LEARNING BY BREWING:
Beer Production Experiments in the Chemical Engineering Laboratory

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The process of brewing fermented beverages such as beer employs several key chemical engineering concepts while simultaneously appealing to many students' interests and curiosity. At the University of California, Berkeley, we have developed a biotechnology track within the unit operations laboratory course (CBE154) that guides students through brewing-related experiments before culminating in a student-designed project to produce and analyze a fermented beverage of their choice. Our main objectives were: (1) to provide students with the opportunity to apply biochemical engineering concepts in an experimental lab setting; and (2) to encourage student creativity in solving an open-ended engineering problem.

The analysis of beer and its production has been used in classrooms and labs as a practical interdisciplinary topic that encompasses a wide range of concepts and tools.[1] Several analytical chemistry techniques can be used to analyze the various components and properties of beer,[2,3] as well as the chemical reactions that occur during beer production.[4] Courses that focus on biology may instead study the functional properties of yeast cells during fermentation or amylases present in malt grains.[2,4,5] In engineering courses, the brewing process itself may be the focus.[1]

Student groups following the traditional CBE154 curriculum at UC Berkeley complete a total of six laboratory experiments (over the course of a semester) that probe various chemical engineering topics. Each experiment is allotted four lab sessions (two weeks) of four hours each. In contrast, student groups on the biotechnology track complete only two of the traditional experiments (on mass transfer and the distillation of ethanol-water mixtures), substituting the traditional heat transfer and kinetics modules for Immersion Heat Exchanger (IHX) and Yeast Fermentation Kinetics (FERM) experiments, respectively. IHX and FERM introduce students to some of the key processes and theoretical concepts of brewing before they plan and execute their self-designed brewing projects (PROJ). We made every effort possible to ensure that the brewing track was as rigorous and broad as that completed by the other students, but with a focus on the concepts relevant to the biotechnology industry.

Heat exchangers are a ubiquitous unit operation in chemical processes, and the beer-brewing process is no exception.

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For a thorough overview of brewery usage of engineering technology and heat-transfer operations, see Chapter 10 in *Handbook of Brewing*.[6] In the beginning stages of beer production at the homebrew scale, malt extract and hops are mixed with water and boiled to produce the hot wort. During this time, flavors and sugar are extracted into the solution that later becomes the beer. After this process, the wort must be cooled from nearly 100 °C to less than 25 °C prior to adding the yeast (known as pitching) and initiating fermentation. Wort chilling must take place rapidly to avoid contamination by competing microorganisms, such as wild yeast or bacteria, which could spoil the beer.[6,7]

At the homebrewing scale, immersion heat exchangers are commonplace due to their affordability and simple operation. During the IHX lab, students use brewing equipment (heating kettle, coiled immersion heat exchanger, etc.) to cool boiling water, characterize the heat transport properties of the heat exchangers, and develop a model to predict cooling time. They also assess the economics and water usage of the process to inform their choice of operating conditions on brew day.

The FERM lab asks students to consider the applications of yeast growth and fermentation to beer production, with similar principles governing many industrial biotechnology processes related to the production of medicines, biofuels, and beverages. Students investigate the conversion of dextrose to ethanol by brewing yeast—*Saccharomyces cerevisiae*—undergoing anaerobic fermentation.[8,9]

In the absence of oxygen, yeast perform anaerobic metabolism of glucose to produce CO_2 and ethanol:

$$\text{cells} + C_6H_{12}O_6 \rightarrow CO_2 + C_2H_5OH + \text{more cells} \qquad (1)$$

The simplified reaction above does not count the other by-products produced by cells undergoing anaerobic metabolism. *S. cerevisiae* also performs anaerobic fermentation in the presence of oxygen if the substrate concentration is high enough, typically above 0.4 wt %.[10] This is called the Crabtree effect. Under conditions relevant to beer brewing, the Crabtree effect guarantees that substrate consumption produces the desired product, ethanol. Upon completion of the FERM experiment, students determine the stoichiometric and kinetic parameters for the strain of *S. cerevisiae* provided in the lab, and develop a mathematical model to predict substrate, product, and cell concentrations during anaerobic fermentation.

The fermentation project has been offered to four three-membered student teams every semester for the past two years. Students use the techniques and knowledge gained during IHX and FERM experiments to design and execute their final project: the production and analysis of a 2.5-gallon batch of a fermented beverage of each group's choosing. Students must determine what processing steps to undertake, which measurements to make, what information is needed from literature, and if any additional experiments are required. In addition to the heat transfer and kinetics topics covered in IHX and FERM, students must also: consider the extraction processes and chemical reactions at each phase of beer production; determine how these processes affect the final taste, smell, and color of their product; and quantify their success at achieving their desired product by using various analytical techniques. Students also evaluate their process from an economic and environmental standpoint. On the final day of lecture, the rest of the class and the chemical engineering faculty are invited to taste and rate the beverages from all teams.

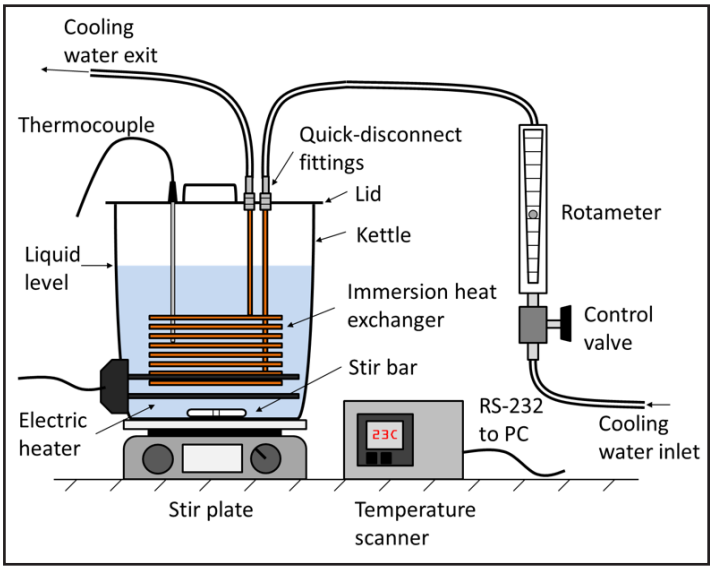

Figure 1. *Schematic of the experimental apparatus for the IHX experiment. K-type thermocouples connected to the temperature scanner are omitted for simplicity.*

Students who complete the fermentation project are exposed to a variety of techniques and theories that are relevant to careers in biotechnology and brewing industries, including cell culture, fermentation kinetics, reactor scale-up, systems design, and more. By incorporating environmental and economic concerns as well, students gain an appreciation for the additional complexities that must be considered when designing and producing a consumer good.

APPARATUS AND METHODS

Immersion heat exchanger (IHX)

The immersion heat exchanger consists of metal tubing wound into a number of coils and immersed into a tank of liquid. Here, we consider a system where an immersion heat exchanger cools the hot working fluid (water) in the kettle (Figure 1). Students vary the cooling water flow rate and the heat exchanger surface area, and analyze the resulting temperature versus time data for the hot working fluid and the cooling water outlet.

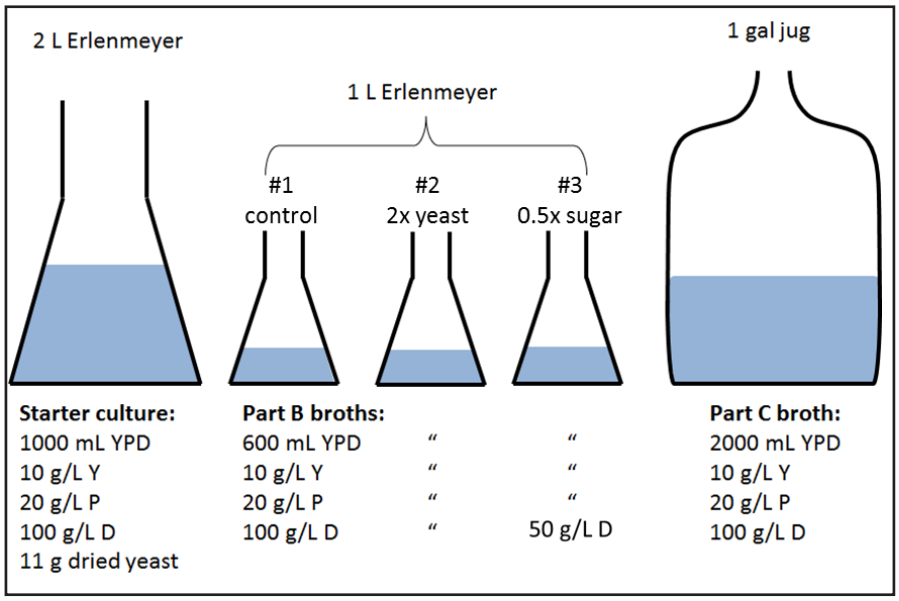

Figure 2. *Schematic of the five final solutions required for the FERM experiment. Excess YPD broths are provided for initial hydrometer measurements (discussed below). The labels above each container indicate the type of glassware used while those below indicate the final solution volume along with the concentration of all solutes.*

Water partially fills a kettle (Blichmann BoilerMaker G2) resting on a stir plate (IKA C-MAG HS 10). An electronic immersion heating element (Blichmann BoilCoil) inside the kettle first boils the water, and is then turned off during cooling. A stir bar with variable speed control establishes a circulating flow pattern to improve fluid mixing within the kettle.

Three custom kettle lids are used during the experiment. The lid employed during heating has only one opening for temperature-probe insertion. The two lids utilized during cooling each have an immersion heat exchanger of different size fastened to the lid. The immersion heat exchangers are fashioned from copper tubing with 0.315" ID and 0.375" OD coiled into either six or nine loops of 7" ID. Thus, each heat exchanger has a different surface area. The cooling water inlet and outlet for the heat exchangers are fitted with quick-release connections enabling straightforward swapping of heat exchangers with the water line.

A high-pressure industrial cold-water line provides cooling-water supply for the immersion heat exchangers. As shown in Figure 1, Tygon tubing connects the water supply to a control valve and rotameter (Blue-White F-400N) for flow measurement and control. Downstream of the rotameter, Tygon tubing and quick-disconnect couplings join the tubing to the heat exchanger inlet and outlet. Tygon tubing directs the cooling water exiting the heat exchanger to the sink for drainage or, potentially, collection.

The thermocouples (K-type) required for temperature measurement and subsequent data analysis are not shown in Figure 1 for simplicity. Two thermocouples are permanently attached at the cooling water inlet and outlet. There are up to six other thermocouple wires and probes for use in this experiment; students are encouraged to decide the best placement for their needs. The Omega TC-08 data logger and Omegasoft program enable real-time data monitoring and logging to a data file for future analysis.

Yeast fermentation kinetics (FERM)

The FERM experiment consists of three segments. First, students develop a calibration curve relating hemocytometer cell count to spectrophotometer absorbance at 600 nm and to dry cell weight (part A). Next, students perform three batch fermentations on a 500 mL scale to determine conversion and product yield for the fermentation reaction under varying initial substrate and yeast concentrations (part B). Finally, students run a 2-L reactor equipped with a mass flow meter to monitor the CO_2 production rate continuously during fermentation (part C). The experimental setup consists of a flow setup to measure the flow rate of evolved CO_2 along with a bench of analytical equipment and accompanying labware.

Students require a concentrated yeast starter solution for their calibrations and batch fermentations, as well as yeast-free cell-culture solutions—comprised of water, yeast extract (Y), peptone (P), and dextrose (D)—for their batch fermentations. Figure 2 details the required solution compositions and volumes. All YPD solutions and glassware must be sterilized in an autoclave to avoid contamination. The dextrose must be autoclaved separately from the yeast extract and peptone when formulating the YPD broth, or else the broth discolors. These broths are mixed the day they are needed in order to minimize the chance of contamination. The starter culture broth is inoculated with yeast (freeze-dried Nottingham Ale Yeast from Lallemand) roughly 24 hours before the first day of the lab.

Students pitch the YPD broths with the appropriate amount of yeast cells by adding the appropriate volume of the starter culture. The initial amount of yeast influences the fermentation rate and the degree of competition amongst yeast cells for resources required for cell growth. This initial cell count thus governs the available reaction pathways and, ultimately, the products produced. When brewing beer, for instance, pitching too few yeast cells can lead to acetaldehyde formation, imparting an undesired green apple taste.[1] The initial yeast concentration therefore plays a critical role in the final

taste of a fermented beverage. A brewer's rule of thumb is to pitch ~106 yeast cells per mL solution per 0.004 specific gravity (SG) increase above 1.000 (called degree Plato, °P) of the starting sugar solution, in this case the YPD broths.[10,11] SG is measured using a hydrometer, discussed below. Students use this guideline to calculate the amount of yeast to pitch; for the 500 IL flask in which they double the initial yeast concentration (part B, flask #2; Figure 2), they use double the recommended amount.

An iodine-based sanitization solution (IoStar) is used to sanitize the wine thief, a long tubular device used to take samples out of a vessel, as well as any other equipment that comes into contact with the yeast cultures or YPD broths.

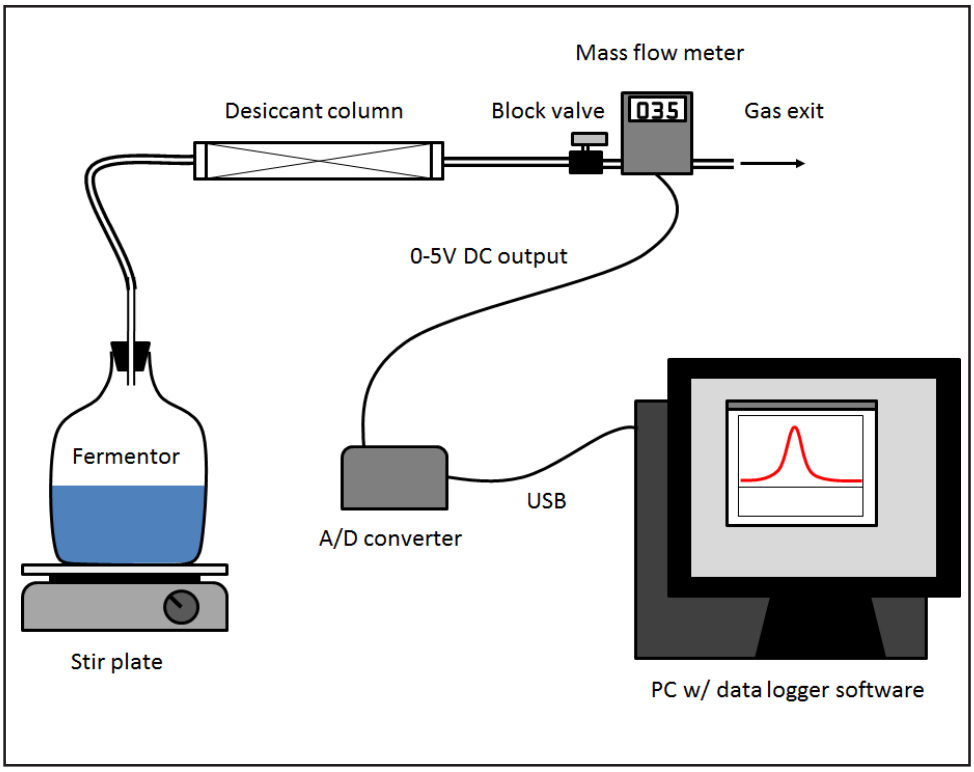

Figure 3. *Schematic of the fermentor setup for measurement and logging of evolved-gas flow rate (part C of FERM experiment).*

Microscope and hemocytometer

A light microscope (Fisher Scientific) and hemocytometer (Hausser Bright-Line 3110) enable quantification of the number of cells in a cell culture solution. The measurement technique requires only 10-15 µL of aqueous cell suspension. The hemocytometer is a thick glass slide containing two chambers, each with a laser-etched grid of lines for counting cells. The microscope stage illuminates the grid from below for visualization and quantification of the number of cells within each square. A cover slip sits exactly 0.100 mm above the chamber, creating a defined volume within the chamber when it is filled with fluid.[12]

Spectrophotometer

Relative to the hemocytometer, the spectrophotometer (Thermo Scientific Genesys 20) offers a faster, simpler method for quantifying cell concentration by optical density (OD). However, OD must be calibrated with cell count (discussed above) or dry cell weight (discussed in Data Analysis section). Samples for spectrophotometer analysis (~ 1 mL) rest in small plastic cuvettes placed in the sample holder, and are analyzed at 600 nm.[13]

Hydrometer

Hydrometers are weighted, hollow, glass cylinders designed to sink to varying depths in aqueous solution, thus leaving a scale exposed indicating the depth of submersion. As the solution density increases, the buoyant force per submersion depth of the hydrometer increases and more of the hydrometer will remain exposed. We calibrated the hydrometer to report specific gravity (SG) of aqueous sugar solutions; a calibration curve relating SG to the glucose concentration in solution is provided to students. SG measurements with the hydrometer require ~80 mL of sample in a 100 mL graduated cylinder, and enable determination of initial and final reactant concentrations. The hydrometer is calibrated for a particular fluid temperature, so sample temperature must be recorded for subsequent correction.[14]

Gas chromatograph

The compositions of ethanol-water liquid mixtures are determined using a gas chromatograph (GC; SRI 310 TCD, Porapak N column) fitted with a pre-column containing glass wool to adsorb any solutes, such as sugars or proteins, within the fermentation broth, and protect the chromatography column from fouling. Using a micro-syringe, students inject a 1 µL sample through a rubber septum into a flash vaporizer port. The vaporized sample enters the column oven at a temperature of 141 °C. PEAK software outputs a TCD chromatogram containing two peaks that correspond to ethanol and water. Using a calibration curve provided to them, students convert the relative peak areas into mole fractions.

Flow setup

The fermentor, a 2-L glass vessel, contains the cell culture and rests on a stir plate to ensure consistent mixing during fermentation (Figure 3). The fermentor exit is partially sealed with a rubber stopper, forcing gas to exit through a stainless-steel tube and into a mass flow meter before exiting into the room. A desiccant column precedes the flow meter and removes moisture from the gas to protect the flow meter. The mass flow meter (Omega Engineering FMA-A2305) outputs a 0-5V DC signal proportional to the measured flow rate, and an analog-to-digital (A/D) converter (DATAQ DI-145) transforms the voltage into a digital signal logged continuously by the computer software (WinDaq) until fermentation is complete. A calibration curve relating the volumetric flow rate of CO_2 to the output voltage is provided to students.

Final brewing project (PROJ)

Students are free to choose any fermented beverage producible within the project budget ($50 per group; paid by the department), equipment, and time constraints (one ~4-5 hour lab period for brewing and ~2 weeks for fermentation). Suitable beer recipes yielding 2.5 gallons of product can be provided to readers upon request.

Briefly, the process generally involves steeping a small amount (~8 oz.) of crushed malt and/or other grains in water at 65 ˚C to extract flavors into the wort. Next, the grains are removed and the mixture is heated to 100 ˚C before adding the liquid malt extract, providing the bulk of the sugar source for the yeast, and the hops, which add flavor. Proper timing of hops addition is important to extract the desired flavors. After boiling the mixture (typically 1 hour), the IHX setup is used to cool the wort to room temperature before transferring it to a 3-gallon carboy for inoculation with yeast. Fermentation lasts up to two weeks. Once complete, students carbonate their beverage, if appropriate, by transferring their product to a keg and exposing the beer to high-pressure CO_2 under refrigeration for 24 hours (details provided upon request). An iodine-based sanitization solution (IoStar) is used to sanitize all equipment coming into contact with the product.

Unlike industrial all-grain brewing, we use malt extract to simplify and shorten the brewing process. However, this eliminates some industrially relevant steps, as well as opportunities to study enzymatic degradation of malt starches. If other courses have more time, they could employ all-grain brewing to introduce more chemical engineering ideas such as surface-kinetic mechanisms of cellulases.

Technical assessment of beverage production requires a number of measurements before, during, and after processing. Students choose which measurements are both useful and feasible. The analysis of heat transfer phenomena during wort cooling (IHX) and the quantification of sugar, yeast, alcohol, and CO_2 performed in the FERM experiment provide a convenient starting point. The calibration curve relating specific gravity, SG, to sugar concentration, c_s [g/mL], is different for malt-based media than it is for YPD media. Brown[15] determined that the specific gravity of aqueous malt solutions obeys the following relationship:

$$SG = 1 + \frac{K_0 c_s}{1 - S_1 c_s} \qquad (2)$$

where the so-called solution factor, K_0, and its constant, S_1, are properties of the particular solute and solvent, but remain independent of composition. Using dry malt extract (DME), we determined that $K_0 = 0.3815$ mL/g and $S_1 = 0.1327$ mL/g. Several additional analytical techniques not explored during FERM or IHX are possible to quantitatively assess the taste and appearance of the final product,[16-19] and students are encouraged to be creative.

SAFETY

A graduate student teaching assistant is present in the labs at all times. In addition to the traditional personal protective equipment used by all students in the lab, the following safety precautions should also be considered.

Immersion heat exchanger (IHX)

Be aware of hot surfaces and safely handle all hot equipment with insulated gloves. Students should be vigilant to prevent splashing and contain all spills or leaks quickly to prevent contacting electrical equipment with water. Power down equipment immediately if it becomes covered in water.

Yeast fermentation kinetics (FERM)

Household bleach diluted in water (10-20% by volume) is used to deactivate all yeast solutions prior to sink disposal. Students should avoid skin or eye contact with either the bleach solutions or the IoStar sanitizer. A flammable 70% ethanol solution is used to sanitize lab surfaces. Yeast fermentation produces significant amounts of gaseous CO_2 capable of pressurizing and exploding the fermentation vessel if venting is not enabled.

Final brewing project

In addition to the safety concerns for FERM and IHX, instructors must also consider the safety of the product analysis techniques students choose to use. Students must properly sanitize all lab equipment and materials via heat treatment, spraying surfaces with a 70% ethanol solution, or sanitizing with an iodine solution (IoStar) in order to prevent contamination during fermentation. Additional practices to ensure the safe production of a consumable are given by the Brewer's Association's Good Manufacturing Practices (GMPs).[20] Generally, the low pH, along with the ethanol and hop acids present in the wort, prevent the growth of dangerous microbes; instead, contamination typically leads to off-flavors that are harmless, albeit unwanted.[21,22] The kegging process involves pressurized CO_2; thus, users must ensure that CO_2 canisters, kegs, and lines are properly rated and secured.

DATA ANALYSIS

Immersion heat exchanger (IHX)

The specific objectives of this experiment are to:

i. Measure the temperature-time history for cooling three gallons of water at various cooling-water flow rates with one immersion heat exchanger.

ii. Construct a mathematical model to predict the wort and cooling-water exit temperatures as a function of time.

iii. Utilize a minimization scheme to fit experimental data (i) to the model (ii), with the overall heat-transfer coefficient, U_o, as the fitting parameter.

iv. Predict the wort-cooling behavior as a function of cooling-water flow rate and heat exchanger area to identify which operating conditions would cool the wort to 24 °C fastest.

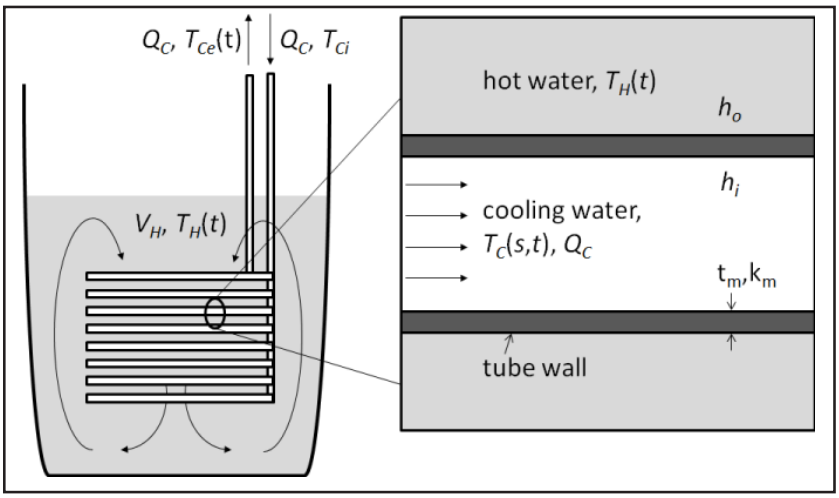

Figure 4. *Schematic diagram of an immersion heat-exchanger cooling down a tank of hot water. The tank contains volume V_H of hot water at transient temperature $T_H(t)$ while cooling water at flow rate Q_C enters the chiller at constant temperature T_{Ci} and exits at transient temperature $T_{Ce}(t) > T_{Ci}$. The panel to the right shows a local cross-sectional view across the tube. The local temperature driving force T_H-$T_C(s,t)$, the inner and outer heat-transfer coefficients, h_i and h_o, respectively, and the tube wall thickness and conductivity, t_m and k_m, respectively, set the local heat-transfer rate between the two fluids.*

Kern[23] presents an analytical solution for the case considered in Figure 4. A transient energy balance on the hot water (assuming adiabatic walls, constant V_H, and perfect mixing in the tank) yields an ODE for $T_H(t)$

$$\rho_H V_H C_{PH} \frac{dT_H}{dt} = -U_o A_o \Delta T_{LM} \qquad (3)$$

with initial condition

$$T_H(0) = T_{H0} \qquad (4)$$

where ρ_H and C_{PH} are the mass density [kg/m^3] and specific heat capacity [J/kg/K], respectively, of the hot water (assumed to be constant), U_o is the overall heat-transfer coefficient [W/m^2/K] based on the outer surface, $A_o = \pi D_o L$ is the outer surface area [m^2] of the immersion heat exchanger in contact with the water, D_o is the outer diameter [m] of the tube, and L is the total length of the tube. T_{H0} is the initial water temperature in the tank and ΔT_{LM} is the log-mean temperature difference between the cooling water and the hot water:

$$\Delta T_{LM} = \frac{(T_H - T_{Ci}) - (T_H - T_{Ce})}{\ln\left[(T_H - T_{Ci})/(T_H - T_{Ce})\right]} \qquad (5)$$

Eqs. (3)-(5) describe the time-dependent heat-loss rate from the hot water to the cooling water through the immersion chiller. Energy balance requires that the heat lost by the hot water in Eq. (3) be absorbed by the cooling water:

$$U_o A_o \Delta T_{LM} = \rho_C Q_C C_{PC} (T_{Ce} - T_{Ci}) \qquad (6)$$

where ρ_C and C_{PC} are the mass density [kg/m^3] and specific heat capacity [J/kg/K] of the cooling water, respectively. Rearrangement of Eq. (6) results in an equation for T_{Ce}, which can be substituted into Eq. (3) to yield a separable, first-order ODE for $T_H(t)$, whose analytical solution is:

$$T_H = T_{Ci} + (T_{H0} - T_{Ci}) \exp\left[-\frac{\rho_C Q_C C_{PC}}{\rho_H V_H C_{PH}}\left(\frac{K_c - 1}{K_c}\right)t\right] \qquad (7)$$

Here, K_c is a constant used to simplify the expression. According to Eq. (7), the hot-water temperature decays exponentially towards a steady-state temperature equal to that of the cooling water inlet temperature, T_{Ci}. We can use Eq. (7) to solve for the cooling-water exit temperature, T_{Ce}, which also approaches a steady-state limit of $T_{Ce} = T_{Ci}$. In practical operation, heat loss or gain via other mechanisms besides the immersion heat exchanger necessitates modification of Eq. (3). The result is likely an ODE without an analytical solution. Typically, such modifications have not been necessary to yield models that closely fit student cooling data; however, they do provide opportunity to increase model complexity.

To determine the value of U_o required to model T_H versus time using Eq. (7), the equation is rearranged in terms of dimensionless temperature, Θ:

$$\Theta = \frac{T_H - T_{Ci}}{T_{H0} - T_{Ci}} = \exp\left[-\frac{\rho_C Q_C C_{PC}}{\rho_H V_H C_{PH}}\left(\frac{K_c - 1}{K_c}\right)t\right] \qquad (8)$$

Students use their experimentally determined temperature values to plot $\ln(\Theta)$ versus time. Linear regression of the data

and evaluation of the slope yields U_o (contained within K_c) for each set of data collected at varying cooling water flow rates (Figure 5a). Students are asked to perform a sensitivity analysis to determine how sensitive their U_o values are to the other values in Eq. (8), thus obtaining a rough estimate of the uncertainty in U_o.

Next, students evaluate how U_o scales with cooling water flow rate. U_o accounts for convective heat-transfer at the inside and outside of the tube wall and for conductive heat transfer through the tube wall:

$$\frac{1}{U_o} = \frac{1}{h_o} + \frac{A_o \ln(D_o/D_i)}{2\pi kL} + \frac{A_o}{h_i A_i} \quad (9)$$

Here, $A_i = \pi D_i L$ is the tube inner surface area, D_i is the tube inner diameter, and k_m is the thermal conductivity of the metal tube wall. Note that the subscript "o" in U_o indicates that it is defined with respect to the tube outer surface area. The right-hand side of Eq. (9) is a sum of convective and conductive thermal resistances in series. Thermal conductivities for common metals are readily available, making calculation of the conductive resistance straightforward. The convective resistances, meanwhile, require heat-transfer coefficients relevant to the specific flow conditions within the tube and in the tank around the coils. Convective heat-transfer coefficients are tabulated through the Nusselt number (Nu):

$$Nu = \frac{hL_c}{k_f} \quad (10)$$

where h is the heat-transfer coefficient, L_c is the characteristic flow length, and k_f is the fluid thermal conductivity. Nu correlations for flow inside tubes appear in Chapter 20 of Welty, et al.[24] and Chapter 14 of Bird, et al.[25] The relevant Nu correlation describing the turbulent cooling water flow within the immersion heat exchanger coils is:

$$Nu = 0.023\ Re^{0.8}\ Pr^{0.4} \quad (11)$$

where Re is the Reynolds number and Pr is the Prandtl number. Since Re is proportional to the fluid velocity inside the tube, v, it follows from Eqs. (10) and (11) that h_i scales with $v^{0.8}$.

Heat-transfer correlations for flow around immersed heat-exchanger coils are more specialized and less readily available, however students can determine the value of h_o by substituting the expression for h_i obtained from Eqs. (10) and (11) into Eq. (9) and rearranging to yield:

$$\frac{1}{U_o} = \frac{A_o D}{0.023\left(\frac{D}{v}\right)^{0.8} Pr^{0.4}\, kA_i} v^{-0.8} + \frac{1}{h_o} + \frac{A_o \ln(D_o/D_i)}{2\pi kL} \quad (12)$$

A plot of $1/U_o$ versus $1/v^{0.8}$ yields a line whose y-intercept can be evaluated to determine h_o (Figure 5b); h_o should be constant assuming the stirring speed in the kettle is constant, and values should be lower than h_i due to the slower fluid motion and, hence, thicker thermal boundary layers on the outside of the coils. In practice, however, h_i and h_o values are comparable, an artifact of external heat losses through the kettle sides and lid that lead to overestimates of h_o.

Finally, students are asked to extend their model to predict cooling time and cost when using heat exchangers of different surface area or varying the cooling water flow rate. They must also choose operating conditions that they will use later in the semester to cool their hot wort.

Yeast fermentation kinetics (FERM)

The specific objectives of this experiment are to:

i. Develop a calibration curve to relate dry cell weight (DCW) to measurements of cell count from the

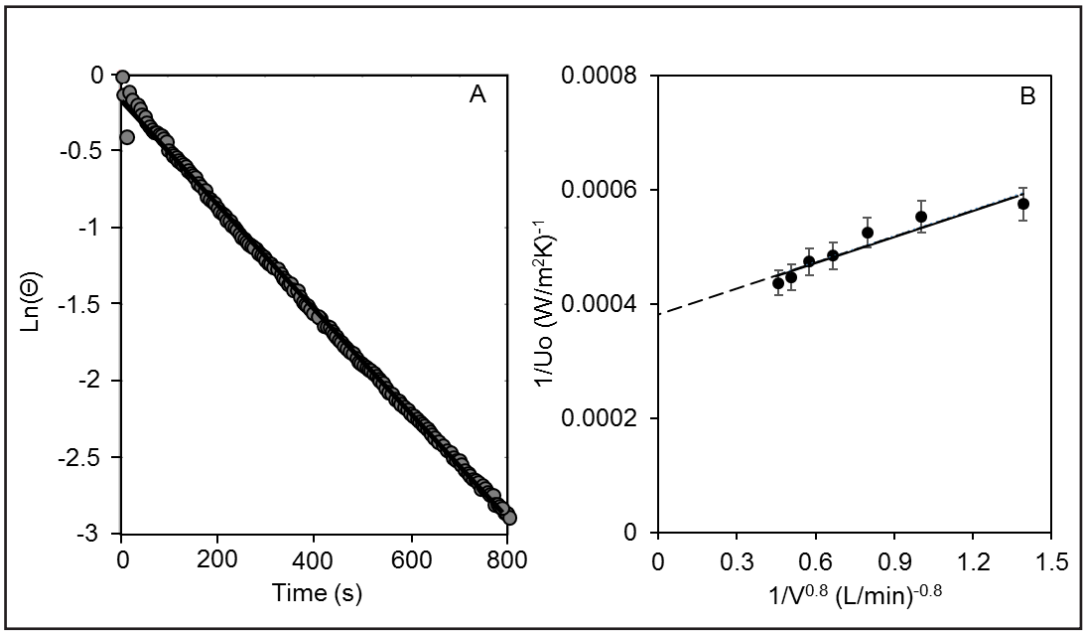

Figure 5. *Sample student data obtained from the IHX experiment. (A) A linear plot of ln(Θ) versus time at one cooling water flow rate. A linear fit (black line) to the data (gray dots) yields U_o from evaluation of the slope. (B) Plotting $1/U_o$ versus $1/v^{0.8}$ for all cooling water flow rates (black circles) with a linear fit (black line) yields h_o from evaluation of the y-intercept.*

hemocytometer and optical density (OD) from the spectrophotometer.

ii. *Perform fermentations on a 500 mL and 2-L scale and quantify the amount of substrate consumed along with biomass, ethanol, and CO_2 produced.*

iii. *Analyze the results from (ii) to determine stoichiometric and kinetic parameters for the given strain of* S. cerevisiae.

iv. *Develop a mathematical model to predict substrate, product, and cell concentrations during anaerobic fermentation and compare to experimental results.*

Objective (i) allows interconversion among the various methods for quantifying biomass. Conversion from cell number or OD to DCW is vital for characterizing the yield coefficients utilized in the kinetic model (iv). The calibration curve is created by first performing serial dilutions from a concentrated cell culture provided in the lab (Figure 2) to produce samples between $5x10^6$ to $2x10^7$ cell/mL (~50x dilution), a range of concentrations that yields accurate cell counts using the hemocytometer. Students also measure the OD of these samples at 600 nm using the spectrophotometer, thus correlating OD with cell count.

To correlate DCW with OD, a larger number of cells is required for accurate weight measurements. Students prepare 1x to 5x dilutions of the concentrated cell culture, then evaporate all liquid using a vacuum oven. Once measurements of the DCW are obtained, students can calculate what the DCW would be over the linear range of OD obtained using the 50x diluted samples.

To analyze their fermentations, students quantify the yield of various products using yield coefficients that relate the mass of products created to the mass of reactant consumed. We define yield coefficients for ethanol, biomass, and CO_2 in terms of:

$$Y_{P/S} = \frac{\text{mass ethanol created}}{\text{mass substrate consumed}} \quad (13)$$

$$Y_{C/S} = \frac{\text{mass cells created}}{\text{mass substrate consumed}} \quad (14)$$

$$Y_{CO2/S} = \frac{\text{mass } CO_2 \text{ created}}{\text{mass substrate consumed}} \quad (15)$$

where $Y_{i/j}$ is the mass yield coefficient for creation of species i divided by the consumption of species j; i or j may take on the values of CO_2, P for ethanol (product), C for cells, or S for substrate. For ease of application to mass balance, we present yield coefficients normalized to substrate consumed.

Due to the production of biomass, only about 95% of sugar consumption is directed to the production of CO_2 and ethanol.[26] Yield coefficients for *S. cerevisiae* are estimated or measured by a number of other sources.[27-30] Using the hydrometer, the GC, and the flow setup (see Apparatus and Methods), students determine the sugar concentration (of both initial and final solutions), the final ABV%, and the amount of CO_2 produced, respectively, in order to calculate experimental yield coefficients using Eqs. (13)-(15). According to mass balance, the sum of the yield coefficients should equal unity. At the 500 mL scale (Figure 2; part B), no CO_2 evolution data is obtained, however students can either assume that CO_2 and ethanol are produced in stoichiometric amounts or use the experimentally determined stoichiometry obtained from the 2-L setup (part C).

Using their results, students evaluate how changing the initial sugar or yeast concentrations (part B) or scaling up to 2-L (part C) affected their product yields and overall mass balances. In part C, students use their CO_2 data, the starting sugar and cell concentrations, and the measured yield coefficients (to convert CO_2

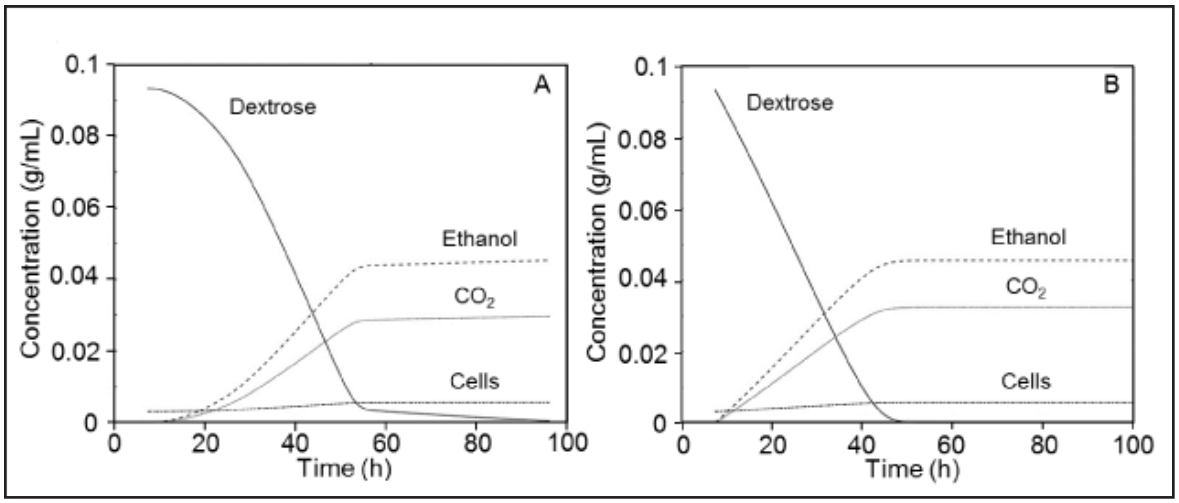

Figure 6. *Sample student data (A) and model predictions (B) of the concentrations of dextrose (solid), ethanol (dash), CO_2 (dot), and cells (dash-dot) versus time in a batch stirred tank reactor (BSTR). In (A), CO_2 evolution is collected continuously using a mass flow meter (see Apparatus and Methods), and the data is smoothed to yield an averaged curve; assuming constant yield coefficients enables extrapolation of the concentrations of all other species throughout the experiment. In (B), Eqs. (19)-(21) and experimental μ_{max} and K_S values are used to construct the model.*

concentration to sugar, cell, and ethanol concentrations) to predict all species concentrations in the bioreactor as a function of time, assuming constant yield coefficients (Figure 6).

Next, students evaluate the kinetics of the fermentation process to develop a model that can be compared to their experimental results. Detailed biochemical models exist that characterize intermediate products and rates within the various metabolic pathways occurring during yeast growth.[30,31] However, we use a simple lumped model that captures the basic physics of fermentation kinetics. The Monod equation,[32] modified to account for the toxicity of the ethanol product to yeast cells, predicts the specific cell grow rate, μ, as:

$$\mu = \left(1 - \frac{C_P}{C_P^*}\right)^n \frac{\mu_{max} C_S}{K_S + C_S} \qquad (16)$$

where μ_{max} is the maximum specific growth rate [hr^{-1}], C_S is the fermentable substrate composition [g/mL], K_S is the Monod constant [g/mL] corresponding to the substrate concentration at half of the maximum growth rate, C_P is ethanol concentration in the broth [g/mL], C_P^* is the toxic concentration of ethanol [g/mL], and n is the unitless toxic power index. The Monod model behaves similarly to the Michaelis-Menten model for enzymatic reactions, and adequately models fermentation processes using fermentable sugar as the limiting substrate.[27,28,30,31] Miller and Melick[28] estimate C_P^* to be 0.093 g/mL (~11 % ABV) and n to be 0.52 for *S. Cerevisiae*.

Using the cell concentration versus time data from part C, students calculate μ at each time point using:

$$\mu = \frac{\frac{dC_C}{dt}}{C_C} \qquad (17)$$

C_C is the concentration of cells [g/mL]. An estimate of Monod kinetic parameters μ_{max} and K_S can be calculated experimentally by first rearranging Eq. (16) as:

$$\frac{\left(1 - \frac{C_P}{C_P^*}\right)^n}{\mu} = \frac{K_S}{\mu_{max}}\left(\frac{1}{C_S}\right) + \frac{1}{\mu_{max}} \qquad (18)$$

By plotting the left-hand side of Eq. (18) versus $1/C_S$, students can extract μ_{max} and K_S from the y-intercept and slope, respectively, of a linear regression of the linear portion of the data (representing cells in the exponential growth phase). This method can introduce quite a bit of uncertainty in extracted parameters, however, since cell growth rate during the batch fermentation process is dependent not only on substrate concentration, but also on other factors such as stir rate, pH, dissolved oxygen, yeast strain, temperature, and ionic or metabolite species concentrations, which may all change over the course of the fermentation process. In addition, kinetic parameters may be sensitive to data manipulation methods, such as smoothing of the CO_2 flow rate data. Nevertheless, students can use Eq. (18) to yield an estimate of the magnitude of each kinetic parameter.

Miller and Melick[28] report μ_{max} and K_s values of 0.45 h^{-1} and 0.13 g/L, respectively, however parameters are dependent on yeast strain, substrate type, and experimental conditions.[27-31] Students are expected to compare their experimentally estimated kinetic parameters to literature values, keeping in mind the limitations of the data analysis methods, the physical meaning of μ_{max} and K_s, and how varying experimental conditions would affect their magnitudes.

With μ_{max} and K_S values, students develop a model to predict transient substrate, product, and cell concentrations during anaerobic fermentation. Transient species mass balance in a batch stirred tank reactor yields ordinary differential equations (ODEs) for their concentrations :

$$\frac{dC_C}{dt} = \mu C_C \qquad (19)$$

$$\frac{dC_S}{dt} = -Y_{S/C}\mu C_C \qquad (20)$$

$$\frac{dC_P}{dt} = Y_{P/C}\mu C_C \qquad (21)$$

Students develop a numerical solution of the coupled ODEs from Eqs. (19)-(21) using MATLAB with either a built-in ODE solver such as ode45 or their own method such as Euler or Runge-Kutta. The solution to Eqs. (19)-(21) is the mathematical model for yeast fermentation kinetics, and is used to predict the CO_2, ethanol, cell, and substrate concentrations in the 2-L bioreactor versus time. The model assumes that cells do not die, substrate is consumed for cell growth only, and that the yield coefficients remain constant over the time period considered. The model does not account for yeast lag time, but the recorded CO_2 versus time data does.

By comparing their model predictions to their experimental results, students should consider the causes for any discrepancies observed between the experiment and model. They can adjust the kinetic parameters of the model to obtain better agreement between theory and experiment, thus gaining an appreciation for how such parameters affect the fermentation process and what differences exist between the assumptions made by the model and the actual experimental conditions.

Final brewing project

Using the techniques and theories examined in FERM and IHX experiments, student groups design and complete a brewing project to produce a drinkable fermented beverage. Students must make the beverage from a technical standpoint —that is, to quantify the inputs and outputs and to describe the physics of the processing steps. Several lab periods are allotted to planning their processing steps, analytical measurements, and any additional experiments, as well as to completing a literature review. After pitching their proposed project to the instructors, students execute their plans over several weeks,

during which they analyze their success in meeting their desired specifications and suggest what improvements to the process, product, or measurements are required. In addition to the analysis techniques used in FERM and IHX, students can quantify bitterness, color, and calorie content by analyzing the amount of isohumolone in their beer, measuring the absorbance of 430 nm wavelength light using a spectrophotometer, and bomb calorimetry, respectively, among other analysis techniques.[16-18,33] Students can also compare their results to the heat transfer and fermentation kinetics models developed in IHX and FERM, respectively, and address any discrepancies they observe.

Finally, students also consider their process from an environmental and economic standpoint. By quantifying the water usage in their process as a ratio of the volume used for processing to the volume of the final product, they can compare their water usage to those reported by the brewing industry and other studies.[34-36] Similarly, the energy usage of their process can be quantified per volume of final product or as the lb of CO_2-equivalent emissions; such values are compared to the energy usage reported by the brewing industry and other studies.[36,37] Students should consider what process improvements would increase their water and energy efficiencies, and what benefits they would expect to gain if they were to scale up production volume. From an economic standpoint, students estimate the production cost per six-pack of their beverage, compare to typical production costs of beverages on the market, and estimate how their costs would decrease if they scaled up to the size of a craft brewery (annual production of less than 186 million gallons).

ASSESSMENT AND CONCLUSION

The fermentation project has been offered to four three-membered student groups every semester for the past two years. While the subject matter alone generates much student interest, instructors strive to select participants who desire to pursue a career in brewing or fermentation-related industries. In fact, several students who have completed the project have no interest in consuming beer or are unable to do so because of their age; such students, along with most other participants, are drawn to the project because of their career interests in biotechnology and the opportunity to apply their engineering knowledge to a tangible consumer good. Many student participants discuss their brewing project work in job interviews, and have gained employment in the brewing or biotechnology sectors.

Students are also drawn to the open-ended structure of the final design project, motivating many to go above and beyond the required analysis questions to explore their process and product. The freedom in the project portion provides students an opportunity to exercise their creativity and intellectual independence. For instance, in addition to the analysis methods outlined in IHX and FERM experiments, some students forge collaborations with the undergraduate chemistry laboratories to use HPLC, bomb calorimetry, and other techniques to analyze their product. Having coursework culminate in the brewing project also motivates students to thoroughly investigate and analyze the preceding IHX and FERM experiments since they know that they will later apply their newly developed skills and theoretical models in their final project. As an added incentive to develop a tasty brew (and get creative in naming and branding their product), peers and chemical engineering faculty are invited at the end of the semester to taste and evaluate the beverages produced by the brewing groups.

Executing the fermentation project in parallel to the traditional lab sequences requires additional coordination to ensure a seamless schedule. Proper timing in preparing the cell culture solutions and sterilized YPD broths is crucial to allow enough time for cell cultures to grow before the lab period while minimizing the chance of contamination. To prevent cross-contamination with chemicals or materials from the other unit operation experiments, we designated a separate lab as "food safe." All fermentation-related materials, equipment, and experiments have been segregated to this lab; any lab equipment not related to the project is prohibited. Students are required to maintain a clean workspace in order to prevent unwanted microbial growth and pests. Students must also thoroughly sanitize all equipment and materials that come into contact with their beverage during fermentation in order to produce a product that is safe and desirable to drink. Instructors should be mindful of any campus alcohol policies, and to obtain the age of brewers and potential tasters to ensure that they are of legal drinking age.

The beer production process, like many other chemical processes, contains unit operations governed by phenomena relevant to chemical engineers. The experiments outlined in this paper provide only a small set of potential experiments related to brewing. More sophisticated heat-exchange equipment or commercial quality bioreactors would enable more ambitious experimental protocols for IHX or FERM. In addition, beer brewing employs packed bed and membrane filtration operations, extractive processes, and temperature-sensitive enzymatic degradation steps that provide even more opportunities to explore chemical engineering fundamentals. Hopefully the experiments presented here can serve as groundwork for instructors to create their own brewing-related projects. We encourage interested instructors to contact the corresponding author for additional information and resources to implement the track we present here.

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