Development of a Biochemical Experiment for the Unit Operations Laboratory Through An Undergraduate Research Project

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Introduction

In the era of rapidly expanding biotechnology based processes, it is necessary to train and educate undergraduate chemical engineering students and broaden their education and knowledge in the fields of emerging technologies such as biochemical engineering.

It is obvious that the successful commercialization of the developments in biochemical engineering depends on the technical advance in biochemistry and biology as well as the education of those who will implement such advances. We believe that the biotechnology and pharmaceutical industries benefit best from chemical engineers who have been trained and educated on how to implement and expand chemical engineering knowledge to biological systems. One way of achieving this is by adding new experiments in biochemical engineering to the undergraduate chemical engineering laboratory curriculum [1] (unit operations laboratory). The overall objectives of this endeavor are: 1) to familiarize the students with the operation of equipment used in biotechnology, 2) to introduce students to the applicability and limitations of biochemical engineering models, and 3) to let students discover the unique problems in working with biological systems.

At the same time, it is crucial to provide research opportunities to our undergraduate students in order to develop their ability to design and conduct experiments, to analyze and interpret data, to design a system, component, or process to meet desired needs, to function in multidisciplinary teams, to identify, formulate and solve engineering problems and to stimulate collaborative learning environments[2]. One approach that can be used in providing research opportunities to our undergraduates, and in broadening their education, is by involving them in the process of developing new experiments that cover emerging technologies for the unit operations laboratory. The benefits of this approach are two fold. First, we provide the opportunity to our students to develop the abilities mentioned above (ABET 2000 criteria)[2]. Second, we expand the unit operations laboratory in a cost-effective manner into areas related to the emerging technologies such as biochemical technology, environmental science and engineering, electronic materials and semiconductors technology, etc.

Hence, the focus of this paper is to discuss the implementation of this approach by considering an example of developing a biochemical experiment through an undergraduate research project.

Outline of the Implemented Approach:

The goal was to develop a multifunctional modular biochemical experiment by undergraduate students as research projects that can be used in classrooms as well as in the undergraduate chemical engineering laboratory courses. The experiment developed by Badino and Hokka (1999)[3] to produce clean fuel via ethanol fermentation process was selected since different measurement and analytical techniques can be used, and various kinetics-transport parameters can be studied. In addition, the set-up can be modified to perform different fermentation experiments in the unit operations laboratory.

The experiment has been designed and developed under the supervision of the laboratory instructor (M. Al-Dahhan) and the laboratory technical assistant (S. Picker) in following stages:

Stage 1 (Summer, 1999): A freshman undergraduate student (C. Weigand) was appointed to achieve the following tasks.

1. Evaluate and understand the Badino and Hokka (1999)[3] experiment.
2. Propose a modified design, if necessary, for the experimental set-up that can be utilized as multifunctional modular biochemical experiment.
3. Identify the required components and equipment. Properly size and order them.
4. Construct the set-up.
5. Perform preliminary testing and troubleshooting of the developed experimental set-up.

These tasks have been performed successfully and efficiently during the summer of. The student obtained valuable design and research experience through the interactions with the supervisors as a member of a team.

The schematic diagram of the developed set-up is shown in Figure 1. Two fermentors (kettle-shaped) consisting of 1000 *mL* beakers with rubber o-rings were used. Fermentor lids were made of stainless steel and were held in place by a lid holder with three springs. The lids were machined to accommodate ports for a thermocouple well, gas exit, inoculation, sampling tube, and tubes for heating/cooling coil ("U" tube). The temperature was controlled via a Fischer hot water bath heated at 34.5°C equipped with a pump that circulated the hot water through the plastic tubing connected to a solenoid valve that was electrically controlled to remain open or closed by a temperature controller based on the recorded temperature. If the temperature was below 30°C, the valve remained open, allowing warm water to flow to the fermentor before circulating back to the hot water bath. The working solution was sufficiently mixed by use of a magnetic stirrer and a Corning stirring plate.

It is known that in a suitable environment, sugars (monosacharides) are transformed to ethanol and carbon dioxide by the action of yeast where cell growth and product generation take place

simultaneously. As implied by stoichiometry, one mole of hexose produces two moles each of carbon dioxide and ethanol as follows:

$$\underset{(\text{hexose})}{C_6 H_{12} O_6} \xrightarrow[30^\circ C]{yeast} \underset{(\text{carbondioxide})}{2\,CO_2} + \underset{(\text{ethanol})}{2\,C_2 H_5 OH} \qquad (1)$$

As shown by the stoichiometry of the ethanol production, equation (1), measurement of carbon dioxide should provide indirect means of determining the amount of ethanol produced in the reactor. Therefore, the CO_2 volume liberated by ethanol fermentation is measured by 1 inch diameter PVC pipes that are filled with water. The pipes are about two feet (0.61 m) long and are connected to the gas exits of their respective fermentors. Each column is connected to a water exit container. The volume of the produced CO_2 is equivalent to the volume of the displaced water. The moles of CO_2 produced can be evaluated using ideal gas law.

Stage 2 (Fall, 1999): A Junior undergraduate student (A. Chen) was appointed to continue the work by performing stages 2 and 3. The tasks set for stage 2 were as follows:

1. Interact with the previous student (C. Weigand) to acquire all the needed information and knowledge about the system.
2. Perform detailed testing and troubleshooting.
3. Overcome the technical and operational problems for yeast formulation.
4. Identify and acquire the needed materials for yeast fermentation.
5. Screen different types of yeast by measuring the produced volume of CO_2 with time which indirectly indicates the rate of ethanol production as mentioned earlier.
6. Develop a step-by-step experimental procedure.

The student successfully and efficiently performed the tasks above.

During the process of screening different types of yeast, it was found that various yeasts differed from one another in terms of their initial rate of CO_2 production. As shown in Figure 2, 1.0 *gm* of Nottingham yeast in Medium 1 took over 4 hours to produce measurable amount of CO_2 while 0.9 *gm* of Lalvin EC-1118 yeast in Medium 2 produces measurable volume of CO_2 in a considerably lesser amount of time. As the initial concentration of sugar is reduced, the production rate of ethanol and CO_2 is reduced (See Figure 2). It was found that as the initial cell concentration (initial weight of yeast) increases the rate of CO_2 and ethanol production increases. Figure 3 shows a higher rate of production of CO_2 and ethanol obtained by 3.6 *gm* of Munton's Brewing yeast in Medium 1. In about one hour the CO_2 production reached a plateau due to all the sugar being converted into ethanol and carbon dioxide. Such conditions would be suitable for classroom as well as for the lab session experiment.

The operation of the set-up indicates that a simple design for the heating we used in this setup is sufficient to maintain the yeast at about 30°C as compared to more elaborated temperature

controlling system proposed by Badino and Hokka (1999) that consists of alternating hot and cold water streams and baths.

Stage 3 (Spring-Summer, 2000): This represents the final stage for the development of the experiment.

The tasks set for the undergraduate student (A. Chen) are as follows:

1. Identify, prepare and perform analytical methods to measure cell, glucose and ethanol concentrations.
2. On selected yeasts, propose an experimental plan to evaluate the effect of yeast type, substrate concentration and initial cell concentration on the ethanol and CO_2 production, cell growth and kinetic parameters. [3,4,5,6,7]
3. Perform the experimental plan developed in (2) above.
4. Analyze and interpret the data.
5. Develop the final report in the form of a laboratory manual.

Tasks 1 and 2 have been achieved while the remaining tasks are in progress. At the end of this stage the experiment will be ready to be introduced to the senior's unit operations laboratory course. It is planned to have the student who performed stages 2 and 3 (A. Chen) serve as teaching assistant for the experiment.

Final Experiment Outline

The Structure of the Laboratory Session

The students are divided into groups of two to three students. Each laboratory session consists of a pre-laboratory week and a laboratory week. During the pre-laboratory week, the students should get acquainted with the set-ups and related background and demonstrate their readiness to conduct the experiment. The tasks required during these weeks are outlined below:

Pre-Laboratory week:

- Review the facility.
- Learn how to operate it.
- Learn the measurement techniques.
- Read the key papers and understand the theory.
- Identify the objectives and the parameters to be investigated.
- Propose the experimental plan.
- Prepare a pre-laboratory proposal (i.e. a pre-laboratory report).
- Demonstrate knowledge of the facility need to conduct the experiment.

Laboratory Week

- Conduct the experiment.
- Collect the required data and perform the necessary measurements to achieve the objectives.
- Interact with the other students who performed the experiment at different operating conditions and obtain the needed experimental data and findings.
- Process, analyze and interpret the data.

- Prepare the final report.

The Experiment Assignments

The overall assignments of this experiment are as follows:

- Investigate the effect of yeast type, substrate and initial cell concentration on the ethanol and CO_2 production, cell growth and kinetics parameters.
- Based on the experimental data and findings obtained by the groups, design a large ethanol fermentor (different amount of ethanol production will be identified for each group). Identify the needed components and equipment along with their specifications.

In order to perform the experiment within the duration of the laboratory session (4-5 hours), each group of students will be asked to conduct the experiment at different operating conditions using selected yeast type and selected two initial concentrations of either the substrate or the cell. However, the obtained information by one group will be available to the others to complement their work for performing the overall analysis and design assignments. Hence, the tentative specific assignments are outlined as follows. [3,4,5,6,7,8,9]:

- Evaluate the ethanol and CO_2 production and the cellular concentration with time.
- Estimate the yield coefficients.
- Estimate the specific growth rate.
- Determine the kinetic parameters of Monod's and Aiba's et al. models. Use fourth-order Runge-Kutta method to predict the variation with time of substrate, cellular and ethanol concentrations. Compare these predicted values with the experimentally measured ones. Discuss the findings.
- By utilizing the findings obtained by other groups at different operating conditions, perform the following:

I. Analyze the effect of yeast type, substrate initial concentration, cell initial concentration on the ethanol and CO_2 production, cell growth and kinetics parameters.
II. Design a large-scale fermentor for ethanol production. This includes identification of the required equipment and process engineering components and their specifications.

Remarks

A biochemical experiment to produce clean fuel via the ethanol fermentation process has been developed and tested at a very reasonable cost by undergraduate students as research projects supervised by the laboratory instructor and the laboratory technical assistant. The developed experimental set-up is flexible enough to conduct short duration experiments (2-4 hours) to study the effect of yeast types, substrate concentration and initial cell concentration on the ethanol and CO_2 production, cell growth and its thermodynamics and kinetics [3,4,5,6,7]. The approach conducted to develop such experiment has been implemented successfully. The project has provided valuable design and hands-on research experience to the involved undergraduate students. The approach stimulates the collaborative learning environment and provides an example of how to expand the curricula to include the fields of emerging technologies in a cost-effective manner along with providing useful opportunities to our undergraduate students.

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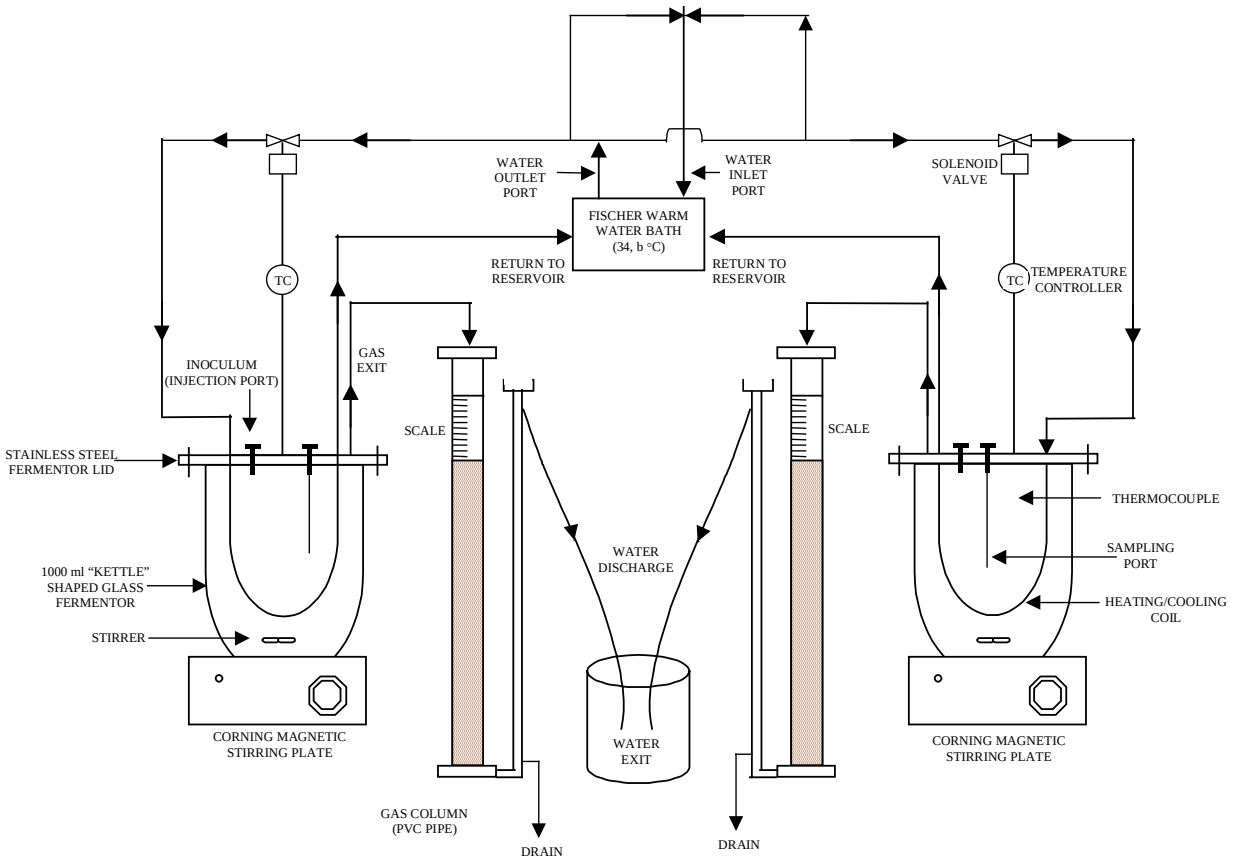

FIGURE 1: Schematic Diagram of the Ethanol Production Experiment

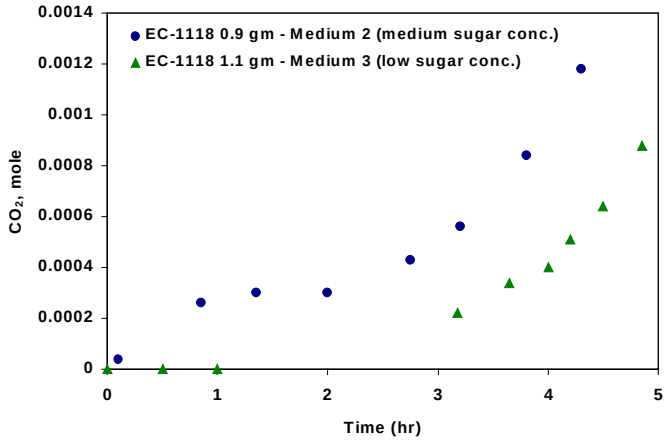

FIGURE 2: The effect of sugar concentration on the CO_2 production by EC-1118 yeast.

Medium 2: (medium sugar concentration) 32.0 *g* sugar and the same KH_2PO_4, yeast nutrient, $(NH_4)_2\ SO_4$ and $MgSO_4 \cdot 7\ H_2O$ concentrations as Medium 1 aliquoted to 750 *ml* with de-ionized H_2O. Medium 3: (low sugar concentration) 16.0 *g* sugar and the same KH_2PO_4, yeast nutrient $(NH_4)_2\ SO_4$ and $MgSO_4 \cdot 7\ H_2O$ concentrations as Medium 1 aliquoted to 750 *ml* with de-ionized H_2O.

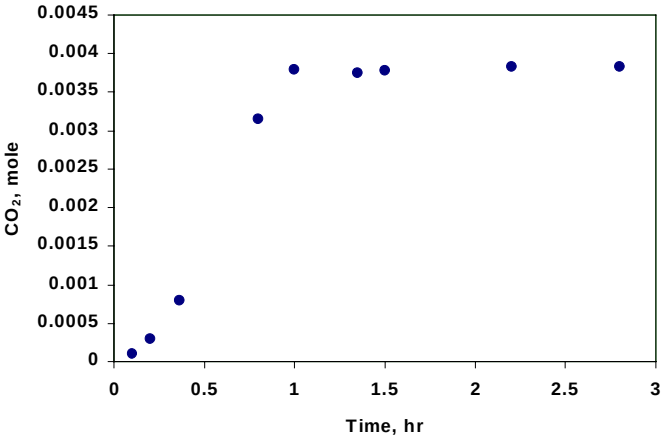

FIGURE 3: Gas production rate obtained by 3.6 g of Munton's Brewing yeast in Medium 1.

Medium 1: (high sugar concentration) 52.8 *g* C&H pure granulated sugar, 4.0 *g* KH_2PO_4, 2.4 *g* yeast nutrient (St. Louis Wine and Beer Making), 1.4 *g* $(NH_4)_2\ SO_4$ and 0.3 *g* $MgSO_4 \cdot 7\ H_2O$ aliquoted with de-ionized H_2O to 750 *ml*.