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A comparison of optical measurement methods for the growth of S. cerevisiae

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A comparison of optical measurement methods for the growth of *S. cerevisiae*

Honors Undergraduate Thesis

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Abstract

Genetic engineering of living organisms provides the opportunity to express and harvest different proteins from cell surfaces. Yeast (*S. cerevisiae*) is one such organism and is capable of being grown on an industrial scale. Cellular concentration is an important parameter to monitor while fermentation processes are underway, in order to control the environment inside the growth medium and maximize yields. Spectrophotometry is a conventional method for measuring concentration, but is limited by a narrow absorbance range, and the need for on-site periodic sampling. A continuous method of measurement, as provided by Bug Labs BE2100 non-invasive biomass monitor, would allow for remote monitoring of cell concentration, and an increased sampling rate. Previous studies on the use of the "BugEye" as a continuous measurement device for *E. coli* growth failed to yield a simple correlation between absorbance measurements using a spectrophotometer and the Bug Unit of the BE2100. This study aims to monitor fermentation of *S. cerevisiae* and produce a useful correlation between optical density from a spectrophotometer and the BugEye output. The results were not conclusive on a definite conversion; however, strong linearity and minimal error was found between the two measurements. Further research is likely to quantify their relationship.

Background

Yeast (*S. cerevisiae*) are capable of being genetically modified to create different sequences of proteins. The proteins are expressed on the surface of the yeast cell and can be cleaved off for use in a variety of applications. Fermentation, a process by which the yeast is grown, can be performed in different systems (shaken flask or bioreactor), and the most important metric for protein yield is the amount of yeast created. Measuring growth in an accurate and precise manner allows for the optimization of other conditions (temperature, pH, dissolved oxygen (dO%)) that provide the best environment for the yeast. Multiple methods exist for measuring the growth of biomass, with different sampling rates (discrete vs. pseudo-continuous) being a major component differentiating them.

The simplest method for measuring growth is a direct measurement of cell mass, where a sample of the growth medium is taken, dried, and weighed for a mass of yeast per sample volume. Care must be taken not to char the cells, so temperature and drying time must be tightly controlled to ensure that there is no damage or loss of mass of the sample. This method isn't widely applicable since the time to prepare, dry, and weigh a sample accurately is often as long as the growth window of the yeast.

Another method using discrete sampling is hemocytometry. A sample of growth medium is appropriately diluted and inserted into a specially constructed chamber, where a background grid provides a reference to count the number of cells present. The chamber is manufactured to a precise volume that can be used along with the number of cells counted and an appropriate dilution factor to compute cell concentration [1]. While the method was originally created to count blood cells, it is an appropriate method for counting cells in general, including yeast.

Problems present with hemocytometry include the presence of foreign particles obscuring or being counted as yeast cells and relying on human observation to quantify the number of cells.

A third method relying on discrete sampling is spectrophotometry. A spectrophotometer utilizes the Beer-Lambert Law to relate absorption of a wavelength of light to the concentration of a solution. The spectrophotometer is zeroed with a blank, a sample of sterile growth medium, and then used to measure the absorption of inoculated medium. The yeast will selectively absorb wavelengths (~600 nm) which can be used to determine the optical density (OD) of the solution. It is important to note that OD is a dimensionless quantity representing absorbance and is *proportional* to a concentration value. A spectrophotometer works in a range between 0-1 OD, and samples with absorbances higher than 1 must be diluted for accurate measurement. Spectrophotometry provides a superior measurement of concentration; foreign contaminants are less likely to interfere with/absorb a particular wavelength of light, and the cells in solution are at no risk of thermal damage/loss from absorbing light in solution.

A common drawback to these discrete sampling methods pertains to the need for periodic sample taking and measurements that consume time that could be allocated to more important tasks. The repetition of the sampling process also increases the risk of introducing human error. Continuous methods of measurement are available for monitoring concentration of cells in solutions without the need for human intervention. The recording of data in a pseudo-continuous manner (up to once a minute) readily supplies more data for analysis as well.

Impedance Spectroscopy is a method of continuous measurement characterizing the conductive behavior of a solution as cells grow. A capacitance meter is suspended inside the growth medium and measures the permittivity and conductivity of the solution over time, which directly corresponds to the amount of yeast in the solution. Measurements of impedance can be

complex to interpret, however; changes in pH from respiring yeast, and any conductive ions in the medium can affect impedance of the solution [2].

Fluorescence spectroscopy is a second technique for continuously measuring the amount of growth in solution. NADH, a molecule central to cellular respiration, fluoresces at 445-460 nm, resulting in visible violet/blue light, while the oxidized form NAD^+ does not fluoresce. As NADH is produced during respiration, where ATP is synthesized in the cell, and the ratios of NADH to NAD and NADP are tightly controlled by cellular processes, the concentration of *living* yeast cells [3] can be easily determined by monitoring the intensity of fluorescence.

Thirdly, Raman Spectroscopy is a continuous method of measurement that uses the scattering of light to determine the composition of a sample. A laser is beamed into a relevant solution, where some light is absorbed and then re-emitted at a different wavelength. These different wavelengths are separated with a filter and analyzed over time. The intensity of the scattered light changes with the concentration of different species in solution, providing a non-destructive method of measuring not only yeast, but other chemical components such as glucose and ethanol that are compatible as well [4]. However, difficulties lie in the processing of signals, and determining if chemical components are interfering with each other's scattering.

For this experiment, continuous measurements are provided by the Bug Labs BE2100, also known as the "BugEye". The BugEye is attached to the outside of the bioreactor vessel, shines infrared light into the vessel, and measures the absorbance of infrared light at a wavelength of 850 nm, using multiple laser emitters and sensors for an accurate average. This works analogously to the spectrophotometer; however, the measurement of the BugEye claims accuracy between a range of 0.1-300 OD [6]. Although the BE2100 outputs a signal in "Bug Units", it is calibrated with yeast biomass at known OD values, suggesting that a correlation

could be drawn between Bug Units and other units of measurement. The output of the BugEye is affected by multiple factors, including sparge and stir rates, which must be accounted for when using the raw output from the sensor.

Previous work has been conducted over the correlation between BugEye output and OD measurement for *E. coli* grown in a bioreactor system. Moreland [5] suggests that the correlation between OD and Bug Units is non-linear. Their observed range of OD is between roughly 0.1 and 1.7, and the BugEye output is measured at 500, 750, 1000, and 1250 RPM. The correlation between the two adopts a sigmoidal or logistic curve, which behaves more linearly with increasing RPM. The correlation between RPM and Bug Units, however, is linear. Of note from this experiment is that the observation of *E. coli* is conducted for only 12 hours; the system does not complete a logarithmic phase of growth.

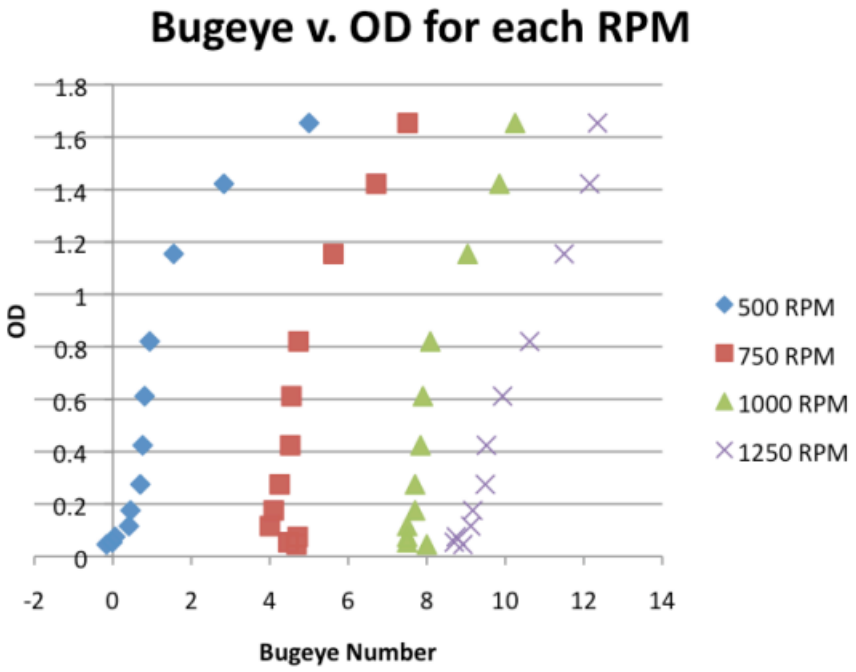

Figure 1. Average Bugeye Number vs. OD for E. coli, from H. Moreland (2015) [5]

Objective

This experiment aims to correlate the behavior of the BE2100 non-invasive biomass monitor with conventional spectrophotometry for the growth of *S. cerevisiae* in a bioreactor system. The sparging inside the vessel will be controlled, and raw output of the BugEye sensor will be recorded at different stir rates (100, 250, 500, and 750 RPM) to be compared with measurements of OD from a spectrophotometer. These measurements will be used to build a model between all three parameters and evaluated for future use in observing and optimizing the growth of yeast using a bioreactor.

Materials

Growth Medium:

Minimal yeast growth medium is prepared in the following manner, using per liter of solution:

- 6.7 g casamino acids
- 5 g yeast nutritional base (YNB) + ammonium sulfate mix
- 20 g of sugar* [Medium Dependent]

Both SD and SG mediums are used in this experiment; dextrose and galactose are used respectively as the choice of sugar. Casamino acids and YNB are mixed together with roughly 900 mL of nano-pure water, and autoclaved. The sugar is dissolved with nano-pure water separately in a sterile environment, and not autoclaved to prevent caramelization. Both mixtures are then combined, and additional water is added to produce 1 L of the desired growth medium.

Equipment:

The bioreactor used in this experiment is an Applikon 1 L bioreactor. All associated controls, probes, and equipment were controlled and continuously monitored by BioXpert software provided by Applikon. pH was monitored by an Applisense pH probe but not controlled. Temperature was monitored by a stainless-steel probe and controlled using a heating jacket to keep temperature at 30°C. Dissolved oxygen content (dO%) was monitored using another Applisense dO% probe, and air was continuously sparged into the medium at 1 L/min with additional pure oxygen supplied as necessary to maintain saturated conditions in the medium. Stirring was provided by an attached motor and modulated by the dissolved oxygen controls. Transfer of sterile media into the bioreactor is achieved using a Cole-Parmer Masterflex Peristaltic Pump.

OD was measured using both the Bug Labs BE2100 biomass monitor, and a Beckman Coulter DU800 spectrophotometer. The BE2100 measures absorbance at 850 nm, and the spectrophotometer measures absorbance at 600 nm. Raw Bug Eye output is captured and recorded by BE2100 control software simultaneously with the BioXpert software.

Figure 2. A photo of the DU800 spectrophotometer.

Figure 3. A photo of the Bug Labs BE2100.

Procedure

Seed Growth:

A seed culture of EBY100-GFP inoculates 10 mL of SD medium and is incubated on a shake-plate at 30℃ and 250 rpm for 48 hours. Ideally the seed reaches ~10 OD, to promote immediate log phase growth in the reactor.

Equipment Preparation:

The bioreactor, growth medium, transfer tubing, and other misc. glassware were autoclaved at 121℃ for a minimum of 27 minutes, for sterility.

Preparations:

The growth medium is pumped into the bioreactor using sterile tubing. The reactor is assembled and probes for temperature, pH, dO%, are inserted into the medium. A heat jacket is attached to the outside of the glass vessel, and the BugEye camera is strapped on over a viewing port. Environmental controls are applied for temperature (30℃) and dO% (90%) to promote respiration and aerobic growth. Aeration of the sterile media proceeds for a minimum of 12 hours to calibrate the oxygen probe to saturated conditions. Antifoam is added as necessary to reduce volume loss by surface foaming.

Experimental Procedure:

The seed culture is removed from incubation and the OD is measured with the spectrophotometer. This measurement is used to compute the starting OD when the seed is added to the bioreactor. After adding the seed to the bioreactor volume, growth is continuously observed by the BugEye, and the system is allowed to ferment until the stationary phase is reached, typically after 3-5 days. At irregular intervals, roughly 2-3 times a day, the stir rate is fixed at 100, 250, 500, and 750 RPM and the BugEye output is recorded at each. Additional antifoam is added when foam is observed on the surface. A sample of medium from inside the reactor is taken and used to prepare a cuvette. A blank cuvette with SG medium is used to calibrate the spectrophotometer, and the OD is recorded from the sample cuvettes. If OD is outside the range of the spectrophotometer (0-1) then a dilution is performed by a factor of 10 and measured for an accurate reading.

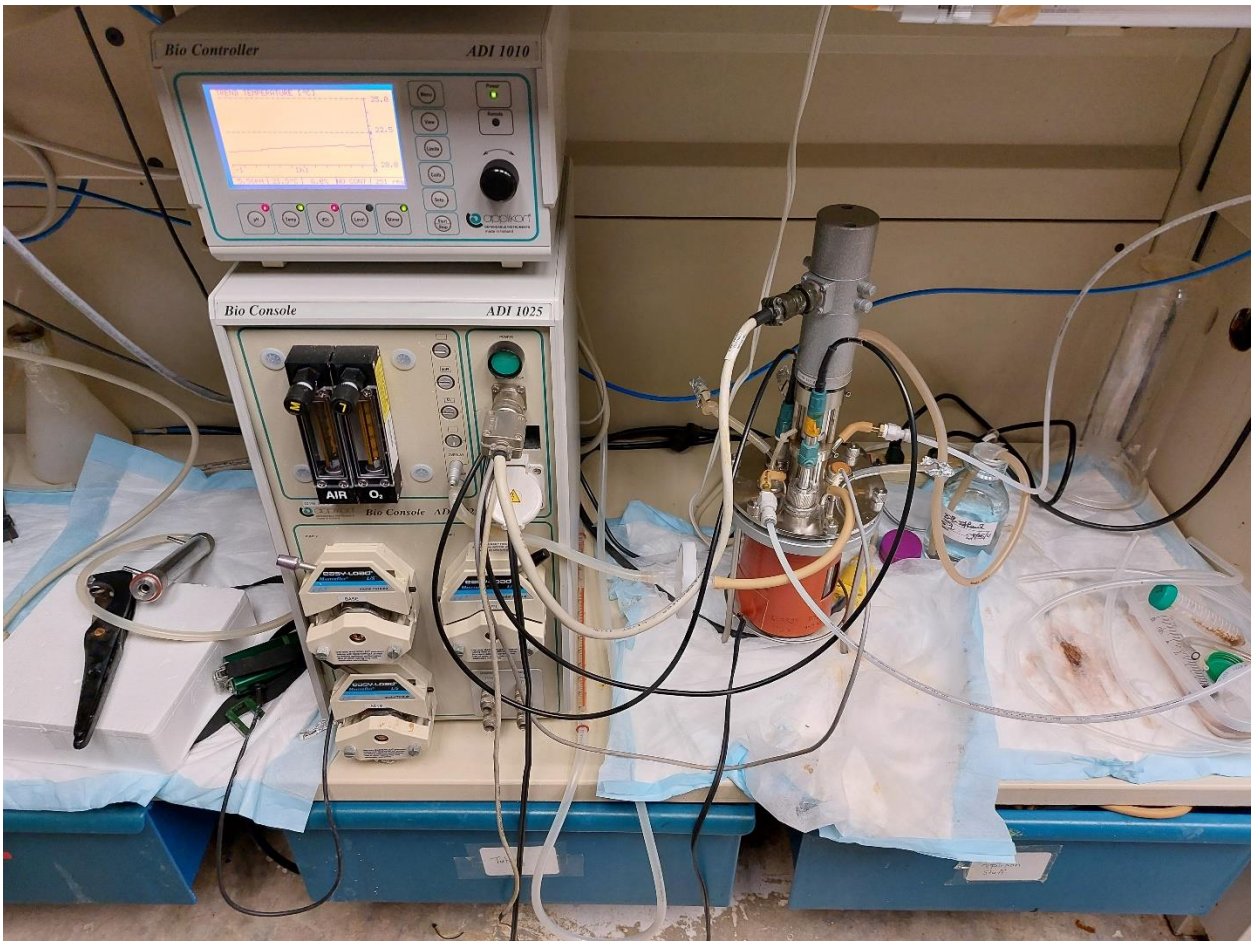

Figure 4. A photo of the fully assembled bioreactor system. The BugEye sensor is not strapped onto the vessel.

Data & Results:

Duplicate trials were attempted using the bioreactor to grow *S. cerevisiae*. Both the OD measured by the spectrophotometer and the Bug Eye output are recorded and shown by Figures 5, 6, and 7. Each point is a measurement taken at a time measured in hours after the start of fermentation. For OD, raw spectrophotometer data is recorded for both dilute and undiluted solutions, even though absorbances above 1 are inaccurate. A corrected, "actual" OD is compiled from both sample sets as shown in Figure 8.

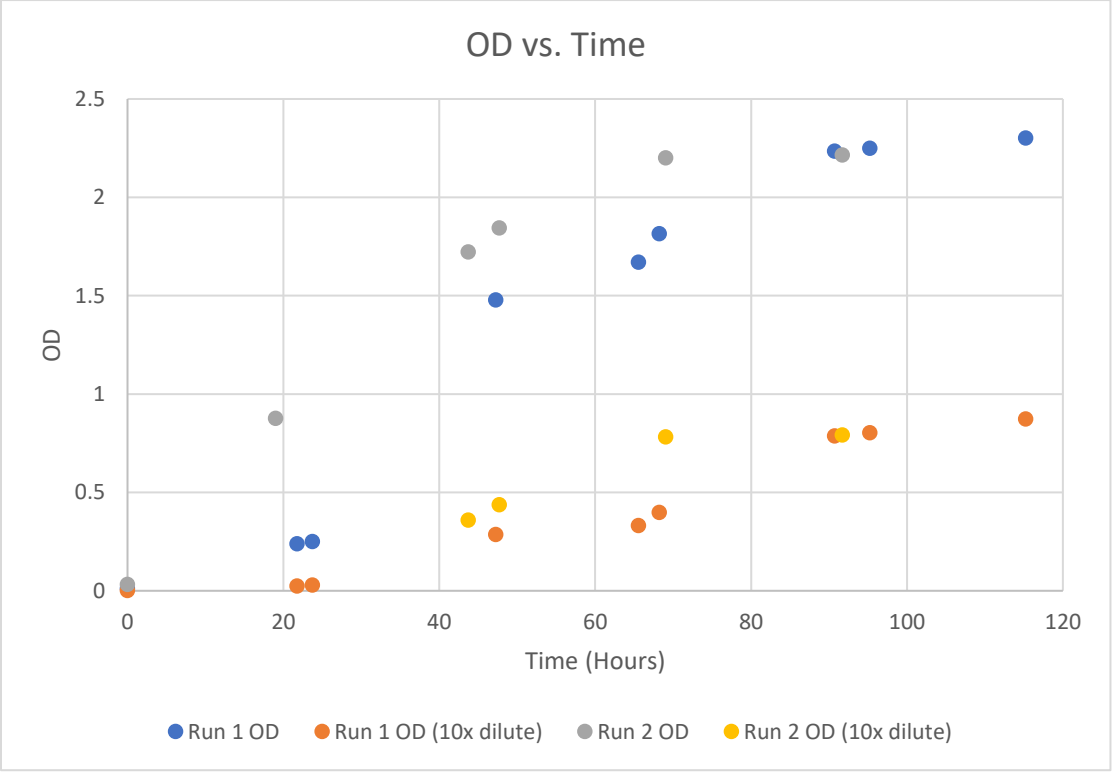

Figure 5. A plot of OD over time for both experimental trials3

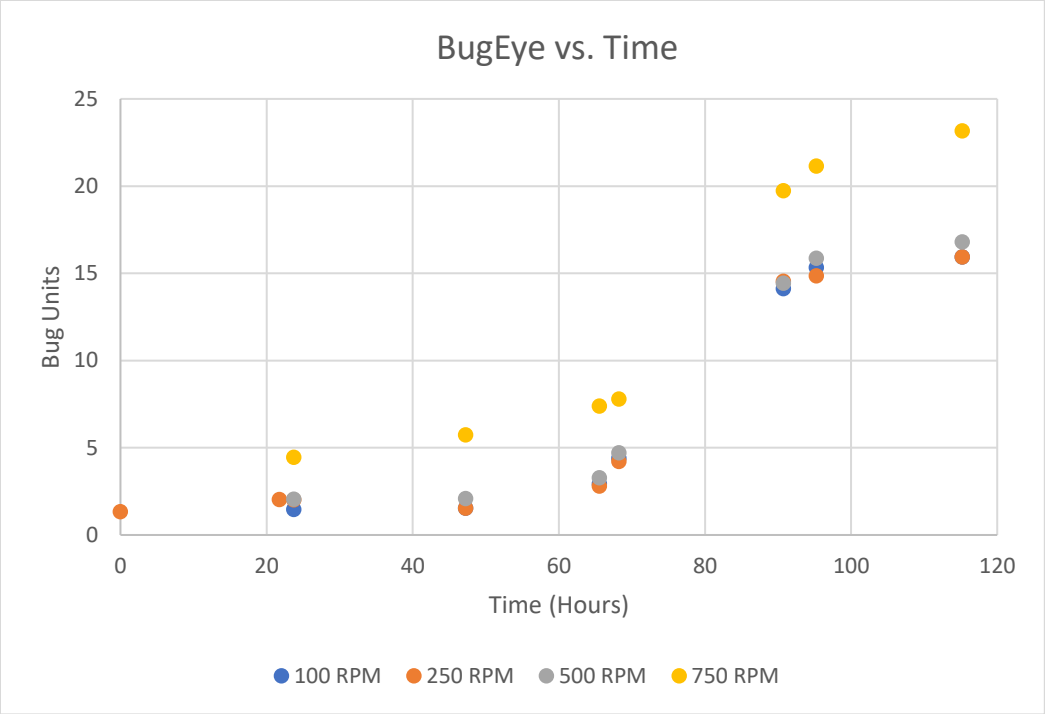

Figure 6. A plot of raw BugEye output for trial 1.

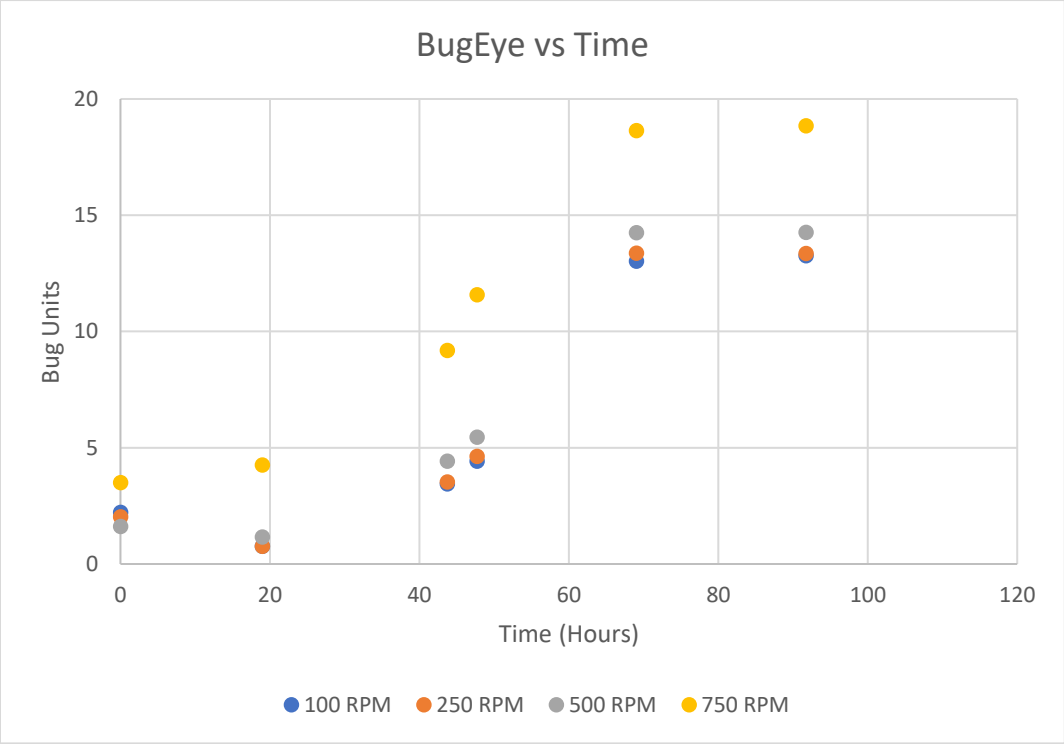

Figure 7. A plot of raw BugEye output for trial 2.

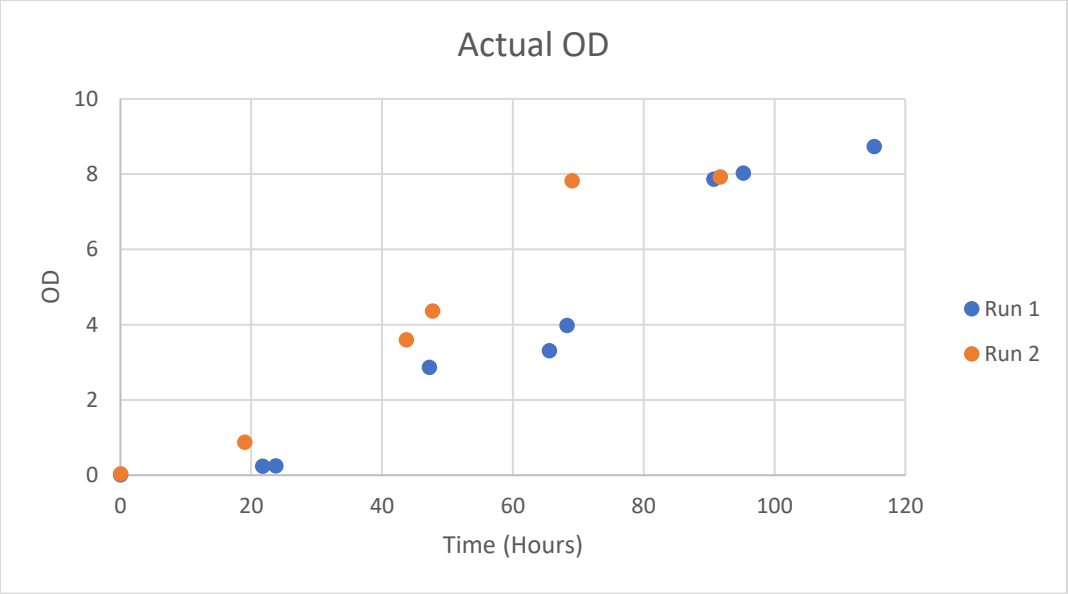

Figure 8. Actual OD of media, computed from diluted spectrophotometer measurements.

Both methods of measurement present sigmoidal growth curves, which match the expected growth curve for yeast fermentation. The plots suggest that Bug Units and OD tend to increase with each other. The trend between them appears to be linear in nature; a comparison between the recorded OD and BugEye output is shown in Figures 9 and 10. The linearity of the data increases when excluding measurements taken with OD < 0.4, as shown in Figures 11 and 12. Although accuracy is claimed in a range extending to 0.1 OD, changes in absorbance and concentration were not recorded by the BugEye until around 0.3-0.4 OD in practice. This linear behavior between OD and Bug Units for *S. cerevisiae* versus *E. coli* is possibly explained due to a difference in cell size, as the average cell size of yeast (1-10 μm) is an order of magnitude larger than *E. coli*. (0.1-1 μm). Furthermore, a completion of the log-phase of growth is reached in this experiment, whereas Moreland [5] finished testing inside the log-phase, which may have eliminated more linear behavior as OD increased.

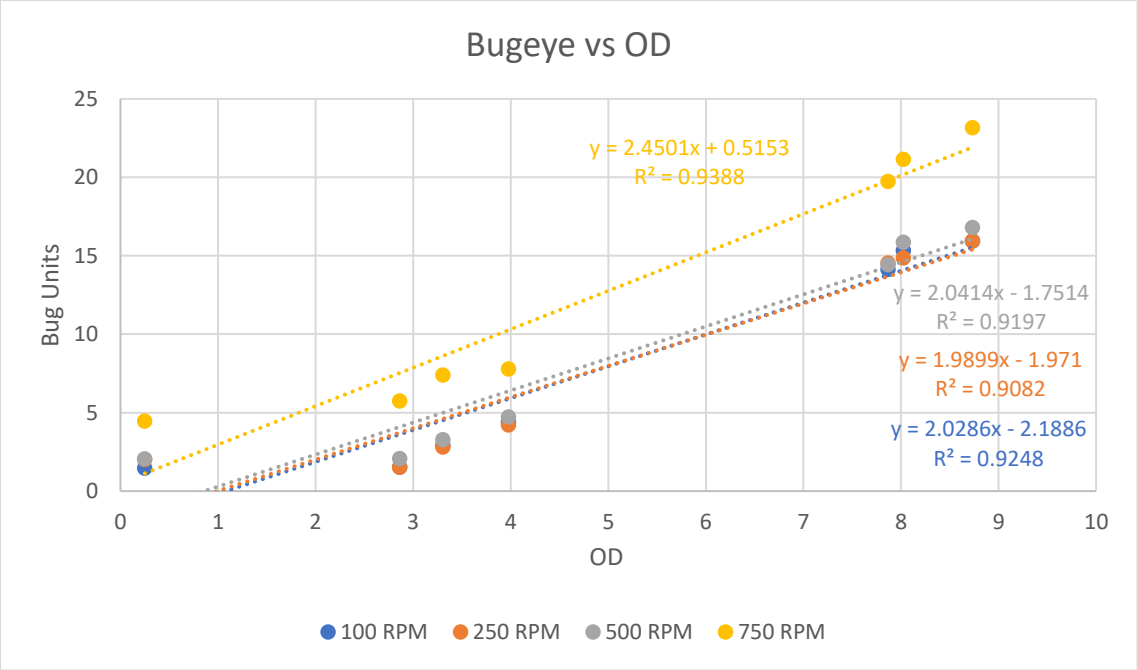

Figure 9. Bug Units versus OD for trial 1, at different RPM.

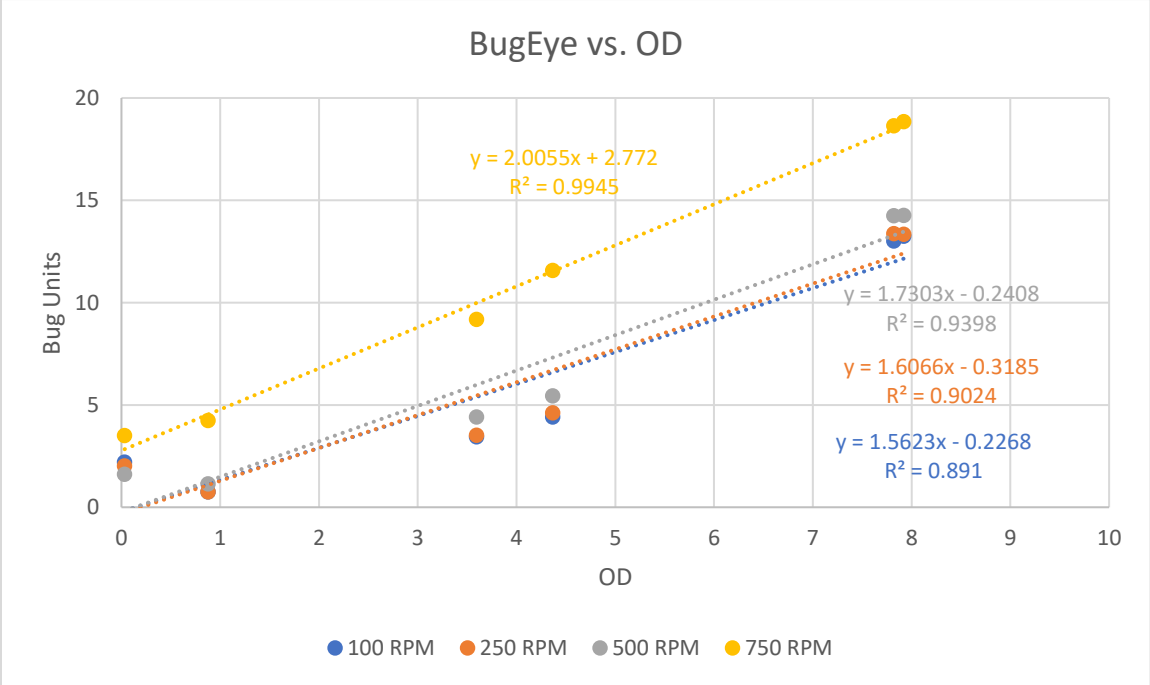

Figure 10. Bug Units versus OD for trial 2, at different RPM.

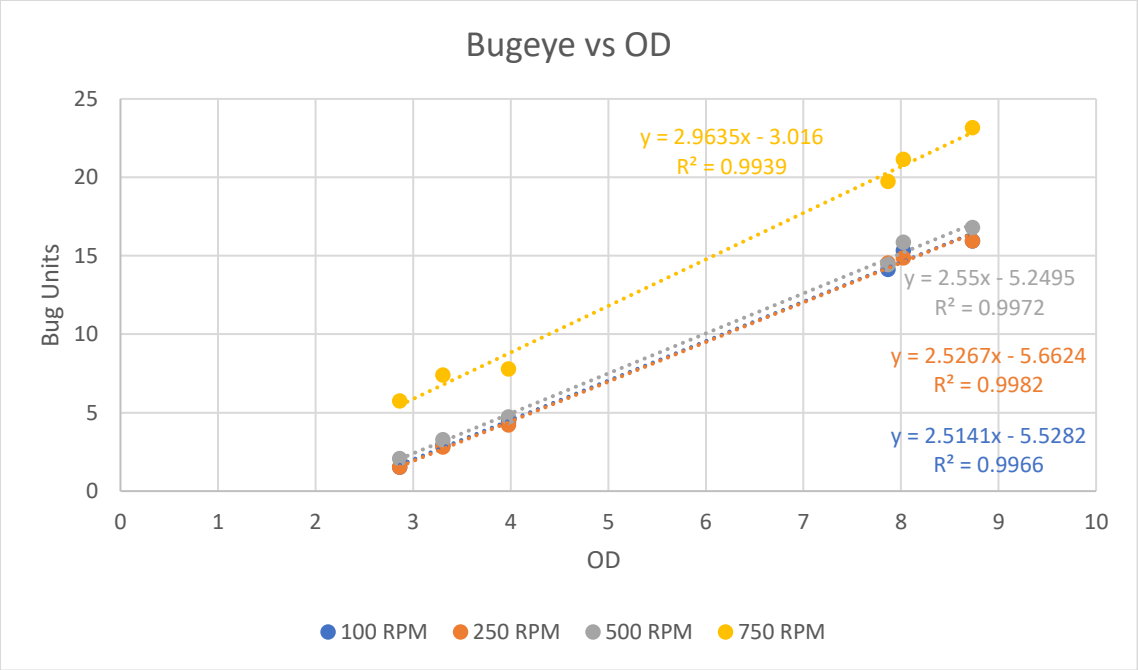

Figure 11. Bug Units versus for trial 1, excluding OD < 0.4

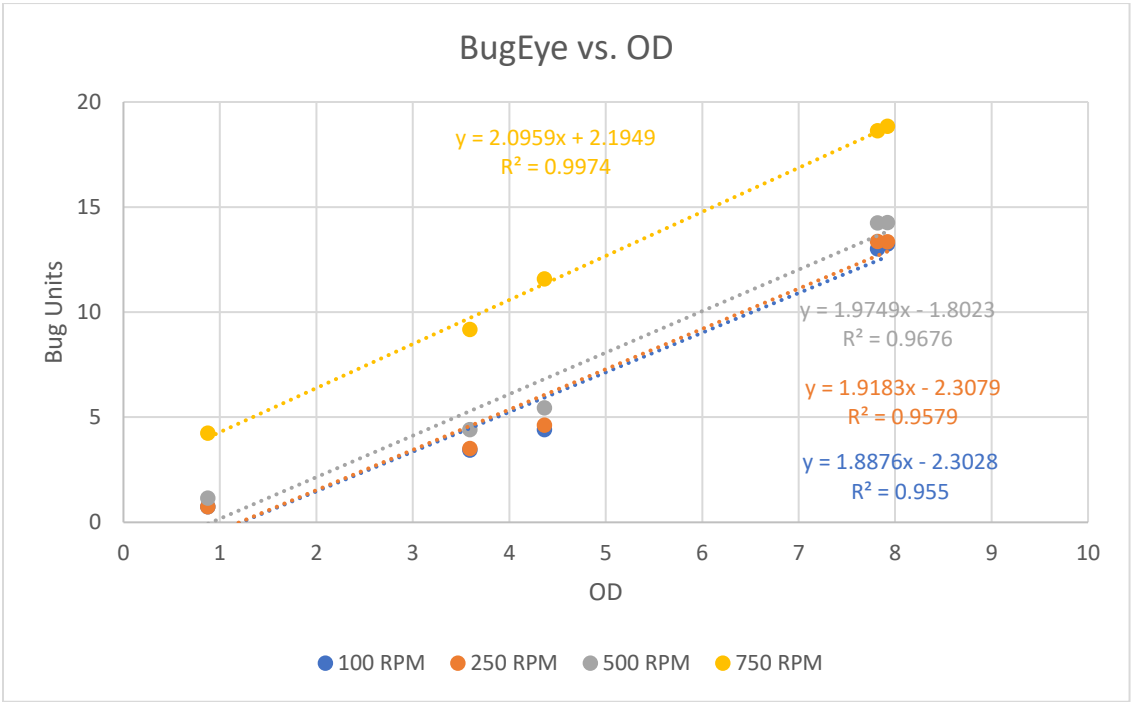

Figure 12. Bug Units versus for trial 2, excluding OD < 0.4

These results are promising for developing a future model of BugEye behavior, however no clear determination on concentration can be made from only Bug Units yet. Trials 1 and 2 differ in recorded Bug Units for the same range of OD values, complicating a potential relationship between the two. Variation is likely due to inconsistent foaming behavior and volume loss during the experiment, which may change the medium composition and directly affect the absorbance measured. Further experimentation is necessary to determine the behavior of the BugEye sensor given a specific optical density.

Additional plots of the behavior of RPM versus the BugEye or slopes of the trendlines are given in the appendices. An exponential relationship is displayed between increasing RPM and the recorded Bug Units, as well as the slopes of the trendlines between Bug Units and OD, in contrast to the linear behavior measured by Moreland [5].

Conclusions

While the Bug Labs BE2100 can observe changes in concentration of yeast undergoing fermentation in a bioreactor, an exact conversion between OD and Bug Units is still unknown. High linearity between increases in OD and Bug Units suggests that the BugEye can determine precise concentrations of cells in solution, and that interference/noise from sparging/stir rate is negligible; in practice, a different range of Bug Units is obtained for different trials for similar OD ranges. Although these results cannot be used to build a model of BugEye behavior, it is likely additional experimentation could reveal the reason for discrepancies or provide more consistent measurements between trials, producing an accurate conversion to OD.

References

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[5] Moreland, H. (2015). A study on the BE2100 Noninvasive Biomass Sensor as an instrument for measuring optical density during fed-batch fermentation at various stir rates. Biological and Agricultural Engineering Undergraduate Honors Theses Retrieved from https://scholarworks.uark.edu/baeguht/24

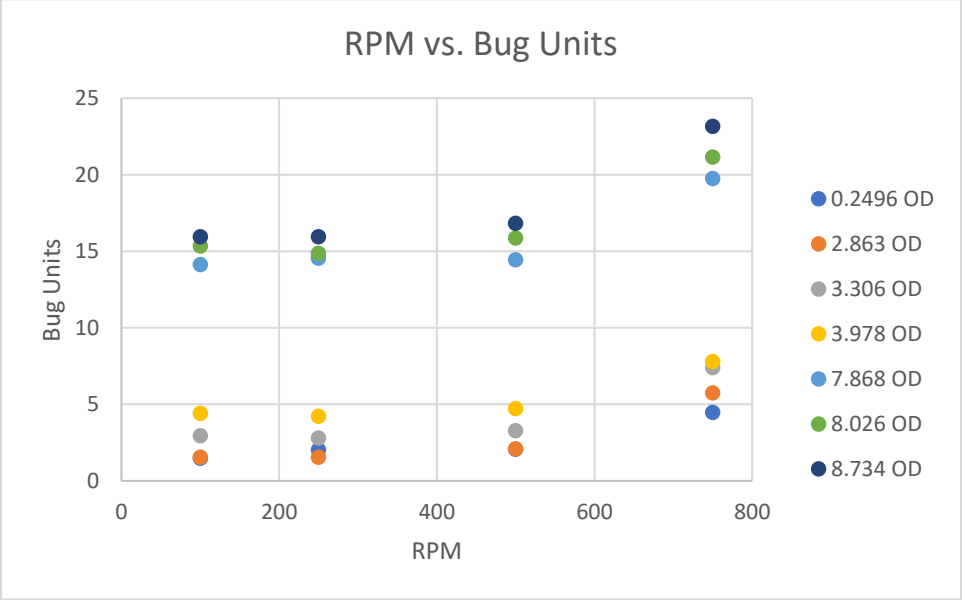
[6] Bug Lab. (2015) Operator's Manual BE2x100

Appendix

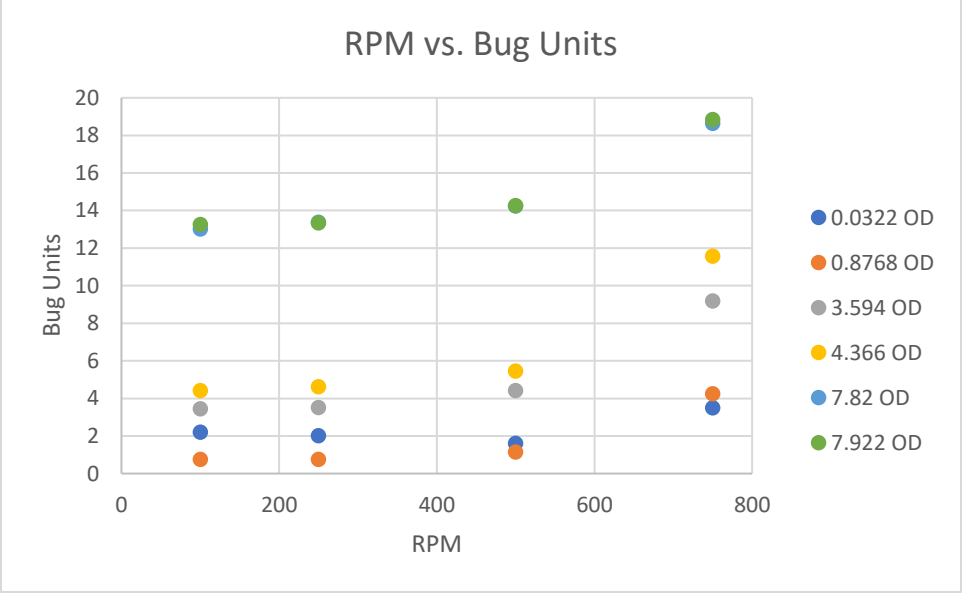
Appendix 1

RPM vs Bug Units

Trial 1



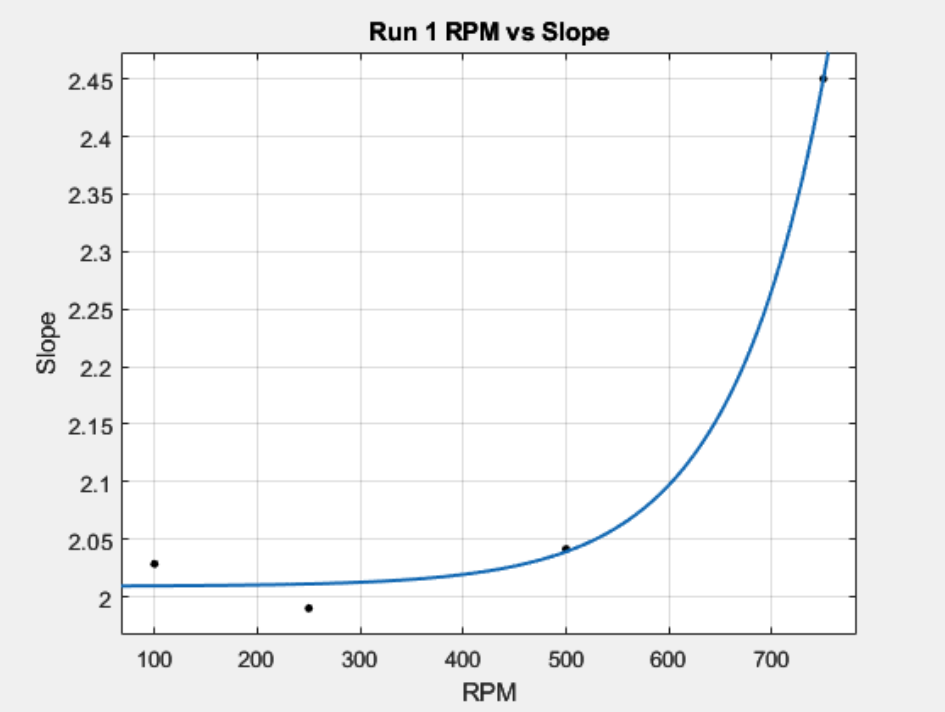
Trial 2



Appendix 2

RPM vs Trendline Slope

Trial 1

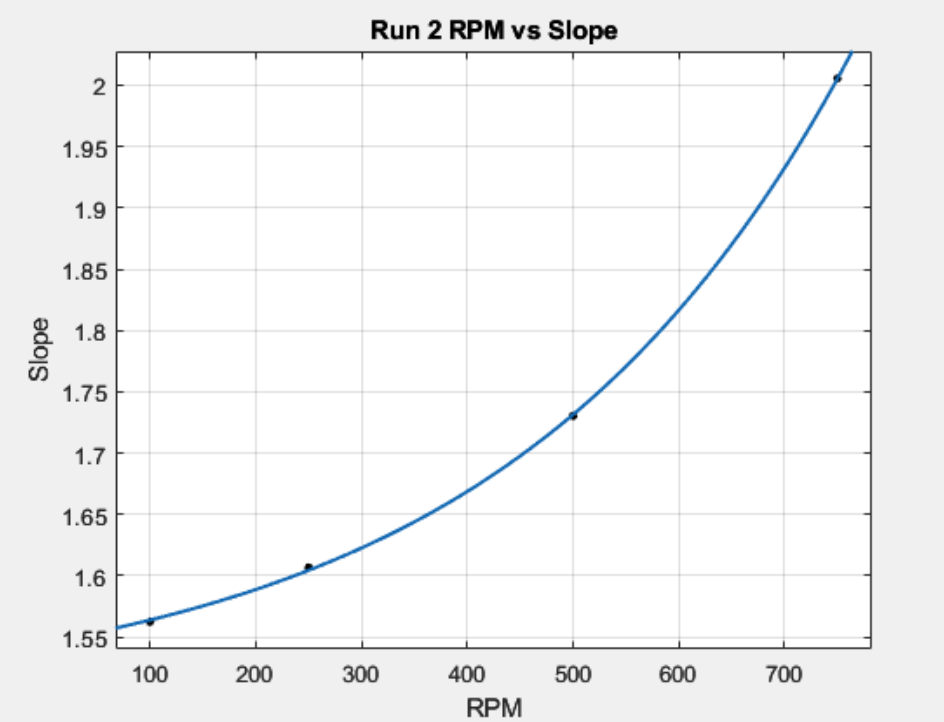


Fit name: Run 1 RPM vs Slope

General model:
f(x) = a*(b^(x))+c
Coefficients (with 95% confidence bounds):
a = 0.0001402 (-0.006511, 0.006791)
b = 1.011 (0.9474, 1.074)
c = 2.009 (1.727, 2.291)

Goodness of fit:
SSE: 0.0008183
R-square: 0.9942
Adjusted R-square: 0.9825
RMSE: 0.02861

Trial 2



Fit name: Run 2 RPM vs Slope

General model:
f(x) = a*(b^x)+c
Coefficients (with 95% confidence bounds):
a = 0.05087 (-0.01386, 0.1156)
b = 1.003 (1.002, 1.005)
c = 1.495 (1.392, 1.597)

Goodness of fit:
SSE: 8.547e-06
R-square: 0.9999
Adjusted R-square: 0.9998
RMSE: 0.002924