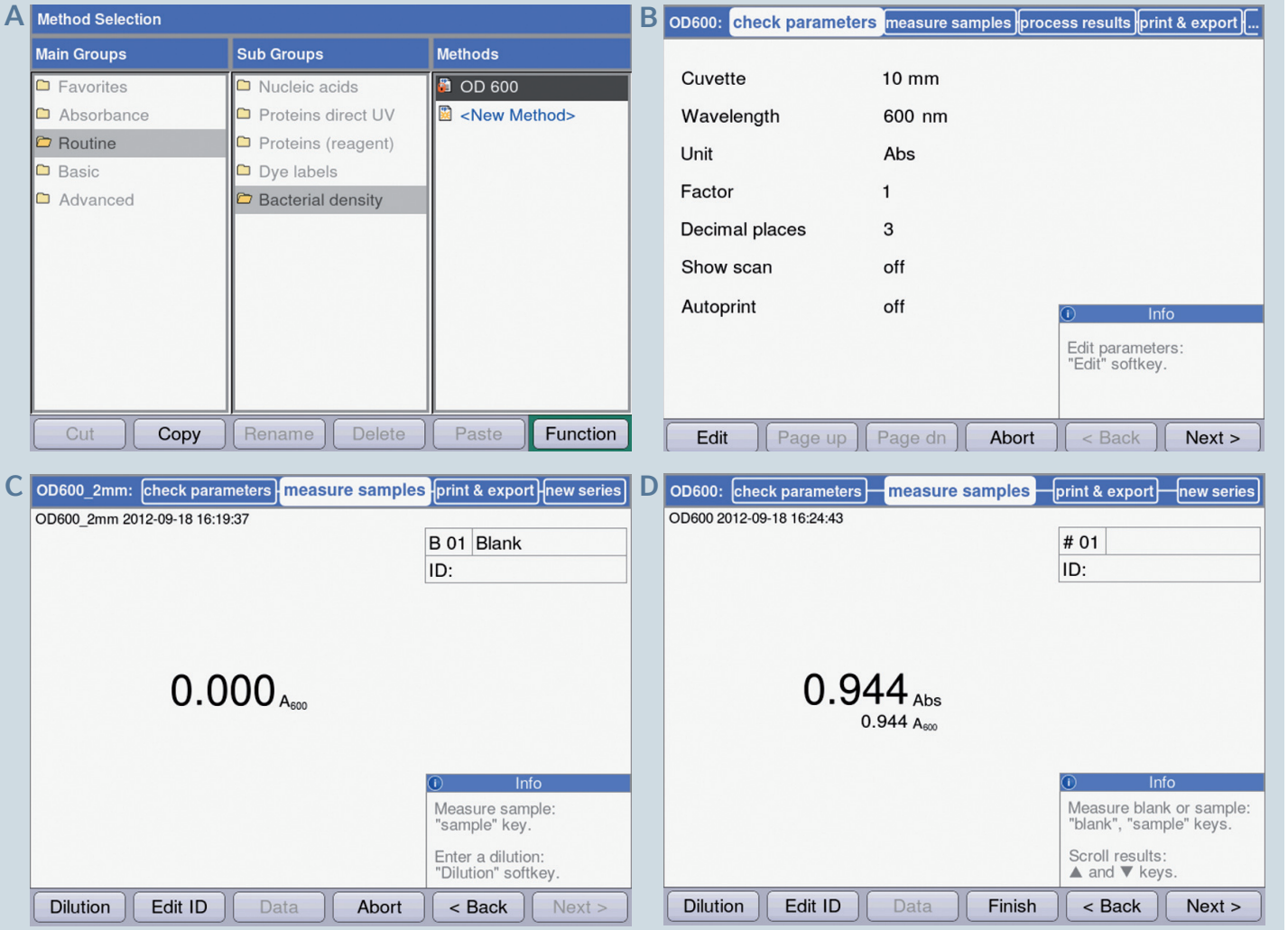

Figure 5: Parameter selection on the BioSpectrometer for use of the 10 mm light path in the method OD 600
A) Selection of method OD600.
B) Verification of parameters such as path length and wavelength.
C) Display for blank measurement.
D) OD600 measurement example of a bacterial culture using the Hellma fiber optic probe on the BioSpectrometer

Results and Discussion

Comparison of optical density values obtained with a plastic cuvette with those obtained using a fiber optic probe shows only minimal differences between their measurement results. Slight deviations are to be expected for multiple measurements, as OD measurements are simply measurements of turbidity, and motion within the culture needs to be taken into consideration. Table 1 shows the example of measurement after 3.5 h. The standard deviation is small for both measurement systems. It is further evident that the deviation of the average of the results obtained with the probe, compared with those of the PMMA macro Cuvettes, is small: a mere 1.2 % at the 3.5 h time point.

Table 1: Measurement (OD600) results of the 5-fold determinations using the Hellma fiber optic probe and PMMA macro Cuvettes, respectively.

Time 3.5 h		
Measurement	**PMMA cuvette 10mm**	**Hellma probe 10mm**
1	1.186	1.119
2	1.173	1.159
3	1.144	1.150
4	1.149	1.109
5	1.142	1.185
Average	1.159	1.144
Standard deviation	0.020	0.031
[%] CV	1.693	2.690
Deviation to PMMA cuvette 10 mm	-	0.014
Deviation to PMMA cuvette 10 mm [%]	-	1.243

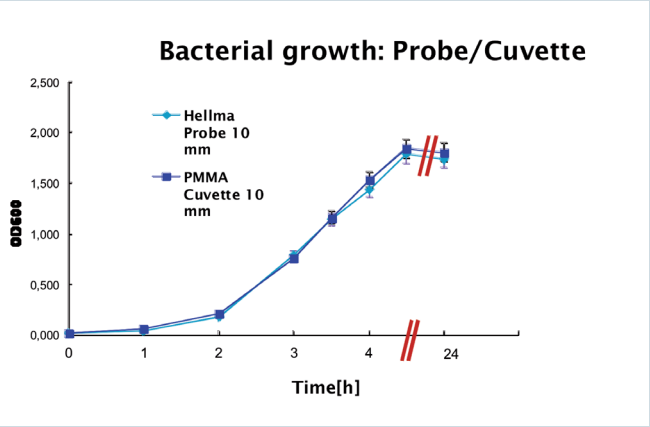

Figure 6: Growth curve of one of the bacterial cultures measured with the fiber optic probe compared with the 10 mm PMMA macro Cuvettes. The 5 h time point is equivalent to the result obtained on the following day after testing the cooling function of the incubator shaker (value = 24 h).

Figure 6 shows the growth curve of one of the cultures measured. The two curves representing optical densities obtained with the fiber optic probe, in comparison with the 10 mm plastic cuvette, are nearly identical up to an OD of approximately 1.2. Only above an OD of 1.4 slight deviations are noted; however, these are small and thus negligible. Due to the congruence of the growth curves it may be inferred that probe and cuvette are equally suited for measurement of bacterial growth.

Following termination of bacterial growth after 4.5 h by keeping the culture in the incubator shaker at 4 °C overnight, the optical density was slightly lower than that measured on the previous day. This is indicative of complete growth cessation, thus demonstrating the suitability of the incubator shaker's cooling and general programming functions for termination of further culture growth following the incubation phase. This also applies during times when it is not possible to transfer the cultures from the incubator to the refrigerator.

If bacterial cultures are very overgrown, it is possible that optical density measurements can no longer be performed. Since OD measurements at very high bacterial concentrations do not follow linear kinetics it is not recommended to simply dilute the culture. Measurement of the diluted suspension will not yield the true OD of the undiluted culture. More accurate results may be obtained with a fiber optic probe of shorter path length (e.g. 2 mm). With this approach, a possible dilution error can be avoided. If a probe with shorter path length is to be used, the respective value is changed in the method parameters for the purpose of OD calculation (figure 7). In the results display, this value is automatically calculated to a path length of 10 mm. The original data are also displayed.

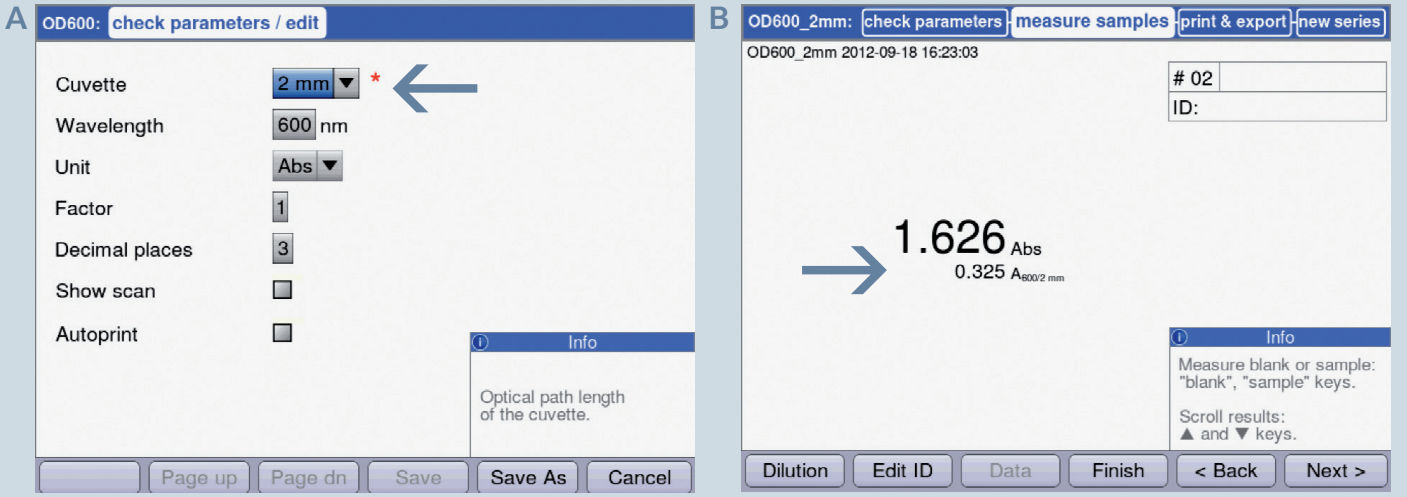

Figure 7: Parameters to be selected and measurement results obtained when using a fiber optic probe with a path length of 2 mm.
A) Selectable parameters: If using a path length of 2 mm, this must be changed in the respective field.
B) Measurement result display: The value displayed in smaller font represents the original measurement with a path length of 2 mm; the value displayed in larger font represents the value calculated for a path length of 10 mm.

Conclusion

The series of experiments performed here was able to show that the alternative use of a fiber optic probe yields equally good results which are comparable to those obtained with a cuvette. Since sample removal from the culture flask is not required for this method, but rather the measurement occurs directly inside the vessel, time is saved,
as well as consumables such as pipette tips and single use cuvettes. A further advantage is the option to measure higher cell densities due to the exchangeable path lengths included with the probe.

Furthermore, the advantage of the programmability of the incubator shaker Eppendorf New Brunswick Innova 42R could be demonstrated. Since no further bacterial growth occurred in the cooled incubator shaker overnight, this instrument is ideally suited for growth, followed by cooling, of bacterial cultures. If growth parameters of a bacterial culture are known, this incubator shaker may easily be used overnight or over the weekend by programming the desired times, temperatures and revolutions per minute.

Ordering information

Description	Order no. International	Order no. North America
Eppendorf BioSpectrometer® kinetic 230 V/50-60 Hz, European power plug, additional power plug connections available	6136 000.002	-
120 V/50–60 Hz, mains plug for North America	6136 000.010	6136000010
Eppendorf BioSpectrometer® basic 230 V/50-60 Hz, European power plug, additional power plug connections available	6135 000.009	-
120 V/50–60 Hz, mains plug for North America	6135 000.017	6135000017
Eppendorf New Brunswick™ Innova® 42R Incubated/Refrigerated, 3/4'' orbit, 230 V, 50 Hz	M1335-0006	-
120 V, 60 Hz	M1335-0004	M1335-0004
Eppendorf New Brunswick™ Innova® 42R Incubated/Refrigerated, 1'' orbit, 230V, 50 Hz	M1335-0016	-
120 V, 60 Hz	M1335-0014	M1335-0014
Hellma® fiber optic probe	661.668-UV	

Your local distributor: www.eppendorf.com/contact
Eppendorf AG · 22331 Hamburg · Germany
eppendorf@eppendorf.com · www.eppendorf.com

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