

**MEMORANDUM**

**DATE: October 10, 2009**

**TO: Laboratory Group A**

**Mac Stout, Stephen Grobstein, Michael Beus**

**FROM: Tony Butterfield**

**Engineering Training Supervisor**

**SUBJECT: Immortal Yeast**

Rapamycin is an immune suppressant and antibiotic discovered in bacteria living in the soils of Easter Island. This compound has been widely studied, but has been in the news lately due to its apparent ability to extend the life span of higher mammals. Please read the literature for details and the hypothesized mechanisms behind rapamycin’s effects (e.g. Drug Discovery Today 12:112-124, Genes and Development 20:174-184, PNAS 95:4264:4269, and many others).

Our client produces a certain alkaloid pharmaceutical by use of an engineered strain of *Saccharomyces cerevisiae*, and they hope to study the effect of increasing their yeast’s cumulative lifespan by use of rapamycin. Your task is to conduct the first round of preliminary testing for this project, using common wild-type yeast.

The growth medium for all experiments should initially contain 10 g/L yeast extract, 20 g/L Bacto Peptone, and 40 g/L glucose. Normally, about 2.0 g/L yeast is used in the inoculums. Be sure to autoclave the medium for 15 min prior to use; a couple stray microbes could cause you problems. Check the literature to decide upon an appropriate concentration for rapamycin needed to test its effect on yeast growth and justify your choice. Note that too high of a concentration will stunt growth, but we want the concentration to be high enough to significantly increase life span.

One of the first values you should estimate is the volumetric mass transfer coefficient of the bioreactor at the operating conditions at which you will run the reactor. This may be done either while cells are growing or with no cells present. We will discuss how to accomplish either method at our preliminary lab meeting.

Next, we are interested in comparing certain kinetic parameters, both in the presence and absence of rapamyocin during the exponential growth phase. Operate with an excess of O2 and assume a Monod expression for the specific growth rate, with glucose as the limiting reactant. Determine the relevant kinetic parameters and yield coefficient during the exponential growth phase for each experimental run.

During the exponential growth phase (when the majority of resources go to reproduction) our client’s yeast produces their alkaloid product at approximately half the rate per unit mass live cells than when in the stationary phase. Therefore, it is also hoped that rapamycin may extend the stationary phase, even if at the cost of some growth. Please describe what effect you observe, if any, of rapamycin on the length of the stationary phase and cell life span.

You may use the HPLC in order to determine glucose concentration, and there is a dissolve O2 sensor attached to the bioreactor. Cell concentrations may be determined by optical density using the plate reader or UV-Vis. If we get the new cell counter up and running, you may use that as well. Be sure to perform calibrations for each analytical method you use. To determine the amount of viable, living cells in a sample you may attempt several methods which we will discuss at your preliminary lab conference; however, please come with some ideas of your own.

Keep in mind that the bioreactor is a particularly tricky piece of equipment to use in our lab, due to the fact that a typical experimental run necessitates more than 3 hours of attention. I will introduce the inoculums to the reactor at about 9AM the day you plan on taking measurements, to avoid the lag phase and assure you obtain data during the exponential growth phase. You may plan to be a part of this preparation, but it is not required. Furthermore, to track the stationary phase, it may be desired to take measurements past the time when the lab is locked up, or on days when there is no lab scheduled. I do not expect you to come in at such times and I will be glad to help by taking measurements when you cannot. In short, your group may need to coordinate with me throughout this project.

As always, contact me with any questions you may have, and I look forward to discussing this project with your team on or before Monday, October 19, 2009. With a bioreactor project, in particular, I recommend we meet as soon as possible.