Bacterial growth curve by OD_{600} and SoloVPE

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Abstract

The increase in the cell size and cell mass during the development of an organism is termed as growth. It is the unique characteristics of all organisms. The organism must require certain basic parameters for their energy generation and cellular biosynthesis. The growth of the organism is affected by both physical and Nutritional factors. The physical factors include the pH, temperature, Osmotic pressure, Hydrostatic pressure, and Moisture content of the medium in which the organism is growing. The nutritional factors include the amount of Carbon, nitrogen, Sulphur, phosphorous, and other trace elements provided in the growth medium. Bacteria are unicellular (single cell) organisms. When the bacteria reach a certain size, they divide by binary fission, in which the one cell divides into two, two into four and continue the process in a geometric fashion. The bacterium is then known to be in an actively growing phase. To study the bacterial growth population, the viable cells of the bacterium should be inoculated on to the sterile broth and incubated under optimal growth conditions. The bacterium starts utilising the components of the media and it will increase in its size and cellular mass.

The dynamics of the bacterial growth can be studied by plotting the cell growth (absorbance) versus the incubation time or log of cell number versus time. The curve thus obtained is a sigmoid curve and is known as a standard growth curve. The increase in the cell mass of the organism is measured by using the Spectrophotometer. The Spectrophotometer measures the turbidity or Optical density which is the measure of the amount of light absorbed by a bacterial suspension. The degree of turbidity in the broth culture is directly related to the number of microorganism present, either viable or dead cells, and is a convenient and rapid method of measuring cell growth rate of an organism. Thus, the increasing the turbidity of the broth medium indicates increase of the microbial cell mass. The amount of transmitted light through turbid broth decreases with subsequent increase in the absorbance value.

This traditional methodology relying on fixed pathlength UV spectroscopy can require several minutes of stagnant time because of the need for careful sample handling, preparation (base-line correction), and (in particular) dilutions needed for bringing samples into the spectrophotometer's linear range. The doubling time of many bacterial cells is very short. Errors created in performing those dilutions and baseline corrections can take longer that the doubling time of bacterial cells and can significantly affect calculated sample optical density.

The SoloVPE is the laboratory implementation of C Technologies, Inc.'s variable pathlength technology using Slope Spectroscopy methods that are based upon the Slope Spectroscopy Equation which is fundamentally derived from Beer's Law. Slope Spectroscopy analysis with variable pathlength requires no sample preparation, no baseline correction and no dilution of even the most highly cell-concentrated samples, saving substantial time without changing any other aspect of the assay.

In this work, the bacterial growth curve by two methods during bacterial cells cultivation is investigated. These are the standard spectrophotometer and the SoloVPE method. For cultivation, *Escherichia coli* cells (*E. coli* K-12 W3110) and YPG medium were used. The bacterial growth curve was determined and standard spectrophotometer and SoloVPE were compared. Additionally, a correlation between these two methods was found. This principle allows a fast and cheap method to analyze bacterial cells samples during bacterial cell cultivation by avoiding baseline corrections and dilutions and getting accurate results faster and with less effort.

Abbreviations

DI-water = Deionized water
EC = *Escherichia coli*
OD = Optical density
OD600 = Optical density at wavelength 600 nm

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1 Introduction

The aim of this study is to detect the bacterial growth curve of *E. coli* by two methods, such as standard spectrophotometer and SoloVPE method. The cell-concentration range, which can be analyzed by those methods, will be tested to show the reasonable statistical certainty and time consumption during measurements. This allows to find the most suitable method for further bacterial cells analysis during bacterial cells cultivation.

Theoretical part

2 Measuring principle

2.1 Bacterial growth curve

The growth curve has four distinct phases (Fig 1)

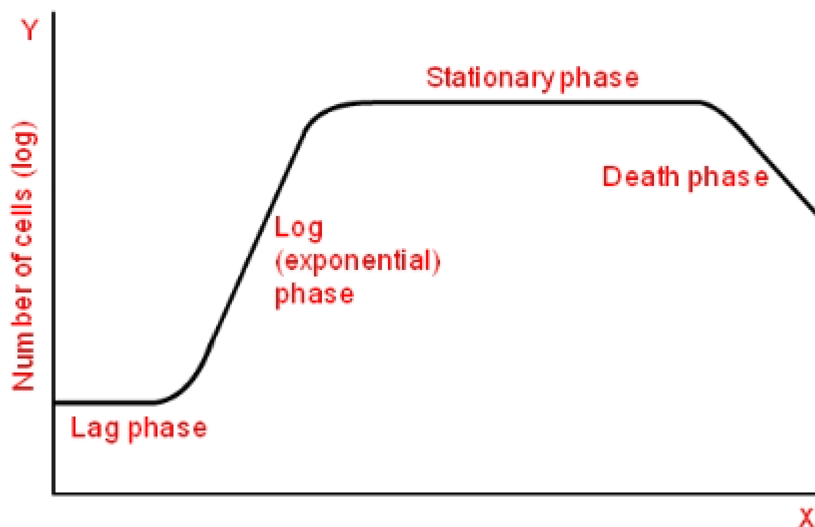


Figure 1: Different phases of bacterial growth

2.1.1 Lag phase

When a microorganism is introduced into the fresh medium, it takes some time to adjust with the new environment. This phase is termed as Lag phase, in which cellular metabolism is accelerated, cells are increasing in size, but the bacteria are not able to replicate and therefore no increase in cell mass. The length of the lag phase depends directly on the previous growth condition of the organism. When the microorganism growing in a rich medium is inoculated into nutritionally poor medium, the organism will take more time to adapt with the new environment. The organism will start synthesising the necessary proteins, co-enzymes and vitamins needed for their growth and hence there will be a subsequent increase in the lag phase. Similarly when an organism from a nutritionally poor medium is added to a nutritionally rich medium, the organism can easily adapt to the environment, it can start the cell division without any delay, and therefore will have less lag phase it may be absent.

2.1.2 Exponential or Logarithmic (log) phase

During this phase, the microorganisms are in a rapidly growing and dividing state. Their metabolic activity increases and the organism begin the DNA replication by binary fission at a constant rate. The

growth medium is exploited at the maximal rate, the culture reaches the maximum growth rate and the number of bacteria increases logarithmically (exponentially) and finally the single cell divide into two, which replicate into four, eight, sixteen, thirty two and so on (That is 2^0, 2^1, 2^2, 2^3.........2^n, n is the number of generations) This will result in a balanced growth. The time taken by the bacteria to double in number during a specified time period is known as the generation time. The generation time tends to vary with different organisms. *E.coli* divides in every 20 minutes, hence its generation time is 20 minutes, and for *Staphylococcus aureus* it is 30 minutes.

2.1.3 Stationary phase

As the bacterial population continues to grow, all the nutrients in the growth medium are used up by the microorganism for their rapid multiplication. This result in the accumulation of waste materials, toxic metabolites and inhibitory compounds such as antibiotics in the medium. This shifts the conditions of the medium such as pH and temperature, thereby creating an unfavourable environment for the bacterial growth. The reproduction rate will slow down, the cells undergoing division is equal to the number of cell death, and finally bacterium stops its division completely. The cell number is not increased and thus the growth rate is stabilised. If a cell taken from the stationary phase is introduced into a fresh medium, the cell can easily move on the exponential phase and is able to perform its metabolic activities as usual.

2.1.4 Decline or Death phase

The depletion of nutrients and the subsequent accumulation of metabolic waste products and other toxic materials in the media will facilitates the bacterium to move on to the Death phase. During this, the bacterium completely loses its ability to reproduce. Individual bacteria begin to die due to the unfavourable conditions and the death is rapid and at uniform rate. The number of dead cells exceeds the number of live cells. Some organisms which can resist this condition can survive in the environment by producing endospores.

2.2 Standard spectrophotometer

This method allows to determine the turbidity or Optical density which is the measure of the amount of light absorbed by a bacterial suspension. The degree of turbidity in the broth culture is directly related to the number of microorganism present, either viable or dead cells, and is a convenient and rapid method of measuring cell growth rate of an organism. Thus, the increasing the turbidity of the broth medium indicates increase of the microbial cell mass (Fig 2). The amount of transmitted light through turbid broth decreases with subsequent increase in the absorbance value.

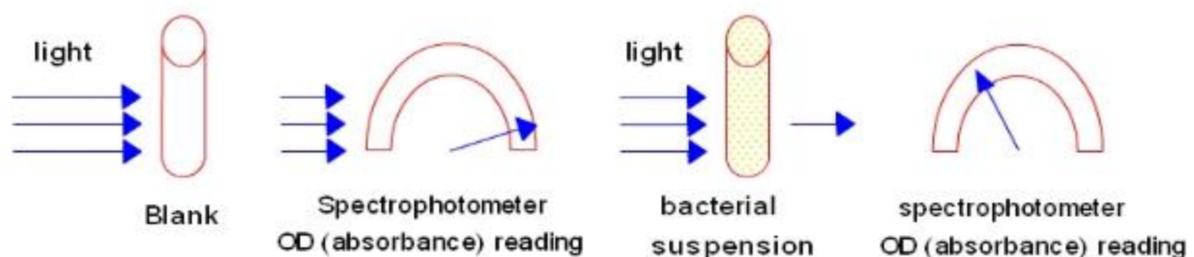


Figure 2: Absorbance reading of bacterial suspension

2.3 SoloVPE

By evolving beyond the limitations of traditional fixed pathlength spectroscopy, the SoloVPE method expanded the mature UV-Vis technique from a 2-dimensional to a 3-dimensional science. Conceptually simple, but analytically empowering, C Technologies' variable pathlength solutions (shown in figure 3) have revolutionized the measurement of concentration by delivering rapid and accurate results while avoiding costly dilution and background correction steps on the widest range of samples which is all made possible by the Slope Spectroscopy technique.

Unlike the single value dependence of legacy UV-Vis methods, the data dense slope method characterizes samples by collecting multiple absorbance data points at several pathlengths to create a section curve (Absorbance vs. Pathlength plot). Light is transmitted through the sample via an optical fiber or fibrette (shown in figure 4). Using a step motor to control the pathlengths from 5 µm to 15 mm (depending on a vessel type, that are shown in figure 5) in as small as 5 µm steps. The section curve is then analyzed in real time to verify linearity in compliance with the Beer-Lambert Law. The linear region of the section curve is directly proportional to the concentration of the sample based upon the sample extinction coefficient. Capable of making spectral and fixedpoint measurements at wavelengths between 190 and 1100 nm and at pathlengths between 5 microns and 15 millimeters, this relationship allows the SoloVPE system to measure low and high concentrated samples directly and report concentration results in less than 60 seconds.

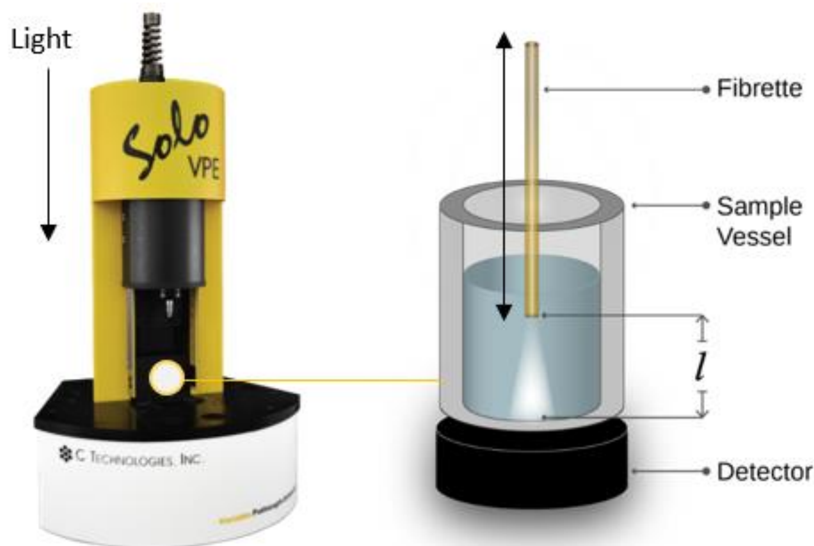


Figure 3: Schematic principle of variable pathlength of SoloVPE method [12]

Figure 4: Fibrettes - Disposable or reusable Solid core UV transmissive silica [12]

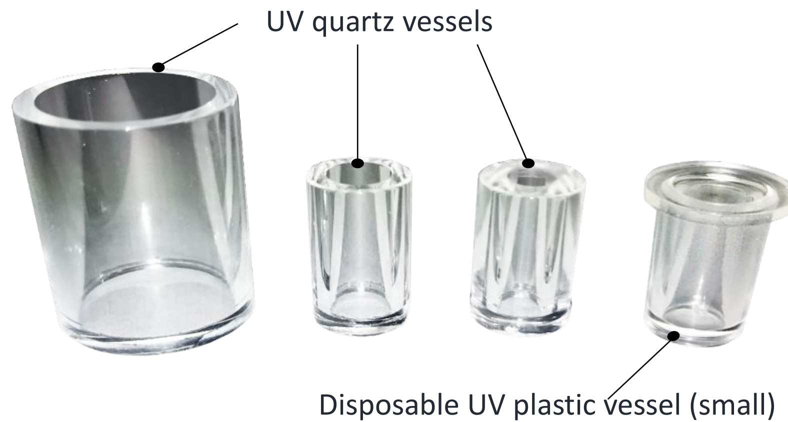


Figure 5: Vessels - Disposable or reusable vessels for SoloVPE method [12]

3 Measuring range, limitations and advantages

3.1 Standard spectrophotometer

The OD measuring range found in the literature of the standard spectrophotometer method is approximately between 0.5 g/L and 1.0 g/L. A wavelength of 600 nm is used to determine the bacterial cell concentration/optical density. The bacterial cells absorption properties are around absorbance of 1. Because bacterial cells, that absorb at 600 nm, will may not absorb at wavelength higher or lower than 600 nm, with higher cell-concentrations it leads to an error in the measuring result.
By using wavelength of 600nm, disposable plastic cuvettes must be used to avoid any interferences with the cuvette during the measurement. Limitations of this method are, as already mentioned, the absorption properties of the bacterial cells and the interferences with cultivation medium, in other words, the need of dilutions and baseline corrections. However, this method is simple to use and non-destructive which allows a fast and reliable measure of bacterial cells growth.

3.2 SoloVPE

The measure range of the SoloVPE method for bacterial cell cultivation is not determined. The great advantage of SoloVPE method is that still OD_{600} and Beer's Law is applied, however it can keep chosen specific concentration or extinction coefficient fixed by changing the pathlength to take linear measurements. Capable of making spectral and fixed-point measurements at wavelengths between 190 and 1100 nm at pathlengths between 5 µm and 15 mm, the SoloVPE Solution is adaptable to a wide range of sample types and concentrations. Finally, it can be said that this method allows a fast analyze of a high range of proteins. However, like all methods, the SoloVPE method has some disadvantages, these are mainly the beginning investment price and consumability of fibrettes and vessels.

4 Experimental plan

To measure the growth curve for *E. coli*, two samples (one from each shake flask) are taken every 30 minutes in order to find the absorbance/optical density (OD) or Slope absorbance at wavelength 600 nm and to test and compare the results detected by two methods, such as standard spectrophotometer and SoloVPE. To compare the results, *E. coli* K-12 W3110 cells and YPG medium (glucose added into the medium before autoclaving) were used. The cultivation was held in an incubator set to 37 °C and 150 rpm.

The comparison study is divided into three parts. The first part examines the growth curve detected by standard spectrophotometer method. In the second part, the growth curve, determined by Slope absorbance using SoloVPE method is investigated. The third part of this study compares results detected by standard spectrophotometer and SoloVPE method and examines the differences between of these methods. Furthermore, the correlation between the two methods is determined.

4.1 SoloVPE settings – Quick Slope

- Slope Mode: **Fixed**

- … (next to the Slope Mode): - Start PL: **3 mm**

- Step PL: **0,15 mm**

- Sample vessel: **SV1-Small**

- Wavelength: **600 nm**

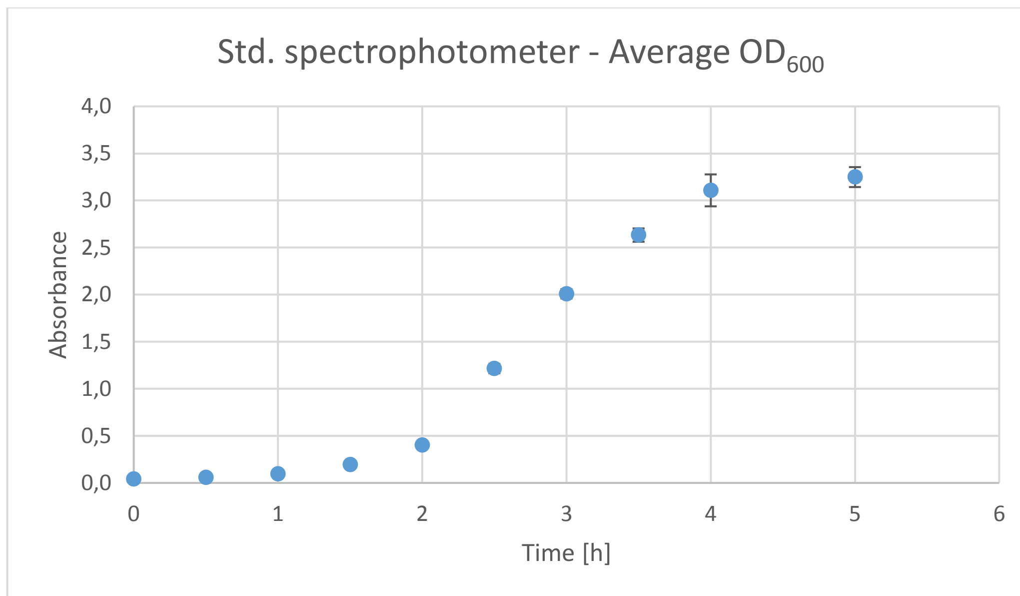
- Extinction coefficient: **1 mL/mg.cm**

5 Results and discussions

5.1 Standard spectrophotometer

Table 1: Absorbance/Optical density of the *E. coli* suspension during bacterial cells cultivation measured by standard spectrophotometer at wavelength of 600 nm

Time [h]	Absorbance		Average absorbance	Standard deviation
	Shake flask 1	Shake flask 2		
0	0,041	0,039	0,0400	0,001414
0,5	0,059	0,054	0,0565	0,003536
1	0,091	0,097	0,0940	0,004243
1,5	0,202	0,183	0,1925	0,013435
2	0,412	0,394	0,4030	0,012728
2,5	1,160	1,090	1,1250	0,049497
3	1,900	1,830	1,8650	0,049497
3,5	2,530	2,430	2,4800	0,070711
4	3,120	2,880	3,0000	0,169706
5	3,180	3,030	3,1050	0,106066



Graph 1: Growth curve of the *E. coli* measured by standard spectrophotometer. Samples taken after 2.5 hour of cultivation were diluted by dilution factor of 10 and then recalculated back to the real OD600.

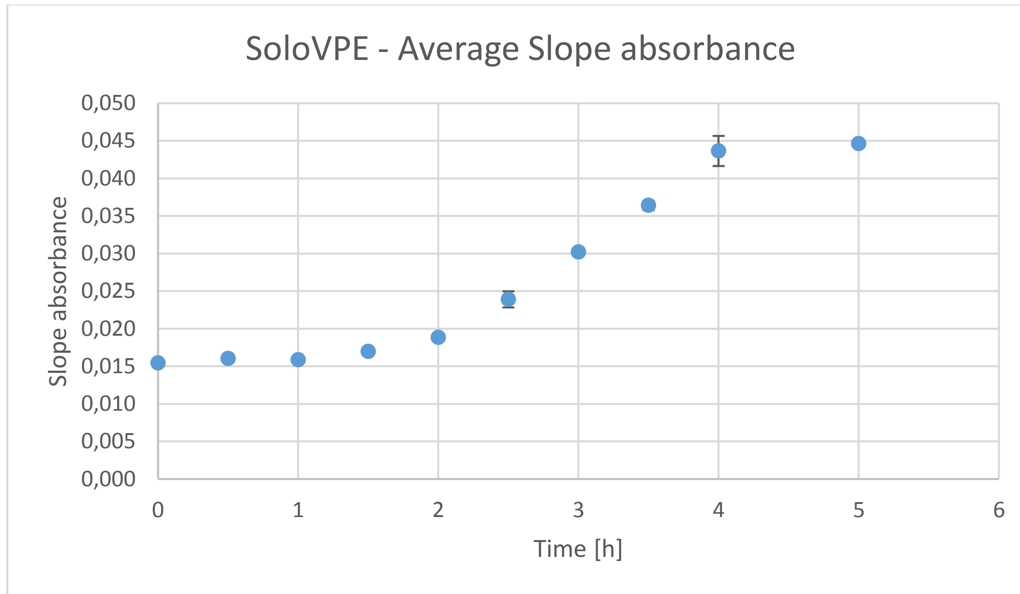
To investigate the OD_{600} for higher concentrations of *E. coli* cells by standard spectrophotometer, dilutions had to be done. First dilution was prepared after 2.5 hours of cultivation. For all dilutions, a 10-fold dilution was performed.

The repetability of the method was tested by repeating the *E. coli* cell cultivation in two or three shake flasks three times. The results are presented in table 2. The standard spectrophotometer method showed a good repetability.

5.2 SoloVPE

Table 2: Slope absorbance of the *E. coli* suspension during bacterial cells cultivation measured by SoloVPE at 600 nm

Time [h]	Slope absorbance		Average absorbance	Standard deviation
	Shake flask 1	Shake flask 2		
0	0,01549	0,01536	0,015	0,0001
0,5	0,01566	0,01636	0,016	0,0005
1	0,01564	0,01608	0,016	0,0003
1,5	0,01702	0,01693	0,017	0,0001
2	0,01871	0,01898	0,019	0,0002
2,5	0,02466	0,02314	0,023	0,0011
3	0,03058	0,02985	0,030	0,0005
3,5	0,03683	0,03595	0,036	0,0006
4	0,04223	0,04506	0,045	0,0020
5	0,04481	0,04444	0,044	0,0003

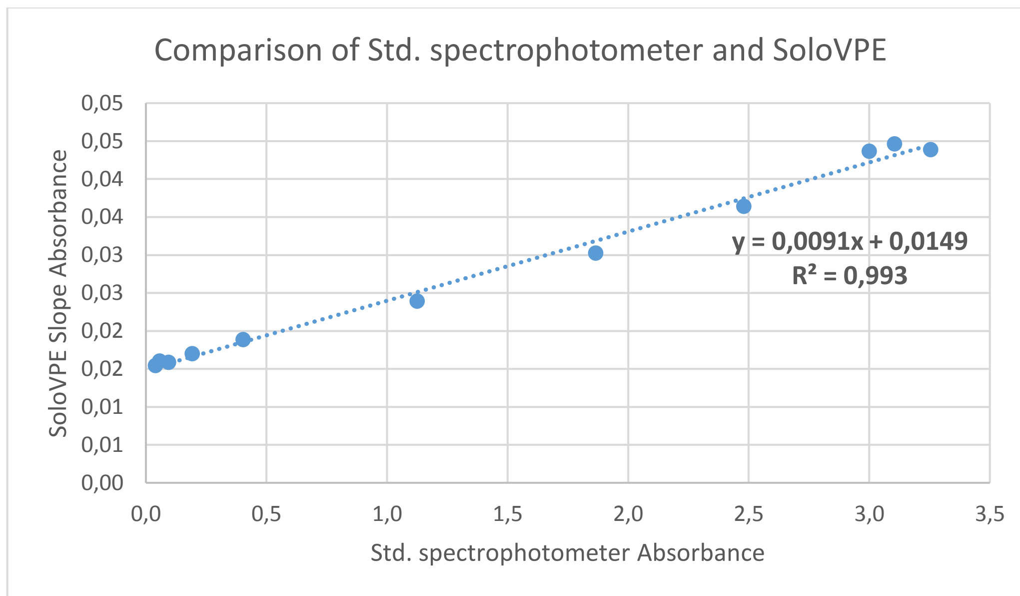


Graph 2: Growth curve of the *E. coli* measured by SoloVPE.

The repetability of the method was tested by repeating the *E. coli* cell cultivation in two or three shake flasks three times. The SoloVPE method shows a good repetability.

5.3 Comparison of Standard spectrophotometer and SoloVPE

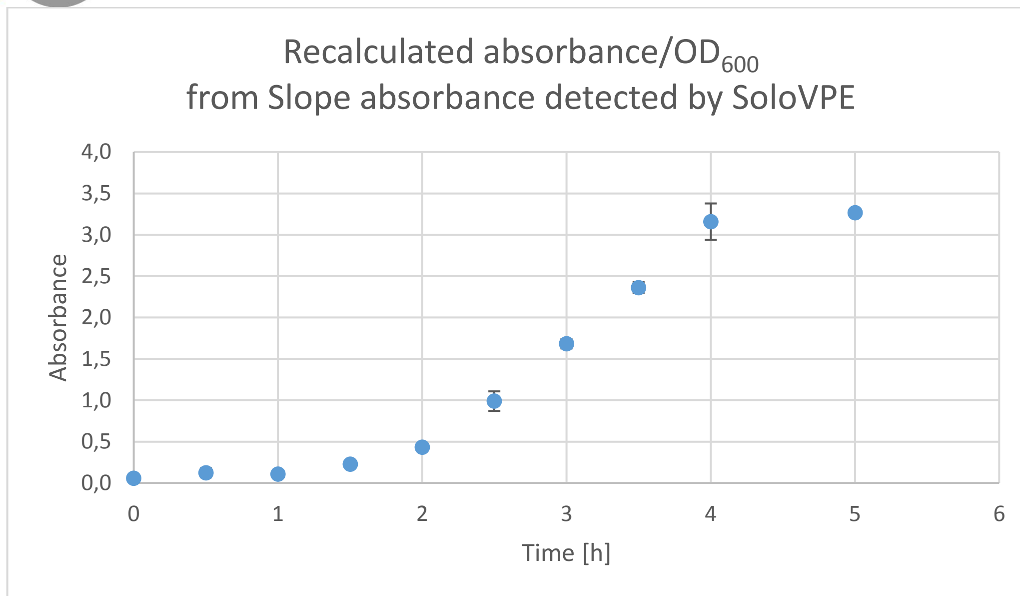
Additionally, the results measured by two different methods, standard spectrophotometer and SolovPE, were compared. Both bacterial growth curves had the same shape. A correlation between these two methods was determined. Furthermore, a recalculation from SoloVPE results to OD_{600} (Standard spectrophotometer) results by the linear regression function was done. It was found, that with larger differences in R^2 (R^2 97,5 % or lower) it is impossible to recalculate results detected by SoloVPE (Slope absorbances), because the values are very small and the RSD of 2,5 % or higher has a significant impact on the recalculations.



Graph 3: Correlation between results detected by standard spectrophotometer and SoloVPE

Table 3: Recalculation of results detected by SoloVPE (Slope absorbance) during *E. coli* cultivation into results detected by standard spectrophotometer (Absorbance/OD_{600}) using a function (y = 0,0091x + 0,0149)

Time [h]	Absorbance		Average absorbance	Standard deviation
	Shake flask 1	Shake flask 2		
0	0,065	0,051	0,058	0,0101
0,5	0,084	0,160	0,122	0,0544
1	0,081	0,130	0,105	0,0342
1,5	0,233	0,223	0,228	0,0070
2	0,419	0,448	0,434	0,0210
2,5	1,073	0,905	0,989	0,1181
3	1,723	1,643	1,683	0,0567
3,5	2,410	2,313	2,362	0,0684
4	3,003	3,314	3,159	0,2199
5	3,287	3,246	3,266	0,0288



Graph 4: Growth curve of the *E. coli* from recalculated results

The repetability and accuracy of determined function was tested by recalculation all SoloVPE results (Slope absorbances) from three different *E. coli* cultivations. All measured data were recalculated and growth curves that were plotted from the recalculated OD_{600} showed identical shapes. The function shows a good repeatability and precision and can be used for recalculation Slope absorbances (SoloVPE results) to standard absorbances (OD_{600}) values, that are detected by standard spectrophotometer.

6 Conclusion

It can be concluded that the *E. coli* cells grew as described in the literature. After 3 hours of cultivation, due to the strong interferences with high cell-concentrated samples, dilutions had to be performed to determine the OD_{600} by standard spectrophotometer. The SoloVPE method allowed to measure cell suspension without using any dilutions and baseline corrections.

The difference in growth curves between these two methods can be described by detecting different parameters by each device. The standard spectrophotometer detects the absorbance, but the SoloVPE detects Slope absorbance with a unit Abs/mm, that is determined from ten measurements of absorbances in ten different pathlenthgs. Overall, the to growth curves are very similar in shape.

Additionally, a correlation between these two methods for bacterial cell quantification during bacterial cell cultivation was found. Furthermore, a SoloVPE results were recalculated into OD600 (standard spectrophotometer) values by using a function that was determined by linear regression.

Finally, it can be said that SoloVPE method is suitable for measuring the optical density of cells during bacterial culture. This method is less time consuming and can be recalculated to standard OD600 values by using one simple function.

7 Sources

[1] https://vlab.amrita.edu/?sub=3&brch=73&sim=1105&cnt=1

[2] https://www.sciencedirect.com/science/article/pii/S0960982210005245

[3] https://jb.asm.org/content/189/23/8746