Effect of Increasing Glucose Concentrations on the Growth Rate of *Tetrahymena thermophila*

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Abstract

The protozoan, *Tetrahymena thermophila*, is an effective model organism due to its short generation time of approximately two hours. It has displayed even faster growth rates in the presence of nutrients, such as glucose, by phagocytizing and storing them in food vacuoles. The aim of our study was to expose *T. thermophila* to various glucose concentrations, treatments of 0.2%, 2%, 4%, and 6% so we could produce an effective synthetic medium that would supply abundant *Tetrahymena* cells for experimentation. We hypothesized that growth rate would be greatest at 4% glucose with a decrease at 6% glucose due to differential oxygen pressures resulting from the saturation of glucose within the media. In our study, we placed the *T. thermophila* in an SPP medium varying the glucose concentration with each treatment. We then determined cell density after initial cultivation at 1, 3, 5, and 7 hours using a haemocytometer. Greatest growth rate was found at 2% glucose with 21,844 cells/mL and lowest in the 6% glucose with 8,220 cells/mL. Using a one-way ANOVA test, we found our p-value to be statistically significant; however, because the growth rate was highest for 2% rather than the predicted 4%, we could not reject our null hypothesis.

Introduction

Tetrahymena thermophila is a unicellular eukaryotic protozoan that inhabits freshwater environments (Collins 2012). These organisms are cultured in laboratory settings as model representatives of eukaryotic cells, and are beneficial research organisms due to their rapid doubling time. The rate at which these cells double is enhanced when they are cultured in synthetic nutrient rich media in the lab (Kiy and Tiedkte 1992). According to Asai and Forney (1999), the optimal growth for *T. thermophila* is 35℃.

Tetrahymena take up nutrients through the process of phagocytosis (Cassidy-Hanley 2012). During phagocytosis, *T. thermophila* ingests nutrients through its oral apparatus and stores it in vacuoles for digestion (Collins 2012). *Tetrahymena* takes around 20 seconds to one minute to form food vacuoles. Food vacuoles cannot form during cell division (Nilsson 1977).

Evans and Witty (1980) indicated that the rate at which an organism grows is dependent on the contents of its medium. Glucose was increased in differential concentrations to evaluate the growth rate of *T. thermophila.* Glucose has shown to increase growth rate in prior studies in the case of Kiy and Tiedkte (1992) and Szablewski *et al*. (1991). However, at a certain concentration, the uptake of nutrients from the media will begin to have negative effects on the cell from lack of oxygen tension in food vacuoles (Blum 1969).

The purpose of our experiment was to determine if 4% can be considered an optimal glucose concentration for media in future experiments. Our null hypothesis postulated that a 4% glucose concentration, there will be a decrease or no difference in average growth rate of *T. thermophila* compared to 0.2%, 2%, and 6% glucose concentration. Our alternate hypothesis suggested that at 4% glucose concentration, there will be a greater average cell density *T. thermophila* compared to 0.2%, 2%, and 6% glucose concentration.

The premise on which these hypotheses were based was that increased glucose available for consumption via phagocytosis will increase the rate of cell division. However, at a certain concentration, the uptake of nutrients from the medium will begin to have detrimental effects on the cells because of lack of oxygen necessary for respiration (Blum 1969). We predicted an increasing positive growth rate from 0.2% to an optimal 4% glucose concentration and a lower or negative growth rate for the 6% glucose medium (Figure 1) where reduction in oxygen pressure within the media negatively affected the cellular mechanisms required for respiration.

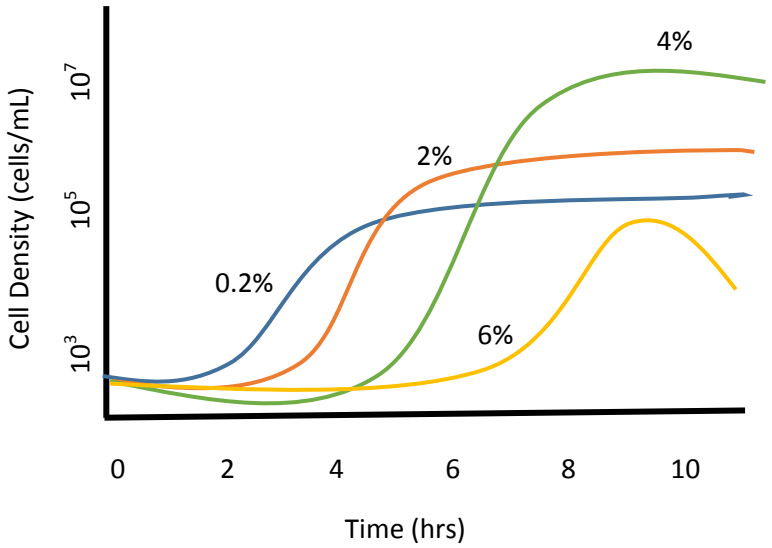

Figure 1. Expected rate of growth for *T. thermophila* in enriched glucose media measuring cell density over time. Increasing densities can be seen from 0.2%, 2% to an optimum 4% glucose concentration and a decreasing density in the 6% enriched media.

Methods

Materials

We started with 50 mL of stock cell culture of *T. thermophila* cells suspended in SSP growth medium (Figure 2) and four 20 mL synthetic nutrient media samples each containing differential glucose concentrations at 0.2%, 2%, 4%, and 6% glucose in the nutrient mix (Figure 3). The control treatment was the SSP growth medium containing 2% protease peptone, 0.1% yeast extract, 33 μM $FeCl_3$, and 0.2% glucose.

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Figure 2. *Tetrahymena thermophila* live cell stock culture (suspended in SSP medium)

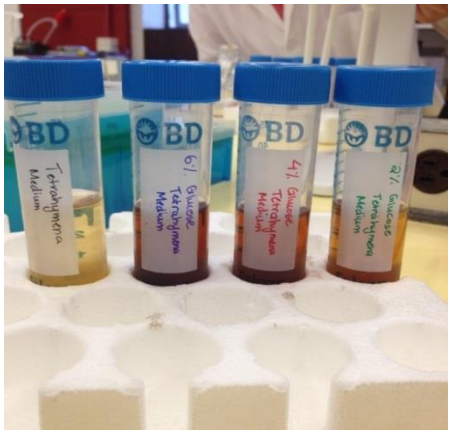

Figure 3. Glucose media (Left to Right: 0.2%, 6%, 4%, 2%)

Stock Culture Cell Count

To begin, we conducted an initial cell count of the stock culture. In a small tube, we took 100 µL of the culture and added 20 µL gluteraldehyde to fix the cells. From this mixture, we pipetted 20 µL and placed it on a Fuchs Rosenthal haemocytometer with a coverslip holding the sample between the two surfaces. Using a Zeiss Axiostar Plus compound microscope at 100x total magnification, we counted the number of cells within three 1mm x 1 mm boxes and calculated an average of cells per square. We then calculated the number of cells per milliliter by using the average number of cell per square and taking into account the 5 x 10^3 dilution factor of the box size in addition to the 1.2 dilution of the fixative added in a 100:20 ratio of culture to fixative by multiplying these factors to the average number of cells per square. From this, we calculated the number of cells in the initial 50 mL culture of *T. thermophila* by multiplying the number of cells per milliliter we found by 50.

Treatments Setup

After obtaining the initial cell count, we transferred 10 mL of the stock culture to four labeled 15 mL test tubes. We centrifuged the tubes for 5 minutes at full speed to concentrate the

cells and removed the medium by decanting all of the supernatant. In the same tube, we added 5 mL of the differential sterile nutrient media and mixed the concentrated cells with the new media. For each of the test tubes, we counted the cells.

Replicates Setup

Using the concentrations from the four tubes, we calculated the number of cells in the medium and the amount of nutrient medium required to obtain an initial concentration of 30,000 cells/mL (Figure 4). We estimated the average cell count for each treatment and determined the amount we needed to obtain 30,000 cells/mL per replicate. We prepared 2 milliliters per replicate. For example, in our 0.2% glucose treatment, we estimated a cell count of 44,687 cells/mL; thus, we took 0.671 mL of the 0.2% medium containing cells and added 1.329 mL of the 0.2% glucose medium to each replicate.

Figure 4. Replicates for 0.2%, 2%, 4%, and 6% treatments respectively

Data Collection

We began our data collection for time 0 by conducting initial cell counts for each

replicate. Each replicate was tested using the cell count procedure similar to the initial dilution of the cells. Between counts, we incubated our replicates at 35℃ in a water bath and samples were taken at 1, 3, 5, and 7 hours after our initial setup.

Data Analysis

We determined growth rate by calculating the slope of each replicate by graphing the cell density *vs*. time. We found that replicate 2 resulted in a decrease in growth in two of our treatments; thus, we removed it from our data analysis. We averaged the growth rate of the three remaining replicates to find the average growth rate and the 95% confidence intervals for each treatment. Finally, we used one-way ANOVA to statistically analyze the data by using the treatment type as the dependent variable.

Results

The averaged cell densities across all four replicates are 12999 cells for 0.2%; 21844 cells for 2%; 11080 cells for 4%; and 8220 cells for 6%. We observed the greatest cell density in the 2% glucose solution with a total of 32438 cells/mL after the seventh hour and the lowest cell density at 6% glucose solution with a total of 12281 cells/mL. Figure 5 shows the average growth rate cross the replicates of each treatment. At 0.2%, the mean growth rate is 3440 cells; at 2%, the mean growth rate is 4274 cells; at 4%, the mean growth rate is 441 cells; and for 6%, the mean growth rate is 432 cells. There is a divide between the high growth rates of 0.2% and 2%, and the significantly lower growth rates of 4% and 6% glucose concentrations. The one-way ANOVA statistical test from this data gave a p-value - 9.7571×10^{-7} ($p<0.05$) which is statistically significant.

Along with the values obtained, the features of the media were also significant. The initial *T. thermophil*a wild-type culture was a translucent yellow color; however increasing

glucose concentrations increased the intensity of the color of the solution. The 0.2% solution was the lightest and gradual increases in brown colouration was seen in the 2%, 4%, and the 6% being the darkest. In addition, we observed clumping of the cells during microscopic analysis. The *Tetrahymena* cells that we observed were approximately 40-50 µm in size with a circular shape and contained several food vacuoles.

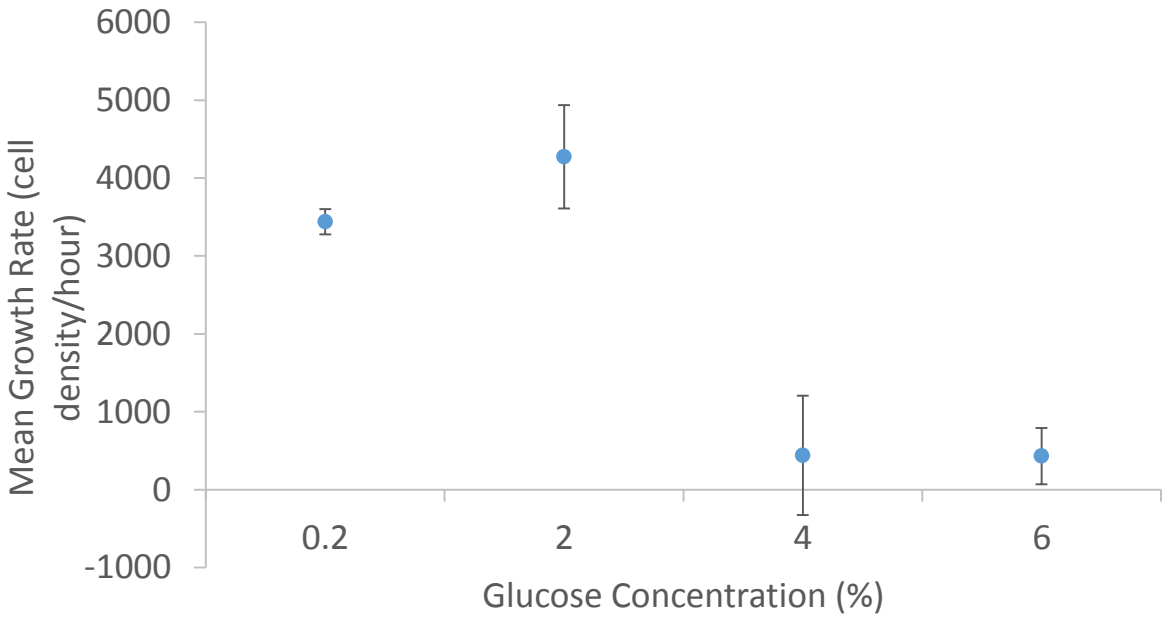

Figure 5. Mean growth rate of *Tetrahymena thermophila* of the four treatments at 0.2%, 2%, 4%, and 6% glucose concentration. Error bars show 95% confidence intervals. n=11.

Discussion

Based on the results of the one-way ANOVA ($p < 0.05$), we reject the null hypothesis that there will not be a difference or have a decreased average growth rate of *Tetrahymena* in the 4% glucose control in comparison to the 0.2%, 2%, and 6% treatments. This statistical analysis suggests there is a significant difference between 4% glucose and the other differential glucose concentration treatments. However, as evident in Figure 5, the averages from the replicates of

each treatment indicate that the highest growth rate was not at the predicted optimum of 4% glucose.

In contrast, experimental data show that 0.2% and 2% glucose media had a higher growth rate than 4% enriched glucose media. This comparison refutes the evidence that the null hypothesis can be rejected, as it does not support the direction of our alternate hypothesis. From this evidence, we cannot support the alternate hypothesis thus, with all the information considered, we fail to reject the null hypothesis and do not lend support to the alternate hypothesis.

In order to interpret the higher growth rate observed at the 2% glucose concentration, we compared our data collection and methods to the past experiments on glucose enriched media used for mass cultivation. Growth of *T. thermophila* under differential glucose conditions was tested by Kiy and Tiedke (1992), who found that with increased glucose concentrations, the doubling time of *T. thermophila* is reduced. Specifically, at 4% glucose, the concentration of cells at the end of the test period is significantly higher than 0%, 1%, 2%, and 6% treatments cite. The results collected may have been obscured by the differences in the timelines: our experimental sampling lasted seven hours in contrast to the 35 hours of growth utilized by Kiy and Tiedke (1992). Evidence indicates that there is an initial decrease in the growth rate at 4% concentration between 0-7 hours which is consistent with the data collected in our experiment.

In wild-type *Tetrahymena,* growth is enhanced through the production of food vacuoles (Seaman, 2007). The rate of cell division is determined by the formation of food vacuoles at the oral apparatus, and the having the nutrients that are needed for growth present (Rasmussen 1973). Nutrients are taken up into the oral apparatus through the process of phagocytosis, and ultimately ingested into a formed food vacuole for digestion (Collins 2012, Suhr-Jensen and

Orias 1979). Within the cytoplasm, this structure is subject to the activity of acid phosphatase that stimulates the breakdown of ingested material (Rasmussen 1973). With an increase in nutrient composition within the synthetic media, additional food vacuoles can be produced, which plays a role in increased cell division.

Kiy and Tiedke (1992) also observed an increased mortality rate in the medium that contained an enriched synthetic nutrient media with 6% glucose, consistent with the low average growth rate of the 6% glucose treatment in our own experimentation. But if increased food vacuole production were beneficial, then why did the 6% glucose media have a slower growth rate? The biological mechanism that prevents the higher glucose concentration from increasing cell growth is not well understood. Szablewski *et al.* (1991) suggested that the amount of glucose has a direct impact on the growth rate of *Tetrahymena*, however, the amount added to the medium may also play a role in metabolic processes that could act as a limiting factor to growth (Blum, 1970).

When an increased concentration of glucose is added to the medium, a lower oxygen tension, or pressure, results from the saturation of the carbohydrate within the nutrient medium (Blum, 1970). In a similar species *Tetrahymena pyriformis*, it was found that the lack of oxygen within the medium stimulated the cells to undergo anaerobic processes for energy production in addition to having a negative effect on several enzymes in the gluconeogenic pathway used to make glucose *de novo*. These two factors may have contributed to the lower growth rate of the 6% enriched media as the cells did not produce enough ATP from the anaerobic metabolism to foster rapid division. Though glucose is used to supplement culture media for increased cell division, high concentrations can have a negative effect on cell growth.

With this in mind, this experiment also had sources of uncertainty that may have played a role in the results we obtained. The initial dilution of the cells had a lower cell density than we would have hoped as some cells may have been poured out with the supernatant after centrifugation. The initial low cell count may have played a role in the higher growth rate of 0.2% and 2% treatments. Future experimentation that would benefit the research in culture media could include a study over a longer time in addition to increasing concentrations of oxygen and the effects of cell division based on the oxygen tension within *T. thermophila.*

Conclusion

In this study, we found that the medium containing the 2% glucose concentration produced the greatest cell density of *T. thermophila* cells. Thus we failed to reject the null hypothesis. Although, we hypothesized that 4% glucose concentration would produce the greatest cell density, we were still able to create an effective medium for rapid *T. thermophila* growth. The control medium that contains 0.2% glucose concentration had a lower cell density compared to the 2% concentration; therefore this synthetic medium had a faster growth rate and has promise for an improved culture medium.

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