SAMPLE POPOSAL

Chemical Engineering 4905

The following sample proposal is not intended to represent the scope and depth of the projects proposed by or assigned to students. It is an edited and slightly altered student proposal and may contain some incorrect statements and formatting, and may describe questionable experimental procedures. The report is intended to illustrate only the organization and elements of an acceptable proposal as discussed in class, in the grading rubric, and in the lab handbook. This document focuses primarily on issues specific to proposals. For greater detail about issues applicable to technical writing in general, see the sample formal report in the Projects Lab Handbook.

The comments in the margins of the proposal are intended to call the attention of the student to required proposal content. A student's proposal should not contain such comments in the margin.

The proposal should be bound in some kind of cover.

Proposal for Continuous Cell Concentration Analysis System

By

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Professor Kevin Whitty

Assigned: February 9, 2010

Due: March 5, 2010

Submitted: March 5, 2010

Comment [T1]: Title page.
Includes a concise and descriptive title.
This page is not labeled with a page number, and it should not be, but it is Page i.

Comment [T2]: Proposal authors.

Comment [T3]: Recipient.

Comment [T4]: Relative dates.

Comment [T5]: This proposal should include the author's signatures at the bottom. For example:

_____ _____
John Doe Jane Doe

Centered and with handwritten signatures above the line.

Table of Contents

Comment [T6]: Lists the accurate section number, title and page number of each major section of the proposal.

This is Page ii and is labeled as such.

List of Appendices

Comment [T7]: It would be best to instead include the appendices in the Table of Contents, particularly when there is only one.

List of Tables

Comment [T8]: Lists the accurate table number, title and page number of each table. Make sure the table title is the same in this table as it is in the body of the text.

List of Figures

Comment [T9]: Lists the accurate figure number, title and page number of each figure. Make sure the figure title is the same in this table as it is in the body of the text.

EXECUTIVE SUMMARY

The bioreactor unit at the University of Utah currently has no sensory equipment to indicate cell concentration. The result of this is that one must guess when the best time is to take samples. Additionally, it must be assumed that refrigerated storage will slow down activity enough to be inconsequential to analysis results when samples cannot be immediately processed. Manually taking more samples and processing them immediately can generate better data and get closer to finding cell growth transition phases. However, this comes with an increased cost of employee time and, in the case of the cell counter, significant reagent material costs.

By building UV/VIS spectroscopy profiles of yeast cells and its media to generate absorbance vs. concentration curves at ideal wavelengths our team intends to run a flow through cell on a continuous read UV/VIS spectrometer and translate this into real time cell concentration. This should enhance reactor performance analysis by clearly indicating the growth phase of reaction, and automatically collecting data to estimate cell growth kinetics. This can reduce the number of samples needed as well as the time and material expenses required to collect and analyze them. Ideally, this will pave the way to creating an automated, dedicated, real-time concentration analysis instrument based on UV/VIS absorption.

This project will be pursued in three phases. The first step is preliminary work to establish UV/VIS absorbance profiles of media, yeast cells, and active reaction culture. With this, preferred wavelengths can be ear-marked and customized equations to relate composite absorbance to cell concentration built. This will be accomplished using known concentrations of target compounds analyzed by existing UV/VIS and fluorescing concentration analysis equipment. The second part requires contrasting this gathered absorbance data with sample comparison tests on the existing continuous read UV/VIS spectrometer. Using this comparison data and performing subsequent test runs will allow tuning of the previously generated equations. In the third step the accuracy of the real-time concentration analysis system will be quantitatively benchmarked, and its ability to indicate primary shifts in bioreactor cell growth phases (particularly lag to exponential and exponential to stationary) will be evaluated. The concentration accuracy will be compared against that of the cell counter and other instruments.

Upon conclusion of this project the strengths and weaknesses of the real-time cell concentration system will be documented; and suggestions will be made for design of an improved, custom-built system headed by Professor Butterfield.

Comment [T10]: Throughout the document the font should be 12 pt, except for captions, text within figures, and footnotes which may be as low as 9pt. Single spacing should be used.

Comment [T11]: Executive Summary. Meant to concisely summarize the proposal. The summary should fit to one page.

Comment [T12]: Briefly explain the problem(s) to be solved by the proposed work.

Comment [T13]: Explain the potential benefits of the proposed project.

Comment [T14]: Briefly describes the methods to be used.

TECHNICAL BACKGROUND

Every substance on this planet absorbs some wavelength of light. The chemical makeup of the substance dictates which frequencies of light are absorbed and which are reflected. For the purpose of this project, UV/VIS spectrometry will be used to continuously measure the absorbance of a growing culture of microorganisms. This measured absorbance is related to concentration by Beer's Law described below:

> Beer's law relates the absorption of light to the properties of the material through which light is traveling. The law states that there is a logarithmic dependence between the transmission, T, of the light through a substance and the product of the absorption coefficient of the substance, α, and the distance the light travels through the material, *l*. The absorption coefficient can, in turn, be written as a product of either a molar absorptivity of the absorber, ε, and the concentration c of absorbing species in the material, or an absorption cross section, σ, and the density N of absorbers.[1]

For liquids, Beer's Law is described by the following equation:

$$T = \frac{I}{I_0} = 10^{-\alpha l} = 10^{-\varepsilon l c} \quad (1)$$

where I_0 and I are the intensity of the incident light and the transmitted light, respectively. In practice I/I_o is given as a percentage referred to as percent transmittance. The other popular choice of quantifying spectroscopy results is termed absorbance. Transmittance and absorbance are related via the following equation:

$$A = -\log_{10}\left(\frac{I}{I_0}\right) \quad (2)$$

where A is absorbance. Thus 100% transmittance equals an absorbance of zero and 10% transmittance gives an absorbance of 1. In practice, most spectroscopy instruments start to lose accuracy progressively as absorbance values increase past. Solving Beer's law equation in terms of absorbance shows why it is often the more popular choice of measurement in spectroscopy due to eliminating the exponential factor with the resulting equation of:

$$A = \varepsilon \ell c = \alpha \ell \quad (3)$$

Currently, no cell counting capabilities are present on the bioreactor. A sample must be taken whenever the concentration is desired and run through a cell counter. During the extraction and analyzing process the cells will continue to grow and multiply; thus increasing error associated with the collected data. There are several solutions to this problem and they are as follows:

1. Place the samples in a refrigerator, which effectively ceases any growth.
2. Add a small amount of toxin to the system that will terminate the growth of the cells in the sample.
3. Install an instrument to analyze cell concentration continuously, eliminating any lag time between sample extraction and analysis.

Comment [T15]: This is page 1. In total the proposal should be no more than 15 pages, single spaced, excluding tables of contents, nomenclature and reference, executive summary and appendices.

Comment [T16]: This section functions as an introduction to the topic at hand. Note that the first page of the Technical Background is Page 1 of the proposal and is numbers as such.

Enough technical background should be given so that a non-expert reader will be able to understand the engineering problems addressed by the proposal.

Comment [T17]: This quote is copied from Wikipedia. It would be best to find a more authoritative, less ephemeral source for a professional proposal.

Comment [T18]: Enough background is given so that a non-expert reader will be able to understand the importance of solving the problem.

Also, the authors explain why the proposed approach is better than alternatives.

The refrigerator solution is not effective at instantly stopping cell growth and any time spent out of the refrigerator will result in a near instantaneous return to regular cell growth. The addition of a toxin has been proven to be an effective inhibitor of cell growth but the chemical can interfere with and reduce the accuracy of sample analysis. Additionally, the lab would have to maintain a constant supply to allow students to use this method. The third and final solution is the most complex; but it is also the most effective option. If enabled correctly it can display a real-time graphical representation of how cells are growing inside the bio-reactor. This would allow users to take samples at precise and crucial times during the different growth phases of the cells.

Microorganisms have four main phases associated with their life cycle; lag, exponential, stationary and death. The lag phase is the ramp up period where the cells are getting accustomed to their environment. Next is the exponential phase where the cells rapidly grow and multiply. During this phase, glucose and oxygen are consumed by the cells to reproduce. Oxygen concentration dissolved in the bioreactor solution is maintained with a constant flow of air into the bioreactor. However, the glucose concentration becomes a limiting factor. Cells will only reproduce when there is a sufficient amount of nutrients (glucose) to present in the reactor.

After most of the glucose is depleted the cells enter a stationary phase. In this phase the net growth rate is zero or the growth rate is equal to the death rate.[2] This is the same phase where specialty chemical production is possible. By limiting a primary nutrient after cells have grown to a sufficient population, a cell culture is forced to pursue alternate chemical metabolic pathways thus changing what they produce. The death phase, as the name implies, is where the cells begin to die off and cease production of any substances. A graph showing the primary growth phases of microorganisms over time is shown below:

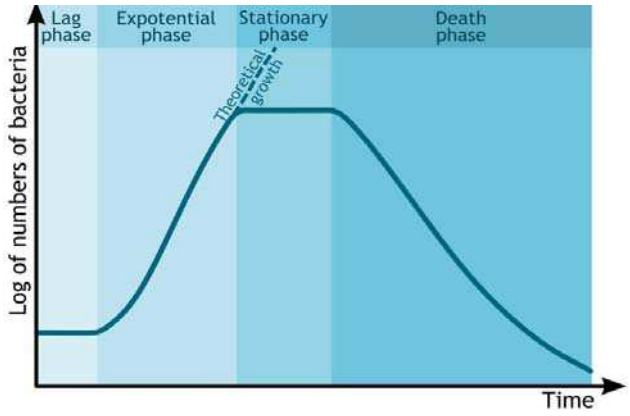

Figure 1: Bacterial Growth Phases

The growth rate of microbes is directly related to cell concentration and is characterized by the net specific growth rate, defined as:

Comment [T19]: References are given. The authors show they have adequately reviewed the literature and found all approaches that have been tried in the past to solve the problem(s) addressed by the proposal.

Comment [T20]: Figure title is below the figure and matches the title in the Table of Figures.

$$\mu_{net} = \frac{1}{X}\frac{dX}{dt} \tag{4}$$

where X is cell mass concentration, t is time, and μ_{net} is net specific growth rate. Integrating Equation (4) yields:

$$X = X_0 e^{\mu_{net}(t-t_{lag})} \tag{5}$$

where X and X_0 are cell concentrations at time t, t_{lag} is the time in the lag phase. Equation (5) gives us a value of cell concentration at any time (t).

Yield coefficients are needed to describe how cell concentration changes with substrate concentration and can be defined as:

$$Y_{X/S} = -\frac{\Delta X}{\Delta S} \tag{6}$$

where ΔX is the change in cell concentration over time and ΔS is the change in substrate concentration over the same time period.

Oxygen is usually added by sparging air through the bioreactor because it is needed to facilitate growth in the yeast cells. The transfer of oxygen to the cells is most often limited by the transfer of oxygen through the liquid film surrounding the gas bubbles. This rate of oxygen transferred (OTR) from the gas to the liquid is described as:

$$OTR = k_L a(C^* - C_L) \tag{7}$$

where k_L is the oxygen transfer coefficient, a is the gas-liquid interfacial area, $k_L a$ is the volumetric oxygen transfer coefficient, C* is saturated dissolved oxygen concentration, C_L is the actual dissolved oxygen concentration in the broth. $k_L a$ can be found by measuring the dissolved oxygen concentration over time and graphing the data. This will produce a line whose slope is the value for $k_L a$. Equation (7), along with oxygen uptake rate (OUR), can be used to ensure oxygen is not the rate-limiting factor. The OUR is a measurement of the amount of oxygen taken in by the cell broth over time and can be defined as:

$$OUR = q_{O_2} X = \frac{\mu_g X}{Y_{X/O_2}} \tag{8}$$

where q_{O_2} is the specific rate of oxygen consumption, Y_{X/O_2} is the oxygen yield coefficient. The OUR and OTR can be compared to determine if oxygen transfer is the rate-limiting step. If $OUR = OTR$, oxygen is the rate-limiting step.

The exponential phase of the life cycle is crucial as its kinetics directly determine how fast the majority of the biomass is produced. It is often described by the Monod Equation as follows:

$$\mu_g = \frac{\mu_m S}{K_S + 1} \quad (9)$$

where μ_m is the maximum specific growth rate when $S \ll K_S$. K_S is the saturation constant and is equal to the concentration of the rate-limiting substrate when the specific rate of growth is equal to one-half of the maximum and S is the substrate concentration. Equation (9) derives from the premise that a single enzyme system with Michaelis-Menten kinetics responsible for the uptake of S is a rate-limiting step, and therefore also governs growth rate of a cell culture.[2] Equation (9) describes substrate-limited growth only when growth is slow and population density is low.

OBJECTIVES

This project has a set of quantitative and qualitative goals. Listed in order of importance they are:

1 The real time cell concentration analysis system will clearly indicate major growth phase changes from lag to exponential growth, exponential growth to stationary, and as secondary consideration "possibly" stationary to death phase accurate within 3 minutes of phase change or better. This accuracy is to be verified by the characteristic cell growth phase comparison of data generated by the system itself as well as cross compared to plots generated by data analysis of samples drawn from the reactor near likely transition points.

2 The real time cell concentration analysis system will determine exponential growth rate accuracy within 10% of the YC-100 cell counter. This comparison will be based on samples taken in the leading portion of the exponential growth phase and analyzing them using the YC-100's ability to distinguish dead cells from total cells when determining concentration.

3 The real time cell concentration analysis system will function reliably with no requirement for a person to attend to it making software adjustments or physically alter parts during operation.

4 The real time cell concentration analysis system will serve as a prototype furthering the efforts to create an analysis instrument dedicated to this purpose from the ground up.

5 The real time cell concentration analysis system will process recorded absorbance data into concentration automatically in a manner that is clear and easy to understand.

Comment [T21]: Listed in this section are clear, quantitative goals that the project, if approved, must achieve in order to be considered successful.

These goals must be feasible and supported by the technical background.

Comment [T22]: Goals are specific and the means of determining success are made clear.

STATEMENT OF WORK

The work done to complete this project consists of three primary phases broken down into, pre-experimentation, configuring and tuning, and performance evaluation. Steps and procedure are broken down in the following alpha-numerically bulleted list followed by an estimated time table. Options to systematically reduce experimental load as well as contingency plans are outlined as well should unexpected delays and obstructions occur or if aspects of the experiment fail outright.

1 Pre-Experimentation
- A UV/VIS Absorbance Profiling
  - a Material Preparation
    - i. Create 500mL bioreactor media for a yeast culture following sterilizing SOP and combining 10g/L yeast extract, 20g/L Bacto Peptone, and 40g/L glucose with DI water and agitating until no solids are visible and refrigerate.
    - ii. Create 500mL "raw yeast" solution by following sterilizing SOP and combining 10g/L active yeast culture with DI water and agitating until an even suspension exists with no visible clumps and refrigerate.
    - iii. Run a "standard" yeast cell culture bio reaction using 1.5 L of media as defined in step 1.1Ai, and an inoculum of 2g/L yeast. Controller settings should be 6.5pH, 120ccm air sparging, 500rpm agitation, and temperature setting at 37^{o}C. Taking samples after inoculum is introduced and every 30minutes after, labeling appropriately and refrigerating immediately.
  - b Material Processing
    - i. Using sterilizing SOP create labeled dilution sets using media from step 1.1Ai and yeast solution from step 1.1Aii in percent concentrations of 100, 85, 70, 55, 40, 25, and 10.
    - ii. Process dilution set and bioreaction samples through Lambda 35 UV/VIS spectrometer using SOP and analyze from a wavelength of 1000 to 200nm
    - iii. Process dilution set and bioreaction samples through Synergy-HT Bio-Tek, Microplate Reader using SOP and analyze from a wavelength of 1000 to 200nm.
    - iv. Process bioreaction samples through YC-100 Cell Counter using SOP and performing dead and total cell counts.
    - v. Dilute bioreaction samples with DI water to 29% of original concentration and reprocess on Lambda 35, and the Microplate Reader.
- B Data analysis
  - a Compare wavelength profiles generated from steps 1Abii to 1Abv to determine a wavelength were yeast cells absorb strongly but media does not, consider this "ideal wavelength".
  - b Calculate absorbance vs. concentration for media and yeast cells at ideal wavelength to determine absorbtivity constant ε for both. If notable waste or side product absorbance is determined to exist in bioreaction samples, consider calculating absorbtivity constant ε for waste product as well.
  - c Calculate Yx/s and if using waste product absorbance Yx/w from bioreactor sample results.

Comment [T23]: This section gives a clear, step-by-step, written plan of work.

This example was created before CH EN 1705. Now, the statement should also indicate who will be primarily responsible for which tasks. It is should also be clear for which tasks CH EN 1705 students will be responsible and the time requirements on each.

Comment [T24]: The plan of work contains sufficient explanation and technical acumen so that the reviewer would be convinced the plan is sound and feasible.

d Build Beer's Law equation relating absorbance in terms of yeast cell concentration using Yx/s (and if including waste product absorbance Yx/w) to keep all concentration parameters in terms of yeast cell concentration.

2 Configuring and Tuning
- A Flow through cell pump configuration
  - a Setup "blank" bioreactor filled with 1.5L media and settings as per step 1Aaiii without inoculum.
  - b Connect sterilized peristaltic pump and lines to bio reactor and flow through cell in Spectronic 21 UV/VIS spectrometer.
  - c Set to read absorbance of media and start pump.
  - d Adjust pump speed so that air bubbles do not get trapped in tubes but also do not get pushed down far enough to interfere with UV/VIS light path.
  - e Verify stable absorbance signal and lack of air buildup in fluid lines for 10minutes, record optimized pump setting.
- B UV/VIS Absorbance Offset Analysis
  - a Run 100%, 55%, and 10% concentrations of media and yeast solution created in step 1Aai and 1Aaii through the flow through cell of the Spectronic 21 and compare absorbance values at the ideal wavelength chosen in step 1Ba to those obtained via the lambda 35.
  - b Generate offset values to the equation created from step 1Bd for use in the continuous read Spectronic 21.
  - c Create 3 randomized known concentrations of media and yeast solution and test analyze with spectronic 21 to verify beer's law equation functionality.
- C Tuning Reactor Run
  - a Connect Spectronic 21 to bioreactor and run bioreaction as in step 1Aaiii.
  - b Take samples every 1hr and attempt to process absorbance of the "ideal wavelength" (IW) immediately on Lambda 35, Microplate Reader, and yeast cell concentration on the YC-100 Cell Counter.
  - c Process data with equation and verify functionality, adjust equation as needed to make a better fit of data.
  - d Encode equation into OPTO software receiving signal from spectronic 21 to automatically generate plot and yeast cell concentration values

3 Performance Evaluation
- A Initial Run – Maiden Voyage
  - a With Spectronic 21 connected to the bioreactor and enabled. And with OPTO software configured and running. Perform a yeast reaction as in step 1Aaiii.
  - b Take samples every 1hr.
  - c Attempt to process samples immediately for IW absorbance on the Lambda 35, and Microplate reader, as well as cell concentration on the YC-100 Cell Counter.
  - d Compare data analysis, attempt to build bioreaction growth phase curves with each instruments data set if possible and compare to automatic concentration data and plot generation from customized OPTO software.
  - e Evaluate accuracy and ability to indicate phase transition clearly.

f Further adjust equation or software parameters for better clarity and/or accuracy if necessary.

B Evaluation Series To Establish Statistics (as far as time allows)
- a Run a series of three bio reactions as described in step 1Aaiii with the spectronic 21 and customized software connected and enabled.
- b On each run attempt to specifically collect samples in target growth regions of the bioreaction. 2 samples from the lag region, 5 at the beginning of the exponential growth phase 10 minutes apart, and 2 samples in the static phase.
- c As in step 3Ac through 3Ae evaluate yeast cell concentration accuracy and growth phase transition indication.
- d Generate approximate 95% confidence error for both concentration accuracy and growth phase transition time.

C Deviant Bioreaction Test (if time)
- a Run a bio reaction with parameters notably different from 1Aaiii with spectronic 21 and OPTO software connected and enabled to the bioreactor.
- b Take samples and analyze as in steps 3Bb and 3Bc to verify robustness of real time concentration analysis configuration.

D Deviant Absorbance Test (if time)
- a Run a bioreaction as in step 1Aaiii with an impurity present at an initial known concentration and absorbance
- b Calculate theoretical absorbance offset in equation and enter option in software to enter offset values for compounds beyond media and cells absorbing notably at the IW.
- c Perform bio reaction and analyze as in steps 3Bb and 3Bc for equation robustness.

Table 1 Estimated Project Time Table

Week	Date	Project Steps Completed
0	3/2/2010	1Aai, 1Aaiii
0	3/4/2010	1Abii, 1Abiii
1	3/9/2010	Complete up to 1Abv
1	3/11/2010	Complete up to 2Bc
2	3/16/2010	Complete up to 2Cd
2	3/28/2010	Complete up to 3Af
Facilities Closed		
3	3/30/2010	Work on 3B
3	4/1/2010	Work on 3B
4	4/6/2010	Complete up to 3Bd
4	4/8/2010	Build Report of Work

Should the project fall behind certain aspects can be treated as "optional" and skipped in order to restore the schedule within reason. The following items can be treated as optional to reduce the experiment load in 4 stages should the indicated steps not be achieved according to the schedule given by Table 1:

Comment [T25]: The proposed work schedule is reasonable and achievable.

Specific dates are given for completion of each major phase of the project.

Comment [T26]: Contingency plans are given to account for significantly probable roadblocks the group may face if they are allowed to put their plan to actions.

Experiment Reduction 1 The earliest aspect of the experiment that can be treated as optional and stripped from the experiment without changing the overall design early is sample analysis on the Microplate Reader. This analysis is arguably redundant since both the Lambda 35 and Microplate Reader use UV/VIS spectroscopy for analysis. If the project has not yet completed step 1Abv on schedule, Microplate Reader analysis will be dropped from all parts of the experiment.

Experiment Reduction 2 If step 2Cd is not completed on time it can be skipped so long as the OPTO software can still communicate enough information to clearly indicate the cell culture growth phase transitions.

Comment [T27]: Watch for inconsistent formatting, such as this odd gap.

Experiment Reduction 3 If step 3Af is not completed on time the entire step 3A can be skipped as it is to a degree a refinement of step 2C.

Experiment Reduction 4 If step 2B is not completed on time then error analysis of concentration accuracy must be estimated from previous data or left out of the experimental results and explained.

Should certain aspects of the experiment fail with no solution found to be reasonable in terms of time and/or resources the following contingency options are to be considered:

Contingency Plan 1 Should the bioreactor and its control system fail, the experiment will be carried out skipping bioreactor runs and be tested on a "dummy" system composed of a sterilized large beaker covered with cheesecloth using media and inoculum identical to what would be used in the bioreactor and allowed to run its course at room temperature without sparging or pH control. While not ideal this should still allow the real time cell concentration system to be developed.

Contingency Plan 2 Should any one of the three analysis instruments fail (lambda 35, Microplate Reader, YC-100), it must be dropped from the experiment and the other two instruments should be focused on.

Contingency Plan 3 Should the delivery loop mechanism (pump and flow through cell) of the spectronic 21 fail, the experiment will change to be focused on analyzing why it failed and what is needed to circumvent it, possibly moving on to design a customized flow through cell or filtration system if time.

Contingency Plan 4 Should the spectronic 21 instrumentation fail, the project should be re-focused to attempt working with T. Butterfield's prototype sensor instead, either by substituting it into the already outlined experiment or by following direction of Mr. Butterfiled in analysis and design of the prototype sensor system itself.

Any equipment or experiment failures outside the scope of the pre-existing contingency plans will be dealt with as needed and outlined clearly in the final experimental report.

The total estimated cost of this experiment is broken down and listed in Table 2. The format of this table was provided by Dr. Eddings and is available at the University of Utah's Chemical Engineering Projects website (http://www.che.utah.edu/projectslab/Lecture_Material/material.php).

Table 2: Budget Calculation

Comment [T28]: The proposed budget includes all required categories, is correctly calculated, and is realistic and consistent with the schedule.

	Budget Calculation for "Continuous Cell Counter Project"			
		Labor	Weekly	Dollar
A. Senior Personnel (list names below)		Weeks	Rate*	Amount
1	Stephen Grobstein	4.5	$ 1,250.00	$ 5,625.00
2	Christopher Brau	4.5	$ 1,250.00	$ 5,625.00
3	James Johnson	4.5	$ 1,250.00	$ 5,625.00
4				$ -
5				$ -
6				$ -
A. Subtotal				**$ 16,875**
	** calculated on Labor_Rate_Calc sheet (see tabs at bottom of this sheet)*			
		Labor	Weekly	Dollar
B Other Personnel		Weeks	Rate*	Amount
	Engineering Staff	0.5	$ 1,000.00	$ 500.00
	Other Professionals (Technician, Programmer, etc.)	1.5	$ 807.69	$ 1,211.54
	Secretarial/clerical	0	$ 461.54	$ -
	Other			$ -
B. Total Salaries (A+B)				**$ 18,587**
C. Fringe Benefits			Calculation	
	Percentage	33%	**$ 6,134**	
C. Total Salaries Wages and Fringe Benefits				**$ 24,720**
D. Equipment (itemize and dollar amount for each)			Item Cost	
	Cell Counter Cartridges		$ 900	
	Tubing		$ 25.00	
D. Equipment Subtotal				**$ 925**
E. Travel			Subtotal	
	Domestic			
	Foreign			
E. Total Travel				$ -
F. Other Direct Costs			Subtotals	
	Materials and Supplies		$ 209	
	Publication Costs			
	Consultant Services			
	Computer Services			
	Subcontracts			
	Other			
F. Total Other Direct Costs				**$ 209**
G. Total Direct Costs				**$ 25,854**
H. Indirect Costs				
	Percentage	49.5%		
H. Indirect Costs				**$ 12,798**
I. Total Direct and Indirect Costs				**$ 38,652**
	This represents total actual cost of the project			
J. Cost Sharing				$ -
K. Net Cost of Project to Sponsor				**$ 38,652**

CAPABILITIES

The work load for this proposal has been evenly distributed between three group members; Christopher Brau, James Johnson and Stephen Grobstein. Stephen and Chris both have a high degree of experience in bio-engineering and are pursuing careers in that field. As a whole, the group has an array of experience working with bio reactors and bio-processes. This includes professional experience, extracurricular learning opportunities and experience gained from the curriculum at the University of Utah and University of Minnesota.

Stephen Grobstein has taken part in several extracurricular research opportunities. These include participation in Advanced Bio Fuels' Fuel Ethanol workshop. A program aimed at developing engineers' knowledge and understanding of advanced bio fuel technologies. Stephen has also worked with a USTAR professor studying Great Salt Lake algae strings. Steve has firsthand experience working with the BioFlo 110 Modular Benchtop Fermentor as he has researched the effects of rapamycin on growth phases of yeast. From the course of this research Stephen and his previous experimental group concluded that an on-line cell counting mechanism would be an invaluable addition to the current bioreactor module.

Christopher Brau has amassed plenty of related experience while working for Thermal Fischer. As a chemical engineer Chris has exceptional insight into bio-processes as he is also double majoring in Bio-Chemistry from the University of Minnesota. In his professional experience with HPLC, Chris has worked extensively with UV/VIS spectroscopy, with much attention directed towards absorbance profiling of organic compounds and molecules. He has also had hands-on experience working on industrial Bio-Reactors and has assisted in the design of a bio-reactor control system.

James Johnson has worked with an industrial rotating bed contactor used for the biological treatment of waste water at Silver Eagle refinery; testing affluent water samples from the RBC unit to determine bio-activity and degradation during the water treatment process. In his time as a laboratory technician at Silver Eagle, James gained a working knowledge of the operation of many analytical devices; such gas chromatography and x-ray spectroscopy. James has also gained much practical experience while working at FLSmidth Minerals. Here has had been familiarized with various flowsheet operations such as filtration, sedimentation and flotation. He has assisted in the mechanical set-up of bench and pilot scale testing equipment for each of these processes.

Comment [T29]: Team management structure should be identified. In this sample proposal there is too little detail on which tasks will fall to which individuals. In carrying out their project, however, each group member did take on different responsibilities. Consideration of such division of labor should be demonstrated.

This proposal was written before we had 1705 students working with 4905 students. Now, a 4905 team member should be clearly indicated as the one primarily responsible for managing the 1705 student. The roles of all 1705 student team members should also be clearly explained.

Comment [T30]: The relevant experience and qualifications of key team members are listed.

If a 1705 student has important experience relevant to the project, then that experience should also be described.

EQUIPMENT AND FACILITIES

The existing Bio-reactor located in the Chemical Engineering Laboratory, is the BioFlo 110 Modular Benchtop Fermentor, manufactured by New Brunswick Scientific. It consists of a 2 liter jacketed vessel in which cell growth occurs. It is operable as either a CSTR or batch reactor. The BioFlo reactor has advanced control capabilities as it is equipped with several control elements such as ph, gas flow, temperature, agitation, and anti-foam control. The unit also consists of: three peristaltic pumps, used for the addition of concentrated acid and base solutions and anti-foaming agent; two probes, for measuring ph and dissolved oxygen; and a gas flow mixer with rotameter, for controlling the flow from compressed nitrogen and oxygen tanks. Selection of the various operational modes is made by accessing the primary control unit; seen below at the top of the bioreactor:

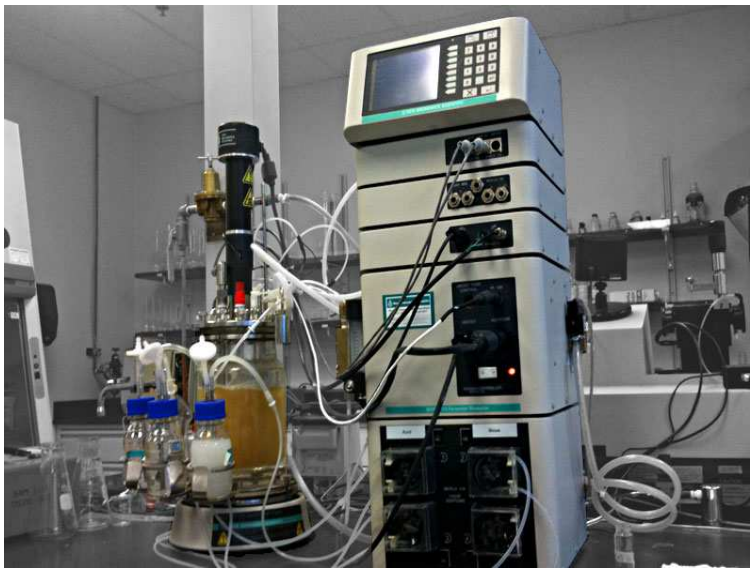

Figure 2: BioFlo 110 Modular Benchtop Fermentor

While this unit is highly advanced, there are still limitations that arise when conducting research with this equipment. Currently the BioFlo reactor has no means of measuring cell concentration in real-time. Cell concentrations are determined by sampling the reactor and measuring the number of cells in the sample with the NucleoCounter Y-C 100 cell counter. This device (depicted below) employs cutting-edge technology enabling the rapid and accurate analysis of culture samples.

Comment [T31]: In this section all the needed equipment and materials (including chemicals) should be identified and adequately described.

Note that this project did not require purchase of any major pieces of equipment. If it did, an accurate price quote should be given in this section.

Comment [T32]: This is an image from the Projects Lab web site and, as such, it should have a reference.

Figure 3: NucleoCounter Y-C 100 Cell Counter

The principal drawbacks of using this cell counting method are that samples must be taken and processed intermittently; this means that a judgment call must be made to decide the best time to take samples. The sheer volume of samples that would be required to determine growth characteristics for a cell culture dictate that a preferred method of tracking cell concentrations be developed.

It is proposed that a new method using continuous UV/VIS spectroscopy with a flow through cell be employed to determine cell concentrations. With this method, cell solution will be pumped from the bio reactor to a flow-through cell by a peristaltic hose pump. Absorbance from the flow through cell will be measured continuously using the Bausch & Lomb Spectronic 21 continuous read UV/VIS spectrometer. The Spectronic 21 and flow through cell are depicted below:

Figure 4: Bausch & Lomb Spectronic 21

Figure 5: Flow-Through Cell

It is the goal of our group to correlate this absorbance data to fit cell concentration profiles. Correlating absorbance and cell concentration profiles will involve running batch tests in the bioreactor and sampling the cell culture every half hour. Samples will then be analyzed using the cell counter to determine the concentration of live cells. Absorbance of these samples will be measured over a spectrum of wavelengths

by both the P.E. Lambda 35 UV/VIS spectrophotometer and the Synergy-HT Bio-Tek, Microplate Reader; both of which can be seen below in the following figures:

Figure 6: P.E. Lambda 35 UV/VIS spectrophotometer

Figure 7: Synergy-HT Bio-Tek, Microplate Reader

While the real-time concentration's measured by the UV/VIS may not be as reliable as the measurements from the cell counter, it will offer valuable information about the growth characteristics of the cell culture. The on-line measurements will allow for the determination of the type of growth that is taking place in the bioreactor and give insight as to when samples should be taken for analysis on the Nucleo Y-C cell counter.

ANTICIPATED BENEFITS

Currently the only way to determine if the cells are entering into a new phase of growth is to record the Oxygen concentration and evaluate it for trends or by blindly take samples at short regular intervals. In the first case accuracy is questionable and actual transition times are very difficult to determine in real time. In the second case it is necessary to process a large amount of samples costing time and resources. In both cases it is necessary to constantly interact with the bio reactor during a given experiment.

With the completion of this project any person or group running the bioreactor will be able to determine when the culture is transitioning between the lag, exponential, and stationary phases of growth within 3 minutes. The transition from the stationary to the death phase is difficult to determine with UV/VIS spectroscopy. This is due to the similarity in absorbance characteristics between living and dead cells. Additionally the overall biomass concentration does not vary significantly during this transition. Fortunately, most experiments done with the bioreactor focus on the first three phases of growth and usually have no interest in the death phase. Having this immediate view of bioreaction progress can enable users to take samples at specific times relevant to the experiment being run. Additionally this view of cell concentration provides an early warning if something goes wrong with the experiment.

This system also records real-time cell concentration data automatically with an error of less than 10% compared to the YC-100. In some cases this accuracy may be sufficient for a given experiment; reducing or eliminating the need to take samples from the culture. This will directly reduce the time, equipment and reagents needed to analyze samples taken from the culture.

In general, the hands-free automated nature of the real-time cell concentration system will free users to pursue other aspects of an experiment instead of manually monitoring parameters and/or taking samples constantly. Additionally it will allow focus on the data of interest and reduce costs associated with analyzing samples.

Comment [T33]: In this section the anticipated benefits of the project are persuasively presented, without exaggeration or false claims.

The claimed benefits should reasonably follow from the information given in the Technical Background section, or from published articles, with proper references where necessary.

Anticipated Safety and Environmental Impact

There are no anticipated safety or environmental concerns with this work.

Comment [T34]: All possible safety and environmental hazards should be identified, and they are not in this example.

The authors state there are no such concerns with this work. However, a leak occurred while running the reactor when no one was present, flooding the room below the Bioreactor. Such possibilities must be included in this section.

Nomenclature

Symbol	Definition	Units
μ_{net}	Specific growth rate	h^{-1}
X	Cell mass concentration	$\frac{g}{L}$
X_o	Initial cell mass concentration	$\frac{g}{L}$
t	Time	h
$Y_{X/S}$	Yield coefficient for cell/substrate concentration	$\frac{g\ X}{g\ S}$
ΔX	Change in cell mass concentration	$\frac{g}{L}$
ΔS	Change in substrate concentration	$\frac{g}{L}$
OUR	Oxygen uptake rate	$\frac{mg\ O_2}{L * hr}$
μ_g	Gross specific growth rate	h^{-1}
Y_{X/O_2}	Yield coefficient for cell/O_2 concentration	$\frac{g\ X}{g\ O_2}$
OTR	Oxygen transfer rate	$\frac{mg\ O_2}{L * hr}$
$K_L a$	Volumetric transfer coefficient	h^{-1}
C^*	Saturated dissolved oxygen concentration	$\frac{mg}{L}$
C_L	Actual dissolved oxygen concentration.	$\frac{mg}{L}$
S	Growth rate limiting chemical species	$\frac{g}{L}$
K_s	Substrate saturation constant	$\frac{g}{L}$
K_I	Inhibitor saturation concentration	$\frac{g}{L}$
μ_m	Maximum specific growth rate	h^{-1}
$k_L a$	Volumetric Mass Transfer Coefficient	h^{-1}
A	Absorbance	-
I	Intensity of Incident Light	Candelas

Comment [T35]: Every symbol that appears in the text must appear in this table and be defined. The dimensions of each must be given. If a quantity is dimensionless, a hyphen is used to so indicate.

Symbols should be listed alphabetically, and Greek letters should be separated from Latin to make it easier for the reader to find a symbol of interest. Such organization was not used in this sample report.

References

1. Wikipedia: http://en.wikipedia.org/wiki/Beer-Lambert_law
2. Shuler Michael L., and Kargi Fikret, *Bioprocess Engineering Basic Concepts*, 2nd Edition., Prentice Hall PTR, Upper Saddle River, NJ (2007).

Comment [T36]: All references used in the text are listed in an appropriate order and style. All the needed information is accurately included.

In general, this proposal would benefit from inclusion of more supporting material in the Technical Background. Two references (particularly when one is to Wikipedia) does not lend enough confidence in the author's understanding of the work done by others in the area of their proposal.

APPENDX A: MSDS

Comment [T37]: Appendixes contain supplemental information.

This proposal does not include preliminary data, though the authors did have such data. Raw preliminary data should be included in an appendix.

MSDS for Sodium Hydroxide

General

Synonyms: caustic soda, soda lye, lye, white caustic, aetznatron, ascarite, Collo-Grillrein, Collo-Tapetta, sodium hydrate, fotofoil etchant, NAOH, STCC 4935235, sodium hydroxide pellets, Lewis red devil lye, stamperprep, tosoh pearl

Molecular formula: NaOH
CAS No: 1310-73-2
EC No: 215-185-5
Annex I Index No: 011-002-00-6

Physical data

Appearance: odourless white solid (often sold as pellets)
Melting point: 318 C
Boiling point: 1390 C
Vapour density:
Vapour pressure: 1 mm Hg at 739 C
Specific gravity: 2.12
Flash point: n/a
Explosion limits: n/a
Autoignition temperature:
Water solubility: High (Note: dissolution in water is highly exothermic)

Stability

Stable. Incompatible with a wide variety of materials including many metals, ammonium compounds, cyanides, acids, nitro compounds, phenols, combustible organics.
Hygroscopic. Heat of solution is very high and may lead to a dangerously hot solution if small amounts of water are used. Absorbs carbon dioxide from the air.

MSDS for Sulfuric Acid

General

Synonyms: oil of vitriol, mattling acid, vitriol, battery acid, dipping acid, electrolyte acid, vitriol brown oil, sulphuric acid
Molecular formula: H_2SO_4
CAS No: 7664-93-9
EC No: 231-639-5
EC index No: 016-020-00-8

Physical data

Appearance: Colourless oily liquid
Melting point: -2 C
Boiling point: 327 C
Specific gravity: 1.84
Vapour pressure: <0.3 mm Hg at 20 C (vapour density 3.4)
Flash point:
Explosion limits:
Autoignition temperature:
Water solubility: miscible in all proportions

Stability

Stable, but reacts with moisture very exothermically, which may enhance its ability to act as an oxidizing agent. Substances to be avoided include water, most common metals, organic materials, strong reducing agents, combustible materials, bases, oxidising agents. Reacts violently with water - when diluting concentrated acid, carefully and slowly add acid to water, not the reverse. Reaction with many metals is rapid or violent, and generates hydrogen (flammable, explosion hazard).