FACTORS INFLUENCING BACTERIAL GROWTH OF E. COLI DH5α

Al Humoud. Saqr, Ceballos Giraldo. Mateo, Azimi. Fatema, Chandran Pillai. Amrutha, Thomas. Jerin Moslehimoslehabadi. Elham

1. Introduction

Escherichia coli is one of the common Gram-negative bacteria found around us. It has a simple, completely sequenced genome and a rapid growth rate under optimal conditions, is easy to handle and cultivate; and is relatively harmless which allows them to be one of the most frequently used microorganisms in biotechnology. There are different factors that affect the growth of the bacteria such as temperature, pH, O2 and nutrients. When all the conditions are satisfied, it is said that the bacteria have reached its optimal growth condition. Escherichia coli (E. coli) bacteria normally live in the intestines of healthy people and animals. In this experiment, genetically modified E. coli is used which is non-pathogenic and non-toxic, to investigate the rate of growth under different conditions. By incubating bacteria culture overnight in different media, the influence of different conditions on bacteria can be studied. (Freitag, 2022)

1.1 Growth conditions

Bacteria are usually grown in batch samples which means they are in a controlled environment. The growth of E. coli consists of mainly four phases known as the lag phase in which growth progress is almost zero. The bacteria are not completely inactive in this phase, they grow in size and develop primary metabolites. The next one is the exponential phase or log phase in which bacteria start to grow in an exponential way. The growth rate in the log phase is described as a generation- or doubling time which is the time taken for each cell 2 cycle. An optimal generation time is only possible in an environment with lots of nutrients and sufficient biological space. The end of the log phase is called decelerating phase where the slope on the growth curve flattens out and connects to the following stationary growth phase, see Figure 1. The third phase is called the stationary phase where the number of bacteria is constant since bacterial divisions and deaths are equal. The phase is working this way because the medium is insufficient in nutrients to provide further growth. The final death phase occurs when more bacteria are dying than dividing. The total amounts of bacteria are decreasing because they are lacking survival conditions, and most often nutrients or toxic products are accumulating. (Freitag, 2022)

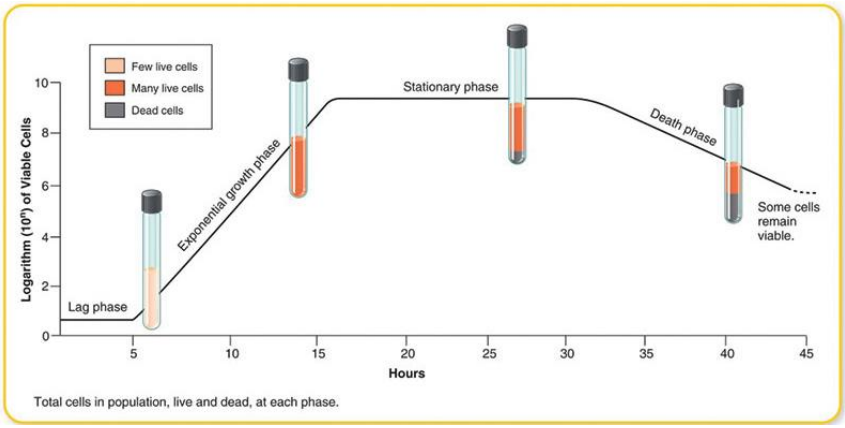

Figure 1. Phases in Bacterial growth. Taken from: https://microbenotes.com/bacterial-growth-curve-and-its-significance/

1.2 Measurement of culture turbidity

To quantify cell growth during the experiment turbidity must be measured. A spectrophotometer is a device that can measure growth by measuring the optical density (OD) which enables a way of counting the number of bacteria in a sample. The method works by sending light at a specific wavelength, which is 600 nm (OD_{600}) in this experiment. The optical density obtained is a number based on the amount of diffracted light. The number is directly correlated to the number of bacteria.

2. Aims and problems

According to a fundamental tenet of microbial development, every microbe has a preferred environment. It can be difficult for researchers to identify the conditions, known as the optimal growth condition, which each bacterium prefers. The aim of this experiment is to analyze E.coli growth in different conditions by measuring its turbidity using spectrophotometry. In order to materialize this aim, different medium with different conditions were prepared. As mentioned in the introduction different factors affect the bacterial growth namely pH, nutrients, oxygen, carbon source, etc. therefore, in this experiment, the composition of the media i.e. the lack and the presence of certain nutrients was tested and evaluated. The media are categorized into three, the first with different pH, and the second with different compositions of c-source, and the third with different percentages of glucose. Additionally, for each medium is replicated, to test the bacterial growth under shaken and not shaken incubation conditions to analyze the effect of the forced addition of oxygen into the medium versus natural means of bacterial oxygen uptake and its effect on their growth.

Few problems arose during the experiment, and they were mostly derived due to human errors. One these problems was that between spectrometer measurements of 2 sample incubated in different solutions (LB and M9 solutions) no appropriate blanking was preformed prompting for the disregarded for one of the measurements, furthermore, time played a restrictive factor in this experiment. The 30-mins time intervals measurement system was approximate and at times it wasn't followed precisely.

3. Materials and methods

The experiment was performed within the span of a day. A 40 ml cell culture of E. coli bacteria was incubated overnight, one day before the experiment, in an LB medium inside an Erlenmeyer flask. An initial qualitative characterization of the smell was performed, and a 1 ml sample of the cell culture was pipetted into a cuvette and its turbidity/optical density was initially measured using a bench-top spectrometer, the reading determined that the optical density (OD_{600}) measurement of the culture was well above the 0.4 units linear limit, this prompted for the dilution of the sample. The sample was diluted a few times and had its optical density measured after each dilution until an appropriate dilution factor of 1:80 was reached that obtained a reading that was within the acceptable OD_{600} range. Based on the newly found dilution factor a sample of 1:80, an appropriate sample of the cell culture was calculated (450 µl) that would produce an OD_{600} of 0.1, this sample was pipetted 12 times into the 6 pairs of flasks carrying different media, further description of each medium can be found in Table 1.

The pipetting process was done carefully, and precise measurements were taken within the sterile area, the flasks were then covered with aluminum foil with an opening allowing the exchange of gases and then removed from the sterile area and transported to an incubation room set at a temperature of 37 degrees Celsius. There, half of the flasks were mounted onto the orbital shaking incubator and the other half were left on the bench top, the shaking incubator was then set at 180 rounds per minute and the flasks were left there and taken out on a 30 minutes basis to measure their optical density. Every 30 minutes, the flasks containing the media would be transported back to the sterile area and samples of each medium were pipetted in 12 cuvettes, each sample’s optical density was then measured with the spectrometer while making sure to blank the spectrometer between measurements of media of different solution due to color difference. In instances where the reading was higher than 0.4 the sample had to be diluted using the same medium by a new dilution factor, this dilution factor was used for all other upcoming readings of the same medium if the reading stayed under the 0.4 limit, otherwise, a new dilution factor had to be calculated.

Table 1. The number of media used their description

Medium Number	Description		Composition
Medium 1	LB Solution at Neutral pH	Shaken	10g Tryptone 5g Yeast extract 10g NaCl 1000 ml ddH_2O
Medium 2		Not Shaken	
Medium 3	LB Solution at 10 pH	Shaken	10g Tryptone 5g Yeast extract 10g NaCl 900 ml ddH_2O
Medium 4		Not Shaken	
Medium 5	M9 Solution	Shaken	Sterile standard M9 medium for E. coli growth
Medium 6		Not Shaken	
Medium 7	M9 Solution with 4% Glucose	Shaken	Sterile standard M9 medium for E. coli growth
Medium 8		Not Shaken	
Medium 9	LB Solution with 10% Glucose	Shaken	Autoclaved standard LB medium for E. coli growth
Medium 10		Not Shaken	
Medium 11	LB Solution with 25% Glucose	Shaken	Autoclaved standard LB medium for E. coli growth
Medium 12		Not Shaken	

3.1 Models for bacterial growth

For the numerical characterization of the cell culture, the model exponential growth kinetics from (Freitag, 2022) were used, see Equations 1, 2, 3.

$$X_t = X_o \times e^{\mu t} \qquad (1)$$

$$ln\left(\frac{X_t}{X_o}\right) = \mu \times t \qquad (2)$$

$$t_D = \frac{ln(2)}{\mu} \qquad (3)$$

These three Equations are used to determine the specific growth rate μ (h^{-1}) based on the time t (h), the initial mass concentration X_o, the biomass concentration in the specific time X_t and the generation or doubling time t_D (h). From Equation 2, the specific growth can be cleared as shown in Equation 4.

$$\mu = \frac{\ln(X_t/X_o)}{t} \qquad (4)$$

Additionally, a bacterial concentration can be calculated using the measurements taken with the spectrometer. This can be done with Equation 5 also taken from (Freitag, 2022).

$$1\ OD_{600} = 8\ \times 10^8\ cells/ml \qquad (5)$$

Using Equations 5 and the medium volumes the overall amount of bacteria can be calculated as can be shown in Equation 6.

$$overall\ amount\ of\ bacteria = bacterial\ concentration\ \times medium\ volume \quad (6)$$

4. Results and discussion

In this section, the calculations of generation time, specific growth, bacterial concentration and overall amount of bacteria were calculated using Equations 3, 4, 5 and 6 respectively.

4.1 Different PH

In this sub-section a total of 4 media were prepared, see Table 1 media 1 to 4. As mentioned in the previous section, measurements were taken on the basis of a 30 minutes intervals in order to measure the optical density of all 4 media, as it is shown in Table 2 and visualized in Figure 2, the optical density measurement for the 4 media are displayed and plotted against time respectively, with media 1 and 2 exhibiting a more observable growth than media 3 and 4. Furthermore, in media 1 and 2 the graphs start to slant away from the x-axis after approximately 1 hour however for media 3 and 4 the readings stayed stagnant at an OD_{600} of around 0.1. Additionally, when comparing the graphs of media 1 and 2 against each other it is observable that medium 1 has registered higher OD_{600} values for the same time intervals when compared with medium 2.

Table 2. Optical Density measurements adjusted to dilution and volume after each measurement for media 1 to 4

Time (min)	Medium 1		Medium 2		Medium 3		Medium 4	
	OD_{600}	Volume (ml)	OD_{600}	Volume (ml)	OD_{600}	Volume (ml)	OD_{600}	Volume (ml)
0	0,100	30,00	0,100	30,00	0,100	30,00	0,100	30,00
65	-	-	-	-	0,133	30,00	0,142	30,00
80	0,168	30,00	0,100	30,00	-	-	-	-
95	-	-	-	-	0,092	29,00	0,093	29,00
110	0,268	29,00	0,235	29,00	-	-	-	-
125	-	-	-	-	0,069	28,00	0,065	28,00
140	0,368	28,00	0,274	28,00	-	-	-	-
185	-	-	-	-	0,103	27,00	0,102	27,00
200	0,740	27,00	0,372	27,00	-	-	-	-
215	-	-	-	-	0,101	26,00	0,093	26,00
230	1,300	26,00	0,608	26,00	-	-	-	-
245	-	-	-	-	0,096	25,00	0,100	25,00
260	1,510	25,75	0,704	25,75	-	-	-	-
275	-	-	-	-	0,107	24,00	0,099	24,00
290	1,950	25,65	0,784	25,50	-	-	-	-
305	-	-	-	-	0,102	23,00	0,110	23,00
320	2,180	25,55	0,864	25,25	-	-	-	-
335	-	-	-	-	0,106	22,00	0,108	22,00
350	2,540	25,45	1,012	25,00	-	-	-	-

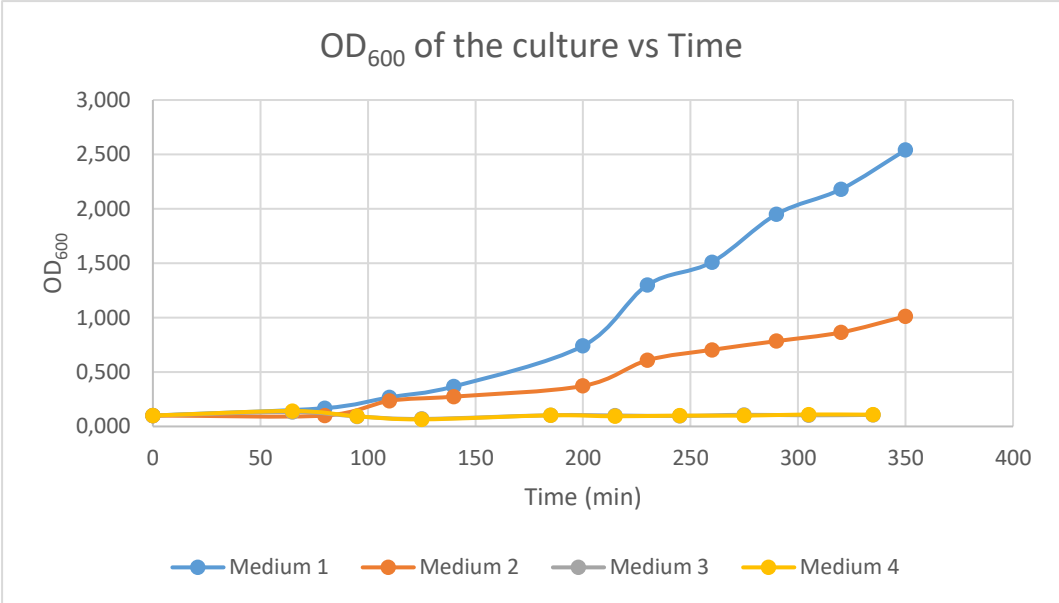

Figure 2. Turbidity through time of media 1 to 4

Moreover, as it can be seen in Table *3* and at nearly 4 hours of growth in Figure *2*, there is a significant increment of bacterial concentration marking the first recognizable point of the exponential phase in this time point. This also can be noticed in Figure *3*, where medium 1 and 2 had a marked exponential growth.

Table 3. Bacteria concentration after approximately 4 hours of cultivation of media 1 to 4

Medium	Time (min)	Bacteria concentration (cell/ml) $\times 10^7$
Medium 1	230	104
Medium 2	230	48,6
Medium 3	245	7,68
Medium 4	245	8,00

In Table 4, the numerical values of the overall amounts of bacteria in each medium are shown. The results were measured using the optical density readings and given the directly proportional relationship between them one can conclude from the readings of media 1 and 2 that a gradual growth occurred followed by an exponential growth thereafter. On the other hand the results also show that for media 3 and 4 growth wasn't uniform, if any can be observed, and the readings can be characterized as random at best. For example after the initial reading the optical density value for both media started decreasing at 65 minutes and until a resumption of a small increase in value at 185 minutes.

Table 4. Overall amount of bacteria media 1 to 4

Time (min)	Overall amount of bacteria $\times 10^9$			
	Medium 1	Medium 2	Medium 3	Medium 4
0	2,40	2,40	2,40	2,40
65	-	-	3,19	3,41
80	4,03	2,40	-	-
95	-	-	2,13	2,16
110	6,22	5,45	-	-
125	-	-	1,55	1,46
140	8,24	6,14	-	-
185	-	-	2,22	2,20
200	16,0	8,04	-	-
215	-	-	2,10	1,93
230	27,0	12,6	-	-
245	-	-	1,92	2,00
260	31,1	14,5	-	-
275	-	-	2,05	1,90
290	40,0	16,0	-	-
305	-	-	1,88	2,02
320	44,6	17,5	-	-
335	-	-	1,87	1,90
350	51,7	20,2	-	-

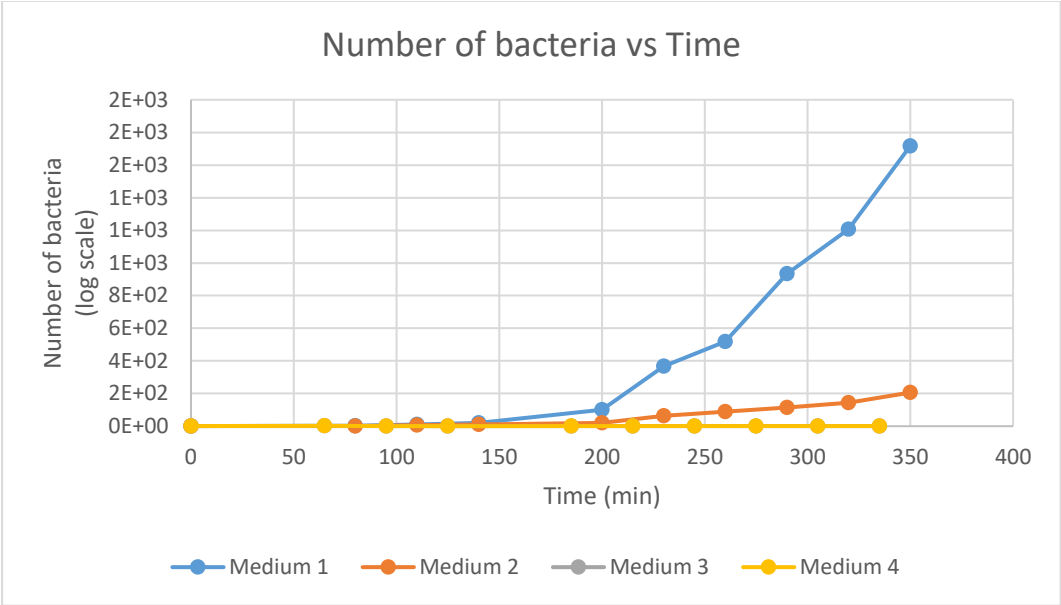

Figure 3. Bacterial growth through time of media 1 to 4

Given the fact that media 3 and 4 didn't produce any growth, there is no porpuse to calculate the values for the specifc growth rate (μ) and the doubling time (t_D), as shown in Table 5 the values weren't included, however for media 1 and 2 the values were calculated with medium 1 having a faster growth rate and a smaller doubling time.

Table 5. Specific growth rate constant and generation time during the exponential phase of media 1 to 4

Medium	μ (h^{-1})	t_D (hr)
Medium 1	0,610	1,136
Medium 2	0,420	1,650
Medium 3	-	-
Medium 4	-	-

E.coli bacteria are considered to be neutrophiles, meaning they prefer pH levels at around 7, from this experiment one can observe that if they are grown in enviroments with pH levels that are higher than that (alkaline), it will hamper their growth potential pulling them away from their optimal growth conditions, this is corraborated by the results of media 3 and 4, furthermore although they are anaerobic strain, their faculative nature requires the presence of oxygen to insure their growth. in medium 1 since it was shaken while being incubated, that introduced more oxygen to the mixture leading to a higher growth rate. (Parhad & N,U, 1974) (Freitag, 2022)

4.2 Lack/Prescence of C-Source

The Table 7 depicts the bacterial growth after 4 hours of incubation, where it is evident that the growth of bacteria reached the exponential phase which can be clearly identified in Figure 4 and Figure 5, where in Figure 5 the medium 5 had a visible exponential growth.

Table 6. Optical Density measurements adjusted to dilution and volume after each measurement for media 5 to 8

Time (min)	Medium 5		Medium 6		Medium 7		Medium 8	
	OD_{600}	Volume (ml)	OD_{600}	Volume (ml)	OD_{600}	Volume (ml)	OD_{600}	Volume (ml)
0	0,100	30,00	0,100	30,00	0,100	30,00	0,1	30,00
65	-	-	-	-	0,104	30,00	0,162	30,00
80	0,147	30,00	0,163	30,00	-	-	-	-
95	-	-	-	-	0,108	29,00	0,105	29,00
110	0,109	29,00	0,094	29,00	-	-	-	-
125	-	-	-	-	0,113	28,00	0,111	28,00
140	0,123	28,00	0,103	28,00	-	-	-	-
185	-	-	-	-	0,133	27,00	0,121	27,00
200	0,556	27,00	0,114	27,00	-	-	-	-
215	-	-	-	-	0,216	26,00	0,159	26,00
230	0,840	26,00	0,456	26,00	-	-	-	-
245	-	-	-	-	0,276	25,00	0,193	25,00
260	2,680	25,75	0,676	25,75	-	-	-	-
275	-	-	-	-	0,326	24,00	0,228	24,00
290	3,210	25,65	0,816	25,50	-	-	-	-
305	-	-	-	-	0,391	23,00	0,279	23,00
320	3,580	25,55	0,916	25,25	-	-	-	-
335	-	-	-	-	0,492	22,75	0,295	22,00
350	3,860	25,45	1,004	25,00	-	-	-	-

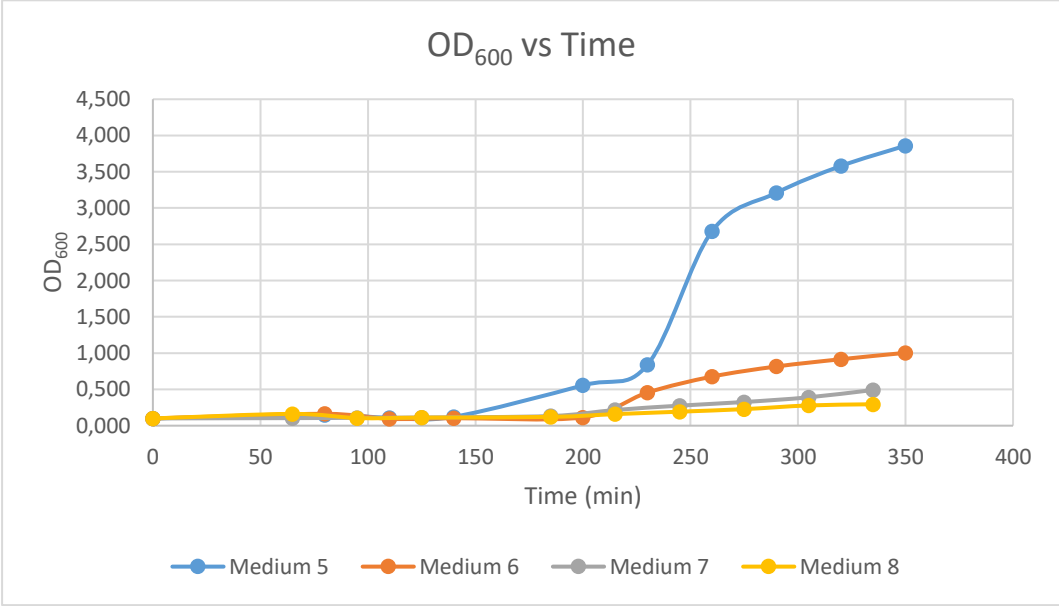

Figure 4. Turbidity through time of media 5 to 8

Table 7. Bacteria concentration after approximately 4 hours of cultivation of media 5 to 8

Medium	Time (min)	Bacteria concentration (cell/ml) $\times 10^7$
Medium 5	230	672
Medium 6	230	36,5
Medium 7	245	22,1
Medium 8	245	15,4

In Table 8, the overall amount of bacteria during different intervals of time are shown. For medium 5 and 6, the table gives a picture of a drastic increase in the bacterial growth, whereas, in medium 7 and 8, there is only a narrow increase of growth overtime.

Table 8. Overall amount of bacteria media 5 to 8

Time (min)	Overall amount of bacteria $\times 10^9$			
	Medium 5	Medium 6	Medium 7	Medium 8
0	2,40	2,40	2,40	2,40
65	-	-	2,50	3,89
80	3,53	3,91	-	-
95	-	-	2,51	2,44
110	2,53	2,18	-	-
125	-	-	2,53	2,49
140	2,76	2,31	-	-
185	-	-	2,87	2,61
200	12,0	2,46	-	-
215	-	-	4,49	3,31
230	17,5	9,48	-	-
245	-	-	5,52	3,86
260	55,2	13,9	-	-
275	-	-	6,26	4,38
290	65,9	16,6	-	-
305	-	-	7,19	5,13
320	73,2	18,5	-	-
335	-	-	8,95	5,19
350	78,5	20,1	-	-

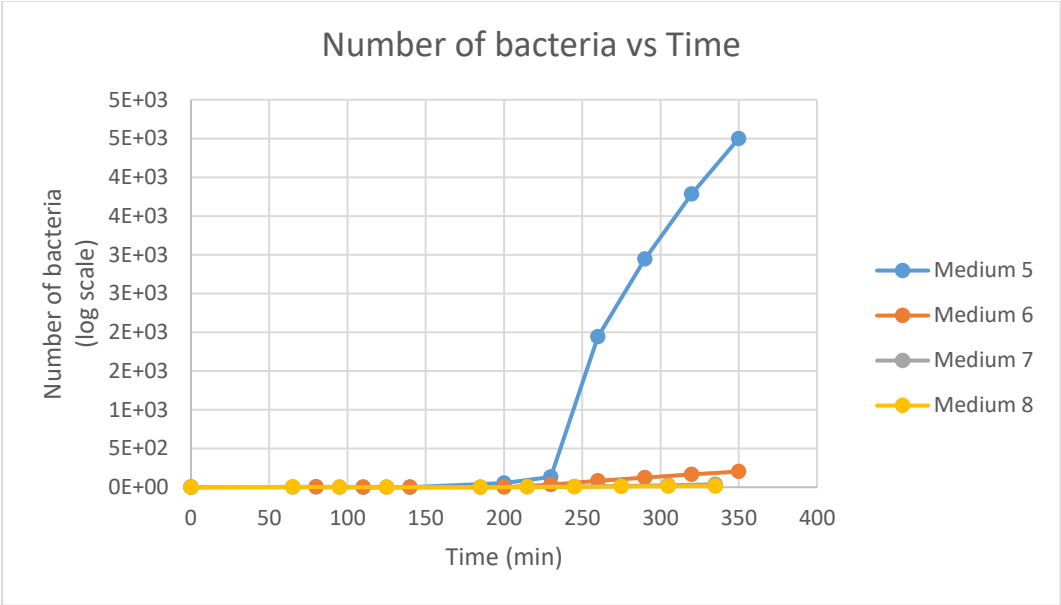

Figure 5. Bacterial growth through time of media 5 to 8

While measuring specifc growth rate (μ) and the doubling time (t_D) in *Table 9*, medium 5 needs less time over medium 6 to show an exponential growth. However, it is unable to calculate the same for medium 7 and 8 due to the lack of exponential growth in these mediums.

Table 9. Specific growth rate constant and generation time during the exponential phase of media 5 to 8

Medium	μ (h^{-1})	t_D (hr)
Medium 5	0,64	1,10
Medium 6	0,35	4,33
Medium 7	-	-
Medium 8	-	-

From the result it is understood that most of the growth occurs in medium 5 which contain M9 pH 7 (minimal medium without glucose) whereas medium 6 shows minimal bacterial growth when compared to medium 5 which is an unshaken medium of M9. This shows that the oxygen concentration play vital role in the growth of bacteria due to the aerobic nature of E.coli.

In Medium 7 and 8 which contain 4% Glucose (shaken and not shaken), the bacterial growth is almost in a steady state. This is due to the facultative aerobic metabolism of glucose which lead to the excretion of acetic acid that results in the reduction of desired product (Freitag, 2022). Overall, it is evident that pH7 with minimal level of Glucose is an optimal condition for the bacterial growth.

4.3 Different Glucose

The overall amount of bacteria for medium 10 should have grown better than medium 9 because of the access of more oxygen. However, the data in

Table 11 it is shown that they grew almost similarly. In case of excess of glucose as the aerobic metabolism of glucose lead to excretion of the acetic acid which is detrimental for bacterial growth, the overall amount of bacteria for medium 11 have increase more than medium 12.

Table 10. Optical Density measurements adjusted to dilution and volume after each measurement for media 9 to 12

Time (min)	Medium 9		Medium 10		Medium 11		Medium 12	
	OD_{600}	Volume (ml)	OD_{600}	Volume (ml)	OD_{600}	Volume (ml)	OD_{600}	Volume (ml)
0	0,100	30,00	0,100	30,00	0,100	30,00	0,100	30,00
25	-	-	-	-	0,085	30,00	0,209	30,00
40	0,101	30,00	0,200	30,00	-	-	-	-
55	-	-	-	-	0,038	29,00	0,126	29,00
70	0,051	29,00	0,073	29,00	-	-	-	-
85	-	-	-	-	0,075	28,00	0,170	28,00
100	0,088	28,00	0,084	28,00	-	-	-	-
115	-	-	-	-	0,095	27,00	0,208	27,00
160	0,124	27,00	0,124	27,00	-	-	-	-
175	-	-	-	-	0,732	26,00	0,904	26,00
190	0,287	26,00	0,500	26,00	-	-	-	-
205	-	-	-	-	0,242	25,00	1,220	25,00
235	-	-	-	-	0,644	24,00	1,416	24,00
250	0,984	24,00	0,916	24,00	-	-	-	-
265	-	-	-	-	2,000	23,00	3,570	23,00
280	2,880	23,00	2,720	23,00	-	-	-	-
295	-	-	-	-	1,360	22,00	1,200	22,00
310	1,010	22,00	1,030	22,00	-	-	-	-

Table 11. Overall amount of bacteria media 9 to 12

Time (min)	Overall amount of Bacteria × 10^9			
	Medium 9	Medium 10	Medium 11	Medium 12
0	2,40	2,40	2,40	2,40
25	-	-		
40	2,42	4,80	-2.04	5.02
55	-	-		
70	1,18	1,69	0.882	2.92
85	-	-		3,81
100	1,97	1,88	1.68	3.81
115	-	-		
160	2,68	2,68	2.05	4.49
175	-			
190	5,97	10.4	15.2	18.8
205	-	-		
220	-	-	4.84	24.4
235	-	-		
250	18.9	17.6	12.4	27.2
265	-	-		
280	53.0	50.0	36.8	65.7
295	-	-	2,57	
310	1,9917.8	18.1	23.9	21.1

In *Table 12* it is shown that after 4 hours the bacteria concentration of medium 12 had the lowest value among other media which means the secretion of the acetate to the medium reduce the bacterial growth. Although for medium 12 this value does not follow a reasonable trend as it should be, it seems after 4 hours the concentration of acetic acid reached its maximum value.

Table 12. Bacteria concentration after approximately 4 hours of cultivation media 9 to 12

Medium	Time (min)	Bacteria concentration (cell/ml) ×10^7
Medium 9	250	7,87
Medium 10	250	7,33
Medium 11	250	5,15
Medium 12	250	1,13

Figure 6 and Figure 7 shows that medium 12, which has 25% glucose and is not shaken, has the best growth. (Expect the last reading, which seems to be a human error.) Overall, it is expected that the medium with 10% glucose and shaken should have the best condition for bacteria growth, but this is not shown in the figures, as many human errors may have happened. In addition, as the measurements of these mediums were done last, environmental circumstances may have affected the result.

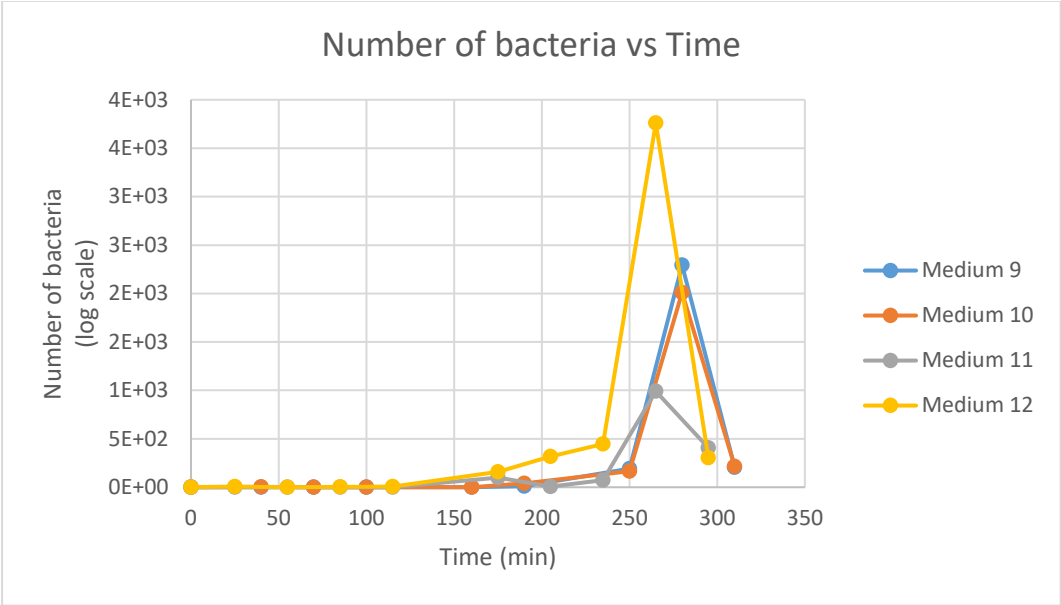

Figure 6. Bacterial growth through time of media 9 to 12

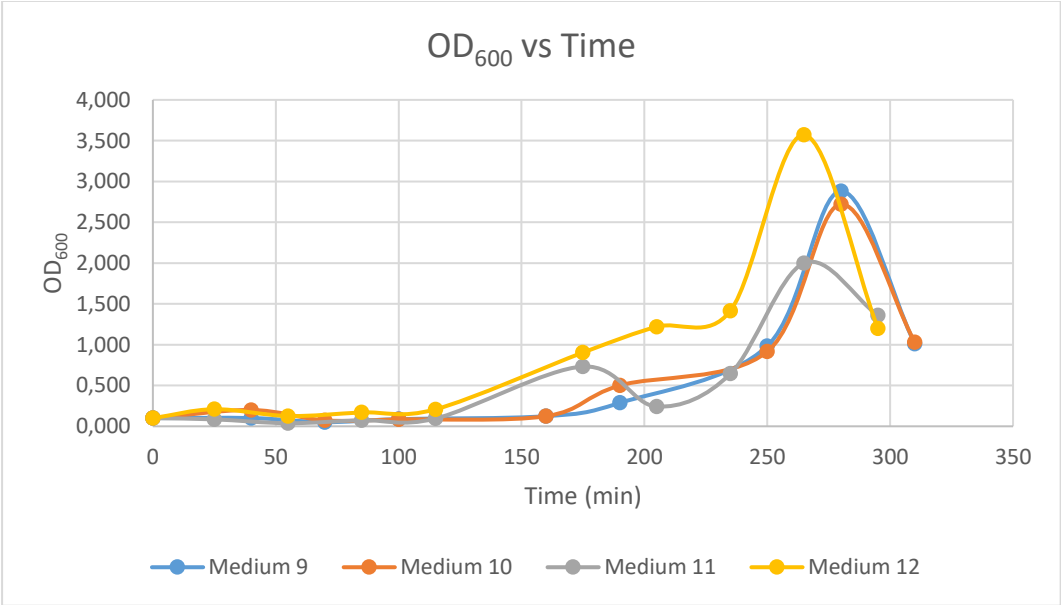

Figure 7. Turbidity through time of media 9 to 12

From *Table 13* it is shown that the specific growth rate for medium 12 is larger than other values which should not be. However as mentioned before the excess of glucose cause overflow metabolism which is common for facultative organisms that have a portion of their anaerobic enzyme set up active in aerobic conditions, and it happens when the glucose content exceeds a critical value.

Table 13.Specific growth rate constant and generation time during the exponential phase of media 9 to 12

Medium	μ (h^{-1})	t_D (hr)
Medium 9	0,55	1,26
Medium 10	0,53	1,30
Medium 11	0,48	1,46
Medium 12	0,68	0,68

In cell culture, there can be too much or too little glucose in the media and the results are greater than just slow cellular metabolism or overpopulation. If the glucose level in cell culture media is too low, the cells will undoubtedly starve but there are other issues at play. In instances where certain bacteria are being cultured, a lack of glucose is exactly the type of hostile environment that can cause endospore formation. It is also necessary to take into account the osmotic pressure brought on by the variation in glucose concentration across the plasma membrane. When there is an osmotic change, molecules will penetrate the membrane, and in low glucose environments, water molecules may enter the cell at quantities that are too great for the cell to handle, leading to lysis.

There may not be the same concerns with a nutrient deficiency if the media's glucose concentration is too high, but there are still issues. In the culturing of mammalian cells, this is especially true. Generally, the excess of glucose in medium lead to secrete of acetic acid to medium which reduces the growth rate of bacteria. In other words, acetate itself is a growth inhibitor. But in this case, a moderate increase in cell mass is observed, which might be because of some errors that were made during the experiment such as incorrectly dealing with spectrophotometry and spectrophotometer, other contamination, and messed up different cultures which lead to recording the wrong data. In addition, Studies have shown that high glucose in cell media can decrease proliferation rates and increase apoptosis. This experiment shows, that although glucose as a nutrient is necessary for bacterial growth, the amount of this glucose is also important, as low levels of glucose cause the death of bacteria e to starvation, and high levels of glucose cause apoptosis. (Scientificbio 2022)

5. Conclusion

After investigating the effects of pH, glucose and oxygen on the growth of E.coli DH5α can be concluded that the optimal growth condition has to be rich in oxygen, as demonstrated in the experiment, for the successful media, the shaken ones demonstrated better growth-wise in comparison to their non-shaken counterparts, furthermore, a neutral pH would ensure an appropriate bacterial growth, this can be noticed in the case that bacteria was grown in alkaline conditions, media 3 and 4, it failed to produce any recognizable growth. Finally, glucose plays an important role in cell growth as anticipated with no or low glucose levels the maximum growth rate and the lowest doubling time as the case of media 1 and 5.

6. References

Freitag, R., 2022. *Biotechnology practical course.* Bayreuth: Chair for Process Biotechnology.

Parhad, N. M. & N,U, R., 1974. Effect of PH on Survival of Escherichia Coli. *Water Pollution Control Federation vol. 46, no. 5*, p. pp. 980–86. JSTOR.

Mayo Clinic. (n.d.). E. coli - Symptoms and causes. [online] Available at: https://www.mayoclinic.org/diseases-conditions/e-coli/symptoms-causes/syc-20372058#:~:text=Escherichia%20coli%20(E.%20coli).

Backlöf, K., Ejdebäck, M. and Karlsson, D. (n.d.). *Bachelor Degree Project in Cell and Molecular Biology 15p Autumn term* 2011 GROWTH CONDITIONS AND EXPERIMENTAL SETUP FOR

BACTERIAL GROWTH AND THE FADING OF PHENOLPHTHALEIN IN ALKALINE SOLUTION. [online] Available at: http://www.diva-portal.org/smash/get/diva2:505443/FULLTEXT01.pdf.

Aryal, S. (2019). *Bacterial growth curve and its significance | Basic Microbiology*. [online] Microbe Notes. Available at: https://microbenotes.com/bacterial-growth-curve-and-its-significance/.

Understanding the role of glucose in Cell Culture Media (no date) Scientific Bioprocessing. Available at: https://www.scientificbio.com/blog/understanding-the-role-of-glucose-in-cell-culture-media/ (Accessed: November 24, 2022).