Kinetic Measurements of Antibiotic Susceptibility Studies with a Microplate Photometer

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Abstract

In this paper we describe how a filter based microplate photometer, Thermo Scientific Multiskan FC, can be used reliably and easily to measure turbidity of bacterial samples in antibiotic susceptibility studies. The growth curve measurements were performed on a 96-well microplate. The performance of turbidometric measurements on a 96-well microplate and the liquid evaporation from 96- and 384-well microplates during long incubation were also studied.

Introduction

The turbidity of a liquid sample is traditionally measured either with a nephelometer or a photometer. The measure of turbidity (also called optical density, OD) refers to the scattering of light from the particles of the measured sample. When measuring turbidity of a bacterial culture, the amount of scattered light is directly proportional to the number of bacterial cells in the sample.

Bacterial growth curves are usually measured by using a photometer to detect the optical density of a sample. Photometers are designed to measure light absorbance by a sample, but they can also be utilized to study sample turbidity, if a nephelometer is not available. Light absorbance is a completely different kind of phenomenon than light scattering from sample molecules. Therefore photometers are not optimized for turbidometric assays, but still they are suitable for that purpose. Nephelometers are more sensitive in turbidometric measurements, but instead they cannot be used for measuring absorbance.

Using microplates to cultivate bacteria facilitates the simultaneous measurement of a high number of samples. Therefore different growth conditions can be easily tested on a microplate format. A possibility to create a kinetic measurement protocol with specific interval times for kinetic readings, shaking the plate during the intervals and keeping the instrument in elevated temperature during a long kinetic measurement can all be achieved with a good microplate photometer.

Methods

A filter-based microplate photometer, Multiskan® FC, was used in three kinds of studies:

1. Bacterial growth curves

Salmonella Typhimurium was grown in the presence of varying concentrations of antibiotic (erythromycin) on a 96-well plate for 16 hours. Multiskan FC was programmed by a PC software to handle the whole kinetic protocol so that the plate was automatically measured every 15 minutes and it was shaked during the kinetic interval times. The temperature of the instrument was kept at 37 °C throughout the measurement session.

2. Turbidometric performance

Salmonella Typhimurium was grown to late logarithmic phase (OD = 3.19). Then a dilution seriers of ½ dilutions was made from the culture and 300 µl of each dilution was added to the wells of a 96-well microplate with 7 replicates. The OD of the dilution series was measured at 595 nm with Multiskan FC, and with a cuvette spectrophotometer for reference.

3. Evaporation studies

The idea was to study how much the liquid in the wells evaporates when the plate is incubated for hours inside the instrument in elevated temperature. The 384-well plate was filled with water (50 µl / well) and the plate was incubated at 37°C inside Multiskan FC, both with and without a lid, for 24 hours. Evaporation from a 96 well plate (300 µl water / well) was followed by incubating the plate at 37 and 50°C without a lid. The liquid evaporation was followed by weighing the plate, and by measuring the change in water absorbance at 975 nm at the time points of 0, 1, 3, 7 and 24 hours. The absorbance was measured with Thermo Scientific Varioskan Flash, a multitechnology miroplate reader.

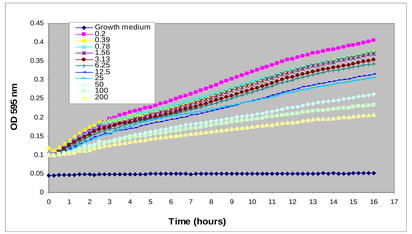
The 96-well plates used in these measurements were Immulon 1B Microtiter plates (cat.no 3355) and the 384-well plates were Microtiter plates (cat.no 95040000, round well, height 10 mm), both from Thermo Fisher Scientific. The maximum height of a plate with a lid can be 15.3 mm with Multiskan FC.

Results

1. Bacterial growth curves

The bacterial growth curves of Salmonella Typhimurium with serial dilutions of erythromycin are presented in figure 1.

FIGURE 1. The growth curves of *Salmonella Typhimurium* at varying concentrations (0.2 – 200 µg/ml) of erythromycin. The differences in the growth speed caused by antibiotic inhibition could be clearly and easily detected by the OD measurement. The SkanIt® Software controlling Multiskan FC automatically created the kinetic curves during the measurement. The software's in-built effective dose (ED) calculation can also be utilized to set the software up to calculate the antibiotic concentration causing the 50% percent (or other user-defined percent) growth inhibition. The ED calculation tool is practical especially in cytotoxicity assays.



2. Turbidometric performance

Table 1 presents the results of OD measurements of a bacterial dilution series. Multiskan FC was compared to a cuvette spectrophotometer of another manufacturer. The photometric linearity of Multiskan FC goes up to 3.0 Abs. The results of turbidometric measurement do not directly correlate to linearity performance of absorbance due to different nature of absorbance and light scattering.

TABLE 1. The optical density (OD) measurements of the bacterial dilution series. The OD of 1/10 dilution of the culture was measured with a cuvette spectrophotometer to be 0.319. Based on this measurement the theoretical OD values for the whole dilution series were calculated. The theoretical values are here compared to the actual measurement values given by the cuvette spectrophotometer (in yellow) and a microplate photometer, Multiskan FC (in green). The sample pathlength on microplate wells is naturally different from the 1 cm pathlength of a standard cuvette. Therefore the microplate measurement values were corrected to correlate to 1 cm pathlength. The calculated differences between theoretical and measured values show that OD values measured with Multiskan FC correlate better to the theoretical values in wider part of the dilution series than the cuvette spectrophotometer.

Dilution	Theoretical OD	OD (cuvette spectrophotometer)	Difference (%) to theoretical OD	OD (Multiskan FC)	OD changed to correlate to 1 cm pathlength	Difference (%) to theoretical OD
1	3.190	1.941	-64.3	1.287	2.253	-41.6
1 / 2	1.595	1.291	-23.5	0.761	1.331	-19.8
1 / 4	0.798	0.894	10.8	0.421	0.737	-8.2
1 / 8	0.399	0.514	22.4	0.229	0.400	0.4
1 / 16	0.199	0.273	27.0	0.113	0.199	-0.4
1 / 32	0.0997	0.138	27.8	0.059	0.103	3.0
1 / 64	0.0498	0.07	28.8	0.029	0.051	1.7
1 / 128	0.0249	0.035	28.8	0.015	0.026	2.4
1 / 256	0.0125	0.021	40.7	0.007	0.013	2.6
1 / 512	0.0062	0.007	11.0	0.004	0.008	17.6
1 / 1024	0.0031			0.003	0.005	41.4
1 / 2048	0.0016	0.003	48.1	0.002	0.003	53.3

3. Evaporation studies

The water weight loss from a 96- and 384-well plate due to evaporation during incubation is presented in table 2.

TABLE 2. Water evaporation during a 24 hour incubation from a 96-well plate (in yellow) at 37 and 50 °C without a lid, and from a 384-well plate (in blue) at 37 °C with and without a lid. The results are presented as water weight loss in grams and as a percentage of the initial weight. A long incubation time clearly causes the liquid to evaporate when the plate is not covered with a lid. But the amount of evaporation without a lid is still not significant for example at a 7 hour time point either with 96- or 384-well plates. The lid obviously inhibits evaporation efficiently, therefore 24 hours at 37 °C causes only < 9 % of the plate liquid to evaporate on a 384 well plate.

Time (hours)	Water (g)	Water evaporation (g) at 37 °C	Water evaporation (%) at 37 °C
0	29.1		
1	28.9	0.3	1.0
3	28.4	0.8	2.7
7	27.5	1.6	5.6
24	24.3	4.8	16.6
Time (hours)	**Water (g)**	**Water evaporation (g) at 50 °C**	**Weight evaporation (%) at 50 °C**
0	29.2		
1	28.6	0.5	1.8
3	27.5	1.6	5.6
7	25.6	3.5	12.0
24	18.9	10.3	35.3

Water (g)	Water evaporation (g) at 37 °C without a lid	Water evaporation (%) at 37 °C without a lid
19.4		
18.9	0.5	2.5
18.1	1.4	7.1
16.6	2.9	15.9
10.8	8.6	51.9
Water (g)	**Water evaporation (g) at 37 °C with a lid**	**Water evaporation (%) at 37 °C with a lid**
19.4		
19.3	0.1	0.5
19.1	0.3	1.6
18.8	0.6	3.2
17.8	1.6	8.6

The changes of water absorbance due to evaporation on a 96-well plate are presented in figure 2 and on a 384-well plate in figure 3.

FIGURE 2. Evaporation on different parts of a 96-well plate during incubation at 37 and 50 °C without a lid. The effects of evaporation on the wells are presented as a plate shaped graph (y-axis = % of absorbance at 975 nm, x-axis = columns of the plate, z-axis = rows of the plate) to indicate on which parts of the plate the evaporation is the fastest (the absorbance decreases the most). It is clearly seen that evaporation increases towards the edges of the plate.

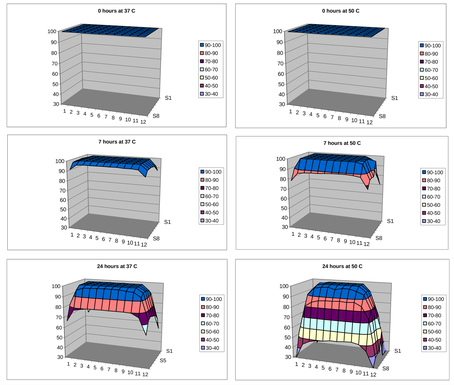
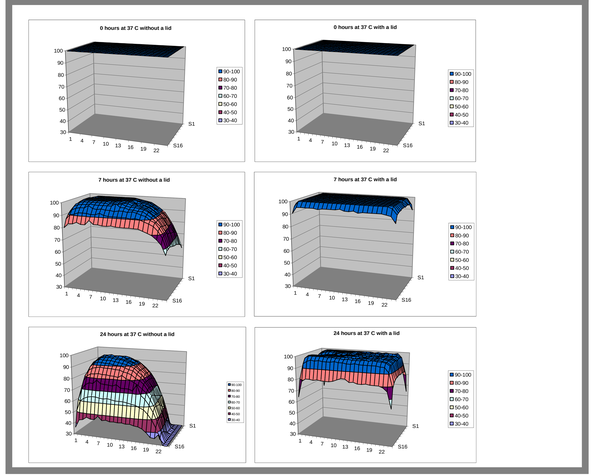


FIGURE 3. Evaporation on different parts of a 384-well plate during incubation at 37 C with and without a lid. The effects of evaporation on the wells are presented as a plate shaped graph (y-axis = % of absorbance at 975 nm, x-axis = columns of the plate, z-axis = rows of the plate). The lid expectedly slows down evaporation very efficiently. Incubation at high temperature may cause water to condensate on the lid and disturb the measurement through the lid. The condensation would have been seen as random increase of absorbance, which clearly was not seen in these results. So the incubator of Multiskan FC has good performance in this sense.



Conclusions

Multiskan FC is well suitable for turbidometric bacterial measurements. The OD of the samples in kinetic measurements can be reliably monitored at a wide OD range. The liquid evaporation from microplate wells in elevated temperature must be taken into account, when the incubation time is long. The effects of evaporation can be minimized e.g by adding the samples to the plate by leaving the edge wells empty. Filling the empty edge wells with water also decreases evaporation from the actual sample wells, as the evaporation occurs primarily from the edge wells.

Further Information

For further information about Thermo Scientific microplate instruments, please contact:

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