AC 2008-1182: COMPETITION BETWEEN STUDENT GROUPS IN THE PROTEIN PRODUCTION CHALLENGE

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Competition between student groups in the protein production challenge

Abstract

Converting biological discoveries into commercial-scale processes requires that graduating chemical engineers obtain an understanding of modern bioprocess principles. This paper describes the development and implementation of a five week long protein production project into a multidisciplinary upper level elective course on bioprocess engineering.

The protein production project was developed and implemented during the fall 2006 semester and repeated during the fall 2007 semester. A key element of the protein production project was the competition between student groups. Group performance was rated using a production rubric, and the team with the highest score was guaranteed an "A" on the project. The rubric included equipment rental costs and production bonuses for producing large quantities of protein of high purity. The equipment rental costs were carefully determined to encourage continued student experimentation in the laboratory. The competitive nature of the project captures students driven by achievement or instrumental types of motivation, which are not necessarily engaged by traditional problem-solving homework assignments.

Assessment data, including comments on student course evaluations and comparison of student final exam performance with and without the project, will also be discussed.

Introduction

This paper describes a five-week laboratory project integrated into a Bioprocess Engineering elective course. Biological products span the entire range from "high volume, low value" to "low volume, high value." In 2006, 4.9 billion gallons of ethanol was produced from corn, for an approximate value of $5.25 billion.[1] In 2001, high fructose corn syrup sales in the U.S. totaled $2.6 billion.[2] Industrial enzymes sales worldwide were $1.6 billion in 1998, with 25-30% used for detergents.[3,4] An order of magnitude greater than these markets was the protein therapeutics market in 2006, amounting to $67 billion worldwide.[5] To mimic the production of a protein therapeutic, the laboratory project developed for the course centered on the production and purification of a colorful protein. In an attempt to maximize the benefit of the laboratory project, students were presented with an open-ended research challenge that incorporated an aspect of competition. The remainder of this section explains the reasoning behind this framing of the project.

In an inductive approach to learning, students are presented with a specific challenge, and in their attempts to meet the challenge learn general principles.[6] When structured properly, laboratory projects may be classified as inductive learning experiences. Laboratory projects have been shown to enhance development of discipline-specific skills and general research skills. Additionally, longer-term laboratory projects may allow students to develop skills associated with undergraduate research experiences. Undergraduate research has been shown to increase

student satisfaction with education and serve as an "educational and personal-growth experience with many transferable experiences."[7,8]

The specific nature of student involvement in a course has a significant impact on student success and satisfaction.[9-12] Biggs and Moore classify four types of motivation:[12]

- intrinsic: natural curiosity or interest in the activity itself
- social: please the professor or peers
- achievement: enhance position relative to others
- instrumental: gain rewards beyond the activity itself

Students motivated by intrinsic and social drivers will naturally engage in their education. Positive competition has been used in lecture settings to appeal to students motivated by achievement or instrumental drivers.[13]

In the case of the protein production challenge:

- In order to foster an inductive environment that mimics an "undergraduate research" experience, the project was given an open-ended structure in which students were challenged to develop their own optimized experimental procedure.
- In order to motivate all students to engage in a laboratory project, particularly those motivated by achievement or instrumental drivers, an element of competition was integrated into the project.

Materials and Methods

Development of recombinant bacteria capable of expressing colorful proteins, and expression of colorful protein DNA (with *E. coli* BL21(DE3) cells transformed with pET21d [DsRed2, EGFP] or pET11a [flavodoxin] plasmid containing the subcloned colorful protein DNA) was performed using standard recombinant DNA techniques[14,15]

Students were provided with a basic protein production protocol for each protein (Appendix A). The students were free to modify the protocol as they saw fit to enhance their performance on the project.

Protein Production Project

During the final five weeks of the semester, students work in the laboratory to produce a colorful protein through recombinant DNA methods. During the early weeks of the project, students become familiar with the basic skills required in the laboratory and are free to perform experiments to optimize protein expression and purification. The course instructor is available for consultation and assistance in the lab. The final week is production week, where students have one chance to produce and purify a protein for a grade.

Graduate students are required to participate in the project, and perform the project individually. Undergraduate students have the option of participating in the project, and perform the project as part of a three or four member team. This is consistent with norms at the institution for dual-enrolled graduate/undergraduate courses; students taking courses for graduate credit are required to display a more in-depth mastery of course material. Undergraduates that participate in the

project can have one of their two in-semester exam grades replaced by their grade on the project, which encourages most students to participate. Undergraduates who do not participate in the project are still responsible for the material, as it appears on the final exam, but do not have a graded deliverable in place of the project.

At the end of the project, each production team submits a production laboratory report that details the materials and equipment used, and the status after each major stage of production. This report is graded. In addition, the team that scores the highest on the protein production rubric is guaranteed an A on the project. The protein production rubric is available in Appendix B. This arrangement creates an environment of friendly competition, as each team attempts to generate the highest score on the production rubric, but can also earn a good grade by writing a strong report.

The dollar values listed in the protein production rubric were based on benchmarking runs performed during Summer 2006 and selected to encourage continued student experimentation in the laboratory. For a typical protein production, protein production and one step purification costs approximately $255 ($210 for production, $45 for purification). Producing 1 mg of protein is a routine task, and results in a bonus of $275. One efficient protein separation step can lead to bonus for both purity goals, resulting in additional bonuses totaling $700. In two years of offering the project, no team or student has failed to produce a positive score on the rubric, with the winning team achieving the production and both purity goals. The average level of performance achieved by individual graduate students and teams of undergraduate students has been comparable.

Assessment

The Bioprocess Engineering course has been taught three times: twice with the protein production project (Fall 2006, Fall 2007) and once with a literature review project (Fall 2005). During the Fall 2006 offering, all students participated in the project, which improved the grades of eight of the eleven undergraduate students. During the Fall 2007 offering, 15 of 17 students participated in the project, which improved the grades of nine of the 14 undergraduate students.

One of the questions on the course evaluation asks “What were the best features of the course?” In both the Fall 2006 and Fall 2007 offerings, the protein production project was mentioned on seven of the eleven evaluations that provided answers for this question (in Fall 2006, one student left this question unanswered; in Fall 2007, four students left this question unanswered).

Two common questions have appeared on the final exams for all three years. The final exam was worth 30% of the course grade. The first is an enzyme kinetics problem, which is unrelated to the project, and can be considered as an indicator of student variability independent of the project from year to year. The second is a question about bioseparation techniques, which is related to the project, and can be considered as an indicator of project impact on student mastery of discipline skills. These final exam questions can be found in Appendix C.

Final exam solutions on these two problems for all students over the past three years were independently evaluated by a faculty member not involved in the course. All identifying aspects

of the student solutions (names, dates, instructor scores) were removed and student solutions were assigned a random number to be used for identification purposes by the evaluator. The solutions were evaluated using existing rubrics, where student solutions are evaluated on multiple topics using a four point scale (4 is high, 1 is low).[16,17]

Each question was rated from 1-4 (4=best) with respect to indicators such as "Formulates appropriate solution strategies," "Identifies relevant principles, equations and data," "Systematically executes the solution strategy," and "Solutions based on chemical engineering principles are reasonable." For the negative control question, solutions were scored using Goal 1, Objective 1, Questions 1, 2, 3, and 4; Goal 1, Objective 4, Question 2; Goal 2, Objective 3, Questions 1, 2, 3, 4; and Goal 2, Objective 5A, Question 1, 2, and 4. For the positive control question, only Goal 1, Objective 4, Question 2 and Goal 2, Objective 3, Questions 2, 3, and 4 were used, as the positive control question did not have a numerical aspect. For further details on these rubric questions, see Appendix D.

The results in Table 1 summarize the results of the evaluations. The 2006 and 2007 cohorts are combined since they both had the opportunity to do the project.

Table 1: Summary of performance on final exam questions.

Cohort	# Students	Avg. Rating* Kinetics Question	Avg. Rating* Separations Question
2005	15	3.69	3.66
2006/7, w/ project	26	3.49	3.52
2006/7, no project	2	3.67	3.17

**Each solution was evaluated with respect to 12 indicators. The results presented are the average of all ratings.*

The fact that the students who did the paper project performed better on the separations (project-related) question than those who did the lab project, in itself, could be construed as evidence that the laboratory project was ineffective. However, the 2005 class out-performed the 2006/2007 cohort on both questions with respect to every single indicator measured, suggesting that it was simply the stronger class for reasons independent of the project.

The students who did the paper project performed better on the kinetics (non-project) question than the separations question, while the students who did the lab project performed better on the separations (project) question. This observation is consistent with the laboratory project being more effective at achieving desired outcomes than the paper project, but the differences noted are much too small to be statistically significant.

As expected, the 2007 students who opted not to complete a project performed below average on the project-related question, though they were above average on the kinetics question. The number of such students, however, was too small to demonstrate anything conclusively.

Summary

A competitive protein production project has been developed and implemented in a multidisciplinary senior and graduate student Bioprocess Engineering course. The competitive nature of the project, where the team that scores the highest on the production rubric is guaranteed an A, has motivated 23 of 25 undergraduate students to perform the project over the last two years. The protein project has been mentioned as the best feature of the course on over half of the course evaluations over the past two years. By appealing to all types of motivation, a high level of student involvement and satisfaction were observed.

Future assessment data based on independent, rubric-based evaluation of final exam solutions over the past three years will determine if participation in the project improved student comprehension of course material related to the project.

Acknowledgements

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Appendix A

Basic DsRed2 production protocol:

Growth

1. From a plate of fresh transformants or a frozen stock, grow a 5 mL overnight culture of BL21 (DE3) + pET21d+DsRed2 in LB + ampicillin at 37 C.
2. For a labeled growth (4L), inoculate 100 mL of M9 + carbenicillin with the 5 mL overnight and grow overnight at 37 C. (For an unlabeled growth, use LB + amp in place of M9 + carb).
3. Inoculate a 1 L culture (M9) with the 100 mL culture, such that the initial OD600 = 0.1.
4. Monitor the OD600 as the culture grows at 37 C. For a labeled growth, induce protein expression by adding 1 mL of IPTG stock when the OD600 = 0.6. For an unlabeled growth, induce at OD600 = 0.8.
5. For a labeled growth, harvest the cells by centrifugation (6000 g for 15 mins) after an overnight expression at 30 C. Discard the supernatant and freeze the cells at -70 C.

Primary Purification:

1. Resuspend the frozen cells in 50 mM Tris, 200 mM NaCl, pH 8.0. Try to resuspend in a small volume as possible. (shoot for 50 mL total for 4 L growth)
2. Lyse the cells by lysozyme and freeze-thaw. Add lysozyme to 100 µg/mL from filter-sterilized stock. Incubate at 30 C for 1 hr, then subject to three or more freeze-thaw cycles between -70 C and 30 C.
3. Centrifuge to pellet cell debris (24000 G for 30 minutes). Save the supernatant.
4. Dialyze against 25 mM Tris, 20 mM NaCl pH 8.5 in non-CE YM-15 dialysis tubing.

Secondary Purification:

1. Load the liquid onto the DEAE sepharose column (equilibrated with 25 mM Tris, 20 mM NaCl, pH 8.5). Elute with a gradient of 25 mM Tris, 20 mM NaCl, pH 8.5 to 25 mM Tris, 300 mM NaCl, pH 8.5. Hint: when setting up the chromatography method, use a normal post-load wash (1 column volume), a normal gradient (3 column volumes), and a normal high-salt wash (1 column volume). Pool the fractions that contain dsRed.
2. Concentrated in an Amicon with a YM-30 filter against three buffer exchanges of 10 mM Tris pH 8.5.
3. Concentrate further in an Amicon YM-100 mini-filter to remove additional impurities.

Notes:

- Monitor presence of DsRed2 by red color or by fluorescence (excitation = 563 nm, emission = 582 nm)
- Quantify DsRed2 concentration by using E = 33710 $M^{-1}cm^{-1}$ at 280 nm, or E = 43800 at 561 nm (Bevis, B.J. and Glick, B.S. Nature Biotech 20: 83)
- Recipe for 1 L M9 media: 100 mL of 10x M9 stock, 1 g of NH4Cl, bring to 980 mL adjust to pH 7.4, autoclave; for 2 liters of media: 8 g glucose, 1 g MgSO4, 2 tubes thiamine stock (each tube = 1 mL of 10 mg/mL), 2 tubes FeSO4 stock (each tube = 1 mL of 10 mg/mL), 200 uL 1 M CaCl2, and 160 mg carbenicillin. Bring to 40 mL with distilled water and cold filter into media. 1 L of 10x M9 = 128 g of Na2HPO4*7H2O, 30 g KH2PO4, 5 g NaCl.

Basic EGFP production protocol:

Growth

1. From a plate of fresh transformants or a frozen stock, grow a 5 mL overnight culture of *E. coli* BL21/DE3 + pet21d+EGFP in LB + ampicillin at 37 C.
2. For a labeled growth, inoculate 50 mL of M9 + amp with the 5 mL overnight culture and grow overnight at 37 C (for an unlabeled growth, use LB + amp in place of M9 + amp).
3. Inoculate a 1 L culture with the 50 mL culture, such that the initial OD600 = 0.1.
4. Monitor the OD600 as the culture grows at 30 C (37 C for unlabeled). For a labeled growth in the fermenter, induce protein expression by adding 1 mL of IPTG stock to 4 L of media (final IPTG = 0.25 mM) when the OD600 = 1.5 (for an unlabeled growth, induce at an OD600 of 0.8).
5. For a labeled growth, harvest the cells by centrifugation (6000 g for 15 min) after 3 hours of expression (for an unlabeled growth, harvest after 4.5 hours of expression). Discard the supernatant and freeze the cells at -70 C.

Primary Purification

1. Resuspend the frozen cells in 50 mM Tris, 200 mM NaCl, pH 8.0. Try to resuspend in as small a volume as possible (shoot for 50 mL total).
2. Lyse the cells by French press.
3. Centrifuge to pellet cell debris (24000 G for 30 min). Save the supernatant.
4. Dialyze against 10 mM sodium phosphate, 20 mM NaCl, pH 6.5 in non-CE YM-8 or YM-15 dialysis tubing.

Secondary purification

1. Load the liquid onto the DEAE sepharose column (equilibrated with 10 mM sodium phosphate, 20 mM NaCl, pH 6.5). Elute with a gradient of 10 mM sodium phosphate, 20 mM NaCl, pH 6.5 to 10 mM sodium phosphate, 300 mM NaCl, pH 6.5. EGFP will stick to the column and elute during the gradient. Hint: when setting up the chromatography method, use a normal post-load wash (1 column volumes), a normal gradient (3 column volumes), and a normal high-salt wash (1 column volume). Pool the fractions that contain EGFP.
2. Concentrate the pooled EGFP to around 100 uM.
3. Load the EGFP onto the Superdex 75 column (equilibrated with 10 sodium mM phosphate, 300 mM NaCl, pH 6.5). The Kd for EGFP is around 100 uM, so it should elute from the column as a monomer, due to the dilution during size-exclusion. We have tried concentrating the protein to 1 mM before loading, but the protein still runs as a monomer. This aspect is still being investigated. Pool the GFP-containing fractions, concentrate, and examine for purity.

Notes

- monitor presence of EGFP by green color or by fluorescence (excitation = 488 nm, emission = 507 nm)
- quantify EGFP concentration by using E = 19970 $M^{-1}cm^{-1}$at 280 nm,
  or E = 55000 $M^{-1}cm^{-1}$at 488 nm (Patterson, G.H. et al., Biophys J 73: 2782 and Tsein, R.Y. Annu Rev Biochem 67: 509), in A=Elc
- recipe for 1 L of M9 media: 100 mL of 10x M9 stock, 1 g of NH4Cl, bring to 980 mL, adjust pH to 7.4, autoclave; add sterile filtered components: 20 mL of 20% glucose (20 g glucose in 100 mL water), 1 mL of 10 mg/mL thiamine, 1 mL of 10 mg/mL FeSO4*7H2O, 2 mL of 1 M MgSO4, 100 uL of 1 M CaCl2. 1 L of 10x M9 = 128 g of Na2HPO4*7H2O, 30 g KH2PO4, 5 g NaCl

Basic flavodoxin production protocol:

Growth

1. From a plate of fresh transformants or a frozen stock, grow a 5 mL overnight culture of *E. coli* BL21/DE3 + petFLV in LB + amp at 37 C.
2. For a labeled growth, inoculate 50 mL of M9 + amp with the 5 mL overnight culture and grow overnight at 37 C (for an unlabeled growth, use LB + amp in place of M9 + amp).
3. Inoculate a 1 L culture with the 50 mL culture, such that the initial OD600 = 0.1.
4. Induce protein expression by adding 1 mL of IPTG stock when the OD600 = 0.6 – 0.8 (final IPTG concentration = 1 mM).
5. Harvest the cells by centrifugation (6000 g for 15 min at 4 C) after 3 hours of expression. Discard the supernatant.
6. Wash pellet with 10 mM Tris, 25 mM EDTA, pH = 8.0 and freeze.

Primary Purification

1. Thaw cells and centrifuge again.
2. Resusupend cells in 5 volumes of 10 mM Tris, 25 mM EDTA, pH 8.0 buffer with 1 mM 2-mercaptoethanol and 50 ug/mL PMSF (phenylmethanesulphonyl).
3. Freeze the cells, then thaw again.
4. Add lysozyme to a final concentration of 0.5 mg/mL, stir suspension in a metal beaker at room temperature for 60 min.
5. Sonicate 3 times for 5 minutes at 50% duty cycle. Be sure to place the metal beaker in an ice-water bath, to keep the suspension cool.
6. Centrifuge (18000 g for 30 min at 4 C) to remove cell debris. Save the supernatant.
7. Add FMN to a final concentration of 50 µM, gently stir the mixture in the dark for 2 h.
8. Centrifuge (18000 g for 20 min at 4 C). Save the supernatant.

Secondary purification

1. Load the liquid onto the DEAE sepharose column (equilibrated with 50 mM Tris, pH 8). Wash with 0.1 M sodium acetate, pH 5.0. Elute with a gradient of 0.1 M sodium acetate, pH 5.0 to 0.1 M sodium acetate, 0.5 M NaCl, pH 5.0. Pool the fractions that contain flavodoxin and examine purity by SDS-PAGE.
2. Concentrate in AMICON concentration with a YM-10 membrane, exchange with 50 mM potassium phosphate, 1mM DTT, pH 6.5.

Appendix B

Protein production project rubric.

FINAL Production Rubric

Negatives	
Item	Cost
Media components	Catalogue price
E. coli BL21 (DE3) + relevant plasmid	$200
Sterilization service (1 autoclave run + associated filter sterilization)	$0.50
Flask rental	$0.01 / hr
Stir plate / Shaker rental	$0.01 / hr
OLD BioFlo rental	$0.10 / hr
Centrifuge rental	$0.10 / hr
Centrifuge bottles	$0.01 / hr
50 mL conical tubes	$0.01 / tube
Chromatography column rental	$0.01 / hr
Chromatography resin	Catalogue price
Purification buffers	Catalogue price
Centriplus units	$9.00 / unit
Akta rental	$1.00 / hr
Gilson peristaltic pump	$0.10 / hr
UV/Vis Spectrophotometer usage (5 min minimum)	$0.01 /min
Additional consultation with Instructor	$2 / min*

* = each team is entitled to 30 min of time with instructor per week
Free items = harvest of cells from fermenter, freeze-thaw for cell lysis, ring stands + gravity for chromatography
Items not listed above will be charged at market / catalogue price

Broken item charge is 1000x the hourly rental charge

Positives	
Event	Bonus
Produce 1 mg of desired protein	$275
Produce “target yield” of protein	$275
DsRed2 = 20 mg / L of culture	
EGFP = 10 mg / L of culture	
Flavodoxin = 20 mg / L of culture	
Achieve “purity goal #1”	$350
DsRed2 with $A_{561}/A_{280} > 0.3$	
EGFP with $A_{488}/A_{280} > 0.2$	
Flavodoxin with $A_{464}/A_{280} > 0.05$	
Achieve “purity goal #2”	$350
DsRed2 with $A_{561}/A_{280} > 0.6$	
EGFP with $A_{488}/A_{280} > 1.0$	
Flavodoxin with $A_{464}/A_{280} > 0.10$	

Appendix C

Final exam question on enzyme kinetics – negative control:

The following data were recorded during a test of kinetics of an enzyme-catalyzed reaction at 49.6 ºC. The enzyme concentration was 1.6 g/L.

S [mmol/mL]	0.1	0.033	0.01	0.0067	0.005
V [mmol/(mL*min)]	5.13	3.70	1.89	1.43	1.11

Determine the Michaelis-Menten constant (K_m) and maximum reaction velocity (v_m).

Final exam question on classification of bioseparations – project related:

Blanch and Clark group bioseparation operations into four categories based on their general purpose. List the four general categories and provide an example of a bioseparation technique in each category.

Appendix D

Final exam evaluation rubrics.

Goal 1, Objective 1: The Chemical Engineering Program at Rowan University will produce graduates who demonstrate an ability to apply knowledge of mathematics, science, and engineering (ABET - A).

	4	3	2	1
1. Formulates appropriate solution strategies	Can easily convert word problems to equations. Sees what must be done	Forms workable strategies, but may not be optimal. Occasional reliance on brute force	Has difficulty in planning an approach. Tends to leave some problems unsolved	Has difficulty getting beyond the given unless directly instructed
2. Identifies relevant principles, equations, and data	Consistently uses relevant items with little or no extraneous efforts	Ultimately identifies relevant items but may start with extraneous info	Identifies some principles but seems to have difficulty in distinguishing what is needed.	Cannot identify and assemble relevant information
3. Systematically executes the solution strategy	Consistently implements strategy. Gets correct answers	Implements well. Occasional minor errors may occur	Has some difficulty in solving the problem when data are assembled. Frequent errors.	Often is unable to solve a problem, even when all data are given
4. Applies engineering judgment to evaluate answers	Has no unrecognized implausible answers	Has no more than one if any unrecognized implausible answers. If any it is minor and obscure	Attempts to evaluate answers but has difficulty. Recognizes that numbers have meaning but cannot fully relate.	Makes little if any effort to interpret results. Numbers appear to have little meaning

Goal 1, Objective 4: The Chemical Engineering Program at Rowan University will produce graduates who possess a working knowledge of chemical engineering principles including balances, fluid mechanics, transport phenomena, separations, reaction engineering, unit operations, thermodynamics, and process design (AIChE Professional Component).

	4	3	2	1
2. Appropriate chemical engineering principles and equations are identified and implemented	Problems consistently are formulated and solved in a highly efficient manner	Problems are formulated and solved correctly. A few inefficiencies or minor errors may occur	Principles and equations are used, but some breakdowns in implementation result	Use of correct equations and principles is erratic. Many significant errors result

Goal 2, Objective 3: The Chemical Engineering Program at Rowan University will produce graduates who demonstrate the ability to identify, formulate and solve engineering problems (ABET - E).

	4	3	2	1
1. Essential information is distinguished from extraneous data	Consistently uses relevant items with little or no extraneous efforts	Ultimately identifies relevant items but may start with extraneous info	Identifies some principles but seems to have difficulty in distinguishing what is needed.	Cannot identify and assemble relevant information
2. The problem is approached in a logical and technically correct fashion	Student consistently and efficiently applies engineering principles. No conceptual errors and few if any procedural errors exist. Student appears to be sound in all areas	Student can apply principles. Only rare and minor conceptual errors occur. Few procedural errors. Student displays a working knowledge in all areas	Student can apply principles but may need improvement in one or more areas. Some conceptual and procedural error may be evident	Student has significant technical problems in several of the listed areas.
3. The absence of necessary information is recognized and reasonable approximations are made	Consistently identifies and estimates missing information using sound principles. Always recognizes the limitations of the estimation	Generally makes approximations of missing data. Recognizes that some error is introduced by approximating	Will approximate, but may lack reasonable basis for approximating. May fail to appreciate limits imposed by approximations	Becomes frustrated and gives up when data are missing
4. The problem solution is usually correct and always reasonable	Has no unrecognized implausible answers	Has no more than one if any unrecognized implausible answers. If any, it is minor and obscure	Attempts to evaluate answers but has difficulty. Recognizes that numbers have meaning but cannot fully relate to problem.	Makes little, if any, effort to interpret results. Numbers appear to have little meaning

Goal 2, Objective 5, Outcome A: The Chemical Engineering Program at Rowan University will produce graduates who have the ability to use techniques, skills, and modern engineering tools necessary for engineering practice (ABET - K). Students will apply fundamental principles of chemical engineering to solve engineering problems.

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	4	3	2	1
1. Synthesizes and defines problems appropriately	Can easily convert word problems to equations. Sees what must be done	Forms workable strategies, but may not be optimal. Occasional reliance on brute force	Has difficulty in planning an approach. Tends to leave some problems unsolved	Has difficulty getting beyond the given unless directly instructed
2. Acquires necessary data and makes reasonable estimates when necessary	Consistently uses relevant items with little or no extraneous efforts	Ultimately identifies relevant items but may start with extraneous info	Identifies some principles but seems to have difficulty in distinguishing what is needed.	Cannot identify and assemble relevant information
4. Uses sufficient mathematical skills to solve engineering problems	Readily applies calculus, differential equations and algebra to solve problems. Displays clear understanding of advanced math techniques	Applies calculus, differential equations and algebra effectively. May have minor errors or be challenged by higher mathematics	Has difficulty in formulating appropriate differential equations and identifying boundary conditions. Has adequate mechanics in mathematics	Is often limited by inability to solve calculus problems or differential equations