Fermentation of Apple Juice to Cider–An Introductory Chemistry Laboratory

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Abstract: An introductory chemistry laboratory designed for a high school or college environment is presented. The process of cider production is used to teach concepts related to fermentation and to allow students to expand their understanding of mass percent, stoichiometric calculations, and the ideal gas laws. This experiment was developed as part of a continuing curriculum reform effort in our introductory chemistry laboratories to integrate biological and chemical concepts, to reduce "cookbook" procedures, and to encourage students to use higher-order thinking skills. This approach has proven effective in stimulating student interest and in fostering greater student engagement in the learning process.

Introduction

Fermentation is a process that puzzled scientists for many years. Publications in the 19th century illustrate the amount of frustration the fermentation process caused scientists. A satire engineered by Liebig and Wohler cast the biological theory of fermentation in an unflattering light [1]. Thanks to a clever experiment by Eduard Buchner, the dispute over the process of fermentation was settled [2].

Different fermentation processes are used depending on the desired product, and many of these processes have been described in the literature [3–7]. Some forms of fermentation are naturally occurring, meaning the ferment, which is the yeast, mold, or bacteria, is already in the reactant. In other situations, the ferment must be carefully added under specific conditions. Still, the fermentation process is the same; sugar molecules in the substance are converted by the yeast, mold, or bacteria to something else, such as alcohol or lactic acid.

In addition to being used in drinks and food, the process of fermentation is used to make products far more important. Fermentation products have both industrial and medicinal applications. Antibiotics, including penicillin and riboflavin vitamins, are created by fermentation. Citric acid created by fermentation is used in metal cleaners and in food preservation. This acid is naturally occurring in citrus fruits, but it is uneconomical to obtain directly from fruits. By fermenting molasses, the same acid is produced cheaply and efficiently.

This laboratory experiment uses alcoholic fermentation as a means to examine one of the many processes of fermentation and some of the many methods used to assess the effectiveness of the fermentation process. Other experiments have been published that evaluate other aspects of alcoholic fermentation (e.g., the determination of malic and lactic acids in wine) [7]. In alcoholic fermentation, yeast converts glucose to ethanol and carbon dioxide.

$$C_6H_{12}O_6 \text{ (glucose)} \xrightarrow{\text{yeast}} 2\ CH_3CH_2OH\ (l) + 2\ CO_2\ (g)$$

It is well known that using everyday examples to illustrate chemical and biological processes raises the interest of students in science and improves their motivation for understanding scientific principles [4]. The use of alcoholic fermentation in an experiment can be readily related to everyday examples due to the large amount of consumer products available that use the process. When you consider that the majority of the world's adult population has consumed an alcoholic beverage at some time, it can be understandable why experiments based on wine or beer are so popular among students [7].

Problem-based Instruction

The role of the laboratory course has been of ongoing interest to chemistry educators. Historically speaking, laboratory instructional styles have fallen into four distinct categories: expository, inquiry, discovery, and problem-based (Table 1).

It is the student outcome, the teaching approach, and the experimental procedure that generally differentiate the laboratory exercises corresponding to these categories [8]. The approach of an activity is the requirement that a student use laboratory data to either confirm the validity of a principle (the deductive approach) or derive the general principle (the inductive approach). The laboratory exercise is further characterized by whether or not the student is provided with a laboratory procedure and whether the outcome of the activity is undetermined or predetermined. Of the four types of instructional styles, the expository or "cookbook" approach and problem-based instructional styles have been most prominent [8]. In problem-based instruction, students create their own experimental procedure based on the problem they need to solve. It is known that problem-based instruction creates a better understanding of the material covered because students must use higher-order thinking skills [9–13]. For years, instructors have realized the importance of stimulating higher-order thinking in the science laboratory [14,15].

Table 1. Descriptors of the Laboratory Instruction Styles

Teaching Style	Student Outcome	Teaching Approach	Experimental Procedure
Expository	predetermined	deductive	given
Inquiry	undetermined	inductive	student-generated
Discovery	predetermined	inductive	given
Problem-based	predetermined	deductive	student-generated

Table 2. Educational Objectives Achieved Comparing Traditional and Problem-based Laboratory Approaches

Problem-based Laboratory Task	Bloom's Educational Objective Achieved	Traditional Laboratory Task	Bloom's Educational Objective Achieved
choosing an appropriate experimental procedure	analysis (level 4)	understanding the given procedure	Comprehension (level 2)
developing/testing/documenting the experimental procedure	synthesis (level 5)	performing the given procedure	Application (level 3)
optimizing/trouble shooting the experimental setup and procedure	evaluation (level 6)	using given experimental setup	Knowledge (level 1)

This experiment was developed as a part of a curriculum reform with an emphasis on integrating biology and chemistry and in fostering greater student engagement in the learning process. One of our goals was to provide students with an introduction to problem-based instruction without overwhelming them at the introductory chemistry level. In all subsequent experiments that we design, we plan to incorporate one section where students must develop their own procedures; however, some aspects of the laboratory will still follow an expository or "cookbook" approach. We feel that this approach will still foster higher-order thinking skills, while being practical in a laboratory that serves up to 750 students per semester and is taught by teaching assistants. We envision that our experimental designs will provide students the opportunity to operate at all six levels of Bloom's taxonomy (Table 2) [16]. Furthermore, the overall cost of incorporating the experiment into the existing curriculum was also a determining factor because our general chemistry laboratories have high enrollments and a small budget.

In summary, the goals of this laboratory were to introduce the concepts of fermentation, show connections between biology and chemistry at an earlier stage of the students' education, introduce students to problem-based instructional methods, develop an experiment that is interesting with real-world applications, and provide a platform in which students use two methods of analysis to determine the "correct" answer. One method followed more of a "cookbook" approach; whereas, in the other method, students were asked to develop their own procedures (problem-based approach).

Description of the Laboratory Exercise

This experiment was conducted over two 2-h laboratory sessions in a section of 24 students enrolled in the first semester of a general chemistry laboratory. All chemicals required for this laboratory were commercially available, and the equipment needed included 125-mL Erlenmeyer flasks, analytical balances that read to 200 grams, 50-mL graduated cylinders, a barometer, 9-in helium-quality balloons, rubber bands, apple juice (nonpasteurized, not from concentrate), and dry wine yeast.

The week prior to the fermentation laboratory, students were asked to conduct a library or Web search to find a process or procedure, other than the production of beer, that utilizes fermentation. This activity was initiated to force students to think about fermentation and its many uses prior to conducting the experiment. The first session of this laboratory involved a short discussion of this prelaboratory assignment followed by the dissemination of an experimental handout. In addition to an introduction, some procedural information, and a report sheet, the handout contained prelaboratory questions designed to assess their understanding of important chemical concepts in the laboratory and space for the students to supply a written procedure to measure the CO_2 gas collected (see the supporting information for this handout). Answers to the prelaboratory questions and a procedure for measuring the gas were due at the start of the next laboratory meeting. Following this discussion, students utilized the remaining time to prepare their fermentation apparatus. Each student placed 50 mL of apple juice in an Erlenmeyer flask, added some dry wine yeast, and then sealed the flask with a balloon. The flasks were stored at room temperature in their lockers until the next laboratory meeting, one week later.

In the second laboratory meeting, the students used the procedure they developed to determine the volume of CO_2 gas collected in the balloon. Most students collected the CO_2 gas into a graduated cylinder by water displacement; however, one student assumed the balloon to be spherical, measured the circumference of the balloon, and calculated the volume from the radius of the sphere. Another student estimated the size of the balloon when filled with the gas, released the gas, and filled the balloon with water to the estimated size. The student then poured the water into a graduated cylinder to determine the volume. From the volume of gas collected and from the mass difference caused by the production of CO_2 gas, two separate calculations to determine the percent by mass of ethanol in the cider were performed (see the supporting material for the complete experimental procedure). Students were then asked to compare and discuss these two measurements and calculations.

To wrap up the laboratory exercise, the students discussed their results as a group, led by the instructor, and came to a consensus on why there were such large deviations in the percent by mass of ethanol calculations using the two methods described above. This was followed by a general review of the concepts and skills learned. During the remaining time,

Table 3: Student results (percent by mass of ethanol in their prepared cider samples).

% Mass Ethanol from Mass Difference	% Mass Ethanol from Volume Collected
6.10	0.23
6.40	0.17
6.97	0.17
7.30	0.23
6.44	0.25
6.53	0.23
3.20	0.23
6.18	0.24
6.60	0.25
6.85	0.15
6.58	0.23
7.30	0.23
6.60	0.22
5.87	0.024 *
6.70	0.25
6.37	0.20
6.12	0.29
5.81	0.27
5.20	0.09
6.47	0.27
6.28 (av)	0.21 (av)
0.88 (SD)	0.06 (SD)

* The amount of gas collected in this trial was significantly less than other trials.

students were allowed to critically evaluate this laboratory experience.

Results and Discussion

Student Results. The data in Table 3 were compiled from 20 of the 24 students results (4 of the students failed to complete the exercise). Also shown are the mean and standard deviations for the results obtained using both the mass difference and volume of gas procedures. The data show that both procedures for determining the percent by mass of ethanol are fairly precise; however, calculating mass percent of ethanol by mass difference was much more accurate.

During the final laboratory meeting, we asked students to discuss which of the two analyses is more reliable. Several students suggested that a better method of collecting the CO_2 gas should be developed. Students were much more concerned about the large deviation in the results than the instructors. A few students even suggested eliminating the analysis based on the CO_2 gas collected. One of our goals was to keep the experimental setup as simple and cost effective as possible. Using our simple experimental setup, it is difficult to achieve a good seal when attaching a balloon to an Erlenmeyer flask, and much of the CO_2 gas produced during fermentation escapes. In the future, we plan to have one group in each laboratory period design their experimental setup to collect the gas over water during the fermentation process. A 2-L graduated cylinder or flask is necessary to collect the CO_2 gas produced during fermentation. Another of our goals was to have students perform calculations using the ideal gas equation. This goal and our goal to keep the experimental setup as simple and cost effective as possible were accomplished in this experiment.

Assessment of the Laboratory. Curriculum reform efforts require assessment methods that credibly determine improvements in skill development and student learning. It is necessary to develop assessment methods that can assess these skills in order to guide curriculum reform efforts and evaluate success [17]. The data collected from the students participating in this laboratory exercise provides the basis for an observational study to qualitatively determine if this laboratory could offer educational advantages over the current laboratory experiments. The following factors were considered as points of observation:

Student Attitudes. It has been said that education is most effective when students are willing to actively engage in their learning experience; therefore, it is possible that student attitudes impact the degree to which learning can occur [18]. In order to evaluate the student's attitudes, an anonymous, critical essay on the experiment was collected from the students participating in this laboratory experience. It was evident upon review of the essays that the experiment was well received by the students. Here are a few student comments:

> "I was able to see the big picture so there was a deeper understanding of the process of fermentation…."

> "I feel that this should be integrated into other labs as well."

> "We got to do something that has practical applications to the real world. I like doing experiments that show how things work that are used in everyday life."

> "It was a very good, fun, and different lab. I enjoyed this lab!"

> "This lab allows students to connect more passionately to concepts in chemistry. I like also how the lab allows the students some leeway on deciding which method to use to measure the gas."

Laboratory Objectives. For these students, this experiment replaced a previously existing experiment with the same achievement objectives: using the ideal gas law to calculate moles from volume of gas, calculating mass percent, performing stoichiometric calculations, and interpreting data. All students in this section achieved these objectives successfully.

Conclusions

This laboratory exercise was a success because it provided students with an introduction to problem-based instruction without overwhelming them at the introductory chemistry level. We plan to develop all of our introductory chemistry laboratories to incorporate one section where students must develop their own procedures; however, some aspects of the laboratory will still follow an expository or "cookbook" approach. We feel that this approach will still foster higher-order thinking skills, while being practical in a laboratory that serves up to 750 students per semester and is taught by teaching assistants. The success of this laboratory can be attributed to the positive attitudes of students, achieving all the goals of the instructors, and the incorporation of a real-world application to which students can relate. Each student gained knowledge of the concepts of fermentation, how to develop a procedure given limited information, how to calculate mass percent using mass difference and volume of gas collected, and how to interpret data. A few students, however, had negative comments regarding having to develop their own procedures for collecting the CO_2 gas. This attitude illustrates opposition that is commonly encountered when using problem-based instruction and illustrates one of the weaknesses in our current program. Students are too accustomed to expository or “cookbook” instruction and not enough problem-based instruction is utilized. This laboratory experience has been shown to be effective at creating a positive laboratory environment, while developing higher-order thinking skills. In addition, the laboratory integrated biology and chemistry using a topic that has real world applications.

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Supporting Material. The student handout for this laboratory is available as 610032bcs1.pdf http://dx.doi.org/10.1007/s00897000440b.

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