

# Application of Statistics in Bioprocess Engineering Laboratory to Reinforce Students' Ability in Data Collection, Analysis and Interpretation.

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**Abstract:** Bioprocess Engineering is a highly interdisciplinary field of study, which deals with the design and development of equipments and processes for the manufacturing of bioproducts from biological materials. It finds varied applications in the field of agriculture, food, pharmaceuticals, nutraceuticals and chemicals. The course demands practical exposure where-in students can actively experience the interconnection between biology and engineering. The present study was conducted for VI semester undergraduate students of Engineering in Biotechnology for Bioprocess Engineering Laboratory. The laboratory comprises fermentation studies for the production of valuable products, aimed at understanding the relationship between variables and kinetic parameters of the microorganism. The exercise results in the generation of voluminous data, which necessitates for optimization of the process parameters. Collection, analysis and interpretation of the data play an important role in understanding the relationship between process parameter and productivity for optimization of the process. Application of statistical methods to facilitate such learning was performed in Bioprocess Engineering Laboratory. Through this process, students successfully demonstrated their ability in planning & conduct of experiment, choosing proper statistical tools for analysis & interpretation of data to draw meaningful conclusion, and formulating testable hypothesis. As part of this course implementation and framework, the results evaluation and assessment of student learning through defined rubrics combined with feedback are presented. The assessment and feedback revealed the significance of statistical usage in understanding of submerged fermentation process.

**Key words:** Statistical methods, Fermentation, Bioprocess Engineering, Kinetic Parameters.

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## 1. Introduction

Recent developments in genetics and molecular biology have excited world-wide interest in biotechnology. The ability to manipulate DNA has already changed our perceptions of medicine, agriculture and environmental management. Scientific breakthroughs in gene expression, protein engineering and cell fusion are being translated by a strengthening biotechnology industry into revolutionary new products and services. Bioprocess engineering is a highly interdisciplinary field of study, which deals with the design and development of equipments and processes for the manufacturing of bioproducts from biological materials (Marius Henkel et al 2015). It finds varied applications in the field of agriculture, food, pharmaceuticals, nutraceuticals and chemicals. The course demands practical exposure where-in students can actively experience the interconnection between biology and engineering. (Society of Biological Engineers).

In most bioprocesses, there are no true theoretical or mathematical models that can describe the whole process with 100% certainty. Because of this limitation arising from the incredible complexity of cellular metabolism, efficient empirical approaches to explain these processes are necessary to solve bioprocess problems. The statistical methods provide lots of data to enable students to reach meaningful conclusions. However, any problem-solving approach is limited by time, money, and availability of resources. Because there are limited opportunities to generate and collect data, it is critical that the data be rich in information. Given these limitations, a statistical method is one solution for obtaining the information-rich data from the process being studied (Eutimio Gustavo et al 2103)

Bioprocess technologies require effective problem-solving methods because they involve both adjustment of multiple parameters and complications that inhibit application of engineering principles. Additional obstacles for bioprocess research include the lack of an accurate mathematical model equation to describe the whole process, high noise levels, interactions among variables, and complex biochemical reactions. These conditions call for a good

strategy to deal with such a complicated system. Statistically designed experiments use a small set of carefully planned experiments. This method is more satisfactory and effective than other methods, such as classical one-at-a-time or mathematical methods, because it can study many variables simultaneously with a low number of observations, saving time and costs. The statistical experimental design provides a universal language with which people from different areas such as academia, engineering, business, and industry can communicate for setting, performing, and analyzing experiments for research (Kwang-Min Lee and David F. Gilmore 2006).

The present study was conducted for VI semester undergraduate students of engineering in Biotechnology for the course Bioprocess Engineering Laboratory. The laboratory comprises fermentation studies for the production of valuable products, aimed at understanding the relationship between variables and kinetic parameters of the microorganism. The exercise results in the generation of voluminous data, which necessitates for optimization of the process parameters. Collection, analyses and interpretation of the data plays an important role in understanding the relationship between process parameter and productivity for optimization of the process. Application of statistical methods to facilitate such learning was performed in Bioprocess Engineering Laboratory. Through this process, students successfully demonstrated their ability in planning & conduct of experiment, choosing proper statistical tools for analysis & interpretation of data to draw meaningful conclusion, and formulating testable hypothesis.

## 2. Methods

### A. Design of the Laboratory:

The Bioprocess Engineering Laboratory was designed taking into consideration the inputs from industry stakeholders. The Laboratory experiments was categorized into four categories (Demonstration, Exercise, Structured Enquiry and Open Ended Experiment) as shown in table 1. With this, BPE laboratory was framed to address different PI's as shown in the assessment section. To enhance thinking ability, problem solving & analysis skills of students were the main objective in designing the BPE Lab.

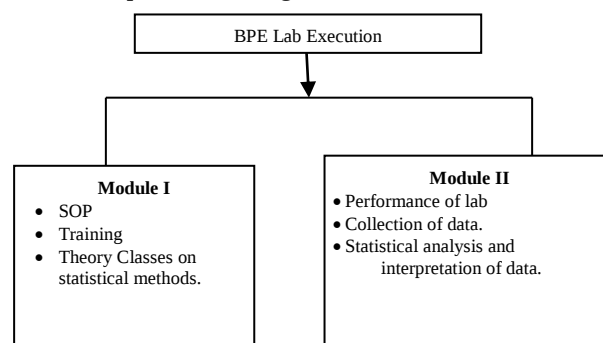
**Table 1. Categorization of BPE lab with rubrics parameter**

| Expt. No. | Name of the Experiment                                    | Type          | Rubrics Parameters                                                                     |
|-----------|-----------------------------------------------------------|---------------|----------------------------------------------------------------------------------------|
| 1         | Study of Lab fermenter                                    | Demonstration | 1. Conduct of experiment                                                               |
| 2         | Determination of thermal death kinetics of microorganism. | Exercise      | 1. Conduct of experiment<br>2. Data Collection & representation<br>3. Analysis of data |

|    |                                                                                  |                       |                                                                                                    |
|----|----------------------------------------------------------------------------------|-----------------------|----------------------------------------------------------------------------------------------------|
| 3  | Batch growth kinetics                                                            | Structured Enquiry    | 4. Interpretation of data<br>5. Verification & conclusion<br>6. Write up                           |
| 4  | Fed Batch kinetics                                                               |                       |                                                                                                    |
| 5  | Determination of kinetic parameters of microorganism                             |                       |                                                                                                    |
| 6  | Kinetics of product                                                              |                       |                                                                                                    |
| 7  | Kinetics of substrate degradation                                                |                       |                                                                                                    |
| 8  | Design an experiment to determine mixing time and power requirement of fermenter | Open Ended Experiment | 1. Identification of problem.<br>2. Identification of tools/parameter<br>3. Selection of procedure |
| 9  | Determination of K <sub>La</sub>                                                 |                       |                                                                                                    |
| 10 | Solid state fermentation                                                         |                       |                                                                                                    |
| 11 | Design an experiment to study the effect of mass transfer on microbial growth.   |                       | 1. Define complex open ended problem<br>Identification of parameters<br>2. Selection of procedure  |

### B. Execution of BPE Laboratory:

The present study was conducted for VI semester undergraduate students of engineering in Biotechnology for Bioprocess Engineering Laboratory. The laboratory comprises fermentation studies for the production of valuable products, aimed at understanding the relationship between variables and kinetic parameters of the microorganism. The BPE Laboratory was executed in two modules as represented in fig.1.



**Fig.1 Modules of BPE Lab**

In module-I, students undergone training on 1) Standard Operating Procedures (SOP) of analytical instruments like Spectrophotometer, Centrifuge and Rotary Flask Shaker 2) Training on Laboratory fermenter was given and 3) Classes on basis Statistical methods was conducted.

In module-II, all laboratory experiments were performed and Analysis of the results was performed using basic

statistical methods like Correlation & Regression, Hypothesis testing (t-Test), ANNOVA and Turkey test.

### C. Assessment of the BPE Laboratory:

Rubrics-based assessment was practiced for all the experiments of lab as shown below.

1) *Demonstration*: In demonstration experiment, working principle, SOP and demonstration of laboratory fermenter was given to students. In this experiment the assessment parameters considered were conduct of experiment (PI Code 4.1.3) and SOP skills (PI Code: 13.2.2). Demonstration experiment was evaluated for 5 marks out of 80marks.

2) *Exercise Experiments*: The assessment parameters considered were Conduct of experiment (PI Code:4.1.3.), Data Collection & representation(PI Code:4.3.1.), Analysis of data(PI Code:4.3.2.), Interpretation of data(PI Code:4.1.4.), Verification & conclusion(PI Code:4.3.4.),Write up(PI Code:10.1.2.). Six exercise experiments were evaluated for 30 marks out of 80 marks. Rubrics for assessment of demonstration and exercise experiments are as follows,

**Table 2: Rubrics for exercise experiments**

| Rubrics parameter                           | Inadequate (up to 25%)                                                                                                                                                   | Average (up to 50%)                                                                                                                                                                         | Admirable /Good (up to 75%)                                                                                                                                                     | Outstanding (up to 100%)                                                                                                                              |
|---------------------------------------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-------------------------------------------------------------------------------------------------------------------------------------------------------|
| <b>Conduct of experiment</b>                | Inadequate quality of experimental work. No proper demonstration of team work and SOP.                                                                                   | Experiments were conducted. But team work and SOP need to be effective.                                                                                                                     | Adequate conduct of experiment. Team work demonstrated however, SOP need to be strengthened                                                                                     | Proper conduct of experiment. Demonstration of team work and followed SOP                                                                             |
| <b>Data Collection &amp; representation</b> | Raw data, including units, are not recorded in a way that is appropriate and clear. Data representation is not according to the format and not at all clear for analysis | Raw data, including units, are recorded although not as clearly or appropriately as they might be. Data representation is per the format with some logical error and not clear for analysis | Raw data, including units, are recorded although not as clearly or appropriately as they might be. Data representation is per the format required and not so clear for analysis | Raw data, including units, are recorded in a way that is appropriate and clear. Data representation is per the format required and clear for analysis |
| <b>Analysis of data</b>                     | Statistical methods were Completely misapplied or absent.                                                                                                                | Statistical methods were attempted Some methods                                                                                                                                             | Statistical methods were attempted. Most methods                                                                                                                                | Statistical methods were fully and correctly applied.                                                                                                 |

|                                      |                                                                                                                                                                                                       |                                                                                                                                                                                                            |                                                                                                                                                                                                                            |                                                                                                                                                                                                      |
|--------------------------------------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
|                                      |                                                                                                                                                                                                       | were applied but with significant errors or omissions.                                                                                                                                                     | were correctly applied but more could have been done with the data.                                                                                                                                                        |                                                                                                                                                                                                      |
| <b>Interpretation of data</b>        | The results are not interpreted in a logical way or compared with literature values. The limitations and weaknesses are not discussed, nor are suggestions made as to how to limit or eliminate them. | The results are interpreted and compared with literature values, but not clear. The limitations and weaknesses are discussed with no suggestions                                                           | The results are interpreted and compared with literature values, but not as fully as they might be. The limitations and weaknesses are discussed, but few or no suggestions are made as to how to limit or eliminate them. | The results are fully interpreted and compared with literature values. The limitations and weaknesses are discussed and suggestions are made as to how to limit or eliminate them.                   |
| <b>Verification &amp; conclusion</b> | No model equation selected for verification and conclusion were not addressed. Berkeley Madonna software not used for verification of results                                                         | Model equation selected is not appropriate for verification and conclusion of experiment is not clear addressed. Berkeley Madonna software used for verification of results but no meaning full conclusion | Model equation selected is appropriate for verification and conclusion is not so clear it should be. Berkeley Madonna software used for verification of results                                                            | Model equation selected is appropriate for verification and conclusion is clearly represented. Berkeley Madonna software used for verification of results                                            |
| <b>Write up</b>                      | Report contains many distracting mistakes, making it generally difficult to follow and poorly organized. Figures, tables and graph are hard to understand, and are not                                | Report is generally clear, but distracting errors and flow make it difficult to follow at times and organization of report is weak. Figures, tables and graph are hard to                                  | Report is logical and easy to read, and may contain a few errors causing minimal reader distraction and organized strongly. All figures, tables and graphs can be                                                          | Report is virtually error-free, and contains few if any reader distractions and clearly organized with excellent transitions. All figures, tables and graphs are easy to understand, and are clearly |

|  |                           |                                                                  |                                                           |                     |
|--|---------------------------|------------------------------------------------------------------|-----------------------------------------------------------|---------------------|
|  | adequate to link to text. | understand, are not all linked to text. Several need improvement | understood with information given and are linked to text. | linked to the text. |
|--|---------------------------|------------------------------------------------------------------|-----------------------------------------------------------|---------------------|

3) *Structured Enquiry Experiments*: The experiments was assessed by considering following rubrics parameter Identification of problem (PI Code: 4.1.1.), Identification of tools(PI Code:4.1.2.), Selection of procedure (PI Code:4.1.2.). Three structured enquiry experiments were evaluated for 30 marks. Rubrics for structured enquiry experiments considered for assessment as follows,

**Table 3. Rubrics for structures enquiry experiments**

| Rubrics parameter                   | Inadequate (up to 25%)                                                                             | Average (up to 50%)                                                                                            | Admirable /Good (up to 75%)                                                                             | Outstanding (up to 100%)                                                                          |
|-------------------------------------|----------------------------------------------------------------------------------------------------|----------------------------------------------------------------------------------------------------------------|---------------------------------------------------------------------------------------------------------|---------------------------------------------------------------------------------------------------|
| <b>Identification of Problem</b>    | Demonstrate a minimal understanding of the problem by identifying objectives and limitation        | Demonstrate a sufficient understanding of the problem by identifying objectives and limitation                 | Demonstrate a clear understanding of the problem by identifying objectives and limitation               | Demonstrate a thorough understanding of the problem by identifying objectives and limitation      |
| <b>Identification of parameters</b> | Identified no parameters with no reference to the literature                                       | Parameters identified but not clearly related to the experiments with very little reference to the literature. | Parameter identified but not clearly correlates to the experimental setup with reference to literature. | Parameters identified according to the experimental setup with clear reference to literature.     |
| <b>Selection of procedure</b>       | Demonstrate d little or no ability to conduct experiments.<br><br>Did not collect Meaningful data. | Demonstrate d some ability to conduct experiments.<br><br>Collected some Meaningful data.                      | Demonstrate d adequate ability to conduct Experiments.<br><br>Collected most of the needed data.        | Demonstrate d superior ability to conduct experiments.<br><br>Collected all the appropriate data. |

4) *Open Ended Experiments*: In Open Ended Experiment, final assessment was done by following rubric parameters; Define complex open ended problem (PI Code: 3.1.1., 3.1.2., 3.1.5), Selection of procedure (PI Code: 4.1.2.). One open ended experiment was evaluated for 15 marks. Rubrics for structured enquiry experiments considered for assessment as follows,

**Table 4. Rubrics for open ended experiment**

| Rubrics parameter | Inadequate (up to 25%) | Average (up to 50%) | Admirable /Good (up to 75%) | Outstanding (up to 100%) |
|-------------------|------------------------|---------------------|-----------------------------|--------------------------|
|-------------------|------------------------|---------------------|-----------------------------|--------------------------|

|                                          |                                                                       |                                                                                                                                               |                                                                                                                                            |                                                                                                                               |
|------------------------------------------|-----------------------------------------------------------------------|-----------------------------------------------------------------------------------------------------------------------------------------------|--------------------------------------------------------------------------------------------------------------------------------------------|-------------------------------------------------------------------------------------------------------------------------------|
| <b>Define complex open ended problem</b> | No clear objectives were determined by no reference to the literature | Design objectives were determined by stating problem but not clearly related to the experiments with very little reference to the literature. | Design objectives were determined by stating problem but not clearly correlate to the experiments with little reference to the literature. | Design objectives were determined by stating problem clearly correlating to the experiments with reference to the literature. |
|------------------------------------------|-----------------------------------------------------------------------|-----------------------------------------------------------------------------------------------------------------------------------------------|--------------------------------------------------------------------------------------------------------------------------------------------|-------------------------------------------------------------------------------------------------------------------------------|

#### D. Feed Back

A formal anonymous feedback was taken from students to identify the gaps and scope for further improvement in learning curve. Sample copy of feedback of student is as shown in table 5.

The questionnaire of Closed-ended structured format on the scale of 10 for each question was framed. 35 students participated in the survey.

**Table 5. Questions for Feedback Survey of the Activity**

| Sl. No. | Questionnaire                                                                                                             |
|---------|---------------------------------------------------------------------------------------------------------------------------|
| 1       | Did the experiments performed in Bioprocess Engineering Lab strengthen skills in the subject?                             |
| 2       | Experiments in Bioprocess Engineering Lab improved hands on experience in operation of fermenter?                         |
| 3       | Implementation of statistics in BPE lab improved the analysis skills?                                                     |
| 4       | Implementation of statistics improved interpretation of results?                                                          |
| 5       | Understanding of microbial kinetics and its implementation in design of fermentation process?                             |
| 6       | Implementation of Berkeley Madonna software improved verification of modelled equation with experimental results?         |
| 7       | Structured enquiry and open ended experiments improved your application of theoretical concepts in lab experiment design? |
| 8       | Improvement of writing skills during the course of lab?                                                                   |

#### 3 Results

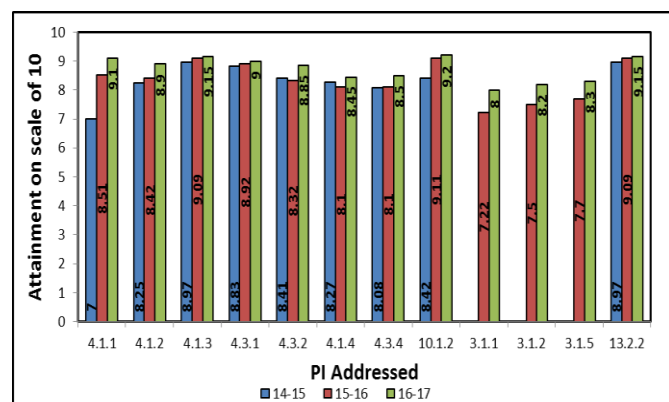
The implementation of statistical methods in BPE lab aimed at strengthening the basic skill-sets and analysis and interpretation skills was instrumental in addressing some of the program Outcomes (POs). The table 6, illustrates the mapping of rubrics framed for assessment to the POs addressed.

**Table 6. Mapping of Rubric Parameters with Performance Indicators**

| Rubrics Parameters                   | PI Code | PI Addressed                                                                                              |
|--------------------------------------|---------|-----------------------------------------------------------------------------------------------------------|
| Identification of Problem/ parameter | 4.1.1   | Define a problem to carry-out investigation with its scope and importance.                                |
| Selection of appropriate procedure   | 4.1.2   | Identify and apply relevant experimental procedure /bioinformatics tools /databases for a defined problem |

|                                   |        |                                                                                                           |
|-----------------------------------|--------|-----------------------------------------------------------------------------------------------------------|
| Conduct experiment of             | 4.1.3  | Use appropriate analytical instruments /software tools to carry-out the experiments                       |
| Data Collection & representation  | 4.3.1  | Use appropriate procedures, tools and techniques to collect and analyze data                              |
| Analysis of data                  | 4.3.2  | Critically analyze data for trends and correlations, stating possible errors and limitations              |
| Interpretation of data            | 4.1.4  | Correlate the experimental outcomes with underlying theoretical concepts and principles                   |
| Conclusion                        | 4.3.4  | Synthesize information and knowledge about the problem from the raw data to reach appropriate conclusions |
| Write up                          | 10.1.2 | Produce clear, well-constructed, and well-supported written engineering documents.                        |
| Define complex open ended problem | 3.1.1  | Recognize a problem statement that assists in the design process                                          |
|                                   | 3.1.2  | Identify and document the process / system requirements                                                   |
|                                   | 3.1.5  | Determines design objectives, functional requirements and arrives at specifications                       |
| Following SOP                     | 13.2.2 | Follow standard operating procedures adhering to laboratory guidelines.                                   |

As part of BPE Lab, the students were exposed to hand-on skills include sub-culturing of microorganisms, inoculums preparation, inoculation, fermentation for metabolite production, understanding the relationship between variables and kinetic parameters of the microorganism, analysis of experimental results using statistical methods, and biochemical estimations pertaining to fermentation



technology process. The attainment of Program Outcomes (PO) was evaluated by mapping the rubrics parameter with Performance Indicator (PI) as shown in the table 6. PI for different experiments was assessed as explained in methodology section based on the rubrics. Based on the mapping of rubric parameters with PI, attainment of various PI on a scale of 10 was measured and represented in Fig.2.

Fig.2: PI attainment for the academic year 2014-15,2015-16&2016-17

The results of the feedback survey are shown in Fig 3. All the respondents strongly agreed that introduction of statistics was useful in analysis of experiments. No

respondents either disagreed or strongly disagreed to the questions in the questionnaire.

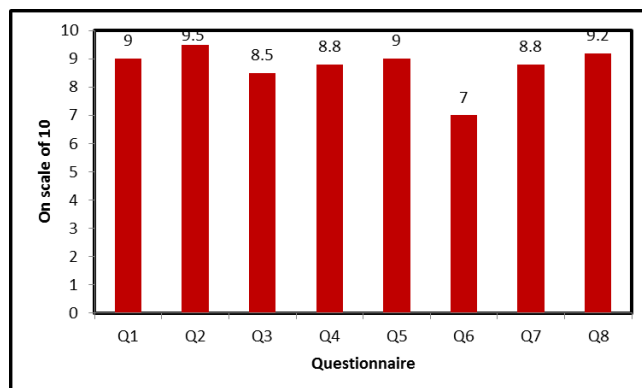


Fig 3. Results of Feedback

The attainment was highest (9.15 & 9.2) for PI 4.1.3,10.1.2 & 13.2.2, which indicated that students are good at conduct of experiments, communication skills and following good laboratory practices, while it was lowest (8.0, 8.2 & 8.3) for PI 3.1.1, 3.1.2 & 3.1.3 respectively, which indicate that scope for enhancement in identification of complex problem skills in students.

The feedback of the students revealed that the implementation of statistics gave them experiential learning. Only limitation observed from the feedback was use of Berkley Madona software used for verification of experimental results with model equation. This limitation was due to lack of time in teaching the operation of software to students.

#### 4. Conclusions:

Based on the experience of implementing the statistical method, we conclude that the students successfully demonstrated their ability in planning & conduct of experiment, choosing proper statistical tools for analysis & interpretation of data to draw meaningful conclusion, and formulating testable hypothesis. Attainment of PI clearly shows scope for improvement in the area of problem definition (PI 4.1.1 & 3.1.1).

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