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Factors Influencing OD600 Measurements

Which factors influence microbial growth and with this varying absorbance values of turbidity measurements using the same photometer?

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. The determination of the turbidity of a microbial sample should be carried out in the same photometer for each repetitive experiment to avoid device related result variations.

Furthermore the OD600 value can vary when the parameters for microbial growth, such as nutrient availability in the medium, temperature, shaking speed in the incubator or glassware used, change. It is therefore important to produce constant parameters for cell growth and carefully check these for each repetitive experiment for reproducible, reliable results.

Introduction

Turbidity measurements to determine the approximate number of bacterial or yeast cells in a culture are common in microbiology and molecular biology laboratories nowadays. This determination is mostly performed using a photometer and measuring the absorbance of a microbial sample in a cuvette at a wavelength of 600 nm. Actually this measurement is not a real absorbance measurement since the microbial cells do not absorb the light, but scatter the light and thereby effect that not all the light emitted by the lamp is detected by the detector of the photometer [1]. So the more microbial cells are present in the solution the less light reaches the detector and vice versa. The photometer calculates the difference between the detected light versus the emitted light and displays the absorbance value. Literature references show that the absorbance value corresponds to an approximate number of microbial cells in a solution, e.g. $1.5x10^8$ viable cells of *Escherichia coli* are present at an absorbance value of 0.5. It must be considered that this number is only an estimation, since also dead cells and cell particles scatter the light and can lead to higher absorbance values. Furthermore the optical set-up of each photometer leads to slightly differing absorbance values, when comparing different photometers [2,3].

For reproducible results it is important to always use the same photometer for repetitive OD600 experiments. Nonetheless when using always the same photometer some factors still influence each measurement and have to be considered when the results of the turbidity measurement vary strongly despite using the same photometer.

Factors influencing turbidity measurements

Many environmental factors influence the growth of bacteria and yeast cells in a solution that are not obvious at first sight, but lead to differing results when turbidity measurements at 600 nm are performed in a photometer. Some of the reasons for differing measurement results have been collected and are displayed in table 1.

Table 1: Factors influencing an OD600 Measurement prior to the measurement or during the measurement using the same photometer

Absorbance value	Factor	Cause	Result
Lower or higher than expected	Nutrient availability in medium	e.g. inaccurate weighing of medium components; evaporation	slower or faster growing microorganisms
Lower than expected	Alteration of sugar components in the medium	e.g. caramelization due to overheating while sterilizing different liquid volumes in one autoclave	slower growing microorganisms
Lower than expected	Temperature accuracy or temperature gradient during incubation	e.g. inaccurate incubator or frequent opening and closing of the incubator door leading to decrease of temperature and the formation of gradients	slower growing microorganisms
Lower than expected	Way to the laboratory/photometer station	Cooling of the culture due to long ways without incubation and shaking; sample removal	slower growing microorganisms
Lower than expected	Cell sedimentation	Moving bacteria; natural sedimentation while the sample stands in the cuvette	A layer of cells on the cuvette bottom
Lower than expected	O_2 gradient in the flask	Inefficient shaking in the incubator	Slower growing microorganisms
Much higher than expected	Contamination	e.g. slow growing microorganisms contaminated by faster growing microorganisms	Desired culture gets overgrown by the contaminant
Lower or higher than expected	Glassware with or without baffles have been used	Different aeration levels of the microbial cultures	Baffled glassware leads to a better aeration of the microbial culture with faster growth and vice versa

Conclusion

To receive reproducible, reliable results for turbidity measurements the same photometer should be used. Additionally growth factors in the laboratory (e.g. medium, temperature, shaking speed) have to be considered carefully and kept as identical as possible to ensure the same growth of microbial cultures over the duration of multiple repetitive experiments. It has to be checked that all instruments used for the experiments such as incubators and shakers work correctly. All reagents and media have to be prepared conscientiously and precisely. The used glassware needs to be clean, sterile, and always the same type of glassware should be used for repetitive experiments. Additionally involvement of all colleagues, when it comes to sensitive experiments where the repeated opening and closing of the incubator door can have an influence, should be considered.

On balance one should always consider that turbidity measurements are an approximation of the actual cell number and that many factors influence the result, even when using the same photometer.

References

[1] Janke S A, Fortnagel P, Bergmann R. Microbiological turbidimetry using standard photometers. Biospektrum, 1999, Vol. 6, 501-502.

[2] White Paper; "OD600 Measurements Using Different Photometers – Why does the absorbance value of turbidity measurements vary using different photometers?", Eppendorf AG, 2015

[3] Harnack K, Spolaczyk R, Janke S A. Turbidity measurements (OD600) with absorption spectrometers. Biospektrum, 1999, Vol. 6, 503-504.

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