Use of Bradford Assay to Analyze Protein Production in Yeast

Braden Thomas, Tram La, Ngoc Tuyen Le
Biology 160: General Biology

Abstract

Our project goal was to find out what the ideal sucrose concentration in yeast was for optimal for the production of protein. We hypothesized that if more sugar was added to yeast, more protein would be produced. The data we collected supports our hypothesis. Having this information would be useful for the food industry and being able to have more protein in the products.

Introduction

The relationship between sucrose concentration and protein production in yeast is crucial in biotechnology. Sucrose, a disaccharide of glucose and fructose, serves as a key carbon source for yeast metabolism. When metabolized, it provides energy and supports growth and protein synthesis. Studies show that increasing sucrose concentrations up to an optimal point enhances cell growth and recombinant protein expression. However, beyond this threshold, excessive sucrose can lead to overflow metabolism, resulting in by-product accumulation (e.g., ethanol), which reduces protein yield and efficiency.(Tyo, K. E., Liu, Z., Magnusson, Y., Petranovic, D., & Nielsen, J. (2014).).The information in this study has an impact on society due to the importance protein has in nutrition.

Hypothesis: Increasing sucrose concentration boosts protein production in yeast up to an optimal level, after which excess sucrose may increase protein yield due to metabolic balances or metabolism. **Research Q: How does the amount of sucrose added to yeast affect protein production?**

Methods

Materials & Location

About the material, we used 1 packet of yeast, water, yellow microcentrifuge tube, sterile glass beads, vortex machine, 1 x PBS solution, Bradford reagent, 1.5 mL cuvettes, Calibrate spectrophotometer, a spectrophotometer, 2 micropipettes, 5 test tube label about 0,10,20,30,40 percent concentration of sucrose and laptop. The experiment was done in lab 110.

Procedure

After preparing the material, we add 1 packet of yeast to 50mL of water and mix well. Add 1mL of yeast to a yellow centrifuge tube with glass beads for stirring. This yellow centrifuge tube is shaken for 1 minute using a vortex machine.

We used a micropipette to add 20 µL of the yeast mixture to a tube labeled PBS. This tube already contained 980 µL of 1x PBS solution. Then, we added 980 µL of Bradford reagent to a cuvette. Next, we took 20 µL of the yeast mixture in PBS solution and added it to the Bradford reagent in the cuvette. We closed the cuvette, inverted it 3 times to mix it well, and let the solution stand. After mixing, the solution turned blue.

Then, we took samples of the solution with sucrose solutions at concentrations of 0%, 10%, 20%, 30%, and 40%, and clearly labeled each cuvette to avoid confusion. Before measuring, we calibrated the spectrophotometer using a cuvette containing only Bradford reagent (without yeast), which was prepared in the lab. Then, we measured the absorbance of the yeast sample at 595 nm using the spectrophotometer and recorded the absorbance values in an Excel spreadsheet. Finally, we plotted a curve showing how the amount of sucrose added to the yeast affected protein production.

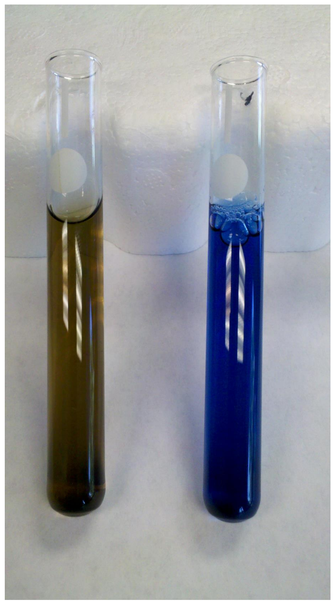

Table 1. Figure on right is the difference between when the bradford assay is added.

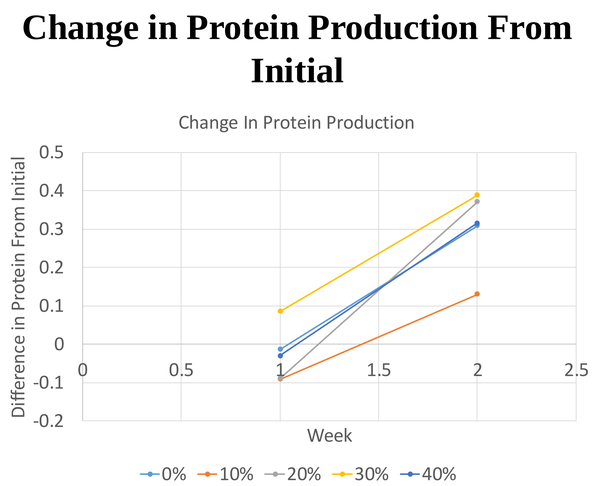

Figure 2. Graph of data showing that on average a higher glucose concentration produced more protein.

Discussion

Our hypothesis is that increasing sucrose concentration enhances protein production in yeast up to an optimal level. Our data support this hypothesis. Between the first and second weeks, protein production increased consistently, with 0% sucrose showing unexpected growth, comparable to 20% and 40% sucrose concentrations. In week one, growth at 0%, 20%, and 40% sucrose concentrations was similar, but 30% sucrose showed notably higher growth, while 10% sucrose was the lowest. In week two, protein production continued to increase across all concentrations, with minimal differences between them. Interestingly, the 20% sucrose concentration grew almost as strongly as the 30% concentration by the second week.

This result is consistent with previous studies showing that sucrose, as a major energy source, promotes cell growth and recombinant protein expression in yeast (Tyo et al., 2014). At higher concentrations, yeast cells can metabolize sucrose more efficiently, leading to higher protein production. However, one difference between our study and previous studies is that we only tested sucrose concentrations up to 40%, while other studies tested higher concentrations. This may be the reason why our results differ from previous studies. For example, the study by Tyo et al. (2014)showed byproduct accumulation at high sucrose concentrations, but we did not test concentrations higher than 40%, so we cannot conclude whether higher concentrations negatively affect protein yield. Factors such as temperature or pH may also have affected our results.

Protein is an essential piece of human life, and yeast is starting to be a part of how we can produce that. Today more research is being put into protein production in yeast (Antonio). Being able to find alternative sources to meat protein is essential for those who can't eat meat since nearly half of all protein consumed comes from meat sources (Pasiakos). Future research should be put into figuring out the ideal environment and other variables for ideal protein production.

Acknowledgements

We would like to give a significant thank you to our instructor, Lauren Maniatis for her instruction and knowledge bestowed upon us. Also, a huge thanks to our lab tech Bethany for being an enormous help in setting up the bradford assay and her instruction in the procedure.

References/ Work Cited

Tyo, K. E., Liu, Z., Magnusson, Y., Petranovic, D., & Nielsen, J. (2014). Impact of protein uptake and degradation on recombinant protein secretion in yeast. *Applied microbiology and biotechnology*, *98*(16), 7149–7159. https://doi.org/10.1007/s00253-014-5783-7

Antonio Carlesso, Raquel Delgado, Oriol Ruiz Isant, Owens Uwangue, Dylan Valli, Roslyn M Bill, Kristina Hedfalk, Yeast as a tool for membrane protein production and structure determination, *FEMS Yeast Research*, Volume 22, Issue 1, 2022, foac047, https://doi.org/10.1093/femsyr/foac047

Pasiakos SM, Agarwal S, Lieberman HR, Fulgoni VL 3rd. Sources and Amounts of Animal, Dairy, and Plant Protein Intake of US Adults in 2007-2010. Nutrients. 2015 Aug 21;7(8):7058-69. doi: 10.3390/nu7085322. PMID: 26308049; PMCID: PMC4555161.