A LAB-SCALE FERMENTATION COURSE WITH AN EMPHASIS ON RECOMBINANT PROTEIN PRODUCTION

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INTRODUCTION

The Golden LEAF Biomanufacturing Training and Education Center (BTEC) at North Carolina State University (NC State) is a cross-disciplinary center that provides educational opportunities to develop skilled professionals for the biomanufacturing industry. Due to the complexities of biopharmaceutical production compared with more traditional products (i.e. small-molecule pharmaceuticals), employees with two-year or four-year degrees comprise a majority of this workforce. Furthermore, the proportion of employees in this industry with Bachelor of Science degrees is growing. Despite drawing from a pool of well-educated candidates, employers commonly find that new hires lack hands-on experience in a laboratory environment and/or familiarity with operations within the industry, including current Good Manufacturing Practices (cGMP). Taken together, employer expectations demonstrate the need to offer students pursuing bachelor's degrees hands-on lab experience related to biopharmaceutical manufacturing.[1]

In this paper we describe a course taught at BTEC that focuses on the production of recombinant proteins by bacterial fermentation. This course, Fermentation of Recombinant Microorganisms, emphasizes the production of therapeutic proteins for the biopharmaceutical industry. As such, it provides hands-on training in upstream processing as well as an opportunity for students to work in teams and report their findings in a written format. All lab sessions use bench-scale equipment, which benefits students in many ways. First, the smaller bioreactors are more tractable and easily manipulated, allowing students to see more of the inner workings of the equipment. Certainly, the theory and operation of a 2 L bioreactor differ very little from that of a 200 L pilot-scale or even 20,000 L production-scale bioreactor. As an introductory course, Fermentation of Recombinant Microorganisms is designed to introduce students to scientific theory and some of the central technologies used in the manufacture of therapeutic proteins. The BTEC curriculum and facilities expose students to much of what industry currently uses to produce biopharmaceuticals.

Additionally, this course design has inherent learning advantages in addition to offering hands-on experience and an introduction to the theory underlying biomanufacturing operations. As a central element of science education, the laboratory offers an environment where inquiry allows students to learn in a distinct way compared to a traditional classroom. Inquiry within this context gives students an opportunity to formulate hypotheses, analyze data, explain findings, and defend their positions, all of which help them retain more material and become more effective learners.[2,3] Research also shows that effective integration of theory and practice improves student attitudes toward learning and, subsequently, their achievement of learning outcomes.[4] The Fermentation of Recombinant Microorganisms course is perfectly positioned to help students develop a variety of technical and professional skills that will make them extremely attractive as potential employees in the biopharmaceutical industry and in the many other industry sectors that use fermentation production processes.

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Bioprocessing is a field that touches on many of the principles typically taught in a chemical engineering curriculum, such as mass and heat transfer, thermodynamics, reaction kinetics, and process control. Indeed, there are many courses described in the literature that specifically cover fermentation in myriad applications. Generally, however, these describe only a single experiment within a larger course[5,6] or an application within the food or fuels industry.[7–9] Unfortunately, a single experiment is limited in the topics that can be covered, and the application of these principles to biomanufacturing is distinct enough from food and fuel that students would benefit from a more targeted course. The closest course to the one presented here is a one-week practicum that uses *E. coli* to produce green fluorescent protein, which offers a nice introduction to bioreactors and their operation.[10] In contrast, this paper presents a course that comprehensively covers the principles and application of fermentation to biomanufacturing and other industries.

COURSE DESCRIPTION

This eight-week course is required for undergraduate students who wish to complete a minor in Biomanufacturing. Students from a variety of NC State colleges, including Engineering, Agriculture and Life Sciences, and Sciences, typically pursue this minor (see Table 1). Additionally, the course is required for Chemical Engineering undergraduates with a Biomanufacturing concentration. Across two semesters, approximately 40 to 60 students take this course, which requires a general microbiology lab as a course prerequisite. The course consists of one hour and 50 minutes of lecture and five hours of lab each week.

Weekly lectures introduce students to the theory underlying the operation and performance of the fermentations. Generally, the lab activities build on the concepts introduced in that week's lecture period. Overall, lab activities follow a progression similar to what occurs during process development for a new biopharmaceutical product: assessing induction, aeration, control, and feeding strategies as well as characterizing and quantifying the cell growth and product formation along the way. Throughout the course students work in groups of two or three students to carry out a series of experiments to characterize and optimize a fermentation process at bench scale. Students select their own lab partners. Lab reports give students the opportunity to cooperatively analyze and explain results and present them in written format.

Together, the labs and lectures equip students to achieve the following learning objectives:

1. describe conceptually the manipulation and optimization of microbial growth in liquid culture
2. explain what makes a recombinant protein expression system suitable for production of a target protein
3. perform studies in a laboratory-scale bioreactor to evaluate cell growth and production of several recombinant proteins
4. describe how oxygen transfer, sterilization, process control, and downstream processing influence aerobic fermentation processes for the production of recombinant proteins
5. analyze data and present findings in written format
6. explain the factors involved in scale up for industrial production of recombinant proteins
7. describe upstream fermentation, downstream processing, purification, and measurement of activity, quality, and quantity of recombinant proteins

CLASSROOM ACTIVITIES

The lecture portion of the course introduces the fundamental theories and principles of fermentation and related technologies. This includes the selection and design of an expression system, liquid-gas mass transfer, sterilization, and process control. More specifically, chemical engineering students see a detailed example of the application of heat and mass transfer and process control, as well as exposure to bench-scale fermentor operations. For non-engineering students this course is often their first exposure to these topics. Subsequent lab

TABLE 1
Distribution of students in the Biomanufacturing minor (2019) by declared major.

College	Major	Number of Students Enrolled in one Semester
Engineering	Chemical and Biomolecular Engineering	19
	Mechanical Engineering	1
	Biomedical Engineering	1
Agriculture and Life Sciences	Biochemistry	6
	Agricultural and Biological Engineering	1
	Bioprocessing Science	3
Sciences	Microbiology	3
	Biological Sciences	5

sessions study the related phenomenon and allow students to apply the lecture material to analyze data and explain their findings. The data from previous lab sessions are used to design experiments later in the session. Lecture topics and a schedule are shown in Table 2. Together, the lab and lecture sessions aim to provide both practical and theoretical knowledge pertaining to fermentation.

LAB ACTIVITIES

Fermentation Conditions

All fermentations are performed with *E. coli* BL21(DE3) that has been transformed with one of two plasmid constructs. Both constructs use the pET27b(+) vector as a backbone, and both strains are maintained with ampicillin selection pressure (Novagen). These plasmids differ in the structural gene they contain and, subsequently, the product each strain produces. The two potential products act as models for products typically made in fermentation: an enzyme and a single-chain antibody fragment. The enzyme, β-glucosidase (CelB) from *Pyrococcus furiosus*, is a model thermophilic enzyme.[11] This particular enzyme is extremely thermostable, a desirable attribute in bioprocessing.[12–14] Furthermore, growth of *P. furiosus* is difficult to achieve in an industrial setting and as such demonstrates the advantages of using a recombinant *E. coli* as the production strain. The second product is a single-chain antibody fragment with affinity for *E. coli*, β-galactosidase. Single-chain antibody fragments have a wide range of uses from diagnostics to therapeutics, serving as a nice counterpart to an enzyme product.[15] Single-chain antibody fragments retain their binding affinity without need of any post-translational modifications. As such, our product again demonstrates the variety of products that are amenable to a bacterial host.

As BL21(DE3) is a lambda prophage strain, induction of the T7 expression system is achieved by addition of isopropyl β-1-D-thioglactopyranoside (IPTG). The expression system is described in Figure 1. Rich, complex media is used in all cases. Luria-Bertani (LB) medium is used for the first four lab

TABLE 2
Schedule of Laboratory Activities.

Week	Lecture Title	Concepts
1	Fermentation Fundamentals for Production of Peptides, Enzymes, Antibodies and Biopharmaceutical Products – Part 1	Applications of recombinant proteins. Value of cells as microbial factories. General process overview. Cell growth kinetics. Media formulation and batch, fed-batch, and continuous kinetics.
2	Fermentation Fundamentals for Production of Peptides, Enzymes, Antibodies and Biopharmaceutical Products – Part 2	Antibodies: structure, function, and applications. Enzymes: relevant examples. Molecular biology of protein expression. Model *E. coli* expression system.
3	Expression Systems for Recombinant Proteins, Metabolic Engineering and Use of "Omics" Tools	Different host cell platforms, bacterial, yeast, insect, mammalian; and their applications. Common issues and their solutions. Principles of metabolic engineering. Genomics, transcriptomics, and proteomics: how we use them to understand physiology.
4	Aeration and Oxygen Mass Transfer	Principles of gas-liquid mass transfer. Oxygen Uptake Rate and Oxygen Transfer Rate. Determination of kLa and its value as a metric and tool. Factors affecting kLa.
5	Sterilization and Inoculum Development	Kinetics of cell death by heat treatment. Quantitation and description of sterilization efficacy. Inoculum health and its effect on fermentation performance.
6	Biosensors and Process Control	Critical control parameters for fermentation: regulatory considerations. Feedback control. Sensor design for bioprocessing. PID control and tuning.
7	Downstream Processing	Isolation and purification of recombinant proteins. Basics of centrifugation, homogenization, chromatography, and tangential-flow filtration.

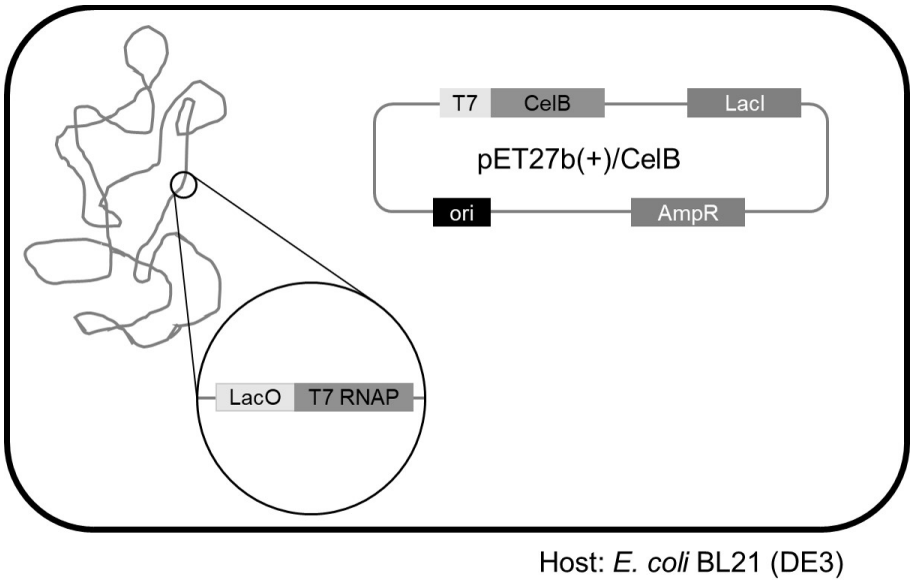

Figure 1. *Expression systems utilized in the Fermentation of Recombinant Microorganisms course. The system uses the lac operon to regulate the expression of a T7 RNA polymerase, which can then act on the T7 promoter in the expression plasmid, leading to production of the protein of interest.*

sessions. A modified optimized LB medium is used for fed-batch experiments to support additional biomass formation. All fermentations are performed using 2 L glass bioreactors with BIOSTAT B plus Sartorius controllers. All experiments use a working volume of 1 L. Prior to inoculation, ampicillin is added to a final concentration of 100 μg/mL along with 100 μL of antifoam 204. In preparation for the class, the appropriate strain is grown overnight in LB medium and the appropriate volume is inoculated into the bioreactor to achieve a starting OD600 of 0.05 (approximately 0.015 g/L). The fermentations proceed at 37°C for two hours until students arrive and begin sampling. Students sample every half hour for three to four hours (depending on the experimental conditions) and use these samples for a variety of analyses. Each lab session, described below, explores different fermentation operating conditions.

Lab Session 1

Lab Session 1 introduces the expression system used in the course. Students evaluate the effect of its induction on cell growth and product formation. The plasmid used simply has the gene of interest inserted into the Multiple Cloning Site (MCS) of that pET27b(+). BL21(DE3) is a lambda-prophage strain that has a gene coding for T7 RNA polymerase under the control of the lac operon. This means that in the absence of inducer (allolactose), expression of T7 RNAP is repressed and the gene of interest is not expressed. Upon addition of inducer (allolactose or its analog IPTG), the lac operon is derepressed and T7 RNAP is expressed, resulting in expression of the gene of interest. The T7 expression system is widely used in research and industry.

Induction of the expression system has the potential to significantly alter the metabolism of the host strain. The copy number of pET27b(+) is around 40. Plasmid abundance combined with the immense strength of the T7 promoter leads to a potentially large metabolic burden from protein expression. This is a known phenomenon, and its demonstration and characterization are the goals of Lab Session 1.[16–19] *E. coli* is most metabolically active during the exponential phase. As such, expression of product throughout the log phase should lead to maximal productivity. On the other hand, induction slows the growth rate of cells, so it is a benefit to induce later in the exponential phase when there is more biomass present for product formation. Therefore, an optimal expression time often lies somewhere in the middle of the exponential phase.[20,21] To explore this phenomenon, students are divided into small groups (two to three students per group), and different groups add inducer (IPTG) at different times during the experiment. Times include 0, 2, 2.5, and 3 hours after inoculation. The addition of no inducer acts as a control and demonstrates the leaky nature of the promoter used for genetic control.

Students take samples every 30 minutes to monitor cell growth as measured by OD600 and every hour to monitor CelB production using an enzyme-based assay. The cellobiose analog para-nitrophenyl glucopyranoside (PNPG) is used as the substrate for the reaction that is carried out at 95°C, and the formation of para-nitrophenol is measured spectrophotometrically. The assay is a modified version of that developed by Voorhorst and colleagues.[11] Data are shared between the groups. Post-lab reports include comparison of growth rates as well as enzyme activities between the different experimental conditions. Optimal conditions for product formation are carried through to following labs.

Lab Session 2

Lab Session 2 is the first activity to explore the role of oxygen transfer during fermentation. In this exercise student groups use different agitation speeds ranging from 200 to 800 RPM. The higher agitation rates are capable of delivering more oxygen into the liquid phase. Again, students collect data on cell growth and enzyme activity to characterize the

performance of their fermentations. Additionally, the Sartorius Multiple Fermentation Control System (MFCS) allows collection and plotting of data for pH, temperature, dissolved oxygen (DO), and agitation speed throughout the run. Reports include comparison of growth rates and enzyme production under the different agitation conditions. Students also discuss dissolved oxygen dynamics under the different experimental conditions.

Lab Session 3

Lab Session 3 acts a continuation of Lab Session 2 to explore oxygen transfer and its importance in recombinant protein expression during fermentation. This lab session compares fermentation under constant agitation to fermentation where DO is controlled at a specific setpoint. Different DO setpoints are used (10%, 20%, 30%, and 50%), and cell growth and enzyme formation are analyzed. These results are contrasted with those generated from a simultaneous fermentation performed using the optimal agitation rate from Lab Session 2. Reports include comparison between fixed agitation and fixed DO as well as the effect of different DO setpoints on growth and product formation.

Lab Session 4

Lab Session 4 explores both fed-batch fermentation as well as the effect of induction temperature on cell growth and protein expression. This exercise uses a different strain of *E. coli* than is used in all previous labs. The host strain is a close analog of *E. coli* BL21(DE3) with an additional pLysS plasmid for the expression of T7 lysozyme, which increases how tightly the expression system is regulated by the host.[22] Further, the pET27b(+) plasmid contains the structural gene for scFv13R4 in the MCS instead of the gene for CelB. This change in product is to expose students to different types of protein products. The media formulation is an enriched LB medium with an extra 5 g/L tryptone and 20 g/L yeast extract. Instead of NaCl, the medium contains 6 g/L Na_2HPO_4, 3 g/L KH_2PO_4, and 1 g/L NH_4Cl. Finally, glucose is added after autoclaving to a concentration of 2 g/L prior to inoculation. As a richer medium is used, optimal induction time and DO controls as determined in previous sessions may not be applicable. Therefore, an induction time of 3.5 hours and a DO setpoint of 30% are used; instructors determine these settings prior to the class period.

Different student groups use different induction temperatures and feeding strategies. Half of the groups reduce the temperature to 30°C after addition of IPTG while half keep the temperature at 37°C to highlight the impact of temperature on growth rate and inclusion body formation.[21] Additionally, half of the groups add 3 grams of glucose to the bioreactor at induction. These conditions are combined so that four distinct sets of conditions are tested: (1) 30°C induction, unfed; (2) 37°C induction, unfed; (3) 30°C induction fed; and (4) 37°C induction fed. Reports include the effect of feeding and induction on cell growth and antibody fragment production. Additionally, students discuss the effect of the above on total protein formation versus target protein formation and provide explanations of the observed effects. As the protein product is not an enzyme, students collect samples for analysis by Enzyme-Linked Immunosorbent Assay (ELISA) as well as Sodium Dodecyl Sulfate PolyAcrylamide Gel Electrophoresis (SDS-PAGE) during the following lab session.

Lab Session 5

During Lab Session 5, students perform ELISA and SDS-PAGE analysis of samples taken from fermentations carried out during Lab Session 4. ELISA and SDS-PAGE are common analytical techniques for a variety of proteins, especially antibodies. Four samples from the run are analyzed, one from immediately prior to IPTG addition and one collected every hour after that for three hours. The ELISA is a typical sandwich type, which uses a biotynilated *E. coli* β-gal to bind to the streptavidin plate and anti-cmyc horseradish peroxidase (HRP) conjugate as the enzyme linked reporter. Themofisher's TMB substrate solution is used for the enzyme reaction, and a 0.1 M sulfuric acid stop solution is used to increase sensitivity and reproducibility. Bovine Serum Albumin (BSA) is used to generate a standard curve for the ELISA method. SDS-PAGE is run using a Bio-Rad Any kDa Tris-Glycine pre-cast gel. Electrophoresis is performed at 125 mV and continues until the dye front reaches the bottom of the gel. The sample taken before induction serves as the control for SDS-PAGE analysis. Students use digital photos of gels stained with BioSafe Coomassie for their independent analysis.

Hazards

Lab activities in this course employ minimally hazardous materials. Solutions are prepared for students prior to their arrival in the laboratory to minimize chemical handling. Acetate buffer is an irritant, and dilute sulfuric acid is both a corrosive and irritant. The recombinant *E. coli* BL21(DE3) strain used in all labs is a Biosafety Level 1 organism. As such, good laboratory practice and general biosafety guidelines should be adhered to, including precautions for the handling of recombinant DNA. The SDS-PAGE lab uses precast polyacrylamide gels to remove the hazard of monomeric acrylamide. Bioreactors are also set up prior to student use. The major hazard from these units is the spinning agitator, which is isolated within the unit upon student arrival. The vessels used in this class are unable to accumulate pressure by design. Finally, students wear personal protective equipment, including a lab coat, gloves, and safety glasses, at all times in the lab.

STUDENT ASSESSMENTS

The various means of assessment and their percentage of the final grade are shown in Table 3 and described below.

TABLE 3
Relative weight of each assessment with numbers of each assessment noted parenthetically.

Assessment	Contribution to Final Grade
Lab Report (3)	40%
Quiz (3)	30%
Final Presentation (1)	20%
Professional Conduct	10%

Lab Reports

Each group completes three lab reports throughout the term for a total of 40% of their final grade. The rubric dictates a research journal style with abstract, introduction, materials and methods, results, discussion, and conclusion sections as well as a reference list and polished figures and tables. Students submit reports as a group, but each student is required to generate their own individual discussion section for each report that is worth 20% of the grade. Each group only generates a small fraction of the data needed to complete the report. As such, students experience the collaborative nature of research and the importance of taking good records and clear communication.

Quizzes

Throughout the semester students take three quizzes, each of which covers material from the previous two lab and lecture sessions. Quizzes ask three to five short-answer questions, and students are given 30 minutes to complete the assessments.

Final Presentations

Halfway through the term, the groups select a topic to research, and they deliver a 10-minute oral presentation on that topic during the final meeting of the class. The focus is on current and new technologies across different areas in the biomanufacturing industry.

Professional Conduct

The final 10% of a student's grade is a combination of attendance and professional etiquette. Etiquette includes the tone and timing of email correspondence with instructors, the ability to work as a team member to complete lab tasks and assignments, interaction with support staff, and adherence to safety guidelines. These criteria are assessed by the instructor and teaching assistants, with problematic occurrences being noted throughout the session.

COURSE EVALUATION

Student Self-assessment

To help determine the efficacy of the course in enabling students to achieve the learning objectives, a pre/post-course survey design was employed. Students anonymously completed surveys during the first week of class and within a week after course completion. Roughly 25% of the 80 students polled elected to complete the survey. Surveys were generated using Qualtrics software and delivered by email. The pre-survey asked students to rank their knowledge of six topics that are directly related to the course learning objectives and are covered in depth during the eight-week class. The post-course survey then asked the same six questions. Table 4 shows student responses both before and after course delivery.

TABLE 4
Pre- and post-course survey responses.

How would you rate your current knowledge of the following concepts:	Number of Responses (Pre-course – Post-course)				
	Poor	Fair	Good	Very Good	Excellent
Systems for expression of recombinant proteins	1 – 0	3 – 0	10 – 5	4 – 4	0 – 1
Cell growth kinetics	1 – 0	4 – 0	12 – 1	1 – 5	0 – 3
Process monitoring and control	3 – 0	7 – 1	6 – 4	1 – 3	0 – 1
Sterilization kinetics and corresponding technologies	7 – 0	8 – 1	1 – 4	2 – 4	0 – 1
Gas-liquid mass transfer kinetics	8 – 1	2 – 3	8 – 2	0 – 4	0 – 0
Processes that support fermentation operations	5 – 0	10 – 1	1 – 2	2 – 5	0 – 1

Initially, very few students ranked their knowledge of any subject above "Good". Less than 25% of students ranked their knowledge as "Very Good", and no students chose "Excellent" at the beginning of the course. The average ranking across all items increased in the post-course survey relative to the pre-course survey (p-value < 0.05 using a Welch's t-test). However, the data collected were anonymous, so there was no way to match individual pre-course and post-course survey responses. As a result, the within student differences and relative change from the pre-survey rankings cannot be determined. It also is important to note that the number of post-survey responses is fewer than on the pre-survey, which may bias the inferences made from these data.

Formative Assessment

Student self-assessment only demonstrates the participants' perceived aptitude. Indeed, it is known that many factors can contribute to inaccurate student perceptions.[23–25] Performance on formative assessments, therefore, is another crucial indicator of the success of achieving desired outcomes. Table 5 illustrates how the assessments align with the learning objectives. Students' responses on lab reports, presentations, and quizzes are used to inform on student learning.

Quizzes

Figure 2 demonstrates that student quiz scores show the highest variability among the formal assessments. 5% of students earned a perfect score on all quizzes, and only 5% of students scored below a C average. These lower-performing students received penalties for hastily or entirely unanswered questions. This could possibly be attributed to time constraints, but it is more likely due to a lack of proper preparation and/or understanding. Unfortunately, performance is seen to be worse on the later questions of these participants' quizzes, making it difficult to discern between the possible causes. More importantly, these results cannot be used to draw conclusions about understanding of those specific topics. Other students' responses do give some insight into learning, however.

Generally speaking, conceptual understanding was strong, while important details seemed to elude some students. For example, almost all students were able to correctly draw a growth curve and label growth phases. When asked to calculate an average growth rate from example data, however, many students failed to use only data from the exponential phase, and as such computed erroneous responses. Additionally, when students were asked to describe the role of the five components of an expression system, around 25% of the students provided the wrong answer for at least one, usually the importance of the host-strain attributes. Students answered questions asking for descriptions of protein structure, feedback control, and sterilization dynamics very well, with the typical outliers. Another conceptual area with varied comprehension was the role of process conditions on oxygen transfer and utilization. Students easily described the relationship between gas transfer coefficient and conditions we tested in lab. However, those conditions that we discussed only in class, such as viscosity and temperature,

TABLE 5
Alignment of assessments to learning objectives.

Learning Objective	Assessment	Outcome
Describe conceptually the manipulation and optimization of microbial growth in liquid culture	Quiz 1, Laboratory Reports	83% showed satisfactory or better understanding
Explain what makes a recombinant protein expression system suitable for production of a target protein	Quiz 1 and Quiz 2	91% showed satisfactory or better understanding
Perform studies in a laboratory-scale bioreactor to evaluate cell growth and production of several recombinant proteins	Laboratory Activities, Laboratory Reports	100% met this objective
Describe how oxygen transfer, sterilization, process control and downstream processing influence aerobic fermentation processes for the production of recombinant proteins	Quiz 2 and Quiz 3, Laboratory Reports	83% showed satisfactory or better understanding
Analyze data and present findings in written format	Laboratory Reports	100% met this objective
Explain the factors involved in scale up for industrial production of recombinant proteins	Not directly assessed	Not directly assessed
Describe upstream fermentation, downstream processing, purification and measurement of activity, quality, and quantity of recombinant proteins	Quiz 1, 2, and 3, Laboratory Reports	94% showed satisfactory or better understanding

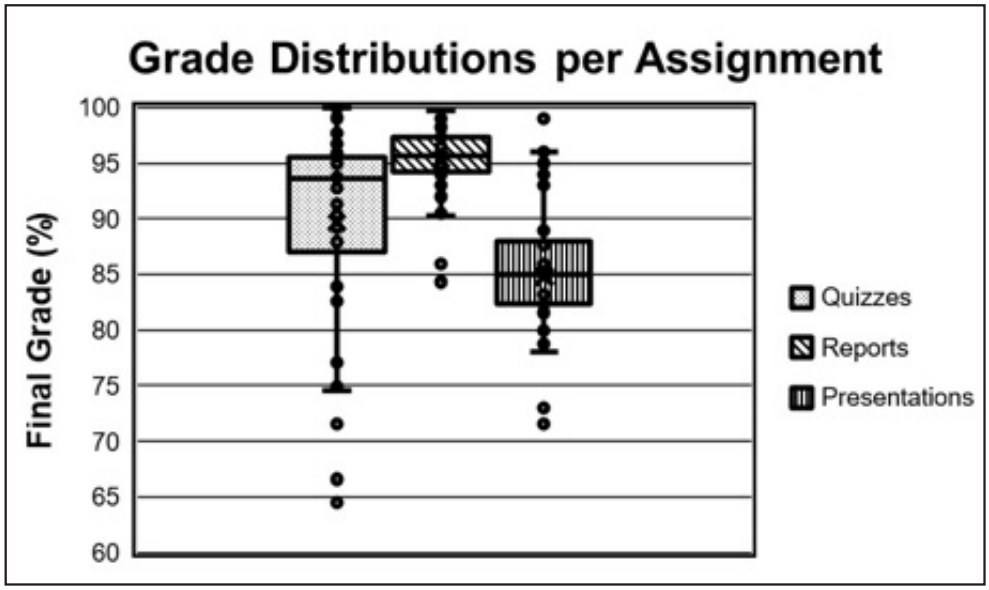

Figure 2. *Distributions of cumulative grades from the three formative assessments.*

proved more difficult to describe accurately by a majority of students. Mirroring replies to cell growth dynamics, nearly all students properly answered more generalized questions on the theory of gas transfer and oxygen utilization. Altogether, general concepts were well understood, with some students failing to fully understand important details.

Lab Reports

Of all assessments, students achieved the highest grades on their lab reports. Table 6 is a grading rubric for lab report submissions. Completion is weighted more heavily than strict accuracy on these reports, though students are more heavily penalized for making similar mistakes in future lab reports. The process of analyzing the data and preparing the text is the exercise here, and unless students completely omit required portions or fail to correct mistakes between submissions, grades are generally high.

The Results and Discussion sections require the most thought and analysis, and together they account for 40% of the grade. Aside from points lost for omission of required data, the most common mistakes seen in the Results section are poor figure/table formatting and erroneous calculations for enzyme activities or growth rates. In the Discussion section students are asked to explain the results they see and indicate whether they were expected. Additionally, more specific questions may be asked, such as "Discuss the effect of aeration on growth and protein expression" or "Are specific activities measured for different cell lysis methods similar? Explain why or why not." In general, students did well at providing scientific answers to these questions. For example, the following is a typical response, which shows good understanding of the principles and provides a scientific explanation for observed results:

> "From the results it can be seen that aeration drastically increases cell biomass and growth. Flasks 3 and 4 had a much larger biomass than Flask 1 and 2. This can be contributed to the greater access to oxygen. The oxygen allows the cells to perform aerobic respiration and therefore make more ATP, when compared to anaerobic respiration. The extra ATP will be used to duplicate and increase growth rates. The aeration also has an indirect effect on the enzymatic activity. Since there is a larger biomass, there are more cells to make the enzymes, this causes the higher enzyme activity in the agitation versus no-agitation."

Another response:

> "The data suggested that aeration contributed to an increase in growth. This was due to increased oxygen supply to the aerobic bacteria, increased access to nutrients, evenly distributed nutrients, and no settling of bacterial cells on the bottom of the flask."

This second response explains the observed trend but does little to provide a thorough scientific explanation. Providing feedback to the student encourages them to go deeper in subsequent lab reports. The subsequent response from this same student, shown below, could be clearer, but does provide a scientific rationale for the trend that was observed.

> "The heating method produced a higher specific enzyme activity, which means that a higher proportion of CelB enzymes was produced per mg of total protein. This means that the heating method was more efficient at producing CelB enzymes even though the mechanical method produced more total protein overall. The heating method must do a better job of lysing the cells and destroying the cell parts that produce the other proteins besides CelB in the total protein concentration."

In the third and final report submitted, this same student provided the following explanation, which provides a more complete scientific rationale:

"Extra glucose means extra energy, if ample oxygen is present to be the final electron acceptor at the end of the electron transport chain. Extra energy, in the form of ATP, allows *E. coli* cellular metabolism to speed up and they can multiply faster. This is proven by the higher growth rates discussed in the results section. Extra energy can also be used to express more proteins, and thus more antibody fragments can be expressed."

This particular student showed more improvement than most. Indeed, despite continuous encouragement by instructors to explain more thoroughly (even if the explanation is incorrect), students often have the tendency to "play it safe" with their discussions. For example, many students point out that mechanical cell disruption leads to higher protein concentrations because heat denatures protein but do not mention that the target product is thermostable, which leads to high specific enzyme activity from heat-lysed samples. Another tendency is for students to use human error as a catch-all explanation for more unexpected results. Reports do give students an opportunity to thoughtfully analyze collected data as opposed to simply describing results.

TABLE 6
Grading scheme for lab reports.

Weight	Section	Guidelines	Deductions
10	Abstract	Experiment summarized and justified. Highlights all major findings.	• Omission of experimental design (-2) • Omission of major results (-2) • Inclusion of unnecessary details, i.e. methods or irrelevant results (-1)
10	Introduction	Hypothesis explained and experiment described. Justify the value of the study and the specific data to be collected.	• Omission of explanation of experimental design, including expression system and model product (-1) • Lack of justification of the value of the study (-1) • Omission of information about experimental setup (-1) • Absence of explanation of hypothesis (-1)
20	Materials and Methods	Details about how all data was collected and analysis was performed.	• Copying lab protocol, or writing style is a list instead of a description (-2) • Omission of an assay or data point collection (-2) • Egregious errors or misrepresentations of the activity (-2)
20	Results	Present the data collected. Include figures and tables. Highlight interesting findings and point out general trends within the data.	• Omission of specifically requested data (-2) • Units missing or incorrect (-1) • Figures improperly formatted or labelled (-2) • Figures and tables only, no explanation of figures (-2)
20	Discussion	Provide scientific explanations for the results. Explain whether hypotheses were me. If hypothesis was not confirmed explain why. Answer specific questions related to observations.	• Omission of discussion of specific topics or results (-2) • Missing or superficial explanation of unexpected results (i.e. attributing all oddities to experimental error, -2) • Simple restatement of general results without discussion of their meaning (-2)
10	Conclusion	Restate major findings. Explain the value of the work. Provide suggestions for improvement or future studies.	• Value of work not explained (-1) • No suggestions for future work (-1) • Summary inaccurate or incomplete (-1)
10	Title, References, Appendix, Report Format	Title should reflect the experiment. Data that is discussed but not included in results appears in appendix. 2 column, journal style format.	• Title doesn't describe dependent and independent variables (-1) • Missing information in appendix (-1) • Improper format (-1 each error) • Missing or extraneous references (-1)

Presentations

Presentation skills require significant practice to improve. This assignment aims to give students an opportunity to improve an important skill – making technical presentations. The average score for presentations is the lowest of all the assessments. The rubric is shown in Table 7. Indeed, at this point in their career, students are novice public speakers. Additionally, they have to speak on topics that we do not directly discuss in class. Because of this, the grades from this exercise offer little insight into what material or skills students learned in the course but do provide a genuine form of feedback to students.

OVERALL OUTCOMES: STUDENT FEEDBACK

Students described their reasons for taking the course and their intentions after graduation during the pre-course survey. Interestingly, exactly half of the 36 students enrolled for the course to complete a specific degree requirement including minor and concentration, with the rest taking it as an elective. 75% of students indicated an intention to join the workforce immediately upon graduation while the remainder hope to pursue graduate study. These demographics further highlight the variety of students who take this course and find it useful.

The post-course survey also asked students to rate the value of the course material for future endeavors. Additionally, the survey asked about the specific benefits of the laboratory portion of the course. Tables 4 and 8 show those responses. In general, replies indicate that the students believe that the material covered in this course will be useful to them in the future. Additionally, all replies agreed that the lab modules supplemented the topics covered in lecture, and all but one response agreed that the lab introduced material that would have been challenging to deliver as a lecture.

TABLE 7
Grading rubric for final presentations.

Criteria	Grade Scale of 1-5
Presentation skills Good use of language, proper addressing of the audience (voice, eye contact, posture) clear delivery of ideas, good response to questions.	
Organization Clear slides, clear objectives, logical development of topic, good use of time, and well balanced participation of all team members.	
Knowledge base Proper background info given, irrelevant info excluded, students show a clear understanding of the topic	
Critical thinking Initial question or prompt properly answered, competing explanations or theories were presented, considered, and addressed, appropriate recommendations or conclusions suggested	
Overall impression Students convey a professional impression and maintain audience interest throughout the presentation.	

TABLE 8
Student post-course assessment of potential utility of course topics.

In your opinion, how likely are you to use what you learned about the following topics over the course of the next 3 years in future courses or job duties?	Number of Responses					
	Very Unlikely	Unlikely	Neutral	Likely	Very Likely	Did Not Respond
Growth of microbes in liquid culture	0	0	1	6	3	0
Design and function of expression systems	0	2	3	1	3	1
Lab-scale bioreactor operation	0	2	1	4	3	0
Process monitoring and control	0	0	0	4	6	0
Commercial sterilization	0	2	0	4	4	0
Bioprocess scale-up	0	2	3	2	2	1

CONCLUSIONS

Our experience demonstrates the successful development and implementation of a laboratory-based course for the introduction of bench-scale fermentation. This course educates students from a wide range of academic backgrounds on the fundamental concepts of fermentation, especially as they apply to the production of recombinant proteins. Survey responses combined with performance on quizzes and lab reports indicate successful achievement of course objectives. Furthermore, many students indicate that the hands-on lab portion of this course was an effective supplement to course lectures and, in many cases, made otherwise difficult material easier to grasp. Finally, self-reported aptitude was high in certain subject areas. Overall, this course effectively enables students to acquire processing and technology-related knowledge and skills that are widely applicable, hopefully equipping them to begin careers in industries ranging from biopharmaceuticals to bulk chemical production.

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