LABORATORY REPORT

GROUP NUMBER: w3

EXPERIMENT NUMBER: 1

TITLE: Cell Growth Kinetics

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EXCELLENT REPORT- OVERALL GRADE 10.0

ROLE ASSIGNMENTS

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ABSTRACT

The exponential growth rate constant of *Saccharomyces cerevisiae* in an aerobic culture at an agitation rate of 200 RPM, a temperature of 30^{o}C and an air flow of 0.75 SLPM was determined. This was done by taking 2.5 mL samples every 10 minutes for a period of four hours from the original stock and measuring their absorption values in a spectrophotometer at a 550nm wavelength. A semi-log plot was made of concentration versus time and the slope of that line, $2.7E^{-3} \pm 0.1E^{-3}$ min^{-1}, was determined as being the growth rate constant at 30^{o}C. Regression analysis showed a linear relationship between the log of concentration versus time (minutes) ($R^2 = 0.993$); the confidence limits for the growth constant fall between $2.6E^{-3}$ min^{-1} and $2.9E^{-3}$ min^{-1}. Growth constants were determined by lab groups 1 (at 25 ^{o}C) and 2 (at 37 ^{o}C) to be $1.9E^{-3} \pm 0.6E^{-3}$ min^{-1} and $3.5E^{-3} \pm 0.5E^{-3}$ min^{-1}, respectively. The measured growth rate constant at 30 ^{o}C was $2.7E^{-3} \pm 0.1E^{-3}$ min^{-1}, which is consistent with a linear relationship between the data obtained by the other groups. LINEAR HOW?

GOOD ABSTRACT- 2.0

RESULTS

Results for the *Saccharomyces cervisiae* in an aerobic culture under 200RPM, 30°C, and 0.75 SLPM airflow are listed and explained below.

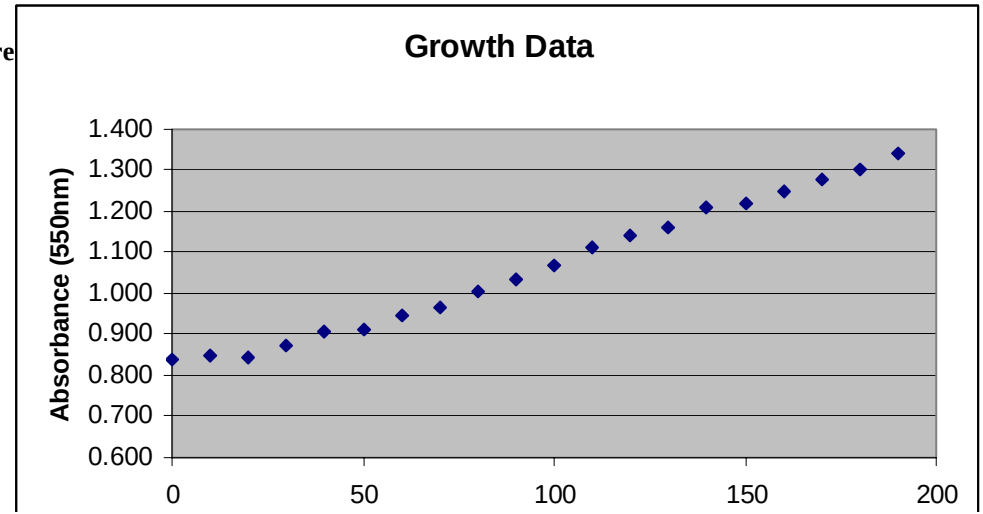
Table 1. Tabulation of Absorbance Values, Time, and Cell Concentrations

Time (min.)	Absorbance	Concentration	Conc. Uncertainty (±)
0	0.840	1.60E+08	0.00E+08
10	0.850	1.62E+08	0.00E+08
20	0.845	1.61E+08	0.00E+08
30	0.875	1.66E+08	0.00E+08
40	0.905	1.72E+08	0.00E+08
50	0.910	1.73E+08	0.00E+08
60	0.945	1.80E+08	0.00E+08
70	0.965	1.83E+08	0.00E+08
80	1.005	1.91E+08	0.00E+08
90	1.035	1.97E+08	0.00E+08
100	1.070	2.03E+08	0.00E+08
110	1.110	2.11E+08	0.00E+08
120	1.140	2.17E+08	0.00E+08
130	1.160	2.20E+08	0.00E+08
140	1.210	2.30E+08	0.00E+08
150	1.220	2.32E+08	0.00E+08
160	1.250	2.38E+08	0.00E+08
170	1.280	2.43E+08	0.00E+08
180	1.300	2.47E+08	0.00E+08
190	1.340	2.55E+08	0.00E+08

$$\text{Concentration} = \text{Absorbance} * (1.9E^{8}) \text{ cells/ml} \qquad \text{(Eq. 1)}$$

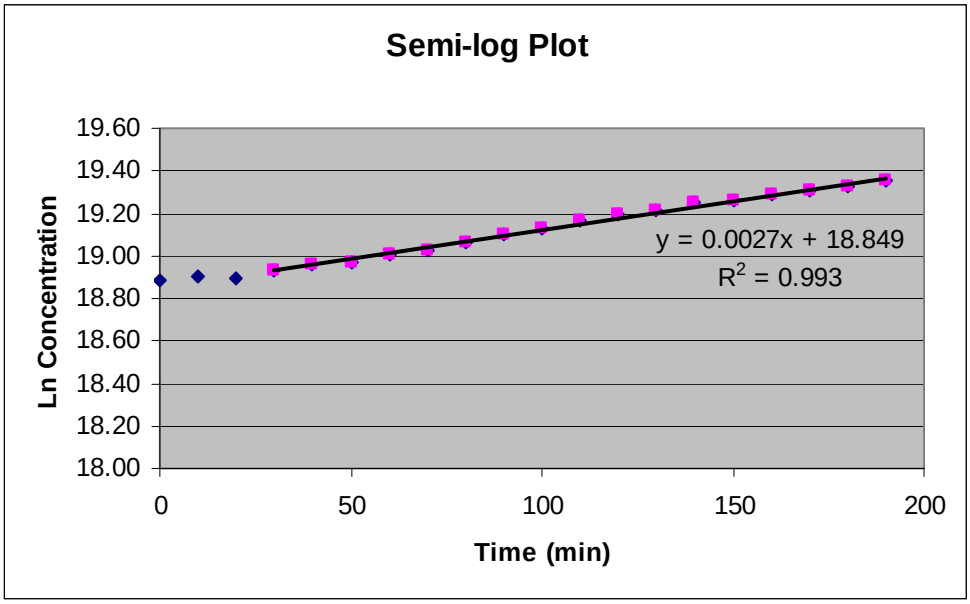
The primary source of uncertainty was due to the spectrophotometer readings ($\pm 1.0E^{-3}$). Because the uncertainty for this machine is so small, the uncertainty does not affect significant digits in the concentration. ARE YOU SAYING THE MACHINE IS GOOD TO 3 SIGNIFICANT DIGITS? IS THERE NO SAMPLING UNCERTAINTY? ALSO, REMEMBER THIS IS YOUR ESTIMATE- THE ACTUAL DATA MAY HAVE MORE SCATTER. HOW MANY DIGITS ARE THERE ON THE MACHINE? WAS IT ROCK STILL- ONCE IN THERE THE READING NEVER CHANGED?

Figure



The end of the lag phase (0-20 min), and part of the logarithmic growth (30-190 min), are evident in the Absorbance vs. Time graph. The stationary phase was never reached due to the reaction rate and time constraints. To determine the logarithmic growth region, a semi-logarithmic regression was utilized as shown in the figure below.
THIS LOOKS AWFULLY LINEAR TO ME, NOT AT ALL LOGARITHMIC, SO I DON'T SEE ANY EVIDENCE AT ALL OF LOG GROWTH. I ALSO SEE 2 POINTS THAT ARE OBVIOUSLY HIGH- DO YOU DISCUSS THEM? DID YOU TRY REGRESSING THIS LINEARLY AND SHOW IT WAS NOT AS GOOD AS LOG?

Figure 2. Semi-logarithmic plot – Ln Concentration vs. Time



Growth in this region is exponential because it is linear on a semi-log plot. ONLY IF YOU BELIEVE THE STATS The exponential growth rate constant, $2.7E^{-3}$ min^{-1} , is the slope of the semi-log plot. The 95% confidence limits for the growth constant falls between $2.6E^{-3}$ min^{-1} and $2.9E^{-3}$ min^{-1}. Using the equation $2 = e^{0.00274t}$, doubling time was calculated to be 253 minutes. Linear regression analysis was by testing several segments of the data that appeared to be in the linear region. WITH YOUR SCATTER NOTHING CAN BE TESTED BY APPEARANCE. The regression analysis for the region from 30 minutes to 190 minutes yielded the lowest standard error for the slope and minimized the 95% confidence interval (see Table 2 below). GOOD!

Table 2. Regression analysis (30 –190 minutes interval)

Regression Statistics	
Multiple R	0.996502655
R Square	0.993017541

Adjusted R Square	0.992552044					
Standard Error	0.0119802					
Observations	17					
	Coefficients	*Standard Error*	*t Stat*	*P-value*	*Lower 95%*	*Upper 95%*
Intercept	18.84858435	0.007141969	2639.136	6.39E-44	18.83336	18.86381
X Variable 1	0.00273939	5.93108E-05	46.18702	1.37E-17	0.002613	0.002866

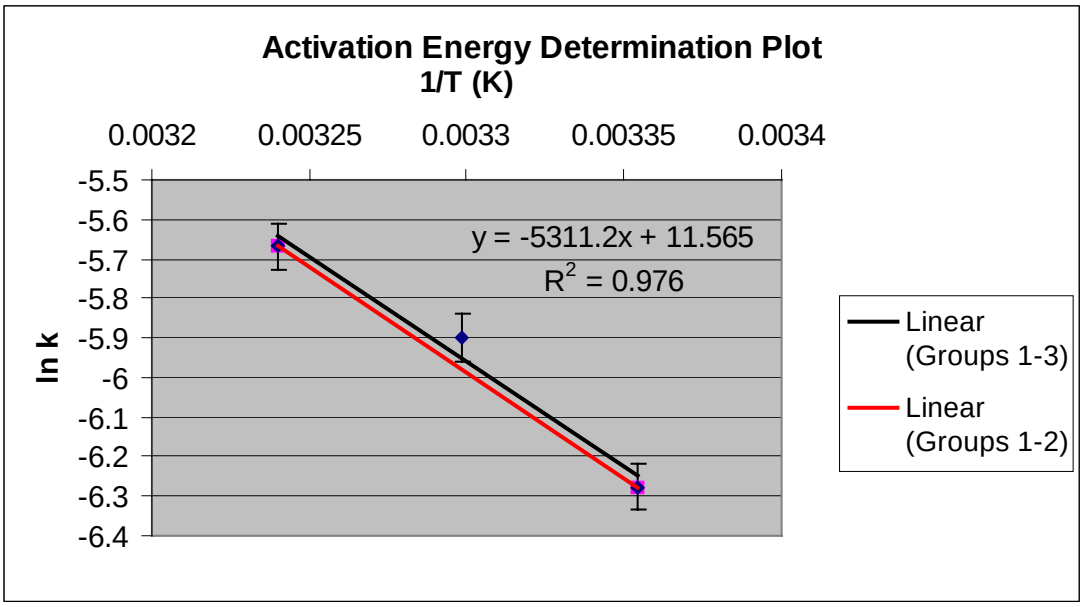
Several intervals were tested to determine the linear region. Regression analysis for the 30–190 minute interval yielded the lowest standard error and minimized the 95% confidence interval for the linear region. A TABLE SHOWING THE DIFFERENT STATS ESPECIALLY CONFIDENCE INTERVALS FOR THE DIOFFERENT REGRESSIONS WOULD BE MORE CONVINCING

Table 3. Cell Growth Kinetics Experiment Data (Groups 1-3)

Group #	*Air Flow*	*Temp. (C)*	*Temp (K)*	*1/T*	*Exp. Growth Constant (min-1)*	*ln k (ln Growth Constant)*	*Precision (+-)*
w1	0.75	25	298.15	0.00335	0.00215	-6.14	0.00062
th1	0.75	25	298.15	0.00335	0.0016	-6.44	0.00012
AVERAGE				0.00335	0.00188	-6.28	
w2	0.75	37	310.15	0.00322	0.00304	-5.80	0.00013
th2	0.75	37	310.15	0.00322	0.00386	-5.56	0.00053
AVERAGE				0.00324	0.00345	-5.67	
w3		30	303.15	0.00330	0.00274	-5.90	0.00013

NEVER AVERAGE. EACH EXPERIMENT IS INDEPENDENT, DONE BY DIFFERENT PEOPLE. REPORT AS SEPARATE DATA POINTS. ALSO, IT INCREASES YOUR N VALUE AND IMPROVES THE POWER OF THE STATISTICS

Figure 3. Determination of Activation Energy for Yeast



The growth rate constants of groups with the other conditions were determined by collecting the results of Bioengineering 210 Lab Groups 1 and 2 (25°C and 37°C, respectively) and averaging the values for each of the conditions. The experimental result for the growth constant at 30°C $2.7E^{-3} \pm 0.1E^{-3}$ – was added, and the data was

plotted on a semi-log plot of ln k vs. $Temp^{-1}$. The slope of this curve equals the activation energy divided be the gas constant. Therefore:

Slope = E_o / R
E_o = Slope*R
E_o = -5311.2*(1.987 cal/mol*K) = -10.55 kcal/mol

There is considerable uncertainty in the slope, and the 95% confidence interval ranges from 3843 to –14469. This allows for E_o values ranging from 7.64 kcal/mol to –28.85 kcal/mol. THIS IS A REASONABLE RANGE- YOU WOULD GET BETTER RESULTS WITHOUT AVERAGING.

Table 4. Investigation of Effect of Cell-Settling and Cell Growth on Absorbance Reading

Time (min)	Absorbance Reading
0 (initial)	1.340
2	1.350
4	1.350
6	1.340
8	1.340
10	1.340

Experimental errors, accounting for the effect of cells settling in the cuvette before spectrophotometer reading, were taken and are shown above. A cell sample was taken and left inside the spectrophotometer for ten minutes.
GOOD RESULTS- 4.0

ANALYSIS

Averaging the growth rate constants obtained by lab groups 1 and 2 at 25°C and 37°C yields growth constant values of $1.90E^{-3} \pm 0.6E^{-3}$ min^{-1} and $3.50E^{-3} \pm 0.5E^{-3}$ min^{-1}, respectively. Qualitatively, when the temperature is lowered, the yeast remains in the lag phase for a longer period of time, WHEN THE CULTURE WAS PREPARED, WAS THE INITIAL PHASE DONE AT THE EXPERIMENT TEMPERATURE OR AT 37 C? and when it does progress to the exponential phase, the graph of the yeast growth verse time climbs more slowly. Since the temperature influences the rate of reaction, the experimental data for the growth constant at T = 30°C would be expected to lie on the line, nearly midway NO- IT IS A 1/T RELATION between the two data points. Quantitatively, if the relationship between temperature and rate of reaction is linear, NO- YOU SHOW IT IS A SEMILOG RELATION VS I/T- THIS IS VERY NONLINEAR. the growth rate constant for group 3 should be $2.84E^{-3} \pm 0.6E^{-3}$ min^{-1}. I THINK YOU GOT THIS VALUE CORRECTLY The measured value was $2.73E^{-3} \pm 1.30E^{-3}$ min^{-1}. Since the uncertainty ranges for the predicted value and measured value overlap, the measured growth rate does not vary significantly from existing data. This reveals a linear correlation between temperature and the growth rate of yeast. WATCH YOUR LANGUAGE- YOU DON'T MEAN THAT.

Figure 3 shows temperature and growth constant data graphed on a semi-log plot of ln k vs. $Temp^{-1}$. The 95% confidence interval cannot be determined for the line-of-best-fit because it is merely connecting two data points. However, when group 3 data is added, regression analysis can be done on the line. WOPULD BE BETTER WITH 5 POINTS! The activation energy (E_o) was determined BEST VALUE to be –10.55 kcal/mol (see Results, Fig. 3), and E_o values within a 95% confidence interval ranged from 7.64 kcal/mol to –28.85 kcal/mol. The accepted range for E_o during growth stage is –10 kcal/mol to –20 kcal/mol. THIS IS NOT ACCEPTED- IT IS TYPICAL FOR MANY ORGANISMS BUT IT MAY NOT AT ALL APPLY TO YEAST Therefore, the data collected in lab contains the accepted range within its confidence interval (i.e., the activation energy is not significantly different). This merely means that the experiments were conducted with less precision than the group of experiments that determined the accepted values, which is not surprising given the fact that the data used to calculate E_o was collected by different BE 210 lab groups at different times. ARE NOT YOU GUYS AT LEAST AS GOOD AS THEY ARE? PROBABLY BETTER!

Two types of experimental errors in the measured variables were analyzed to determine their effects on the results of this experiment. First, failing to properly dry the cuvette after rinsing it between spectrophotometer readings may have left a residue of water inside the cuvette. This would affect the sample by diluting it, resulting in a smaller absorbance reading and thus indicating a smaller yeast cell concentration. The volume of the residual water droplet inside the cuvette can be estimated to be less than 1 μL. As the amount of yeast sample added was 2.5 mL each time, the error resulting from the residual amounts to 1 part per 2500, or 0.01%. This is not a significant error, but should be eliminated if possible. WHY BOTHER IF IT DOES NOT EFFECT YOUR RESULTS? YOU GET NO BROWNIE POINTS FOR BEING HOLIER THAN THOU! However, if the amount of water left from rinsing were 50 μL instead of 1 μL, this would have resulted in 0.5% error. This would have affected the last significant figure of the concentration of the yeast sample, yielding significant error. If this was the case, improper drying would have to be taken into account as a source of error, but since there was typically < 1 μL of water in the tube, it is insignificant. I LOVE YA!

Additionally, it was thought that experimental error might have resulted from cells settling in the cuvette during the time the spectrophotometer reading was being taken. To determine whether or not this occurred, a cell sample was taken and left inside the spectrophotometer for ten minutes, and absorbance readings were taken every two minutes (see Data, Table 4).

The results of this trial can be explained as follows. Expectations are that the yeast cell growth, which would lead to an increase in absorbance reading, would be apparent for the first few minutes, when the temperature of the sample is still suitable for yeast growth. When the temperature of the sample adjusts to that inside the spectrophotometer, however, it is expected that yeast growth would stop since the temperature inside the spectrophotometer is not suitable for yeast growth. The expected increase in absorbance, from 1.340 to 1.350, is observed at time equals 2 minutes. DID YOU USE YOUR

GROWTH CONSTANT TO PREDICT THIS? At time equals 6 minutes, however, the absorbance reading dropped back to 1.340. The dropPROBABLYoccurred because settling cells created a less concentrated region at the point where the spectrophotometer reading was taken, resulting in a smaller absorbance value. Since all readings were taken less than 10 seconds after placing the sample in the spectrophotometer, neither settling nor continuing cell growth had a significant effect on the overall data.

Thus, the possible errors in the conduct of this experiment stem from that of residual water in cuvette (0.01% error) and settling amongst the cells. ONLY LAWYERS WORRY ABOUT WHAT IS POSSIBLE. SCIENTISTS AND ENGINEERS WORRY ABOUT WHAT IS HIGHLY PROBABLE. To eliminate the residual water error, cuvettes could be made to dry faster each time with the use of a blowdryer. The best way to avoid settling error was to take the absorbance readings immediately after placing the cuvette in the spectrophotometer.

Given the time constraints of this lab, the educational goals of accurately determining the growth rate constant and observing cell growth would have been better met by using a different organism. *E. Coli* would have made a good choice because its growth rate at 37^oC (0.0347 min^{-1}) is ten times more than that of *Saccharomyces cerevisiae* at 37^oC (0.0035 min^{-1}). Using *E. Coli* would have allowed groups to observe the entire growth process of the sample, especially at lower temperatures, where growth is not optimal. One drawback to this would be that sampling time would have to be decreased, in order to obtain enough points during the logarithmic growth period. SINCE YOUR GOAL WAS YEAST THE ABOV E PARAGRAPH IS OUT OF PLACE AND SHOULD BE OMITTED.

In conclusion, in this experiment, the exponential growth rate constant of *Saccharomyces cervisiae* was determined by making a semi-log plot and taking the slope of the linear region of the graph. The aerobic culture, being at an agitation rate of 200 RPM, a temperature of 30^oC, and an air flow of 0.75 SLPM, the growth rate constant was determined to be $2.7E^{-3} \pm 0.1E^{-3}$ min^{-1}. A linear relationship existed between the log of concentration versus time. Several sets of regression statistics were taken to determine the linear range of the semi log plot of concentration versus time. The 95% confidence interval provided the best results for the points that lie between 30-120 minutes. The confidence limits for the growth rate constant were determined to be $2.6E^{-3}$ min^{-1} to $2.9E^{-3}$ min^{-1}.

The experimental and educational objectives were both met. Group members measured absorbency levels of yeast as a function of time and quantitatively analyzed both the data obtained in the lab and possible techniques that would upgrade and minimize future error in the apparatus.

EXCELLENT ANALYSIS 4.0

REFERENCES

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