

CHE 194 Biochemical Engineering Laboratory

Spring 2009

R 1600 - 2050

Location of lecture Rm 338

Location of lab Rm 109

Dr. Claire Komives

Engr. 109, 924-4032

Office hours:

MWR 10:30 - 11:50 am

By appointment

Text: There is no required text for this course. Students having completed CHE 192 will find their text helpful: Bioprocess Engineering, Michael L. Schuler and Fikret Kargi, Prentice Hall, 1992. Purchase of this text is not required. Also helpful is the New England Biolabs Catalog. This is free and you can request a copy or just view it on-line at <http://www.neb.com/> A list of useful references will be included with each laboratory procedure.

Prerequisites: Undergrad CHE majors should have taken CHE 192 or have permission from the instructor. Non-CHE majors should have Chem 161 and Bio 3, or Chem 135 or equivalent, or Bio 135 or equivalent. This is a senior/grad course.

Course objectives

To introduce students to the fundamentals of laboratory practice in biochemical engineering. Consideration will be given to protein isolation and purification, basic molecular biology methods, microbial kinetics and energetics, enzyme kinetics, and operation of bioreactors. Biochemical engineering is a multi-disciplinary subject and students are expected to gain an appreciation for the multi-disciplinary nature of the subject.

Notice to students

Students are expected to work in groups relatively independently. Students will not be shown how to do experiments. From the basic principles of the various instruments, gained through reading, the students should be able to run the experiments with relatively little guidance from the professor. The role of the professor is to set up the labs, provide appropriate reading materials ahead of time for the students, and to provide minimal clarification during the lab. Students who have not had previous courses with a wet-lab will have difficulties succeeding in this course.

The first day of class students will be introduced to the course:

- course overview
- lab safety
- lecture on basic principles of UV/Vis spectrophotometry
 - optical density
 - determination of extinction coefficient
 - total protein
- fermentation
- HPLC
- cell disruption
- enzyme kinetics
- ultrafiltration
- subcloning
 - optical density

- o determination of extinction coefficient
- o total protein

Following the intro lecture, students will be given an open notes quiz that counts for 10% of the course grade.

The second class period, students will be challenged with the task to individually make the following measurements with a spectrophotometer:

- 1) pipette a specified volume of water into 3 pre-weighed eppendorf tubes with a micropipettor.
- 2) prepare a set of standards of a compound and determine the extinction coefficient at a specified pH, and perform a scan of the compound at a specified concentration.
- 3) determine the concentration of protein in an unknown solution
- 4) determine the optical density of a solution of bacteria

These basic measurements are essential for many of the future lab modules and thus, students should each be able to complete the experiments individually. Data sheets from these experiments, as provided to the students on the first week of class, must be completed by the end of the second class period to receive credit.

Beginning on the third week of class, students will be assigned to groups and carry out experiments in four different lab modules:

Module I: Molecular Biology

Module II: Fermentation

Module III: Protein purification

Module IV: Protein handling

Course Grade Policy

10% Quiz Day I

5% Unknowns Lab

15% Midterm Exam - Exam on modular labs

20% Final Exam. Th May 21, 5:15 pm, rm 338 Biochemical Process Technology

20% One Major Lab Report on the inquiry experiments

10% Presentation on Inquiry lab

20% Four minor lab reports

Course Policies:

- Students must carefully review and sign the **ACKNOWLEDGMENT OF UNDERSTANDING SAFE LABORATORY PRACTICES AND MSDS SHEETS** at the time of the first meeting. Students are required to follow the safety practices outlined in this document, and students not following safe laboratory practices 3, 4, 14, 15, 25 and 28 will be given an official warning. Upon receiving the third warning, the student will be dropped from the class with an F grade.
- The course will begin promptly at 1600 on Thursdays, but it may not end by 2050, as listed in the schedule. To facilitate beginning on time, students are encouraged to arrive early to the class to turn in homework, etc. Students are encouraged to take advantage of university services for ensuring safety upon leaving the class.

- At the beginning of the first of the two weeks of each of the four modular lab periods, students will be given a quiz on their specific lab. The quiz will not be given credit, but students not receiving 60% or higher on the quiz will be given only 50% credit for that modular lab grade (50% of the grade on the report will be deducted).
- Students must complete every lab. Absence due to illness can only be excused by a doctor's report. There are 14 scheduled lab days and one exam period, in addition to the final exam. **Minor Reports may not be turned in for unattended lab sessions.** Each report is worth 5% of your overall course grade. Arriving late to the class is unacceptable. For every half hour you are late you will lose 20% of the credit on that weeks' minor report. Deduction of credit on reports due to tardiness is up to the discretion of the instructor.
- Reading materials for the laboratory experiments will be posted on the course website at least two weeks prior to the date of the experiments.
- You are encouraged to study together, but your grade will depend on your own mastering of the material. This refers, as well, to the minor laboratory reports. Even though the experiments will be conducted in groups, each student will be responsible for recording their own data and turning in their own report.
- Drop policy: Tuesday, February 3rd is the last day to drop this class without a petition. Petitions **will not** be approved for the following reasons: Taking too many units; poor advising; low grade in this class.
- A course grade of incomplete will be given in the most compelling non-academic circumstance, which must be documented in writing and approved by me no later than 24 hours before the scheduled final examination date.
- Exam scores and course grades will not be given over the phone, as this is a violation of confidentiality.
- Cases of academic dishonesty will result in an F on an exam, and a note to appropriate personnel on campus, including your advisor for addition to your file, and may result in an F in this course. **Cases of manufacturing false data will result in an F in the course.**

I. Technical Description

Module I. Introduction to Molecular Biology Methods: The plan for the molecular biology portion of the course involves subcloning the gene for green fluorescence protein (GFPuv, a protein whose fluorescence has been enhanced from the native protein by DNA shuffling; Cramer, A., et al. (1996) *Nature Biotechnol.* 14:315–319.) from the pGLO plasmid, a plasmid that has both ampicillin as a selection marker for checking the presence of the plasmid, as well as an arabinose promoter which enables user-control of the induction of the gene for GFP by addition of arabinose to the agar or broth. The pGLO plasmid is commercially available from Biorad, and it has the pBAD plasmid as its origin. This plasmid is designed for cloning genes. The sequence of labs is described in detail below in the syllabus. *E. coli* containing the plasmid will be cultured for the students prior to the first lab and a mini prep (purification of the plasmid DNA) will be performed and the purified plasmid stored. The gene for the GFPuv will then be amplified from the plasmid DNA with polymerase chain reaction (PCR) and two restriction sites are to be incorporated on the ends of the gene. (Namely, Xba1 upstream of the gene (5' end) and Kpn1 downstream (3' end).)

The objective of this work is to subclone the GFPuv into a plasmid that has been designed for high expression levels, to enable the purification of the expressed protein after fermentation of the host organisms. The plasmid system chosen for this work is the pET plasmid from Novagen (Moffatt, B.A. and Studier, F.W. (1986) *J. Mol. Biol.* **189**, 113—130.; Rosenberg, A.H., Lade, B.N., Chui, D., Lin, S., Dunn, J.J., and Studier, F.W. (1987) *Gene* **56**, 125—135.; Studier, F.W., Rosenberg, A.H., Dunn, J.J., and Dubendorff, J.W. (1990) *Meth. Enzymol.* **185**, 60—89.) The pET29a plasmid will be cut with the same enzymes as the amplified GFPuv gene. The GFPuv gene can then be ligated into the plasmid and then checked for ligation by DNA electrophoresis. The pET29a-GFPuv plasmid can then be used to transform competent BI21 *E. coli* containing a T7 RNA polymerase for enhanced expression of proteins from the pET expression system. The students do not need to prepare competent BI21 *E. coli* cells because they are purchased directly from Novagen ready for transformation. Kanamycin is used as a selection marker.

This lab in the past was spread over 5 weeks, but this year the molecular biology will be done as a modular lab. Because the groups will do the experiments separately, it is essential that the pre-lab is prepared carefully.

Module II. Fermentation: The first week will involve study and preparation of the vessel and preparation of analytical methods. In addition, students will grow up strains of the pET-GFPuv *E. coli* and analyze the cells for growth and protein production, as measured by optical density and fluorescence, respectively. The analysis of the cells in shake-flask culture will be a preparation for the analysis of the cells growing in the fermentor, which will be done during the second week. The fermentor preparation will include the following: The media and feed solutions will be prepared, a culture flask for growing the inoculum, the pH and dissolved oxygen (DO) probes will be checked to ensure functionality, the base feed pump will be calibrated, the chiller and off-gas condenser will be set up, the vessel can be filled with the autoclavable portion of the media and the sampling device can be tested, and finally, the vessel will be prepared to put in the autoclave. In addition, the offgas analyzer will be calibrated for measurement of O₂ and CO₂, and the Yellow Springs Instrument (YSI) glucose analyzer for analyzing the fermentation broth will be prepared. The BI21De3 cells do not produce any byproducts, so HPLC is not necessary for this experiment. Students are invited to come early during the day of the second lab session when the vessel is inoculated as this must happen early in the day so that the fermentation will be in progress when the students arrive to class. The students can review the initial progress of the fermentation and observe and analyze the fermentation during a 5-hour lab session. Time must be reserved during the second week for inducing the expression of the recombinant protein. The students can then complete the run by spinning down the cells and leaving them prepared for the following weeks washing. (Plan to stay late on the second week to accommodate the growth of the organism.) Analysis of the cell broth includes measurement of glucose, on-line CO₂ and O₂ measurements, optical density, and fluorescence.

Module III. Chromatography: Chromatography is the science that studies the separation of molecules based on differences in their structure and/or composition. In general, chromatography involves moving a preparation of the materials to be separated - the "test preparation" - over a stationary support. The molecules in the test preparation will have different interactions with the stationary support leading to separation of similar molecules.

Test molecules, which display tighter interactions with the support will tend to move more slowly through the support than those molecules with weaker interactions. In this way, different types of molecules can be separated from each other as they move over the support material.

Not all of the common amino acids found in proteins are charged molecules. There are some amino acids that contain hydrocarbon side-chains that are not charged and therefore cannot be purified by the same principles involved in ion-exchange chromatography. These hydrophobic ("water-hating") amino acids are usually buried away in the inside of the protein as it folds into its biologically active conformation. However, there is usually some distribution of these hydrophobic residues on the surface of the molecule. Since most of the hydrophobic groups are not on the surface, the use of HIC allows a much greater selectivity than is observed for ion-exchange chromatography. These hydrophobic amino acids can bind on a support that contains immobilized hydrophobic groups. It should be noted that these HIC supports work by a "clustering" effect; no covalent or ionic bonds are formed or shared when these molecules associate. (The information in this section was copied - not illegally - from a highly recommended web site, which I am sure you will find helpful: http://www.accessexcellence.org/TSN/SS/chromatography_background.html)

The principle objective of this 2-week module is the purification of the GFP produced in the bacteria. The first week of the laboratory will involve using the FPLC apparatus (Fast Performance Liquid Chromatography) that is computer controlled. First, familiarity with the apparatus and the software is to be achieved. The first step of the purification requires *equilibration* of the HIC column. It must be ensured that the column is properly packed, namely that the liquid can flow freely through the column and there are no air bubbles, etc. Once the equilibration procedure is complete, the sample can be loaded and the fractions can be collected. Fractions need to be collected manually since currently the fraction collector does not work. The detector will allow a chromatogram to be stored in the computer. The students should be prepared to design a scale-up procedure using information given in the lecture portion of the course. The fractions containing GFP can be easily visualized with the hand-held UV lamp. These should be pooled and concentrated with the Amicon stirred cell ultrafiltration apparatus. Second, the pooled fractions are run on an ion-exchange column. Please note that the FPLC is old and has not been functioning properly, so we may need to run the system by gravity. In this case, manual fractions will be collected and analyzed with a UV/Vis spectrophotometer.

The second week of the chromatography lab will involve using the HPLC to analyze the purity of the protein. The HPLC has a diode array detector that can take scans along the course of the run. GFP has a particular shape of scan that allows the exact determination of the total amount of protein.

Module IV. Protein Handling: Proteins are at the heart of biotechnology, because of their many and varied functions in living organisms. Most undergraduate laboratory courses do not include protein handling, and this is an important part of laboratory jobs in the biotech industry. The first week will be dedicated to enzyme kinetics. A UV/Vis spectrophotometer is available for measuring the formation of the product of a reaction catalyzed by trypsin, a common protease. The experiment is dedicated to the measurement of the catalytic rate

constant k_{cat} and the Michaelis Constant, K_M of the enzyme for the substrate, *Na-Benzoyl-DL-arginine-4-nitroanilide hydrochloride*. This substrate was designed for measuring the esterase activity of trypsin. The second week is dedicated to the cell homogenization of the cell pellet from the fermentation lab.

II. Schedule:

Class		Group I	Group II	Group III	Group IV	Due
22-Jan	LECTURE	n/a	n/a	n/a	n/a	
29-Jan	class cancelled					
5-Feb	Unknowns lab	n/a	n/a	n/a	n/a	Data sheets
12-Feb	Module lab 1.1	Module I	Module IV	Module III	Module II	
19-Feb	Module lab 1.2	Module I	Module IV	Module III	Module II	
26-Feb	Module lab 2.1	Module II	Module I	Module IV	Module III	Minor report 1
5-Mar	Module lab 2.2	Module II	Module I	Module IV	Module III	
12-Mar	Module lab 3.1	Module III	Module II	Module I	Module IV	Minor report 2
19-Mar	Module lab 3.2	Module III	Module II	Module I	Module IV	
26-Mar	class cancelled					
2-Apr	Module lab 4.1	Module IV	Module III	Module II	Module I	Minor report 3
9-Apr	Module lab 4.2	Module IV	Module III	Module II	Module I	Inquiry proposals
16-Apr	Midterm - Module labs					
23-Apr	Inquiry lab					Minor report 4
30-Apr	Inquiry lab					
7-May	Presentations					Major lab report
21-May	Final exam 7:15 pm					

Module I-1: Run PCR product on a gel to separate from primers and plasmid
Molecular Biology Purify PCR product with Qiagen Gel extraction kit
 Cut pET plasmid with Kpn1 and Xba1.
 Cut gene from PCR product with Kpn1 and Xba1
 Clean fragments from both cuts by DNA electrophoresis/cut bands from gel
 Purify cut insert and pET plasmid with Qiagen Gel extraction kit
 Ligation of GFPuv into pET plasmid (Genechoice kit)
 Transform competent "Novablue" *E. coli* cells with plasmid containing GFP gene and with control plasmid

Module I-2: Grow overnight cultures with antibiotic to select for transformants
 (CK will prepare overnight cultures from your plates)
 Perform mini-prep of pET and pET-GFPuv plasmids from *E. coli* prepared overnight
 Check for insert in plasmid by restriction digest with Kpn1 and Xba1
 DNA gel electrophoresis
 Transform competent BL21De3 *E. coli* cells with pET-GFPuv plasmid

Plate cultures and incubate

Module II-1 Induce protein expression in cultures of pGLO and pET-GFPuv E. coli and
Fermentation compare by measurement of fluorescence
 Prepare fermentor and autoclave
 Autoclave flask for inoculum

Module II-2 Fermentation and analysis

Module III-1 FPLC purification of cell extracts (possibly via gravity separation)
Chromato- 1) HIC with phenyl-sepharose
graphy 2) Ion exchange

Module III-2 HPLC purification of pooled/deionized peaks from Ion exchange

Module IV-1 Measure k_{cat} and K_m of trypsin esterase activity
Protein Handling

Module IV-2 Cell disruption of fermentation broth, analysis of purification factor

III. "Minor" Laboratory Reports

These write-ups are consolidated write-up of typical format of a well-kept laboratory notebook. Prior to beginning the lab, students should prepare the following parts (note this preparation will not be graded): objective, materials used and procedure should be completed PRIOR to actually performing the experiment. The minor lab report to be turned in will include the statement of objective, the results, discussion and conclusion. The week after each module is concluded, the report will be turned in. See schedule table for the exact dates the reports must be turned in. No late reports will be accepted (all late reports will receive zero credit), and the final minor lab report grade (20% of total grade for course) will be determined from the best 3 of 4 scores. See description of objective, results, discussion and conclusion in separate document (Proper Lab Notebooks).

Grading of reports

Point values for each section will be assigned:

Statement of objective	10 pts
Results	30 pts
Discussion	40 pts
Conclusion	20 pts

IV. Lab Prep Worksheets

These can be found on the internet at:

http://www.engr.sjsu.edu/ckomives/Courses/Biochemical%20Engineering%20Laboratory/lab_prep_worksheets.htm

These are not to be turned in for credit. However, you may find them helpful in preparing for the experiment.

V. Major Report

This report is written in a **very specific format** which is designed to give you practice writing a professional experimental report. There is a model report for you to use to write the report, which is posted on the course website. This report will be written in place of a weekly report and it will be written for the experiment carried out on Week V. of the lab course. Please follow the format to get full credit. Take note of the details of the writing, including the tense, person and level of detail appropriate for the report. Grammar is important for this report, as well. The report is done individually and not with your group. Copying from others will not be tolerated, write your OWN report.

The major report must be submitted to turnitin.com (info to be provided) to receive credit. No section of the report should be copied from any printed source, including web sites, books, or student reports.

VI. Inquiry-based experiments

Two lab periods are designed for you to do any experiment you want to do in the lab. These experiments can be done either alone or in teams of no more than two. Teams will turn in only one report, and a significantly higher standard is expected from team reports, namely, the scope of a two-person inquiry experiment should be greater than a one-person experiment because you have two people to do experiments than just one.

You should begin thinking about your inquiry experiments early in the semester because you should turn in a brief proposal a minimum of two weeks before your experiment is to be performed. The proposal should include the objective to be tested, materials and methods, procedure and a hypothesis statement about the expected result. You should clearly state what are the variables you will keep constant and which variable you will be changing during the course of your experiments. You should justify why the variable to be changed is significant to the system under study. You should also describe what mechanisms you must have in place to keep the constant values constant. You should include in the procedure a matrix of the runs you will perform, if appropriate. The grade on these reports will be slightly different than on the other minor reports. You will be graded according to the rubric shown below.

INQUIRY EXPERIMENT RUBRIC*

(NOTE: A score of 0 or 1 points indicates unacceptable performance)

Points	Description
Define Goals and Objectives of the Experiment	
0	No objectives identified
1	Objective identified but Not relevant to experiment OR Contains technical or conceptual errors OR Not measurable
2	Objective is conceptually correct and uses correct technical terminology but may be incomplete in scope or have grammatical errors.
3	Objective is complete, conceptually correct, concise, and uses correct technical terminology but may have grammatical errors.
4	Objective is complete, conceptually correct, concise, specific and clear, and uses correct technical terminology and grammar
Research any relevant theory and previously published data from similar experiments	
0	No theory. Previously published data not included. No computer simulations.
1	Theory section, published experimental data, and computer simulations included but not relevant to the experiment.
2	Theory section includes some of the relevant equations and some discussion relevant to the experiment. Published experimental data or computer simulations relevant to the experiment are included but not used to predict experimental results.
3	Theory section is well written, with equations and some discussion relevant to the experiment. Published experimental data and / or computer simulations relevant to the experiment are included but not used to predict experimental results.
4	Theory section is well written, with equations and discussion relevant to the experiment. Published experimental data are included as well as computer simulations relevant to the experiment. Theory, published data, and simulations are used to predict experimental results.
Select dependent and independent variables to be measured or controlled	
0	Cannot identify variables
1	Can identify variables but cannot distinguish dependent and independent
2	Can identify dependent and independent variables and relationship between them Can identify range for one variable
3	Can identify dependent and independent variables and relationship between them Can identify ranges for both
4	Can identify dependent and independent variables and relationship between them Can identify ranges for both Can identify appropriate increments for measurements
Describe an appropriate protocol for the experiment	
Protocol A – Select appropriate materials/specimens for the experiment to be safely conducted	
0	Has not identified materials for experiment
1	Has identified inappropriate materials or omitted PPE
2	Has a list of materials, including necessary PPE, but is missing key materials necessary for experiment
3	Has a list of materials, including necessary PPE, that is complete but is not clearly organized
4	Has a list of materials, including necessary PPE, that is clearly organized

Protocol B – Select appropriate methods for measuring/controlling variables

- | | |
|---|---|
| 0 | Has not identified methods for measuring/controlling variables |
| 1 | Has identified inappropriate method(s) |
| 2 | Has method(s) listed with no description or incomplete description OR
Has complete descriptions of method(s) that are presented, but list is not comprehensive |
| 3 | Has a comprehensive list of possible methods of measurement and instrumentation with complete descriptions but no discussion of limitations and dynamic range |
| 4 | Has a comprehensive list of possible methods of measurement and testing instrumentation and equipment based on available resources with complete descriptions including a discussion of limitations and dynamic range |

Protocol C – Select appropriate equipment and instrumentation

- | | |
|---|--|
| 0 | Has not identified instrumentation and equipment for measuring/controlling variables |
| 1 | Has identified inappropriate instrumentation and equipment |
| 2 | Has selected appropriate instrumentation and equipment with no justification OR
Incomplete list of instrumentation |
| 3 | Has selected appropriate instrumentation and equipment with incomplete justification |
| 4 | Has selected appropriate instrumentation and equipment with complete justification(for example based on accuracy, sensitivity, reliability, and available resources) |

Select proper range of independent variables

- | | |
|---|---|
| 0 | Ranges not identified |
| 1 | Ranges grossly unreasonable*** OR
Ranges provided with no justification |
| 2 | Range is reasonable* but not adequately justified OR
Range is unreasonable but based on correct theory with mathematical errors |
| 3 | Reasonable* range for all independent variables that are justified based on appropriate but possibly incomplete use of literature, correct theoretical calculations, and equipment/instrumentation limitations. |
| 4 | Optimal** range for all independent variables that are justified based on appropriate use of literature, theoretical calculations, and equipment/instrumentation limitations. |

Determine an appropriate number of data points needed for each type of measurement

- | | |
|---|--|
| 0 | Number of data points not identified |
| 1 | Number of points grossly unreasonable OR
Number of points provided with no justification |
| 2 | Number of points is sufficient to capture mathematical properties in an ideal world, but insufficient in the presence of experimental error or other confounding factors |
| 3 | Reasonable^* number of points for measurements, justified based on some but not all of the following: theory, equipment limitations, and potential error |
| 4 | Reasonable^* number of points for all measurements, justified based on consideration of theory, equipment limitations, and potential error |

* reasonable – pushing the limits of equipment, instrumentation or specimens, or captures some aspects of system behavior but is inadequate for complete analysis

** optimal – range will capture full response of system, is within limitations of equipment, instrumentation, and specimens, and will provide sufficient data for a statistically valid and complete analysis

*** unreasonable – theoretically impossible, or significantly outside the limits of the equipment, instrumentation, or specimens

^* reasonable – a sufficient number of points to capture the mathematical properties of the relationship (e.g. linear versus logarithmic) and account for possible measurement error

^*** unreasonable – insufficient number of points to capture the mathematical properties of the relationship

*Thalia Anagnos, Claire Komives, Nikos J. Mourtos, Kurt M. McMullin, Evaluating Student Mastery of Design of Experiment, Proceedings of the 37th ASEE/IEEE Frontiers in Education Conference, October 10-13, 2007, Milwaukee, WI, pp. TIA1-5.

VII. Course Learning Objectives

Students should be able to do the following upon completion of the course:

General procedures

1. Explain any term on this greensheet to a lay person.
2. Carry out bioprocess and molecular biology experiments taught in the course in a safe manner.
3. Document an experiment in a laboratory notebook format.
4. Determine the total protein in a solution of proteins.
5. Transfer volumes of liquid accurately using micro-pipettors.
6. Prepare buffer solutions at the appropriate pH.
7. Concentrate a protein solution using a stirred-cell ultrafiltration apparatus.
8. Prepare nutrient agar and nutrient agar plates.
9. Sterilize solutions and components using a steam autoclave.
10. Measure optical density of bacteria and estimate the concentration of bacteria in a sample.
11. Streak a nutrient agar plate with a solution of bacteria appropriately to achieve single colonies.
12. Measure fluorescence and optical density in the linear region of the instrument.

Molecular Biology experiments

13. Transform competent *E. coli* cells with a plasmid.
14. Cut DNA using a restriction enzyme
15. Estimate the annealing temperature of a PCR primerⁿ
16. Estimate the size of DNA using gel-electrophoresis
17. Purify a band of DNA from an agarose gel using a commercial kit.
18. Purify plasmid DNA using a mini-prep kit
19. Identify ribosome binding site, start and termination locations of gene transcription, location of PCR primer binding, promoter and other key features of a plasmid.ⁿ
20. Estimate the volumes of insert and vector solutions necessary for a ligation
21. Ligate an insert into a vector using a 5-minute ligation kit
22. Identify the bases on sticky ends of DNA cut with known restriction enzymes
23. Determine whether an insert is present after ligation and transformation

Chromatography

24. Partially purify GFP using an FPLC (or by gravity flow).
25. Analyze the purity of a protein of interest throughout the steps of a purification process.
26. Identify the fractions of eluate that contain the protein of interest.
27. Partially purify GFP using an HPLC.
28. Perform a HIC chromatography step to partially purify a protein
29. Perform an ion-exchange chromatography step to partially purify a protein

Protein handling

30. Measure the k_{cat} and K_M of an enzyme for a colorimetric substrate.
31. Design an experiment to confirm the k_{cat} and K_M of an enzyme

32. Design an experiment to measure the k_{cat} and K_M of an enzyme for a non-colorimetric substrateⁿ
33. Measure the rejection coefficient of a membrane in a cross-flow apparatusⁿ
34. Estimate the molecular weight of a protein using an SDS-PAGE process

Fermentation

35. Prepare a fermentor for autoclaving and autoclave it.
36. Prepare a semi-minimal media for an *E. coli* fermentation.
37. Perform a carbon balance on a fermentation as the material balance for the process
38. Manage the in-flows of glucose, base and air for a fermentor
39. Sample a fermentor.
40. Measure the optical density of a fermentation sample using a UV/Vis Spectrophotometer
41. Measure the glucose concentration of a fermentation sample using a YSI Glucose Analyzer
42. Estimate the oxygen uptake rate, carbon dioxide evolution rate using measurements of O_2 , CO_2 and total airflow of the offgas of a fermentor.
43. Estimate the glucose consumption rate in a fermentor based on glucose concentrations.
44. Identify the *phase* of a fermentation run based on the dissolved oxygen data.
45. Determine if a contaminant organism is influencing a fermentation run.
46. Estimate $Y_{X/S}$, $Y_{X/O}$ and $Y_{P/S}$ in a fed-batch fermentation run

Inquiry experiments

47. Students can design and execute an experiment in the bioprocess lab.

VIII. University, College, or Department Policy Information:

a) Academic integrity statement (from Office of Judicial Affairs):

“Your own commitment to learning, as evidenced by your enrollment at San José State University and the University’s Academic Integrity Policy requires you to be honest in all your academic course work. Faculty are required to report all infractions to the Office of Judicial Affairs. The policy on academic integrity can be found at <http://www2.sjsu.edu/senate/S04-12.pdf>

b) Campus policy in compliance with the Americans with Disabilities Act:

“If you need course adaptations or accommodations because of a disability, or if you need special arrangements in case the building must be evacuated, please make an appointment with me as soon as possible, or see me during office hours. Presidential Directive 97-03 requires that students with disabilities register with DRC to establish a record of their disability.”

c) Cell Phones:

Students will turn their cell phones off or put them on vibrate mode while in class. They will not answer their phones in class. Students whose phones disrupt the course and do not stop when requested by the instructor will be referred to the Judicial Affairs Officer of the University.

d) Evacuation Plan:

Either go out the front door into the Engineering Building hallway - turn LEFT and leave the front door of the building - or exit the back door of the lab onto the driveway. Walk around to the front of the building where everyone should meet to be counted.