

2017

# Microbiology 562: D. Batch Fermentation Module

Kristen DeAngelis

*University of Massachusetts Amherst*, [deangelis@microbio.umass.edu](mailto:deangelis@microbio.umass.edu)

Cecilia Prado

*University of Massachusetts Amherst*, [ceciliaprado91@gmail.com](mailto:ceciliaprado91@gmail.com)

Follow this and additional works at: [https://scholarworks.umass.edu/micro\\_ed\\_materials](https://scholarworks.umass.edu/micro_ed_materials)



Part of the [Microbiology Commons](#)

---

## Recommended Citation

DeAngelis, Kristen and Prado, Cecilia, "Microbiology 562: D. Batch Fermentation Module" (2017). *Microbiology Educational Materials*. 1.

[https://scholarworks.umass.edu/micro\\_ed\\_materials/1](https://scholarworks.umass.edu/micro_ed_materials/1)

This Book is brought to you for free and open access by the Microbiology at ScholarWorks@UMass Amherst. It has been accepted for inclusion in Microbiology Educational Materials by an authorized administrator of ScholarWorks@UMass Amherst. For more information, please contact [scholarworks@library.umass.edu](mailto:scholarworks@library.umass.edu).

# Microbiology 562: D. Batch Fermentation Module

Kristen DeAngelis and Cecilia Prado<sup>1</sup>

07 December 2017

In this module, we set up a fermentation of grains by yeast with hops to brew beer (D1). We make media and pour plates to grow and identify microbial contaminants (D2). Two weeks after setting up the primary fermentation, we prime and condition the fermentation (D3). While we wait for the priming and conditioning to finish, we do a bioinformatics analysis of microbial communities turning ethanol (a product of fermentation) to n-caproic acid, (D4), to illustrate the power of metagenomic sequencing in resolving microorganisms and their potential physiology. Then we identify and examine the microbial contaminants cultured in the primary fermentation (D5). When the beer is finished, we perform a sensory analysis (D6) followed by a field trip to the Berkshire Brewing Company in South Deerfield, MA, where we will tour their brewing facilities and microbiology lab.

Beer is the oldest and most popular alcoholic drink in the world. Humans have domesticated microbial communities to preserve food via fermentation for centuries. Today beer is defined as having four ingredients: grains, hops, yeast and water. In the US, beer is a major industry with thousands of breweries, which is double what it was three years ago. Brew-masters who lead and oversee the brewing process commonly have microbiology training.

Students work in four groups to brew four versions of an American Pale Ale: (1) a control version, (2) a high-pitch rate beer, (3) a beer brewed at high pH, and (4) a control beer brewed at high fermentation temperature. By modifying the conditions of the fermentation and monitoring the microbes and microbial products along the way, students gain a strong foundation in the microbiology of batch fermentation.

## Learning Goals

By the end of this module, students should be able to

- describe how beer is produced, and determine how fermentation conditions are important for a quality product.
- make media, describe the role for different media components, and discuss the advantages and limitations of culturing.
- measure microbes and microbial products, and contrast whether microbial products detected are consistent with what we would expect from microbes present.

<sup>1</sup> Developed with support from Klaus Nusslein, Sue Leschine, and the UMass Center for Teaching Excellence Faculty Development. With thanks for helpful comments from the UMass students in Microbio 562 fall of 2017. This work is licensed under a Creative Commons Attribution-NonCommercial 4.0 International License.



Figure 1: The students enrolled in Microbiology 562: Environmental Biotechnology in the fall of 2017 were the first class to complete this activity.

This curriculum is designed to be conducted over the course of four weeks, in six 3-hour class meetings with no class during the second week to allow time for fermentation. In the fall of 2017, this was Thanksgiving break.

Materials for the six classes are included in this document. There are three types of documents for each of the six class meetings:

1. Handouts for class activities, which in this packet are collated with
2. Data sheets for in class, and
3. Day sheets with order lists and instructions for setting up.

Reading assigned in the first week includes reviews by Bokulich and Bamforth (2013) and Vriesekoop and colleagues (2012). Bioinformatics is introduced in the review by Desai and colleagues (2012). Students spend one class meeting analyzing data presented in the research article by Agler and colleagues (2012). Blog posts on the Great Plate Count Anomaly discuss how media preparation and formulation affects cultivability (2013, 2014).

### References

Matthew T Agler, Catherine M Spirito, Joseph G Usack, Jeffrey J Werner, and Largus T Angenent. Chain elongation with reactor microbiomes: upgrading dilute ethanol to medium-chain carboxylates. *Energy & Environmental Science*, 5(8):8189–8192, 2012.

MicrobiologyBytes blog. The great plate count anomaly. <https://microbiologybytes.wordpress.com/2013/09/17/great-plate-count-anomaly/>, September 2013.

Nicholas A Bokulich and Charles W Bamforth. The microbiology of malting and brewing. *Microbiology and Molecular Biology Reviews*, 77(2):157–172, 2013.

Narayan Desai, Dion Antonopoulos, Jack A Gilbert, Elizabeth M Glass, and Folker Meyer. From genomics to metagenomics. *Current opinion in biotechnology*, 23(1):72–76, 2012.

Gemma Reguera. The "great plate count anomaly" that is no more. <http://schaechter.asmblog.org/schaechter/2014/12/the-great-plate-count-anomaly-that-is-no-more.html>, December 2014.

Frank Vriesekoop, Moritz Krahl, Barry Hucker, and Garry Menz. 125th anniversary review: Bacteria in brewing: The good, the bad and the ugly. *Journal of the Institute of Brewing*, 118(4):335–345, 2012.

# Microbiology 562: D1. Primary Fermentation Set-up

## Batch Fermentation Lab by Dr. Kristen DeAngelis<sup>1</sup>

14 November 2017

This document describes the first lab activity in a six part module exploring batch fermentation. **In this lab, we will set up a fermentation of grains by yeast to brew beer (D1).** On Thursday we will make media and pour plates to grow and identify bacterial contaminants (D2). Two weeks after setting up the primary fermentation, we will prime and condition the beer (D3). While we wait for the priming and conditioning to finish, we will spend one lab doing bioinformatics analysis of microbial communities turning ethanol (a product of fermentation) into n-caproic acid, a fuel precursor and otherwise valuable industrial chemical (D4). We will also spend one lab examining the microbial contaminants that were cultured after the primary fermentation (D5). Once the beer is finished, we will perform a sensory analysis followed by a field trip to the Berkshire Brewing Company in South Deerfield, MA, where we will tour their brewing facilities and microbiology lab.

Beer is the most popular alcoholic drink in the world and a microbial product. The **main objectives** of this module is to answer the question, *what factors in batch fermentation are important for making beer?*

### Learning Objectives

By the end of today's class, students should be able to:

- describe the major ingredients required for the batch fermentation of beer, and explain the role for each ingredient,
- describe the overall process of batch fermentation of beer, and explain why the conditions at each step are necessary, and
- define and use the vocabulary associated with batch fermentation and brewing beer.

Beer requires four major ingredients: water, cereal grain, hops and yeast. Yeast may be classified as top or bottom fermenting yeast depending on the beer style (ale vs. lager).

The brewing process involves two major events: the saccharification of starch derived from the malted cereal grains and the fermentation of the resulting sugar by yeast. A general synopsis is described right below:

1. Mash-in: Malted barley is soaked in hot water to release the fermentable sugars
2. Boiling: the malt sugar solution, or wort, is boiled with hops for seasoning and to prevent contamination

<sup>1</sup> Developed in part by Cecilia Prado, with support from Dr. Klaus Nusslein, Dr. Sue Leschine, and the UMass Center for Teaching Excellence Faculty Development

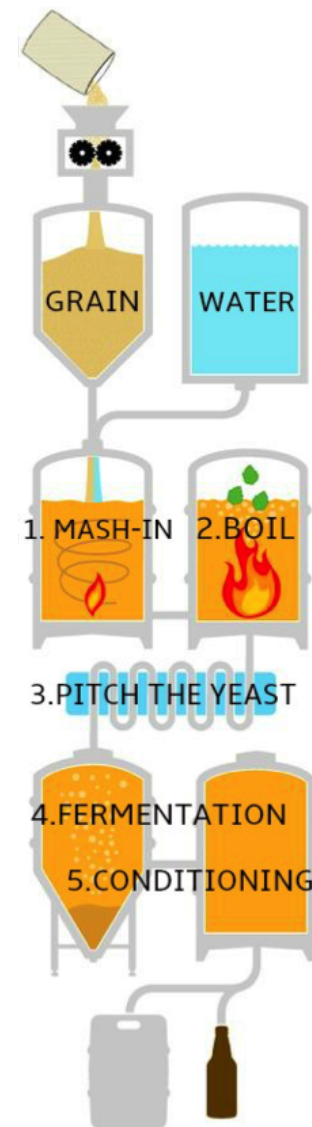


Figure 1: There are five steps in brewing beer. Today we will combine grains and water, mash-in to extract the sugars, boil to sterilize the wort, then pitch the yeast to start the fermentation.

3. Pitching the yeast: the wort is cooled and yeast is added to begin fermentation
4. Fermentation: the wort is fermented by the yeast, releasing CO<sub>2</sub> and ethyl alcohol
5. Priming and Conditioning: when the vigorous or primary fermentation is complete, the beer is bottled with a small amount of added sugar to promote carbonation

### *Setting up the fermentation*

Today, you will prepare a 1-gallon batch of American Pale Ale based on the following recipe. Each group will measure the effect that altering different parameters (mash pH, pitching rate, fermentation temperature) has on the fermentation product.

### *American Pale Ale*

- 1 kg ( 2.2lbs) of 2-row pale malt
- 50 g ( 0.11lbs) of Crystal Malt 40L
- 0.4oz of Cascade Hops (bittering)- 5.5% alpha acids
- 0.4 oz of Cascade Hops (flavoring) -5.5% alpha acids
- Safale US-05 (dry yeast used for most beer types)

As you work through this lab, use the accompanying data sheets to ensure that you collect all the data necessary to complete this lab. Completing the data sheets should be done along side recording all observations in your lab notebook.

By brewing four slightly different batches of beer from the same grains and yeast, we will explore how high pH, high pitch rate (larger starting concentration of yeast), and high fermentation temperature effect the fermentation.

**Table 1** shows the how the materials and methods vary by group.

	Group 1 (Control)	Group 2 (High pH)	Group 3 (High pitch rate)	Group 4 (Temp) (High temp)
Gypsum	1.5 g/gal	1.0 g/gal	1.5 g/gal	1.5 g/gal
Pitch rate	$7.5 \times 10^5 \text{ cells/ml}$	$7.5 \times 10^5 \text{ cells/ml}$	$2.5 \times 10^6 \text{ cells/ml}$	$7.5 \times 10^5 \text{ cells/ml}$
Fermentation Temp	60°F (15.5°C)	60°F (15.5°C)	60°F (15.5°C)	68°F (20°C)

### *Mash pH*

A lower mash pH (between 5.2-5.7) has a direct effect on every step of the brewing process. During mashing, it facilitates starch conversion by increasing enzymatic activity. During the boil, it allows for better hop extraction rates. During the fermentation, it leads to healthier yeast and reduces the risk for contamination.

One way to alter the pH of the mash is by adding mineral salts, such as gypsum ( $\text{CaSO}_4$ ). Adding a gram of  $\text{CaSO}_4$  to our strike water contributes 61.5ppm of calcium ions ( $\text{Ca}^{4+}$ ) and 147.4 ppm of Sulfate ions ( $\text{SO}_4$ ), lowering the mash pH. The calcium concentration of most beers ranges around 50-150 ppm, while the sulfate concentration ranges around 0-150 for moderately bitter beers and 150-350 ppm for very bitter beers.

### *Pitching rate*

The amount of yeast added to the fermentation chamber is the pitching rate. It affects the growth rate of the yeast and the volumetric productivity of the beer fermentation process. A low pitching rate results in a longer lag phase, higher risk of contamination and a longer overall fermentation time. A high pitching rate would initially speed up the fermentation but it could potentially lead to early autolysis.

### *Fermentation temperature*

It is known that temperature has a direct effect on the rate of enzymatic activity. Because of this, fermentation temperature is a key determinant of the final beer aroma composition and quality. Higher brewing temperatures are expected to increase the rate of fermentation and other minor yeast-related pathways, resulting in a greater content of higher order alcohols, esters, sulfur-derived compounds and vicinal diketones (VDKs).

#### **Part A. Single Infusion Mashing**

1. Prepare your strike water by heating 6.88 L (1.82 gal) of water to  $73^\circ\text{C}$  ( $160^\circ\text{F}$ ) in a brewpot with a stir bar.
2. Add the amount of gypsum corresponding to your recipe in the table above.
3. Measure 1 kg of 2-row pale malt and 50 grams of crystal malt into the cheesecloth steeping bag and set aside. Secure the bag in the white plastic fermentation vessel to prevent spillage. This begins the mash-in.

*Finished beers using low pH wort present an improved flavor, clarity, and clarity stability as it ages.*

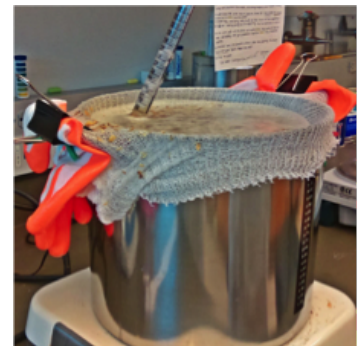


Figure 2: This is an example of how the Mashing is set up. A thermometer is clamped to a ring stand and detecting temperature in the center. The brewpot has a stir bar stirring at the bottom, and is on a hot plate large enough to support the kettle. The brew bag is clamped to the handles and edges of the kettle (with potholders to protect the clamps), to ensure good contact between the grains and hot water. The grain bag is not touching the bottom of the pot.

4. When the strike water is ready, place the steeping bag around the rim of the kettle, securing it with heat-resistant gloves and binder clips (Figure 2). It is important to make sure the bag does not touch the bottom of the kettle, as it may burn.
5. Remove a small sample of the water for a pH reading. Record it in your data sheets.
6. While you are waiting for the strike water, prepare your 96-well plate with iodine test solution, and position the plate over a white piece of paper. Add some soluble starch solution to see what a positive starch test looks like (Figure 3). Use the plate to monitor starch extraction, recording the color in your data sheet.
7. When the strike water reaches 73°C, add the grains by dropping firmly and uniformly from an approximate height of 10 cm (to prevent clumping). Make sure all the grain is soaked and that there are no dough balls forming.
8. With a transfer pipette, take a small sample of the liquid and perform an iodine test. Record what happens in your data sheets.
9. Turn off the heat, cover the brewpot and allow to rest, stirring gently so as not to aerate. Make sure the mash temperature does not fall below 66°C (150°F).
10. After 30 minutes, repeat the iodine test. If the test is still positive, wait another 15 minutes. When all the starches are gone, proceed to the next step.
11. Return the pot to heat and raise the temperature to 77°C (170°C). This is the **mash-out**.
12. Remove the brewpot from heat. Slowly pull the bag out, being careful not to lose any of the grains in the pot. Let the bag drip inside the kettle. Set aside the steeping bag into the assigned bucket. The wort should be the only thing left inside the brewpot.
13. Sample the wort for a hydrometer reading. Use a sterile 25 mL pipette to take wort from the kettle and fill about three-quarters of the test cylinder. Let the sample cool to 20°C. Gently, introduce the hydrometer until it floats by its own (Figure 3). Record the gravity in your data sheets.
14. Sample the wort for a pH reading. Record it in your data sheets.

### Part B. Boiling and Hopping

15. Cover the wort and bring it to a full boil, then uncover and keep boiling for 30 minutes. While the wort is boiling, start preparing an ice water bath large enough to fit the kettle.

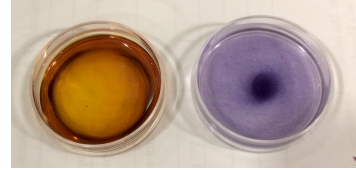


Figure 3: Iodine solution (brown, left) changes color when combined with soluble starch (purple, right).

*Though you need to stir frequently to ensure the brew bag does not touch the bottom of the kettle, stir gently so as not to aerate or stir the boiling wort! It will introduce off-flavors.*

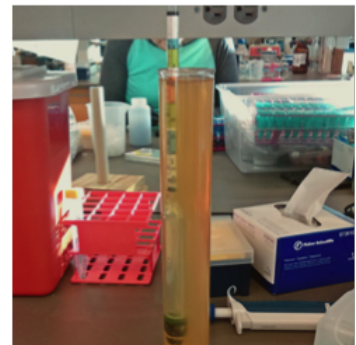


Figure 4: To measure the specific gravity of the wort, gently introduce the hydrometer until it floats. If there is not enough liquid for the hydrometer to float, a reading cannot be obtained. The hydrometer reading needs to be corrected for temperature, since our hydrometers are calibrated to 15°C (60°F).

- Measure 0.4 ounces (11.3 g) of cascade hops (bittering) and add them into the wort.

**Prevent burning wort:** When boiling on a hot plate, wort near the bottom in contact with the hot surface can caramelize and burn, contributing off-flavors to the beer. Stirring gently so as not to aerate should help prevent this. A metal trivet made of a triangle of non-laquered coat hanger can also reduce caramelization of sugars.

- After 30 minutes of boiling have elapsed, remove brewpot from heat. Place the pot inside the ice water bath and let it cool with the lid off until the temperature reaches 50°C. While you are waiting, calculate the IBU.
- When the wort reaches 50°C, put the lid on and continue cooling until the wort reaches 20°C.

AAU = Weight (oz) × % Alpha Acids

IBU = AAU ×  $U$  × 75 / Volume

$U$  = Utilization, or efficiency of the isomerization of the alpha acids; consult a table online to determine  $U$  as a function of boil gravity and time.

### Part C. Transferring the wort

- Cover the top of the white fermenter bucket with three layers of cheesecloth. Secure the cheesecloth with 4 binder clips.
- (Optional) Cool the wort for 20 to 30 minutes.
- After this resting time, aliquot a sample for a hydrometer reading and record it in your data sheets.
- Pour the wort carefully into the fermenter, leaving behind any thick sludge from the kettle. Allow some splashing. Record the total amount of wort collected (mL) in your data sheets.
- Seal the fermenter with its corresponding lid. Do not put the airlock in yet; instead, cover the hole in the lid with a stopper.
- After making sure the fermenter is tightly sealed, place it on the floor and rock it back and forth for several minutes.

**Minimize the transfer of solids:** The white fermenter will collect the wort, which is now a solution of sugars extracted from grains infused with hops. Treat the brewpot gently to allow the solids to settle and remain in the brewpot during the transfer.

Since our hydrometer is calibrated at 15°C, you will need to adjust your results by correcting for temperature.

This step is to provide more dissolved oxygen in the wort that the yeast need to grow.

### Part D. Preparing and pitching the yeast

**Yeast Strain:** Safale US-05 (dry ale yeast)

**Viable cells at packaging:**  $> 6 \times 10^9$ /g

To calculate the amount of yeast cells to add to our wort, perform the following calculation:

Cells needed = (Pitching rate) × (mL of wort) × (°Plato)

Pitching rate is found for your group in **Table 1**. Volume of wort in mL was measured in step 21. °Plato is obtained from the last hydrometer reading, where

°Plato = (Specific Gravity - 1) multiplied by 1000, divided by 4



25. Calculate the mass of cells needed using the equations above, and checking for your pitching rate in **Table 1**.
26. Measure out the mass of yeast needed and place them inside a new Erlenmeyer flask. Add a small amount of warm water (about 50 mL per 2.5 g of yeast). Gently swirl the yeast until they are evenly suspended.
27. Pitch (pour) the yeast into the fermenter, making sure to add it all.
28. Place the sanitized airlock and rubber stopper in the lid. The airlock should be filled to the line with water solution (to prevent contamination).

*Don't forget to aerate the wort before pitching to ensure a quick start to fermentation!*

CO<sub>2</sub> produced by fermenting yeast will accumulate in the headspace of the fermenter and be released through the airlock.

### **Part E. Primary Fermentation**

The vigorous fermentation should start within 12 hours. During this time, the airlock will bubble regularly. For American Pale ale, the average primary fermentation time is 48 hours.

The secondary fermentation will take about two weeks. If the process is interrupted before this time has elapsed, excess carbonation is to be expected. It is important that the fermenter remains closed, since the beer is still susceptible to bacterial infections.

### *Glossary of technical words commonly used by brewers*

**Alpha Acid Units (AAU):** Unit used to measure the bitterness contributed by the total hops used in a small beer batch. Can be obtained by multiplying the weight in ounces by the percent alpha unit of the specific hops.

**Attenuation:** The amount of sugar that was converted to CO<sub>2</sub> and alcohol.

**Conditioning:** A period of time after the vigorous or primary fermentation that continues after bottling. At this point, the yeast have used most of the sugars, and moves on to refine the flavors of the final beer.

**Extracts:** The total quantity of fermentable and non-fermentable soluble products obtained per grain of malt used, before adding the yeast. Calculated by comparing the specific gravity of the wort to the maximum yield of the grains.

**Gravity:** The concentration of malt sugar in the wort. The specific gravity of water is 1.000 at 59°F. The original gravity for most beer worts ranges from 1.035 to 1.055.

**International Bittering Units (IBU):** A measure of bitterness in the beer. Similar to Alpha Acid Units, but it takes into account variables such as the batch size, boil time, and specific gravity of the wort. Equal to the AAU multiplied by factors for percent utilization (wort volume and wort gravity).

**Mash-in:** Soaking crushed grains in hot water for a period of time to allow for the enzymatic conversion of complex starches into simple sugars that the yeast can utilize.

**Mash-out:** Inactivating enzyme activity in the mash by raising the temperature to 170°F. This locks the sugar profile of your wort, and makes it more fluid.

**Plato scale:** Correlates specific gravity with the percent by weight of extract present in solution. Used by many international brewers.

$$^{\circ}\text{Plato} = [(\text{specific gravity} - 1)] \times 1000 / 4$$

**Points per Pound per Gallon (PPG):** Another measurement to calculate the extracted sugars present in the wort.

**Primary Fermentation:** The period of time when fermentation begins, and most of the total attenuation happens. Characterized by intense production of carbon dioxide and krausen (foam forming on top of the product).

**Secondary Fermentation:** The period after the primary fermentation has ended; designated for settling and conditioning of the beer.

# Data Sheet D1 – Fermentation setup

Microbiology 562 - Batch Fermentation Module

## TYPICAL MALT YIELD IN POINTS/POUND/GALLON

Type of Grain	Mass added	Extract Potential	Max PPG*	Typical PPG*
<b>2-row pale malt</b>	2.2 lbs	80%	37	31
<b>Caramel 40L malt</b>	0.11 lbs	29%	29	29

\*PPG: Last two decimals of hydrometer reading

## MAXIMUM AND EXPECTED OG

Type of Grain	Max OG	Typical OG
<b>2-row pale malt</b>		
<b>Caramel 40L malt</b>		

## MASH

Water	Observed value
<b>Volume used (ml)</b>	
<b>CaSO4 added (g)</b>	
<b>Water pH</b>	
<b>Strike water temperature</b>	

Conditions	Observed value
<b>Mash start time</b>	
<b>Mash end time</b>	
<b>Mash-out temperature</b>	
<b>Mash pH</b>	
<b>Hydrometer reading**</b>	
<b>Calculated mash-ing efficiency</b>	

\*\* temperature corrected

## STARCH CONVERSION

Time	Iodine test (1, brown to 5, purple)

Time	Iodine test (1, brown to 5, purple)

## BOIL

Hops	Type	Amount added	Alpha acids (%)	AAU	Estimated IBU	Time of addition
------	------	--------------	-----------------	-----	---------------	------------------

**Bittering hops**

**Flavoring hops**

Conditions	Observed value
------------	----------------

**Boil start time**

**Boil end time**

$$\text{Extraction} = \frac{(\text{PPG}) (\text{lbs of grain used})}{\text{Volume (gal)}}$$

$$\text{Efficiency} = \frac{(\text{Gravity points of wort})}{(\text{Maximum gravity points of recipe})}$$

## FERMENTATION

Wort	Observed value
------	----------------

**Hydrometer reading at 20 degrees Celsius**

**Gravity in degrees Plato**

**Volume collected into fermenter**

**Brewhouse efficiency**

Yeast	Observed value
-------	----------------

**Pitching rate**

**Yeast strain**

**Viable cells at time of packaging (cells/g)**

**Yeast needed (cells)**

**Yeast pitched (g)**

**Date of incubation setup**

**Incubation temperature**

## OBSERVATIONS

## *Microbiology 562: D2. Cultivation of microbial contaminants*

*Batch Fermentation Lab by Dr. Kristen DeAngelis<sup>1</sup>*

*16 November 2017*

This document describes the second lab activity in a six part module exploring batch fermentation. In the previous lab, we set up a fermentation of grains by yeast to brew beer (D1). **Today we will make media and pour plates to grow and identify bacterial contaminants (D2).**

Two weeks after setting up the primary fermentation, we will prime and condition the fermentation (D3). While we wait for the priming and conditioning to finish, we will spend one lab doing bioinformatics analysis of microbial communities turning ethanol (a product of fermentation) to n-caproic acid, a fuel precursor and otherwise valuable industrial chemical (D4). We will also spend one lab examining the microbial contaminants that were cultured in the primary fermentation (D5). Once the beer is finished, we will perform a sensory analysis followed by a field trip to the Berkshire Brewing Company in South Deerfield, MA, where we will tour their brewing facilities and microbiology lab.

### *Learning Objectives*

By the end of today's class, students should be able to

- examine a media recipe and use it to execute selective enrichment of microbes, and
- describe the media components and their role in isolating microbes growing in the batch fermentation.

Today we will prepare Wallerstein Laboratory (WL) Nutrient (WLN) and Differential (WLD) Media for the cultivation and differentiation of brewing contaminants.

WL Nutrient Medium and WL Differential Medium are used simultaneously as a set of three plates. One plate is prepared from WL Nutrient Medium and two plates from WL Differential Medium. The Nutrient Medium is incubated aerobically to obtain a total amount of yeast colonies. One of the differential plates is incubated aerobically for growth of acetic acid and thermophilic bacteria. A second differential plate is incubated anaerobically to scan for growth of lactic acid bacteria and *Pediococcus*.

### *WL Nutrient Medium*

Developed by S.R. Green and P.P Gray developed this media in 1950 after an in-depth analysis of the quality control methods of the fermentation process. The WL Nutrient Medium is used to cultivate any

<sup>1</sup> Developed in part by Cecilia Prado, with support from Dr. Klaus Nusslein, Dr. Sue Leschine, and the UMass Center for Teaching Excellence Faculty Development

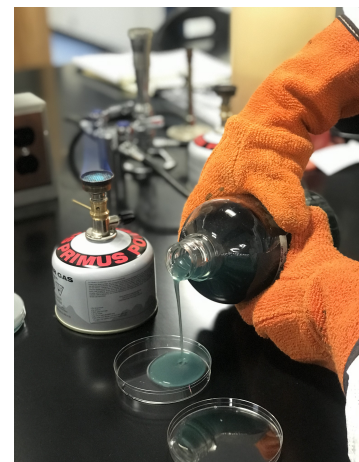


Figure 1: WLN agar media being poured. Photo by M. Orsini, Fall 2017.

yeast, mold or bacteria present during the brewing process. At a pH of 5.5, counts of viable bakers' yeast, and at a pH of 6.5, the medium is suitable for counts of bakers' and distiller's yeast.

### *WL Differential Medium*

Although bacteria can technically grow in WL Nutrient Medium, bacterial colonies tend to be unnoticeable unless the number of yeast cells is small. Because of this limitation, the WL Differential Medium is used to isolate bacteria encountered in brewing and industrial fermentation processes.

**Table 1** describes the media components of WLN and WLD media and their role in illuminating microbial contaminants.

Component	Purpose
Yeast Extract	Source of trace elements, vitamins and amino acids
Peptone	Provides nitrogen, amino acids and carbon
Dextrose	Source of carbohydrates
Monopotassium phosphate	Buffer
Potassium Chloride	Helps maintain osmotic balance
Calcium Chloride	Helps maintain osmotic balance
Ferric Chloride	Helps maintain osmotic balance
Magnesium sulfate	Source of divalent cations
Manganese sulfate	Source of divalent cations
Bromocresol Green	pH indicator
Agar	Solidifying agent
Cycloheximide* WLD only	Inhibits yeast and molds

**Table 2** lists the components of WL media by mass per liter (L).

Component	Mass
Yeast extract	4.0 g
Pancreatic Digest of Casein	5.0g
Dextrose	50.0 g
Monopotassium Phosphate	0.55 g
Potassium Chloride	425.0 mg
Calcium Chloride	125.0 mg
Magnesium Sulfate	125.0 mg
Ferric Chloride	2.5 mg
Manganese Sulfate	2.5 mg
Agar	20.0 g
Bromocresol Green	22.0 mg

### Procedure

Half of the class will prepare WL nutrient media plates while the other half prepares WL differential media plates. Both the WL nutrient medium and WL differential medium are based on the formula described in **Table 2**.

1. Measure 500 mL of distilled water inside a 1L Erlenmeyer flask. Add a stir bar.
2. Place the flask on top of a stir plate, and adjust it to medium speed.
3. While the water stirs, add the indicated amount of each ingredient, except for the agar and except for cycloheximide. See Table 2 for the list of media components. Keep stirring until it is completely dissolved.
4. Place the solution inside a 1L Fisher bottle.
5. Prepare 500 mL of water inside a separate 1L Fisher bottle, and add the amount of agar specified by the recipe. Place the lid on and mix by shaking the bottle.
6. Autoclaving: In order to be autoclaved, the bottle caps must be slightly open to allow steam to escape. In addition, autoclave tape must be placed on the surface of the bottle to indicate when the load is done. The media should be autoclaved for 15 minutes on liquid cycle.
7. While the media is autoclaving, prepare 25 sterile plates.

**Table 3** lists the physical characteristics of WL media

Attribute	Description
Solution	8.0% soluble in water upon boiling
Color	Blue to greenish blue
Opacity	Very slightly to slightly opalescent
Reaction of 8.0% solution at 25°C	pH 5.5

8. After the cycle is done, remove the media.
9. Mix the content of the two bottles by introducing the nutrient solution inside the agar solution. Close the lid and mix by inverting the bottle 1-4 times.

#### bromocresol green



Also known as: 3',3'',5',5''-tetrabromo-m-cresol-sulfonephthalein  
 Type:  $\text{HIn} + \text{H}_2\text{O} \rightleftharpoons \text{In}^- + \text{H}_3\text{O}^+$   
 pK: 4.90  
 Approximate pH range for color change: 3.8-5.4  
 Color of acid form: yellow  
 Color of base form: blue

#### bromophenol blue



Also known as: 3',3'',5',5''-tetrabromophenolsulfonephthalein  
 Type:  $\text{HIn} + \text{H}_2\text{O} \rightleftharpoons \text{In}^- + \text{H}_3\text{O}^+$   
 pK: 4.10  
 Approximate pH range for color change: 3.0-4.6  
 Color of acid form: yellow  
 Color of base form: purple

Figure 2: Color ranges for pH ranges based on bromocresol green and bromophenol blue indicators.

*Use heat resistant gloves to remove bottles from the autoclave, as the bottles will be hot! Liquid might be superheated, so treat bottles gently.*

10. For WL Differential Medium only: Add 4.0 mg of cycloheximide to the media. Close the lid and mix by swirling the bottle 1-4 times. *TA will do this step.*
11. Pour media inside each plate. The most important part is to cover the whole surface of the plate (usually about 20-25 mL). Cover the plates as soon as you are done pouring.
12. Wait until the agar solidifies; then proceed to label and store them: the WLN plates should be stored aerobically at 30°C (yeast). Both sets of WLD plates should be stored at 35°C, one set aerobically and the other anaerobically.

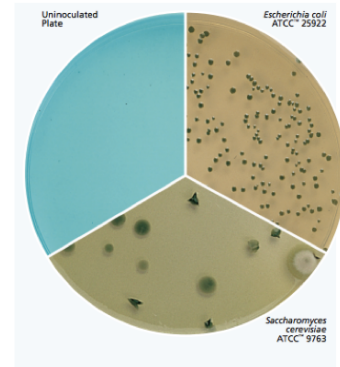


Figure 3: Expected colony morphology of *E.coli* and *Saccharomyces cerevisiae* on WL media. Common brewing contaminants tend to acidify the medium, resulting in a color change (yellowish).



# Data Sheet D2 – Cultivation of microbial components

Microbiology 562 - Batch Fermentation Module

## WALLERSTEIN LABORATORY (WL) MEDIA

Component	Mass required (g)	Added to WLN (g)	Added to WLD (g)	Notes
<b>Yeast extract</b>	4.0			
<b>Casein</b>	5.0			
<b>Dextrose</b>	50.0			
<b>Mastermix of micronutrients*</b>	1.5			
<b>Agar (insoluble until autoclaved)</b>	20.0			
<b>Cycloheximide**</b>	0.004	NA		

\*Monopotassium phosphate, potassium chloride, calcium chloride, magnesium sulfate, ferric chloride, manganese sulfate, bromcresol green

\*\* TA will add to WLD only after autoclaving

## OBSERVATIONS

## *Microbiology 562: D3. Priming and Conditioning.*

*Batch Fermentation Lab by Dr. Kristen DeAngelis<sup>1</sup>*

*28 November 2017*

This is the third lab activity in a six part module exploring batch fermentation. Previously, we set up a fermentation of grains by yeast to brew beer (D1), and made media and poured plates to grow and identify bacterial contaminants (D2). **Two weeks after setting up the primary fermentation, we will prime and condition the fermentation (this class, D3).** While we wait for the priming and conditioning to finish, we will spend one lab doing bioinformatics analysis of microbial communities converting ethanol into n-caproic acid, a fuel precursor and otherwise valuable industrial chemical (D4). We will then spend one lab examining the microbial contaminants detected from the primary fermentation (D5). Once the beer is finished, we will perform a sensory analysis followed by a field trip to the Berkshire Brewing Company in South Deerfield, MA, where we will tour their brewing facilities and microbiology lab.

### *Learning Objectives*

By the end of today's class, students should be able to

- describe how the batch fermentation has changed since setting up the initial fermentation, and
- explain the difference between priming and conditioning in the process of batch fermentation of beer.

In the production of beer, the batch fermentation occurs in three stages: primary, secondary and conditioning. The the first stage, called lag phase or adaptation phase, occurs in the 12-36 hours immediately after pitching the yeast. In the minutes and hours after pitching, yeast use glycogen reserves, lipids, and dissolved oxygen from the wort to grow and begin producing enzymes to break down the more complex carbohydrates. Once the oxygen is consumed, microbes switch to fermentation, consuming sugars from grains made available in the mashing process and producing ethanol. Microbes also consume sugars, amino acids, and other nutrients present in the wort, continuing to produce extracellular enzymes and metabolize their products.

The second stage of batch fermentation occurs over the next 1-2 weeks after pitching the yeast, and is a period of vigorous fermentation. As sugars are converted to ethanol, the gravity of the beer drops two-thirds to three-quarters the original value. During this time, a foamy head called krausen will form at the top of the batch fermentation, comprised of extraneous wort protein, hop resins, and

<sup>1</sup> Developed in part by Cecilia Prado, with support from Dr. Klaus Nusslein, Dr. Sue Leschine, and the UMass Center for Teaching Excellence Faculty Development



Figure 1: This is an example of a wort transfer setup using a cane and tubing to siphon beer with minimal introduction of oxygen and little transfer of insoluble waste products.

dead yeast. Over time, these compounds will settle to the bottom of the fermenter. These compounds taste bitter and should not be transferred to the next stage of fermentation.

Together, these first two stages of microbial activity are sometimes called the primary fermentation, followed up by a secondary fermentation in a secondary glass container. This is because plastic is slightly gas impermeable, so conditions over time become less anaerobic. By transferring to a glass fermenter, oxygen is reduced and insoluble waste products of fermentation are removed.

### *What is priming and conditioning?*

In batch fermentation, beer is conditioned (moved to a secondary fermenter for slow, additional microbial processing of remaining sugars) and primed (where a small amount of sugar is added to the beer to carbonate it). These two processes can occur separately or together, as we are doing in class today.

The third and final stage of batch fermentation is the conditioning. This step takes place two weeks after fermentation started. During conditioning, yeast more slowly ferment the more complex sugars including maltotriose and dextrins. In addition to producing ethanol and carbon dioxide, yeast also produce acetaldehyde, esters, amino acids, ketones-diacetyl, pentanedione, dimethyl sulfide, and other compounds that give beer its depth of flavors.

Today, we will transfer the pre-conditioned beer into a bottling bucket (a glass carboy), along with priming solution (corn sugar and water). They will take a pH and hydrometer reading of their beer, and they will perform serial dilutions to plate on WL nutrient and differential media.

### *Priming and Conditioning*

1. Fill an Erlenmeyer flask with 95 ml of water. Add 23 g of priming sugar. Heat until boiling, then let it cool for a few minutes.
2. Gently pour the cooled priming sugar solution into the bottling kettle (the same kettle used as a brewing kettle, which must be sanitized).

A sanitized siphon will next be used to transfer the beer to the bottling kettle:

3. Place the fermenter (containing the pre-conditioned beer) near the kettle, but your fermenter should be at an elevated position, with your bottling kettle (containing the priming solution) placed below (similar to Figure 1).

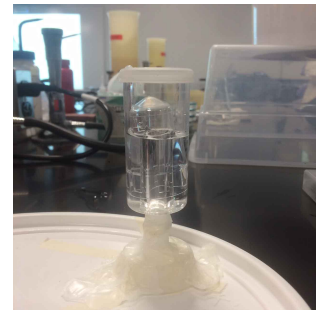


Figure 2: This is a gaslock used to allow gas (CO<sub>2</sub>) to escape during primary and secondary fermentation, while keeping out oxygen or contaminants. We will perform our priming and conditioning in single one-gallon glass bottles secured with screw caps, to prevent gas from escaping, carbonating the beer.

*A siphon is tube used to convey liquid upwards from a reservoir and then down to a lower level, action which is initiated by applying a vacuum using suction or immersion, after which flow continues aided only by gravity.*

4. Attach one end of the hose to the siphon assembly, making sure you have a good seal.
5. Place the other end of the plastic hose into the plastic bucket until the outlet comes in contact with the bottom of the vessel.
6. Gently place the assembly into the fermenter, until the stopper touches the bottom.
7. Pump the siphon to start the transfer. It usually takes only one pump for the beer to start flowing from one vessel to another– let gravity do the rest.

*Don't allow the beer to splash, as it would introduce oxygen to your product. Take your time; quick movements might promote excess carbonation.*

Once the siphon is set up, it is time to collect samples to measure specific gravity and to test for microbes.

8. Aliquot some of the product for a hydrometer reading, and record it on your data sheets.
9. Aliquot 1 ml from the hydrometer cylinder to a sterile microfuge tube. Using sterile water, perform three serial dilutions of the primary fermentation:  $10^{-1}$ ,  $10^{-2}$ ,  $10^{-3}$ .
10. Using sterile technique, streak 100 uL of each dilution onto WLN and WLD plates.
11. For each media type, streak a negative control of sterile water only to check for environmental contamination.

The final step is to bottle the beer into a one gallon bottle.

12. For bottling, place the bottling kettle on an elevated surface. Place the bottle(s) to be used below the bottling bucket (Figure 2).
13. Attach the bottle filler that came with your brewing kit to the other end of the siphon hose (looks like a straw with a black valve in one end).
14. Gently, place the auto siphon assembly inside the bottling bucket, until the stopper touches the bottom.
15. To fill the bottle(s), insert the bottle filler to the bottom of each bottle and transfer your beer, leaving about 1 to 1.4 inches (3-4 cm) of headspace in each bottle (this is necessary for carbonation to occur). The bottle filler only will allow beer flow when it's in contact with the bottom of the bottle.
16. Place a cap on your bottle(s) and incubate it at fermentation temperature for a period of about 2 weeks before refrigerating.

# Data Sheet D3 – Priming and conditioning

Microbiology 562 - Batch Fermentation Module

## BEFORE CONDITIONING

Beer characteristics	Notes
----------------------	-------

**Hydrometer reading**

**Attenuation (%)**

**ABV**

**Color**

**Transparency**

**Aroma**

Priming	Notes
---------	-------

**Amount of corn sugar added (g)**

**Volume water used**

**Incubator temperature**

**Incubation time**

## AFTER CONDITIONING

Refrigeration	Notes
---------------	-------

**Time of refrigeration**

**Color**

**Transparency**

**Aroma**

## OBSERVATIONS

# *Microbiology 562: D4. Bioinformatics of Batch Fermentation*

*Batch Fermentation Lab by Dr. Kristen DeAngelis<sup>1</sup>*

*30 November 2017*

This document describes the fourth lab activity in a six part module exploring batch fermentation. In the first lab, we set up a fermentation of grains by yeast to brew beer (D1). Next we made media and poured plates to grow and identify bacterial contaminants (D2). Two weeks after setting up the primary fermentation, we primed and conditioned the fermentation (D3). **Today as we wait for the priming and conditioning to finish, we will spend one lab doing bioinformatics analysis of microbial communities turning ethanol (a product of fermentation) to n-caproic acid, a fuel precursor and otherwise valuable industrial chemical (D4).** We will also spend one lab examining the microbial contaminants that were cultured in the primary fermentation (D5). Once the beer is finished, we will perform a sensory analysis followed by a field trip to the Berkshire Brewing Company in South Deerfield, MA, where we will tour their brewing facilities and microbiology lab.

<sup>1</sup> Developed in part by Cecilia Prado, with support from Dr. Klaus Nusslein, Dr. Sue Leschine, and the UMass Center for Teaching Excellence Faculty Development

## *Learning Objectives*

After today's lab, students should be able to

- describe the methods for obtaining metagenomic data, how it is sequenced and analyzed,
- describe the difference between phylogenetic and functional diversity in microbial communities,
- evaluate the chemical products and microbial community structure that is consistent with a productive batch fermentation.

Bioinformatics is an interdisciplinary field of study in which computer programming and analysis are used to better understand biological systems. This often includes sequences of DNA (genomes, plasmids, viral and acellular DNA), RNA (ribosomal, messenger, transfer, small interfering, signaling and viral RNA), and proteins. In today's lab, we will study a batch fermentation monitored for microbiology using metagenomic sequencing, and product formation using analytical chemistry methods like high performance liquid chromatography (HPLC).

Metagenomics has changed in the past three decades from sequencing of cloned DNA fragments using Sanger technology to direct sequencing of DNA without heterologous cloning. Though next-generation sequencing runs are not inexpensive, costs of sequencing continue to decline in units of cost per sequence. Work

flow generally consist of sequencing as deeply as is reasonably possible, assembling reads into consensus 'contigs,' and annotating these contigs for function. Metagenomes give phylogenetic information about what organisms are there, and functional information about the functional capacity of the community.

The metagenomic exercise will involve exploration of the dataset described in Alger MT, CM Spirito, JG Usack, JJ Werner, LT Angenent. "Chain elongation with reactor microbiomes: upgrading dilute ethanol to medium-chain carboxylates." *Energy Environmental Science*, 2012, 5, 8189-8192 (doi: 10.1039/C2EE22101B). In this study, a bioreactor is fed beer from a corn ethanol fermentation for months at low pH, removing n-caproic acid over the year-long incubation.

We will explore a set of 10 metagenomes on MG-RAST. Hosted by the Argonne National Lab, MG-RAST is a metagenomic sequence analysis platform, also provides phylogenetic and functional annotation of shotgun sequenced reads, and also serves as a repository for metagenomic data. MG-RAST is optimized for Safari or Firefox. If you are using a different browser, and having trouble interpreting the results, you may want to switch to one of these supported browsers.

This data, along with lots of other metagenomic data, is available in the public domain. There are a few major repositories of public data, including but not limited to MG-RAST (<http://metagenomics.anl.gov>), IMG/M (<http://img.jgi.doe.gov/m/>), MEGAN database (<http://www.megan-db.org/megan-db/>), EBI (<https://www.ebi.ac.uk/metagenomics/>), and others.

**Table 1** Beer to n-caproic acid metagenomic datasets.

MG-RAST ID	Metagenome Name	bp Count	Sequence count	Biome
4480719.3	Beer_CE_Day_44	2,642,577,613	27,029,730	anaerobic digester sludge
4480764.3	Beer_CE_Day_50	2,592,877,521	26,450,676	anaerobic digester sludge
4480776.3	Beer_CE_Day_56	2,393,318,434	24,412,813	anaerobic digester sludge
4480815.3	Beer_CE_Day_62	2,691,922,076	27,452,520	anaerobic digester sludge
4480859.3	Beer_CE_Day_90	2,000,047,524	20,403,845	anaerobic digester sludge
4480861.3	Beer_CE_Day_98	2,383,034,964	24,255,570	anaerobic digester sludge
4480863.3	Beer_CE_Day_112	2,741,965,961	27,922,730	anaerobic digester sludge
4480864.3	Beer_CE_Day_117	2,649,831,957	27,019,804	anaerobic digester sludge
4480865.3	Beer_CE_Day_126	2,303,732,337	23,457,620	anaerobic digester sludge
4480867.3	Beer_CE_Day_132	3,135,396,047	31,928,594	anaerobic digester sludge

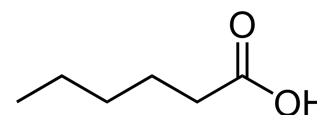


Figure 1: Hexanoic acid, also called n-caproic acid, is a six-chain carboxylic acid derived from hexane. Carboxylic acids are industrially valuable precursors for liquid fuels and chemicals.

### *Procedure*

The Alger et al data are at [metagenomics.anl.gov/linkin.cgi?project=397](http://metagenomics.anl.gov/linkin.cgi?project=397), and consist of 10 metagenomes.

Once you navigate to <http://metagenomics.anl.gov>, you will see that there are some options for links in the upper right-hand corner of the page.

Select the 'analyze' button to be taken to the the analysis portal. Here you can search for the project by ID, project name or keyword. You can also copy and paste the url for the Beer-to-Caproate project.

Next you will need to select a metagenome (or multiple metagenomes) to do any analysis. We will be analyzing ten beer-to-caproate metagenomes.

To create a visualization, first select a data type from the '(1) Data Type' box. The default is 'Best Hit Classification' which is a type of analysis of organism abundance. We will also explore measures of functional abundance.

Second, choose the data and cutoffs you wish to use in the '(2) Data Selection' box. These will vary depending on which data type you are looking at.

Third, and again depending on the type of data, you might have a set of possible visualizations. Pick one of them from the '(3) Data Visualization' box and click the generate button.

You will see the generated visualizations created in separate tabs in this tab-view. In addition to the visualization, the created tabs will display the settings used to create them. Generating a new visualization will preserve the previous visualizations you created.

You can rename the tabs by double clicking the tab-header and entering a new title. You can remove a tab by clicking the 'x' symbol in the top right corner of the tab-header. You can switch between tabs by single clicking the tab-header.

MG-RAST can implement some statistical tests on multiple metagenomes that are not relevant when comparing only two metagenomes. These are the Principle Component Analysis (PCoA) and calculation of p-values for assessing the difference between user-defined groups.

In getting to know MG-RAST, we will aim to answer the following questions in the first hour of class.

1. What are the metagenomes available to this project?
2. What are the quality control cut-offs?
3. Many probability assignments are based on e-values. What are these and why are they important?
4. How many reads were assigned to eukaryotes for the Day 44 samples? What are these?



**Create a new Analysis**

To perform an analysis, you must first load the metagenomic profiles to analyze. A profile holds the abundance values and cutoffs for a list of database sources for a specific dataset. You can select the databases and datasets, as well as a name for your analysis below. Click the -button to load the data from our server.

Profiles are generated on demand. Depending on profile size the initial calculation may take some time. Once computed they will be cached and subsequent requests will download immediately. You can use the -icon in the top menu bar to store profiles on your harddrive and upload them back into your browser cache (without requiring interaction with our server).

Once all required data is loaded you can start the analysis.

selected databases: RefSeq x Subsystems x

available databases: IMG add

metagenomes

Enter filter:  project name:  analysis name:

sequence type: all shotgun amplicon amplicon gene metatranscriptome

project name: beer

beer-to-caproate  
beer-to-caproate  
beer-to-caproate  
beer-to-caproate  
beer-to-caproate  
beer-to-caproate  
beer-to-caproate  
beer-to-caproate  
beer-to-caproate  
beer-to-caproate

Figure 2: Most of the work that we will do today will be from the analysis portal, which looks like this. Notice that the 10 beer-to-caproate metagenomes have been selected based on project name.

Name: \_\_\_\_\_

### 1. Finding public data

Try finding the data from Alger et al on the the MG-RAST server based on some different key word searches (eg., Alger, beer, caproate).

*Which way to search is best? How can you improve the search?*

### 2. Exploring the phylogeny in one metagenome

Let's look at a single community from day 126 when conversion to n-caproic acid was occurring at high rates. Examine a bar chart of the Organism Abundance: Best Hits Classification using first RDP, then SSU as the annotation source using raw data. Make sure you visualize using raw (not normalized) data.

*What can you infer about the taxa that were dominant in the day 126 communities? Does this agree with the paper's conclusions?*

### 3. Exploring organism relative abundance in one metagenome

Now look at the same metagenome (day 126), using at a bar chart of the Organism Abundance: Best Hits Classification using the KEGG annotation, which shows communities based on function, showing raw (not normalized) data.

*How are the community results using KEGG as an annotation source different from RDP? Why?*

#### 4. Exploring community function in one metagenome

Then use the same annotation source (KO, which stands for KEGG ontology) to examine the Functional Abundance: Hierarchical Classification in bar chart form for day 126.

*What can you infer about the community function? How does this agree with what was reported in the paper?*

#### 5. Exploring the phylogenetic community across multiple metagenomes

In the paper, Fig. 2A shows that the relative abundance of an OTU in the family Ruminococcaceae increases from 2 to almost 20% over the course of the first 120 days. Use the tools available to you in MG-RAST and the data from the Beer-to-Caproate project to re-create the data presented in Figure 2A (though you will make a bar chart instead of an x-y plot as in the paper).

*You may need to re-familiarize yourselves with the family Ruminococcaceae ; try doing this using the NCBI taxonomy browser. When looking at your data, make sure you are using the raw reads and not the normalized reads. How does your impression change when based on metagenomics annotated by RDP? SSU?*

#### 6. Comparing multiple metagenomes based on function

Using the hierarchical classification based on functional subsystems as an annotation source, look at all ten metagenomes together in a bar chart. Look for trends in the metagenomes that might correspond to observed trends in central metabolism, as reported in Figure S3 of the paper.

*How do genes associated with different types of fermentation (mixed acid fermentation versus ethanol or butyrate synthesis) vary over time?*

# Microbiology 562: D5. Microbial Contamination of Beer.

Batch Fermentation Lab by Dr. Kristen DeAngelis<sup>1</sup>

05 December 2017

This document describes the second lab activity in a six part module exploring batch fermentation. First, we set up a fermentation of grains by yeast to brew beer (D1). We also made media and poured plates to grow and identify bacterial contaminants (D2). Two weeks after setting up the primary fermentation, we will prime and condition the fermentation (D3). While we wait for the priming and conditioning to finish, we will spend one lab doing bioinformatics analysis of microbial communities turning ethanol (a product of fermentation) to n-caproic acid, a fuel precursor and otherwise valuable industrial chemical (D4). **Today we will examine the microbial contaminants that were cultured in the primary fermentation (D5).** Once the beer is finished, we will perform a sensory analysis followed by a field trip to the Berkshire Brewing Company in South Deerfield, MA, where we will tour their brewing facilities and microbiology lab.

<sup>1</sup> Developed in part by Cecilia Prado, with support from Dr. Klaus Nusslein, Dr. Sue Leschine, and the UMass Center for Teaching Excellence Faculty Development

## Learning Objectives

After today's lab, students should be able to

- describe the sources of microbial contamination in each stage of the brewing process,
- identify and characterize possible beer spoilage contaminants,
- apply physiological data like enzyme assays and differential staining to help identify microbial contaminants.

## Microbial Ecology of Brewing

Beer fermentation is usually performed by a single microbial culture. However, the overall brewing process includes a sequence of microbial constituents with a direct impact on the final product. Microbial organisms capable of beer spoilage may produce off-flavors, acids and non-desirable aromas. They may also contribute to the formation of haze and films. In addition, they could potentially compete with the brewing strain for essential nutrients, which may result in a stuck fermentation or over-attenuation.

The following is a list naming the most common sources of contamination at each stage of the brewing process:

1. **Barley:** Most grain contaminants do not survive the malting and brewing process, but they may leave behind secretory factors such as mycotoxins that could potentially affect the downstream reactions.

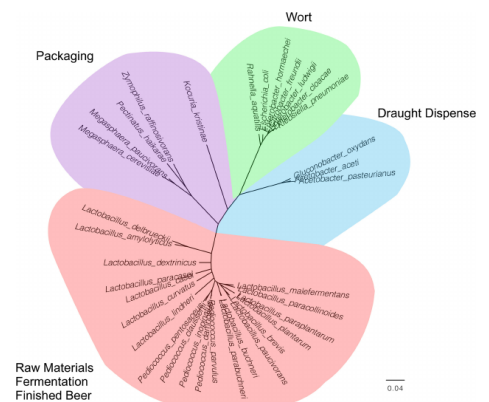


Figure 1: Phylogeny of primary beer spoilage bacteria, Figure 3 from Bokulich Bamforth 2013.

2. **Malt:** Microbial growth may have inhibitory effects on germination, rootlet growth and amylase activity, since unwanted organisms may compete for oxygen with the embryo.
3. **Wort:** Microbial concentration decreases during mashing, with the exception of thermotolerant microbes. During boiling, the most prevalent spoilers are Gram-negative bacteria, since these are aerobic and are not sensitive to hop-derived antimicrobials.
4. **Beer:** After the beer is pitched and fermentation begins, the conditions become hostile to most microorganisms: high ethanol and CO<sub>2</sub>, hop-derived antimicrobials, and low pH. At this stage, many gram-positive strains present a threat to the quality of our beer.

The microbial contaminants of brewing are classified in two categories: Wild yeast and bacteria. The most relevant are described below.

#### Wild yeast

Rogue strains of *Saccharomyces cerevisiae* spoil beer through ester or phenolic off-flavor production, haze or sediment formation or superattenuation, resulting in over-carbonation and diminished body. Other known contaminants belong to the genus *Brettanomyces*, which spoil the beer through the production of 4-ethylguaiacol and 4-ethylphenol, two highly volatile phenolic compounds introducing aromas resembling bandages, sweat and smoke.

#### Lactic Acid Bacteria

Lactic Acid Bacteria (LAB) account for the most predominant bacterial spoilers of beer. They have the ability to grow in low-oxygen conditions, low pH, and high alcohol content. Unlike most gram-positive bacteria, they are not sensitive to hop extracts. The most common belong to the genus *Lactobacillus* and *Pediococcus*. LAB contaminants make beer sour or tart through lactic and acetic acid production. Some strains produce diacetyl. Associated with haze formation. LAB are intentionally introduced into sour beers like German Weissebier, or Belgian sour beers like lambics, witbiers, and Flanders styles.

#### Enteric Bacteria

The most documented contaminants belong to the genus of *Klebsiella*, *Citrobacter*, *Enterobacter*, *Obesumbacterium*, and *Escherichia*. These produce abundant amounts of DMS, organic acids, and 2,3-butadienol, resulting in an unpleasant fruity or vegetable aroma



Figure 2: *Saccharomyces cerevisiae*, the budding yeast which is the main fermenter in beer. Image credit: microbewiki.kenyon.edu

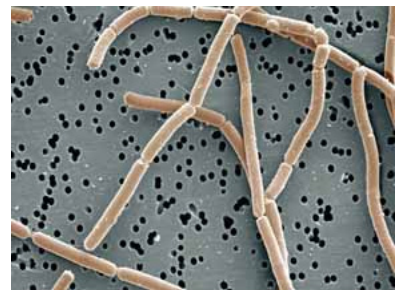


Figure 3: *Lactobacillus*. Image credit: microbewiki.kenyon.edu

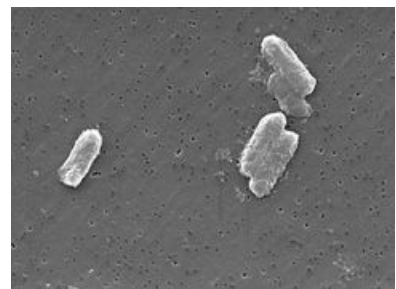


Figure 4: *Citrobacter freundii*, Image credit: microbewiki.kenyon.edu

(acetate, celery-like, parsnip, phenols, cooked cabbage and DMS). Do not usually grow on finished beer.

### Acetic Acid Bacteria

Aerobic and hop-resistant, acetic acid bacteria are found in wort and sometimes in bottled beer. These bacteria produce acetic acid in beer, which contributes to vinegary or cidery flavors. The most notable belong to *Acetobacter* and *Acidomonas*.

### Gram-positive Spore-forming Bacteria

Thermotolerant microbes such as *Bacillus* spp. and *Clostridium* may cause a problem during the mashing step. *Bacillus* spp. can cause excessive acidification and nitrosamine formation, and *Clostridium* can produce high levels of butyric acid in the mash, giving the beer a cheese-like aroma.

### Classification of microbial contaminants in beer

Today, we will examine microorganisms present in our WLN and WLD plates, and use microscopy and physiological tests to classify them.

1. For each of the plates, identify 5-10 isolated colonies to focus on, by circling their footprint with a marker on the bottom of the plate and assigning the colony a name.
2. Record the colony morphology on your data sheet. Observe whether the media change color (an indication of acid production) and record it on your data sheet.
3. Sample some colony biomass and disperse into 50 ul phosphate buffered saline (PBS). Disperse cells in solution.
4. Add 450 ul of PBS to the dispersed cells and break up all cell clumps. Use this cell suspension to perform the physiological tests needed for their identification. Use **Table 1** as a reference; protocols for each test can be found at the end of this manual.

### Protocols

#### Pasteurization Test for Spore Formation

When spore-forming bacteria are subjected to high temperatures, the spore formation process allows them to survive. Streaking bacteria before and after autoclaving can identify spore-forming bacteria.

1. For each colony to be tested for pasteurization, streak 100 ul of the cell suspension onto WLD plates.

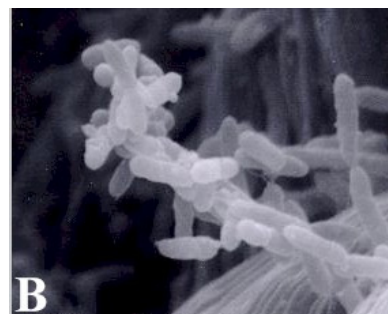


Figure 5: Acetobacter, Image credit: microbewiki.kenyon.edu



Figure 6: Clostridium, Image credit: microbewiki.kenyon.edu

- Heat 100 ul of the cell suspension at – degrees Celcius for – minutes.
- Streak the heated cell suspension onto another WLD plate. Incubate at 30°C overnight, then score the plates. Colonies that grow in the pre- and post-heat treatment are spore-forming bacteria.

### Catalase Test

Catalase is the enzyme that breaks hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) into H<sub>2</sub>O and O<sub>2</sub>. H<sub>2</sub>O<sub>2</sub> is a potent oxidizing agent that can wreak havoc in a cell; because of this, any cell that uses O<sub>2</sub> or can live in the presence of O<sub>2</sub> must have a way to get rid of the peroxide. One of those ways is to make catalase.

- Place a small amount of growth from your culture onto a clean microscope slide. If using colonies from a blood agar plate, be very careful not to scrape up any of the blood agar-blood cells are catalase positive and any contaminating agar could give a false positive.
- Add a few drops of H<sub>2</sub>O<sub>2</sub> onto the smear. If needed, mix with a toothpick. DO NOT use a metal loop or needle with H<sub>2</sub>O<sub>2</sub>; it will give a false positive and degrade the metal.
- A positive result is the rapid evolution of O<sub>2</sub> as evidenced by bubbling. A negative result is no bubbles or only a few scattered bubbles.

### Oxidase Test

This is a test to see if an organism is an aerobe. It determines whether a bacterium produces certain cytochrome c oxidases. The cytochrome system is a component of the electron transport chain, and is only present in aerobic organisms capable of using oxygen as the final electron acceptor. In the oxidase test, an artificial final electron acceptor (N,N,N',N'-tetramethyl phenylenediamine dihydrochloride) is used in the place of oxygen. This chemical changes color to a dark blue/purple when it takes the electron from the last element (cytochrome oxidase) in the electron transport chain.

- Place a small amount of growth from your culture onto a clean microscope slide using a sterile swab.
- Place a drop of reagent onto the culture on the swab.
- Positive reactions turn the bacteria violet to purple immediately or within 10 to 30 seconds. Delayed reactions should be ignored.

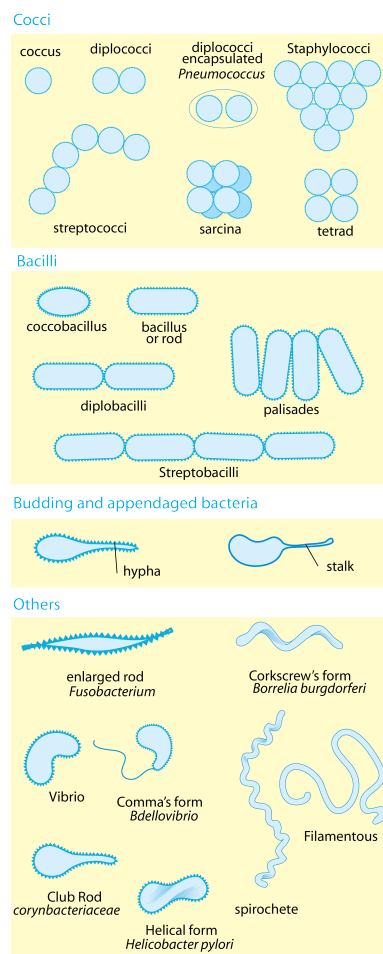


Figure 7: Names for the different cell shapes and arrangements of bacteria.

**Gram-Staining** This test allows for the classification of bacteria into two types: Gram-Positive and Gram-Negative.

1. Apply 10 ul cells to the slide. Air dry, and then heat fix by passing it through a flame a few times.
2. Add about 5 drops of Hucker's Crystal Violet to the culture. Let stand for one minute. Rinse briefly with water. Shake off excess.
3. Add about 5 drops of iodine solution to the culture. Let stand for 30 seconds, wash briefly with water and shake off excess.
4. Tilt the slide and decolorize with solvent (acetone-alcohol solution) until purple color stops running. Be careful not to over-decolorize. Wash immediately (within 5 seconds) with water and shake off excess.
5. Add 5 drops of Safranin O. Let stand for one minute, wash briefly with water and shake off excess.
6. Examine under microscope at both 400x and 1,000x oil immersion.

After completing the physiological tests for at least 5 isolates, use the table below as well as observations about colony morphology to for a hypothesis as to the probable identification of each isolate.

**Table 1** Characteristics of the major contaminants in brewing.

Contaminant	Gram	Catalase	Oxidase	Shape
<b>LAB (smell sharp and fruity)</b>				
Lactobacillus	+	-	-	rod
Pediococcus	+	-	-	cocci
<b>Enteric Bacteria (smell rotten, sulfury)</b>				
Citrobacter	-	+	-	rod
Enterobacter	-	+	-	rod
Hafnia	-	+	-	rod
Klebsiella	-	+	-	rod
Obesumbacterium	-	+	+	rod
<b>Acetic Acid Bacteria (smell sharp and vinegary)</b>				
Acetobacter	-	+	-	rod
Acidomonas	-	+	+	rod
<b>Wild yeast</b>				
Saccharomyces, haploid				Spherical (4um)
Saccharomyces, diploid				Ellipsoid (5-6um)
Brettanomyces				ovoid to vibrio



# Data Sheet D5 – Microbial components of beer

Microbiology 562 - Batch Fermentation Module

## SCANNING COLONIES ON PLATES

Colony name*	Dilution	Media	Morphology	Other observations

\* for example, KD-1

## CLASSIFICATION OF MICROBIAL ISOLATES

Colony Name	Cell morphology	Estimated cell size (nm)	Gram Stain (+/-)	Catalase (+/-)	Oxidase (+/-)	Predicted genus

## OBSERVATIONS

## Microbiology 562: D6. Sensory Analysis of Beer.

Batch Fermentation Lab by Dr. Kristen DeAngelis<sup>1</sup>

07 December 2017

This document describes the second lab activity in a six part module exploring batch fermentation. First, we set up a fermentation of grains by yeast to brew beer (D1). We also made media and poured plates to grow and identify bacterial contaminants (D2). Two weeks after setting up the primary fermentation, we primed and conditioned the fermentation (D3). While we waited for the priming and conditioning to finish, we spent one lab doing bioinformatics analysis of microbial communities turning ethanol (a product of fermentation) to n-caproic acid, a fuel precursor and otherwise valuable industrial chemical (D4). Then we examined the microbial contaminants that were cultured in the primary fermentation (D5). **Today, the beer is finished, and we will perform a sensory analysis followed by a field trip to the Berkshire Brewing Company in South Deerfield, MA, where we will tour their brewing facilities and microbiology lab.**

### Learning Objectives

By the end of today's class, students should be able to

- classify scents from the product of fermentation (beer) and classify them based on their likely chemical composition,
- link the off-flavors in the scent and taste of beer to likely causes including as products of microbial processes.

In today's lab, we will perform a qualitative assessment of our fermentation product, which should be ready for consumption. Learning how to identify off-flavors can provide us with feedback about our technique or the brewing process. We will use proper sensory analysis techniques to develop a flavor profile of our beer in order to perform a comparison between the four batches. This will hopefully allow us to understand the effect that mash pH, fermentation temperature, pitching rate and contamination have on the brewing process.

### Beer Flavor Wheel

The Beer Flavor Wheel is currently the major tool used by sensory analysts as the standard vocabulary for flavor profiling. It was created by Morten Meilgaard in the 1970s and eventually adopted by the European Brewery Convention, the American Society of Brewing Chemists, and the Master Brewers Association of the Americas.

<sup>1</sup> Developed in part by Cecilia Prado, with support from Dr. Klaus Nusslein, Dr. Sue Leschine, and the UMass Center for Teaching Excellence Faculty Development

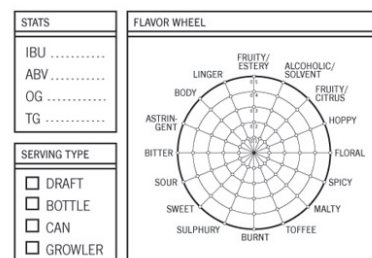


Figure 1: This is an example of a score sheet for rating the qualities of beer. This sheet might be distributed during a tasting or competition, with tasters marking beer qualities and connecting the spokes of the web to get a picture of the flavor profile.

*N.B: The Fall 2017 class included control (group 4), high pitch rate (group 3), low specific gravity (group 2), and low pH and low s.g. (group 1).*

It gives each flavor a descriptive name, grouping them into 14 different classes. The wheel contains 46 first-tier flavor descriptors (familiar terms such as fruity, metallic, or solvent-like). A second-tier breaks out the first descriptions into 76 specific flavors of beer. Only about half of these flavors are commonly found in beer, while the rest are used to classify flavors in defective or specialty beers.

Once the analyst has identified the flavors in a particular beer, a five point system can be used to rate the flavor intensities as Not detectable; Slightly detectable; Detectable; Strong; or Overpowering.

For all beers, the most predominant flavors should correspond to primary constituents: ethanol, hop bittering compounds and carbon dioxide. For specialty beers, hop aroma compounds, caramel and roasted compounds, esters and alcohols (high gravity beers), and short-chain acids may also be in this category.

A complex beer is expected to have prominent peaks of intensity 4 or higher, for flavors in most of the 14 classes. Secondary peaks would correspond to diacetyl, acidic, alcoholic, estery, fruity and glassy. In contrast, flavors in the oxidized, phenolic and sulfuric classes should have negligible intensities.

*The Beer Flavor Wheel was created by Morten Meilgaard in the 1970s and eventually adopted by the European Brewery Convention, the American Society of Brewing Chemists, and the Master Brewers Association of the Americas.*

### *Off-flavors*

The following is a table describing the most common off-flavors present in beer to facilitate their identification. For more detailed information, refer to the Flavor Components in Beer handout from [craftbeer.com](http://craftbeer.com).

**Table 1.** Common off-flavors in beer and their probable causes.

Off-flavor	Characteristics	Common Cause
Acetaldehyde	Precursor of ethanol; resembles green apples or freshly cut pumpkin	The beer is premature and needs more time for conditioning, oxidation of ethanol or bacterial contamination
Alcoholic	Warming prickly flavor, fragrant vinous aroma. Solvent-like in extreme cases	High fermentation temperatures, excessive fermentable sugars, lower mash temperatures, long fermentation time, bacterial contamination
Astringent	Dry grain-like, mouth puckering, tannic, vinegarish-to-intensely tart sensation. Unlike bitterness, astringency is perceived throughout the entire mouth	Using high sulfate water, contamination by LAB or acetic acid bacteria, excessive trub, over hopping, steeping the grains for too long, mash pH exceeding 5.2-5.6 range

**Table 1.** Common off-flavors in beer and their probable causes, *continued.*

Off-flavor	Characteristics	Common Cause
Cidery	Common fermentation product of many yeast strains, levels vary by temperature and recipe	Contamination by acetic acid bacteria, or over priming, or warmer fermentation; acetaldehyde is a known component
Diacetyl	Buttery or butterscotch flavor often with a "slickness" on the palate	Early refrigeration, contamination by <i>Lactobacillus</i> or <i>Pediococcus</i> strains
Dimethyl Sulfoxides (DMS)	Cooked canned corn or celery flavor when present in excessive amounts	High initial temperature, long lag time before fermentation, premature refrigeration, contamination by enteric bacteria
Ester/Fruity	Resemble artificial banana, pear, apple or grapefruit flavors	Higher gravity, excessive trub, bacterial contamination, high fermentation temperature
Light-Struck	'Skunky' or 'catty,' with a sulfur-based corruption of hop flavor	Exposure to sunlight or fluorescent light
Metallic	May resemble the aroma/favor of rusty nails, tin, coins, or blood	Wort contact with aluminum or cast iron or due to hydrolysis of lipids in poorly stored malts
Oxidized/stale	Stale, sherry-wine, rotten fruit or vegetable, cardboard or papery	Old beer, excessive oxygen introduced in the fermenter, excessive aging
Phenol	Described as medicinal, band-aid-like, smokey, clove-like, and plastic-like	Wild yeast contamination, boiling grains, contamination by enteric bacteria
Solvent-like	Pungent, acrid aroma, along with a burning sensation in the back of the mouth	Presence of ethyl acetate from the esterification of ethanol by acetic acid at higher fermentation temperatures
Sour/Acidic	Sour aroma, vinegary or tart	Lactic and acetic acids produced by Enteric, <i>Lactobacillus</i> or <i>Pediococcus</i>
Sulfur	Imperceptible at low levels, at high levels an aroma like that of rotten eggs	DMS formation, SO <sub>2</sub> production from low amounts of mashing and vigorous boil, contamination by enteric bacteria, light-struck beer
Vegetable	Resembles cooked corn, cabbage or broccoli	Low levels of sulfur-derived compounds, bacterial contamination

### *Flavor profile of finished product*

To get a full sense of the flavor profile of a beer is important to consider five different aspects: the appearance of the beer (color, clarity, head retention/foam), its aroma, its flavor, its texture or mouthfeel and the finish of after taste.

By now, you must have four different variations of American Pale Ale. **Table 2** presents a summary of the typical characteristics of this particular style of beer. This can be a useful tool when determining the quality of your product.

**Table 2.** Summary of American Pale Ale Characteristics.

Character	Target range or style
Original Gravity	1.045 - 1.060
Final Gravity	1.010 - 1.015
IBU Range	30 - 45
ABV Range	4.5 - 6.2 percent
Aroma	American hops should be moderate to strong; Hoppy Citrus notes possible; Low matiness should support hops; Fruity esters moderate at most; No diacetyl
Flavor	Clean malt character supports the moderate to high hop flavors; Citrusy flavors are likely; Malt character can be generous, but balance flavors hops. No diacetyl
Appearance	Range from straw-like pale golden to amber; May be hazy if dry-hopped; Brilliant off-white foam with decent retention.
Mouthfeel	Smooth with moderate to high carbonation and medium-light body.

### *Procedure*

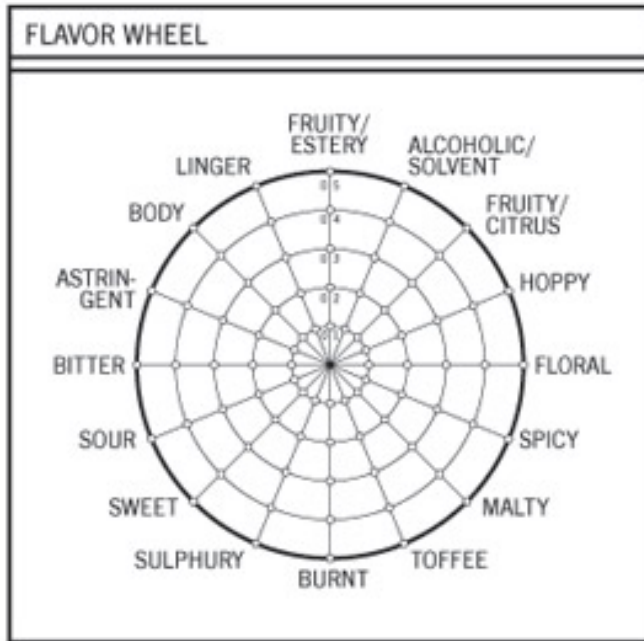
1. Pour a small sample of the finished beer into a glass. This releases the aromas and CO<sub>2</sub>, and facilitates the distinction of the flavors. Make sure the beer isn't too cold, as this makes it harder to taste.
2. Raise your glass and carefully observe the appearance of the beer. Use the datasheet provided in the handouts to record your beer's color, clarity (from brilliant to cloudy) and head retention (whether the foam remains or collapses).
3. Bring the beer closer to you and try to identify the smells that are present. If the beer has no discernible aroma, agitate it by swirling it around in your glass and try again (this will release some carbonation, carrying up the aroma up to your nose). Describe the

aroma composition of your beer by referring to the Beer Flavor Wheel (provided in the handouts) and record it on your datasheet.

4. Take a sip of the beer, swirling it around your mouth before swallowing it. The flavor should be a natural continuation of the aroma, with a few added dimensions such as bitterness. Use the Beer Flavor Wheel to describe the different flavor intensities and record it on your datasheet.
5. Take another sip of the beer, this time paying attention to its texture, weight and carbonation level (mouthfeel). High alcohol beers may have a warming quality, while bitter beers might be astringent. The weight may vary from light and watery to full and heavy. Record your observations on your datasheet.
6. Take another small sip, this time paying attention to the after-taste, or the finish. The flavor of a beer could either be short, or linger between sips. The after-taste can be sweet or bitter, and multiple flavors may be perceived. Take a moment to record your observations, using the Beer Flavor Wheel if necessary.
7. Compare your beer's flavor profile with the one corresponding to the other batches. Record any differences between them, as well as a comparison between your product and the characteristics listed in Table 2.

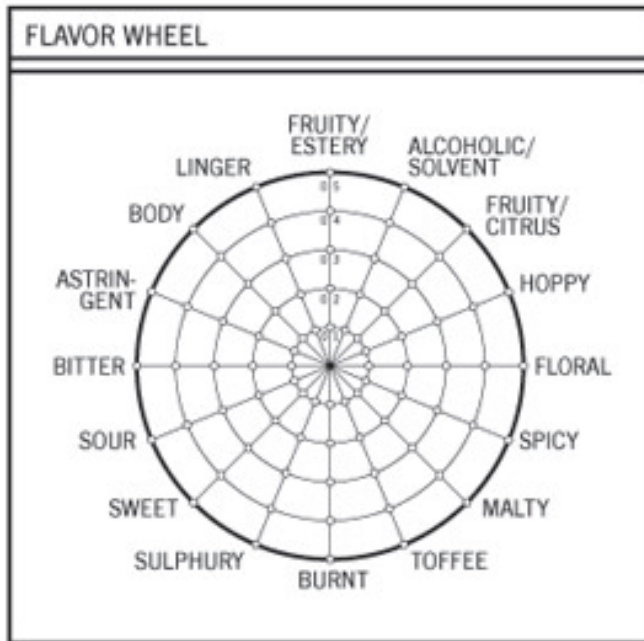
STATS	
IBU .....	
ABV .....	
OG .....	
TG .....	

SERVING TYPE	
<input type="checkbox"/>	DRAFT
<input type="checkbox"/>	BOTTLE
<input type="checkbox"/>	CAN
<input type="checkbox"/>	GROWLER



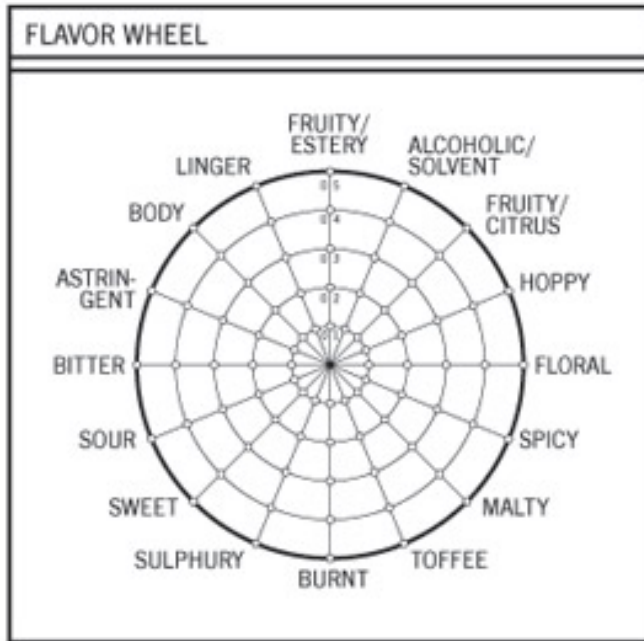
STATS	
IBU .....	
ABV .....	
OG .....	
TG .....	

SERVING TYPE	
<input type="checkbox"/>	DRAFT
<input type="checkbox"/>	BOTTLE
<input type="checkbox"/>	CAN
<input type="checkbox"/>	GROWLER



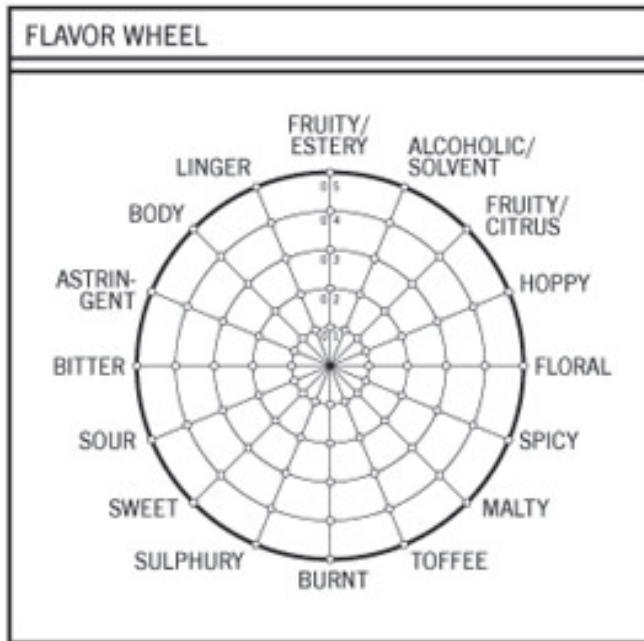
STATS
IBU .....
ABV .....
OG .....
TG .....

SERVING TYPE
<input type="checkbox"/> DRAFT
<input type="checkbox"/> BOTTLE
<input type="checkbox"/> CAN
<input type="checkbox"/> GROWLER



STATS
IBU .....
ABV .....
OG .....
TG .....

SERVING TYPE
<input type="checkbox"/> DRAFT
<input type="checkbox"/> BOTTLE
<input type="checkbox"/> CAN
<input type="checkbox"/> GROWLER





DAY SHEETS: Date & Lab Name: Nov 14, 2017, Primary Fermentation (D.1, Batch Fermentation)

Item	Amount	Location/Note
Brew pot & cover	1/group = 4	
Large heating plate	1/group = 4	
Stir bar for brewpot	1/group = 4	
Water source	6.88 L/group = 27.52	
Scale for 50 g to 1 kg range	1	
Weigh boat	10	
2-row pale malt	1 kg/group = 4	
Crystal malt	50 g/group = 200	
Cascade hops (bittering/flavoring) – 5.5% alpha acids	11.3 g/group = 45.2	
Safale US-05 dry ale yeast	About one package/group	
Gypsum	1-1.5 gal/group = 6	
Steeping bag (cheesecloth)	1/ group = 4	
Binder clips to secure steeping bag to brew pot	2/pot = 8	
Thermometer	1/group = 4	
Clamp to hold thermometer to brew pot	1/group = 4	
Heat resistant gloves for binder clips	2/group = 8	
Transfer pipette to sample strike water for pH	2/group = 8	
pH meter or pH test strips		
Iodine bottle and dropper		
Test dish for iodine test	1/group = 4	
Bucket to set steeping bag in	1/group = 4	
Sterile 25 mL pipettes to sample wort	1/group = 4	
Test cylinder for hydrometer reading		
Ice water bath large enough for brew pot		
Scale for 10 g range		
Fermenter		
Cheesecloth to cover fermenter		
Lid for fermenter		
Stopper for fermenter		
Airlock for fermenter		
Erlenmeyer flasks for pitching yeast		
Warm water for pitching yeast		
Wooden spoons for stirring	1 per group (4)	

Potholders or heat-resistant gloves		
Clear flat-bottom 96-well plates and white paper (for starch test)	1 per person	
Soluble starch solution (for iodine test)	20 ml aliquot per group	

## Notes:

D.1 (Day 1) Fermentation Setup. This full-section lab exercise should occur about 2 weeks before the Priming and Conditioning exercise (D.4). During this full-length lab, the students will set up their batch fermentation. They will use a technique called “brew in a bag”, which involves using a nylon or muslin bag during the mashing step for time and cost efficiency. In addition, the students will scan for microbial contaminants by plating dilutions of wort samples on permissive media.

(TA): Sterile glass 25 ml pipettes, which are not normally available to the lab classes but could be autoclaved for the class or brought over from DeAngelis lab.

(TA): Before class begins, the TA should measure the grains corresponding to each group

(TA): Before class begins (and best the day before), measure 7 gallons of water for the brewing. Each group needs about 1.75 gallons of strike water for mashing. In the past we've filled boiling kettles from the water fountain in the hall, but water can also be purchased.

(TA): We need four large heating stir plates; two are in DeAngelis lab, MTS & Kevin Griffith's lab had the others.

(TA): At the beginning of office hours (1:30 pm), start heating the strike water (the water used for the recipe) to the temperature specified in the recipe. Using the large heating plates, this takes an estimate of 30-40 minutes.

(TA): Sanitize the fermenting bucket. Iodophor is a solution of iodine complexed with a polymer carrier that is very convenient to use. This should be done ahead of time to allow the equipment to dry.

## Recipes:

### Method for sanitizing using Iodophor

One tablespoon in 5 gallons of water (15ml in 19 l) is all that is needed to sanitize equipment with a two-minute soak time. This produces a concentration of 12.5 ppm of titratable iodine. Soaking equipment longer, for 10 minutes, at the same concentration will disinfect surfaces to hospital standards. At 12.5 ppm the solution has a faint brown color that you can use to monitor the solution's viability. If the solution loses its color, it no longer contains enough free iodine to work. There is no advantage to using more than the specified amount of iodophor. In addition to wasting the product, you risk exposing yourself and your beer to excessive amounts of iodine. Iodophor will stain plastic with long exposures, but that is only a cosmetic problem. The 12.5 ppm concentration does not need to be rinsed, but the item should be allowed to drain before use. Even though the recommended concentration is well below the taste threshold, I rinse everything with a little bit of cooled boiled water to avoid any chance of off-flavors.

### Soluble starch solution for iodine test

Dissolve ~0.5g soluble starch (MTS) in 100 ml water. At this concentration, the starch did not fully dissolve.

DAY SHEETS: Date & Lab Name: Nov 16, 2017, Media Preparation (D.2, Batch Fermentation)

Item	Amount (per group, 4 groups)	Location
Yeast Extract	4.0g	
Peptone	5.0g	
Dextrose	50.0g	
Monopotassium phosphate (*)	0.55g	
Potassium Chloride (*)	425.0mg	
Calcium Chloride (*)	125.0mg	
Ferric Chloride (*)	2.5 mg	
Magnesium sulfate (*)	125.0 mg	
Manganese sulfate (*)	2.5 mg	
Bromocresol Green (*)	22.0 mg	
Agar	20 g	
Cycloheximide* WLD only	4 mg	
Scales for weighing chemicals	1 per group (4)	
1L bottles for agar and media	2 per group (8)	

**Notes:**

Half of the class will prepare WL nutrient media plates while the other half prepares WL differential media plates. Students will prepare media and put into the autoclave. While the autoclave is running, we will have a lecture. After the autoclave is done, students will pour plates.

We are making 2L of each media type so that there is enough for plating contaminants today and for plating before and after pasteurization in D5. The remaining plates should be stored until the D5 lab.

(TA): Because it is too difficult for students to all measure out chemicals, TA will make a 10x master mix of chemicals marked with an asterisk (\*), and students will weigh what they need of the mastermix.

(TA): Cycloheximide is toxic to human health, so after autoclaving, the TA will add cycloheximide to WLD media.

(TA): anaerobic plates should be stored in anaerobic incubator before inoculation; aerobic plates should be stored at 4C.

**Recipes:**

10X master mix - make sure this is mixed well so that there is good distribution in medias.

DAY SHEETS: Date & Lab Name: Nov 30, 2017, Priming and Conditioning (D.3, Batch Fermentation)

Item	Amount	Location
Priming sugar	23 g	KMD lab
Siphons, tubing and valved cane	1 per group	
Hydrometer, thermometer, and cylinder		
Boiling kettle		
Glass 1-gallon growlers		
Caps for growlers		
Sterile 1x PBS for plating		
Microwave	One per class	
Erlenmeyer flask (for priming sugar solution)	One per group	
Scale and weigh boats for priming sugar solution	One per group	

**Notes:**

D.3 (Day 14) Priming and Conditioning. This step takes place 2 weeks after fermentation started. Students will transfer the pre-conditioned beer into a bottling bucket (a glass carboy), with priming solution (corn sugar and water). They will take a pH and hydrometer reading of their beer, and they will perform serial dilutions to plate on WL nutrient and differential media.

D.4. Bioinformatics Lab: Omics and the analysis of biofuels fermentation. Students only need their computers for this lab. Therefore, no day sheets are provided.

(TA): Sanitize the brewing kettle, tubing, and siphon.

(TA): Sanitize or autoclave the glass secondary fermenters and caps.

(TA). Locate a 30C incubator for the yeast and 35C incubator for bacteria plus anaerobic incubator.

(TA): Bring the plates to lab; anaerobic plates should be stored in anaerobic incubator before inoculation; aerobic plates should be stored at 4C.

**Recipes:**

DAY SHEETS: Date & Lab Name: Dec 5, 2017, Microbial Contamination (D.5, Batch Fermentation)

Item	Amount	Location
Microscope slides	12 per group	
Hydrogen peroxide	A few drops per group	
Oxidase test strips	One bottle per group	
Hucker's Crystal Violet		MTS
Iodine solution		MTS
Acetone-alcohol solution		MTS
Safranin O		MTS
Microscopes	1 per student	
Phosphate buffered saline		
Heat block (for pasteurization)		

**Notes:**

D.5 (Day 21): Identification of contaminants in finished beer. This full-section lab exercise should occur ~one week after the Day 4 exercise; it will be done on the Tuesday after the D.4 on a Thursday, so only 5 days, in Fall 2017. The students will identify the microbial contaminants that were previously plated. They will use the results from their differential media, as well as microscopy, gram-staining, and catalase and oxidase tests if necessary.

This section of the lab is oriented towards the identification and characterization of possible beer spoilage contaminants. Beer fermentation is usually performed by a single microbial culture. However, the overall brewing process includes a sequence of microbial constituents with a direct impact on the final product. Microbial organisms capable of beer spoilage may produce off-flavors, acids and non-desirable aromas. They may also contribute to the formation of haze and films. In addition, they could potentially compete with the brewing strain for essential nutrients, which may result in a stuck fermentation or over-attenuation.

(TA): We will need access to the microscopes for this lab.

(TA): We need a heat block set up to 80C for pasteurization.

(TA): Bring the rest of the WLD and WLN plates to lab for pasteurization.

**Recipes:**

DAY SHEETS: Date & Lab Name: Dec 7, 2017, Sensory Analysis (D.6, Batch Fermentation)

Item	Amount	Location
Small clear plastic cups	4 per person	

D.6 (Day 28) Quality Assessment/Flavor profile. This full-section lab exercise should occur two weeks after the priming and conditioning. Students will use sensory analysis techniques to assess the quality of their beer. In Fall 2017, this will take place 9 days after priming and conditioning.