The Effect of Temperature on Microorganisms Growth Rate

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ABSTRACT

The objective of this research is to analyse the effect of temperature on microorganism growth. It is important to note that both low and high temperatures influence the growth of microorganisms and therefore it is necessary to know the lower and upper limits and finding out at which temperature the microorganisms grow best. The microorganisms may not grow regardless of being provided with all the necessary nutritional requirements, moisture, oxygen and right environment to thrive in if the temperatures (3, 20, 25, and 30°C) is not favourable for their growth. If the temperatures are below optimum then the microorganisms will have reduced growth, and the same applies to temperatures above optimum. The optimum temperature for the growth of these microorganisms ranges from 23-26°C.

INTRODUCTION

The study is based on the effect of temperature variation in microorganisms which plays the role of converting carbon dioxide to oxygen (Pang et al., 2017). It has been proven that temperature changes in oceans and other bodies of water (as a result of weather changes) can affect the population of microorganisms (Cassim et al.,1998). Thus, this study is going to investigate and determine:

1. The upper and lower limit temperatures for microorganism growth.
2. The optimum temperature for their growth.

In this study, the goal is to determine the optimum temperature necessary for the growth of microorganisms in comparison with temperatures of 3°C and 30°C. The null hypothesis is if the temperatures tested does not influence the growth rate of microorganisms, then temperature does not have an effect on growth rate. The alternative hypothesis is that temperature influences the growth of microorganisms, so the effect of how low and high temperatures affect the growth of microorganisms must be determined.

METHODS

First, rainwater was collected in a large container outside. Then, the number of cells of each treatment was counted within the box drawn on the glass slide. An average of four counts was used. Then, the temperature of the solution was measured. The reading was 20°C. After that

the solution was divided into 16 small clear cylindrical containers of equal proportion. Four of each small container was then placed under different conditions.

Each group of four was then subjected to different temperatures of 3, 20, 25 and 30 °C. A thermometer was placed at each location, to monitor the temperature. Samples were then collected and measured over the course of five days. The number of the microorganism from each container was collected to determine the growth rate in a period of five days. A compound microscope was used to observe and count the microorganisms.

Excel was used to graph the growth of the microorganism. Whereas analysis of variance (ANOVA) where the mean was calculated for the groups and Tukey's test was used for statistical analysis of the data.

RESULTS

After collecting data on the number of microorganisms over five days, the average number of cells in the four test tubes under each condition was calculated. It was noted that there was an increase in the number of cells overall for all temperature treatments although 3 °C treatment had decreased growth 25 °C treatment had the most increased growth rate. One-way ANOVA was used to compare the mean growth rate between each temperature treatment.

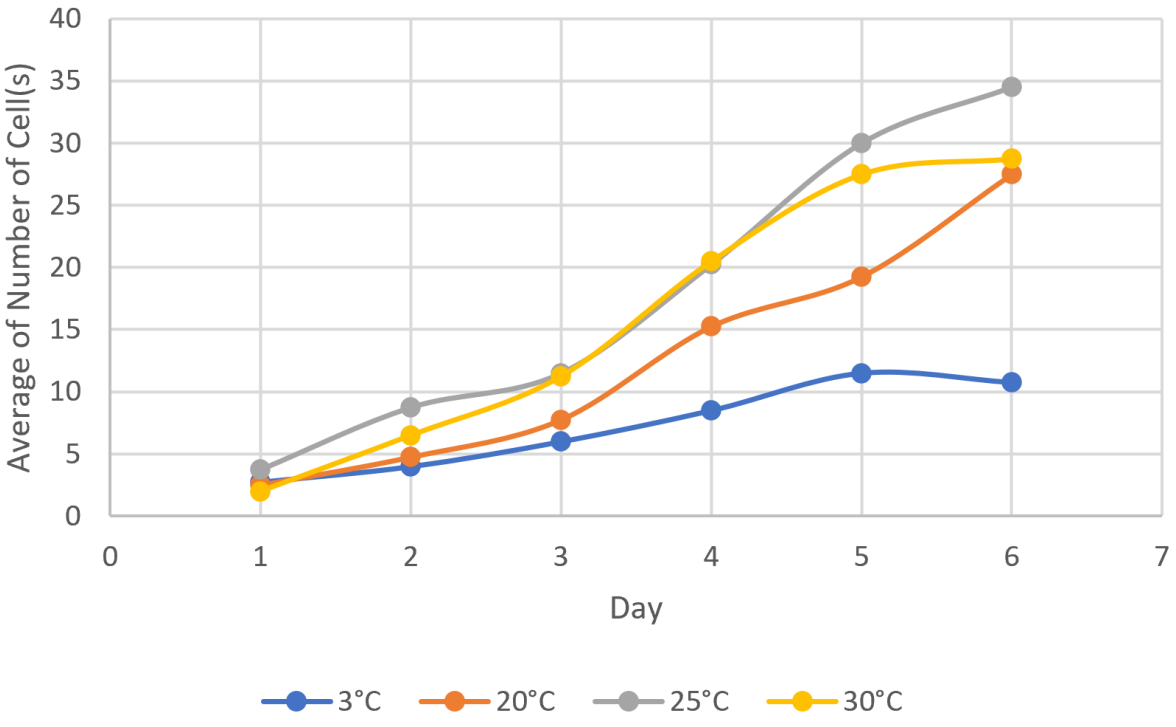

Figure 1. Growth Curve of average of Microorganisms over a period of five days. The four temperature treatments show various degrees of growth progression.

ANOVA Test

The mean growth rate for each of the treatments was compared. By generating a scatter plot and drawing the line of best fit, the mean rate was determined. The slope obtained from each graph from the four replicas was analysed with one-way ANOVA. The degree of freedom was 3 because there were 4 temperature treatments. Whereas the degree of freedom was 12 of the 16 test tubes (Data points). The result of the statistical analysis, one-way ANOVA, was the f-calculated value was 20.551 while the f-critical value was 3.490. Here the f-calculated is greater than the f-critical value. This indicates that the variance between the means of the four treatments is significantly different. Therefore, the null hypotheses that the mean growth between temperatures is the same and can be rejected.

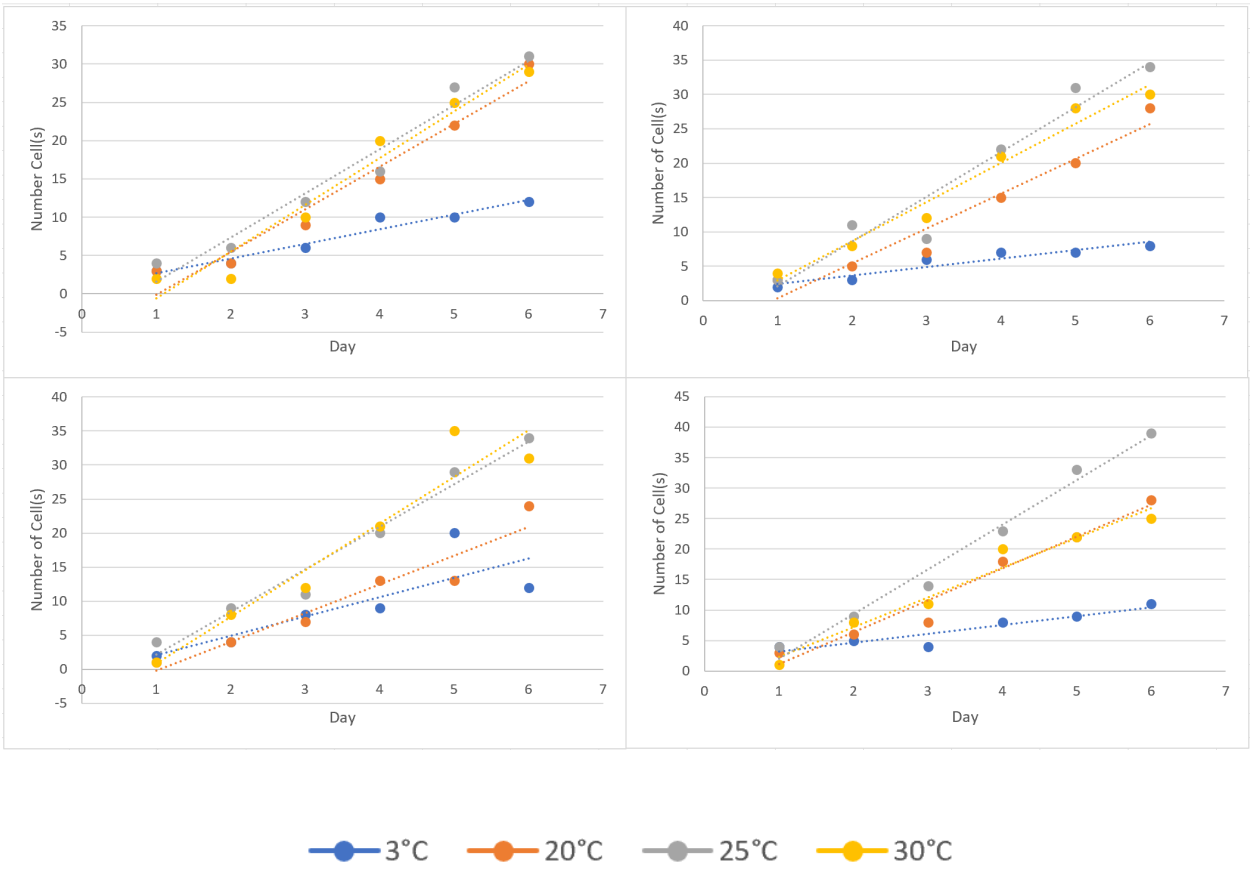

Figure 2. Cell density of the four replicates were plotted. Each graph represents one of the four replica growth trends. A linear line of best fit was used to determine the slope which is also the growth rate. This growth rate was later used for one-way ANOVA.

Tukey-Kramer Test

Tukey's test is used to find means that are significantly different from each other. Tukey-Kramer test was used to determine which mean growth rates were different in the four temperature treatments. Because the null hypothesis was rejected, this test will give the significant difference. There were four treatments while the degree of freedom was 12. The calculated p-values for 3°C treatment growth rate compared with 20, 25 and 30 °C treatment was 0.001, 0.001, and 0.006 respectively. These results are less than the p-value of 0.05, showing that the growth rate between 13 °C and other temperature treatments are different from each other. In contrast 20 and 25 °C have a p-value of 0.900 which is greater than the p-value of 0.05. A similar result was observed between 20 and 30 as well as 25 and 30 °C with p-values of 0.083 and 0.108. Both of this have a greater p-value than 0.05. This shows that the two temperature treatments do not have a significant difference between their growth rates. 3 degrees was the only temperature that had a growth rate that was significantly different from the rest.

In figure 2, the slopes of 20, 25 and 30 °C treatments are more alike the 3 °C slope. In addition, a box plot of the mean growth rates clearly illustrate how 13°C has a mean growth rate that is different from the other temperature treatments as shown in figure 3.

Results for one-way ANOVA showed f-calculated = 20.551 and p-value = 0.00005 with df = 3, df, =16 and n = 16. The difference between the mean growth rates is significantly different. The 3°C treatment's growth rate is significantly different from the other treatments as the calculated p-value was found to be 0.001, 0.001 and 0.006 when compared with 20°C, 25°C and 30°C.

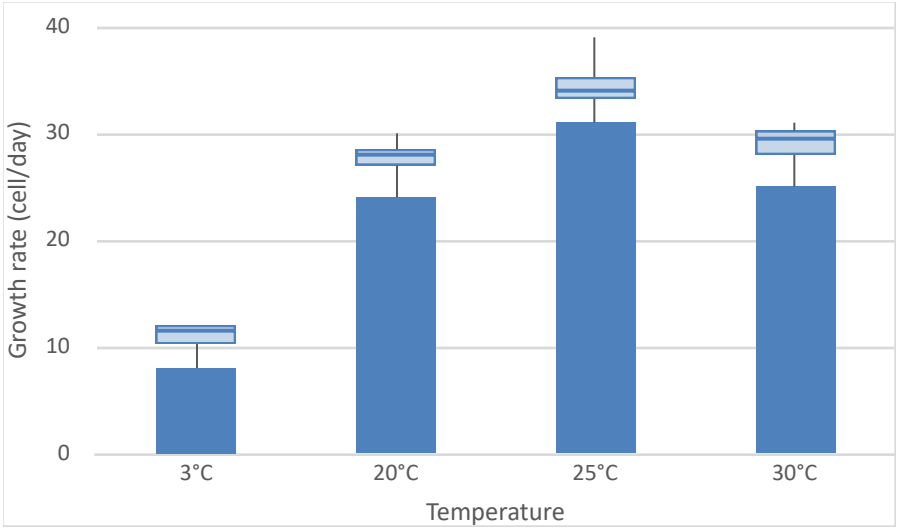

Figure 3. Box plot of growth rates for 4 temperature treatments. n=5. The upper and lower quartile are shown with uncapped whiskers. The median is the horizontal blue line in the boxes.

DISCUSSION

The f-calculated and the p-value were determined using the one-way ANOVA. The null hypothesis for ANOVA is that the mean growth rate of the temperature treatments is the same. Therefore, reject the null hypothesis due the f-calculated value being greater than the f-statistic (Dalgaard, 1998). This was also the same with the calculated p-value being smaller than 0.05 given by ANOVA. Since the mean growth rates of each temperature treatments are different, the null hypothesis that temperature does not affect growth rate was rejected and in agreement with the alternative hypothesis that temperature affects growth rate. A Tukey-Kramer test was used to determine which of the temperature treatments had significant differences from each other (Coleman et al., 2003). The results showed that the calculated p-values for 3 °C treatment growth rate was less than the p-value of 0.05 compared to 20, 25 and 30°C treatment. However, among the 20, 25 and 30°C there was no significant difference between the means of growth rates. Therefore, 3°C treatment is the only temperature treatment that is significantly different from the rest. Thus, it can be concluded that low temperatures have a negative growth rate.

Looking at figure 1, the results indicate microorganisms reaches the highest growth rates at 25°C and 30°C, since previous studies have projected optimal growth rate to range from 27 to 30°C (Wang et al., 2018). However, it was anticipated that there is a lower exponential growth phase as seen in figure 3 for treatments 20°C 25°C, and 30°C, due to an accumulation of biomass in the containers, leading to self-shading (Baranyi, 1994). This concept of self-shading occurs when microbes blocks their light source when biomass becomes high in the given volume. This is possible given that treatments: 20°C, 25°C, and 30°C were observed, to be more pigmented in comparison to the other containers. Given this information, it explains why individual growth rates are similar in those temperature treatments (Cassin et al.,1998). Thus, a lower-than-expected individual growth rate of microorganisms displayed in the results for those temperatures. On the contrary, the lowest growth rate occurred in treatments of 3°C, which aligns with the predictions and previous literature since microorganisms has the best growth rates in temperatures ranging from 27 to 30°C (Silva et al., 2017). Using these results, it can be equated to the effect of refrigeration on the growth of microorganisms. Refrigeration (3ºC) and freezing (-20ºC or less) are commonly used in the food, pharmaceutical, and biotechnology industry. Refrigeration preserves food by slowing down the growth and reproduction of microorganisms and the action of enzymes which cause food to rot. The introduction of commercial and domestic refrigerators drastically improved the diets of many in the 1930s by allowing foods such as fresh fruit, salads, and dairy products to be stored safely for longer periods, particularly during warm weather. It also facilitated transportation of fresh food on long distances. At lower temperature the microorganism activity ceases. As the temperature increases the metabolic activity of microbes increases to some extent after reaching a point again the metabolic activity of microbes decreases. These boundary values define the maximum and minimum temperature at which life can exist. Each species of microbe has its own unique upper and lower limit which is characteristic for that species.

CONCLUSION

My conclusion supports the alternative hypothesis that different temperatures affect the growth of the microorganisms differently between 3, 20, 25 and 30°C. The results show that microbes have a higher growth rate at 20, 25 and 30°C and 30°C than at 3°C. In general, an increase in temperature will increase enzyme activity. But if temperatures get too high, enzyme activity will reduce, and the protein (the enzyme) will denature. On the other hand, lowering temperature will decrease enzyme activity (Bajard et al., 1996). In general, the higher the temperature, the more easily microorganisms can grow up to a certain point. Very high and low temperatures both obstruct the enzyme processes microorganisms depend on to survive.

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APPENDIX

Table 1-4. Data of number of cells collected from the four replicates of each treatment

	3°C	20°C	25°C	30°C
Day 1	3	3	4	2
Day 2	4	4	6	2
Day 3	6	9	12	10
Day 4	10	15	16	20
Day 5	10	22	27	25
Day 6	12	30	31	29

	3°C	20°C	25°C	30°C
Day 0	2	3	3	4
Day 1	3	5	11	8
Day 2	6	7	9	12
Day 3	7	15	22	21
Day 4	7	20	31	28
Day 5	8	28	34	30

	3°C	20°C	25°C	30°C
Day 0	2	1	4	1
Day 1	4	4	9	8
Day 2	8	7	11	12
Day 3	9	13	20	21
Day 4	20	13	29	35
Day 5	12	24	34	31

	3°C	20°C	25°C	30°C
Day 0	4	3	4	1
Day 1	5	6	9	8
Day 2	4	8	14	11

Day 3	8	18	23	20
Day 4	9	22	33	22
Day 5	11	28	39	25

Table 5. The average number of cells of the four replicates

	3°C	20°C	25°C	30°C
Day 0	2.75	2.5	3.75	2
Day 1	4	4.75	8.75	6.5
Day 2	6	7.75	11.5	11.25
Day 3	8.5	15.25	20.25	20.5
Day 4	11.5	19.25	30	27.5
Day 5	10.75	27.5	34.5	28.75