Observations from Morrison and Boyd, *Organic Chemistry*

- The first chemical synthesis by man was the production of ethanol by fermentation
- Ethanol production preceded the synthesis of soap
- **Conclusion:**
  
  *Man’s desire for intoxication precedes man’s desire for cleanliness*
The Secret to Fermentation Is Yeast

- Yeast are unicellular fungi
- For fermentation (beer): *Saccharomyces cerevisiae*
- Originally isolated from the skin of grapes
- Yeast will convert simple sugars to energy for their growth and to by-products:
  - Ethanol (CH₃-CH₂-OH)

Ethanol Is Produced By Biological Fermentation of Sugars

- Organisms from yeast to humans degrade sugars (glucose) for energy using O₂ to produce CO₂
- The energy produced allows production of other carbon compounds for growth
- Under *anaerobic* conditions, yeast will stop the process short, producing *ethanol*, a 2-carbon alcohol

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Ethanol Is Produced By Biological Fermentation of Sugars

- Organisms from yeast to humans degrade sugars (glucose) for energy using $O_2$ to produce $CO_2$
- The energy produced allows production of other carbon compounds for growth
- Under *anaerobic* conditions, yeast will stop the process short, producing *ethanol*, a 2-carbon alcohol

Sidelight: Ethanol is toxic to many cell types, but yeast can exist in >12% (2.5 M) ethanol. Ethanol production may be a yeast anti-biotic/fungal to eliminate competing organisms.

Yeast to Humans: Can Synthesize and Metabolize a Range of Sugars

- Sugar [(CH$_2$O)$_n$ where $n = 6$]
  - is produced by plants and animals
  - and stored as complex carbohydrates (saccharides)
- Both yeast & humans can break disaccharides to simple sugars

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Some Carbohydrates Cannot be Broken Down by Yeast or Humans

- The arrangement of the bonds connecting the sugars is key
- Cellulose has a $\beta$ glucose linkage that resists cleavage

![Diagrams of carbohydrate structures]

Most Sugar in Plants is in the Form of a Complex Carbohydrates, i.e. Polysaccharides

- Polysaccharides in plants:
  - Cellulose – not digestible
  - Polysaccharides in seeds and fruits of plants (starch):
    - Amylose: $1\rightarrow4$ linked glucose
    - Amylopectin: $1\rightarrow4$ and some $1\rightarrow6$ linked glucose

![Diagrams of amylose and amylopectin structures]
Seeds and Fruits Contain Enzymes to Digest Starch

- Amylase enzymes digest both amylose & amylopectin to glucose
  - The enzymes are active in ripening fruit or can be activated by warming (e.g. grains)

Enzymatic Digestion of Starch to Sugars in Plants

- Enzymes (e.g. amylases) breakdown starches:
  - [α-amylase]: Produces disaccharides (e.g. maltose)
  - [β-amylase]: Produces single sugars (glucose) from the ends
  - [Limit dextrinase]: Cleaves at the 1-6 branch points

But some polysaccharide pieces may still remain
Yeast Can Metabolize Simple Sugars Easily For Growth (but Polysaccharides Less Well)

- Sugars (starch)
- Yeast
- \( \text{O}_2 \)
- \( \text{CO}_2 \)
- \( \text{C}_2\text{H}_5\text{OH} \)

Methanol

- What is it?
- How does it form in the fermentation process?
- How does it get in distilled spirits?
Methanol As A By-Product of Fermentation

- Methanol
  - Is a one-carbon alcohol
  - Also distributes across body water and all tissues
  - Is also metabolized in the liver by the same enzymes that metabolize ethanol

- Methanol metabolism produces toxic compounds
  - Formaldehyde – embalming fluid
  - Formic acid – neurotoxic
    - Causes headache, nausea, vomiting in lower doses
    - Is toxic to the optic nerve and can cause blindness (“white lightning”)

Legal Limits for Methanol

- European methanol (MeOH) limit in ethanol (EtOH):
  - 10 g MeOH/L EtOH

- US ATF methanol limit in ethanol:
  - 7 g MeOH/L EtOH → 7 mg MeOH/ml EtOH
  - 2.8 mg MeOH/ml (40% EtOH) → 250 ml = 0.7 g MeOH
  - 1.0 mg MeOH/ml (14% wine) → 750 ml = 0.75 g MeOH

- International Organization of Vine and Wine (OIV)
  - 0.2-0.4 mg MeOH/ml wine
**Methanol Is Not Produced By Yeast**

It Occurs Naturally in Pectin

- Yeast metabolizes glucose to end-products, but not to methanol
- Plants also contain the polysaccharide pectin
- **Pectin** is 1,4-linked α-D-galacturonic acid (oxidized galactose)
- Most of the galacturonic acids in pectin have been methylated
- Pectin found in a variety of fruits
  - Pectin is high in fruit skins (grapes, apples, ...)
  - Potato is has pectin
- Pectin, isolated from fruit, is used as a gelling agent for foods

---

**An Enzyme Release Methanol from Pectin**

- Enzymes in fruits that digest pectin
  - Pectinase – hydrolyzes the 1,4-linkages in pectin
  - Pectinesterase – hydrolyzes the methyl ester on galacturonic acid

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Methanol Content of Fermented and Distilled Beverages

- Grains have very little pectin – the methanol content of beer & distilled whiskeys made from grains is low
- Fruits and some vegetables (potatoes) has significant amounts of pectin
- Methanol is naturally produced during the fermentation of high-pectin fruits and vegetables
- Methanol (bp: 65 °C) is not always/usually separated from ethanol (bp: 78 °C) during distillation
- Fermented fruit beverages (e.g. hard cider and wine) and distilled products (e.g. apple & pear brandy/schnapps) may contain significant amounts of methanol
The Goal for Fermentation is to Get Yeast to Replicate

Replication (growth) requires
- Energy – sugar
- Nitrogen:
  - Amino acids $\leftrightarrow$ Protein
  - DNA
- Phosphate
  - DNA
- Some minerals
  - Calcium
- Some oxygen

- Yeast will not grow on pure sugar alone

Seeds

- Contain everything a plant needs to begin growth
- Nutrients
  - Sugar as polysaccharides
  - Nitrogen as protein
  - DNA and phosphate
  - Some fat (another energy source)

- We can breakdown polysaccharides in seeds that we eat
  - Pancreas secretes $\alpha$-amylase

- But, there is not enough simple sugar in most seeds for yeast to sustain growth
The Trick for Man to Make Beer

- Humans had learned to cultivate grasses using the seeds for food: wheat, barley, etc.
- But grass seeds do not ferment
  - Yeast needs simple sugars

Solution: trick the grass seeds into making simple sugars out of their polysaccharides

Seed Germination Produces Food for the Growing Plant-to-be

- Germination begins when the barley seeds are steeped in water
- Cells begin producing proteins from DNA
  - Including enzymes: amylases
- Next: stop germination by drying the seeds
  - Result: malt
- The malt seeds can also be heated in kilns to produce different types of malt flavors
- Malted seeds are then stored dry until use
What Do You Call a Person That Produces Malt from Grain?

- A maltster
What Do You Call a Person Produces Malt from Grain?

- A maltster

- Note: Brewers are almost never maltsters

Reinheitsgebot: German (Bavarian) Purity Law of 1516

- Brewing beer began in the 700's?

- Duke Wilhelm IV of Bavaria:
  - „Keinem Bier mehr Stücke als allein Gersten, Hopfen und Wasser verwendet werden sollen“

- “No beer ingredients other than
  - barley,
  - hops,
  - water are to be used“

Reinheitsgebot was designed to prevent competition between brewers & bakers for wheat and rye
Reinheitsgebot: German (Bavarian) Purity Law of 1516

◆ “No beer ingredients other than
  ● barley,
  ● hops,
  ● water are to be used”

◆ Yeast was not identified until the 1857 by Pasteur

What the Brewer Does (step 0):
Select Good Water for Taste & for the Yeast

◆ Modern times dilemma: city water has chlorine
  ● Oxidizes & kills yeast
  ● Oxidizes grains & hops
  ● Adds an undesired taste

◆ Cation & anion profile
  ● Some cations add stability & are needed by yeast for growth
    • Calcium (can be added)
  ● Grain adds potassium
  ● Some cations to avoid
    • High sodium (softened water)
    • High iron

◆ Water pH & bicarbonate abundance
◆ Water that tastes bad will make bad beer
  ● E.g. tannin & sulfur compounds
What the Brewer Does (step 1): *Mash the Grain: Produce Simple Sugars for the Yeast*

- Crack (crush) the malted barley
- Soak the barley in water – the “mash”
  - Mash temperature must be optimal for enzyme activation (~150°F or 65°C)
    - Amylases
    - Proteases
- Wait (~60 min) for starch conversion to simple sugars
- Drain off and save the sugar water (the “wort”)
- Rinse the grain to remove residual sugar and add to the wort
- *By-product of the wort: protein, amino acids, phosphate and other nutrients for the yeast*

Barley Is One Determinate that Defines a Beer Style

- Continental beers (e.g. German & Belgian) use Continental Pilsner
- UK beers use British pale malt

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<tr>
<td>Czech Republic</td>
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</table>
What the Brewer Does (step 2): Boil the Wort with Hops

- Boil the wort to sterilize and remove unwanted volatile compounds
  - E.g. dimethyl sulfide

- Add **hops** to the boiling wort
  - Adds taste, aroma
  - Adds antibacterial compounds

- Boil ~60 minutes
- Cool to room temperature

---

**Hops**

- Are the female flower clusters of the species *Humulus lupulus*
  - The flowers are the hop cones

- The hop plant is a perennial vine growing >20 ft high
  - i.e. a weed

- **Cultivated since the 700's**
- **Used to make beer since the 1300's**
  - Displacing "gruit" herb blends

- Hop cones are added to the wort
  - To stabilize beer (antibiotic against bacteria)
  - To flavor beer, imparting a bitter, tangy flavor

- In 1442 Christopher, king of Bavaria, Denmark & Sweden/Norway, required all farmers to have "40 poles" for growing hops

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Hops and *Alpha- & Beta-Acids*

- Yellowish pollen containing hop oil inside flowers
- Hop oil contains both
  - Alpha-acids &
  - Beta-acids
- Alpha- & Beta-acids are a complex set of molecules

### Structures of Hop Alpha & Beta Acids

- Relatively insoluble in water
- The variable part is the blue group
- Different acids give different aromas/taste
- Common groups:
  - -CH\_2CH(CH\_3)\_2 – shown for α & β below
  - -CH(CH\_3)\_2 – cohumulone & colupulone
  - -CH(CH\_3)CH\_2CH\_3 – adhumulone & adlupulone

[Diagrams of n-Humulone (α-acid) and Lupulone (β-acid)]
Boiling Hops in Wort Isomerizes $\alpha$-Acids

- Bitterness is based upon the isomerized $\alpha$-acids
  - $\beta$-acids do not isomerize
- Adding hops late in the wort boil preserves the $\alpha$-acids

Hops Also Define a Beer Style

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<tr>
<td>Belgium</td>
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</table>

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The Hop Profile of Beer Has Several Components

- The aroma components
- The amount of hops added
- When the hops are added to the “boil”
  - Early add: bittering
  - Late add: aroma

What the Brewer Does (step 3): Add Yeast to Cooled Wort and Ferment

- Yeast cells rapidly proliferate
  - Using available $O_2$
  - Using available sugar
- Yeast metabolism produces
  - $CO_2$
  - And ethanol, as an anaerobic by-product
- As available sugar is depleted,
  - Yeast cells stop replicating
  - Yeast cells die or go dormant (flocculate)
  - Ethanol production stops
- Must maintain sanitation from start to finish: don’t grow molds or bacteria too
The Fermentation Conditions Affect Yeast and the Characteristics of the Final Beer

- Yeast produce >500 metabolites

Factors that affect yeast flavor & aroma metabolites

- Grains used and hops
- Strain of yeast
- Temperature of fermentation • Rate of proliferation
- Amount of extra sugars added

Fusel* Alcohols Produced

*Larger than ethanol. Fusel is German for “bad hooch”

- Adds “warming” solvent flavor
- Higher in ale than lager

- Common fusel alcohols:
  - n-Propanol
  - i-Butanol
  - Isoamyl alcohol
  - “Active” amyl alcohol • Both amyls give a banana flavor • Are common in wine
Esters Produced

- Ethyl acetate
  - Fruity solvent taste

- Isoamyl acetate
  - Banana flavor

- Ethyl caproate
  - Apple flavor

- Greater in ale than lager
  - ↑ with fermentation temperatures

Ketones Produced

- Acetone
  - Solvent taste

- Vicinal diketones (VDK)
  - gives a “slickness” to mouth feel
  - buttery, butterscotch flavor
  - Taste threshold: 0.1 ppm

- Diacetyl
  - 2,3-butanedione

- 2,3-Pentanedione

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**Organic Acids Produced**

- Can give a bad taste/smell
  - Acetate
    - Vinegar
  - Propionate
  - Lactate
    - Sour
  - Butyrate
    - Bad, rubbery
  - Caproate
    - Bad, sour milk

**Phenols Produced**

- Phenol
- 4-Vinyl guaiacol (4VG)
  - Normally undesirable. Tastes:
    - Medicinal
    - Plastic, band-aid, "phenolic"
    - Smoky
  - Exceptions:
    - Hefeweizen – adds clove
    - Rauchbier – adds smoke
  - 4VG is produced from yeast with a "phenolic off-flavor" (POF) gene
    - Malt & hops have ferulic acid
    - POF codes for ferulic acid decarboxylase
    - Many cultivated yeasts do not have the POF gene
    - Wild yeasts have the POF gene
Sulfur-Containing Compounds Produced

- **Hydrogen sulfide**
  - Rotten egg smell

- **Dimethyl sulfide (DMS)**
  - from S-methyl methionine
  - cooked corn or cabbage smell

- **Dimethyl sulfoxide (DMSO)**
  - Bacteria reduce DMSO to DMS

- **Sulfur dioxide**
- **Mercaptans**

- **Volatile S compounds lost**
  - During wort boil
  - By CO₂ production during fermentation
    - Lager (colder fermentation) has more volatile S compounds than ale

The “Lightstruck Flavor” (LIF) in Beer

- **LIF** is a **skunky** smell
- Occurs in beer exposed to sunlight
  - Clear bottles bad
  - Brown bottles much less
- Not present in beer lacking hops
  - Hops play a part in producing LIF
- The skunky mercaptan culprit: 3-methylbut-2-ene-1-thiol (MBT)
  - Present in
    - Skunk spray
    - Durian fruit

- **But how is it produced?**
Photochemical Production of the Precursor Radical to form MBT

- Demonstrates photoactivation of riboflavin (RF) and e- transfer to iso-α-acids
- Produces a 2-methylbut-2-ene radical
  - Known to react with H₂S to form MBT:

\[
\begin{align*}
\text{H}_2\text{S} + \text{CH}_2\text{CH} = \text{CH}_2 \rightarrow \text{H}_2\text{S} + \text{CH}_2=\text{CHCH}_3
\end{align*}
\]

The American Homebrewers Association Beer Judge Scoresheet (BSS)

- The Score sheet has a section for descriptor tastes and aromas

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Some of the Negative Descriptors on the Beer Scoresheet and the Compounds that Cause Them

- **Acetaldehyde**
  - Green apple
- **Diacetyl**
  - Butter, butterscotch
- **DMS (dimethyl sulfide)**
  - Sweet cooked canned corn
- **Estery**
  - Fruits & roses – esters & amyl alcohols
- **Grassy**
  - Fresh cut grass or green leaves
  - cis-3-hexanal by bacteria on barley (before the wort)
- **Light-struck**
  - Skunky aroma – MBT
- **Metallic**
- **Musty**
- **Oxidized**
  - Stale, cardboard, sherry-like
- **Phenolic**
  - Spicy (clove, pepper), smoky, plastic – phenols
- **Solvent**
  - Fusel alcohols, ethyl acetate
- **Sour/acidic**
  - Clean – lactic acid
  - Vinegar-like – acetic acid
  - Can be bacterial contamination
- **Sulfur**
  - Rotten eggs, burning matches – H₂S
    - Bacterial contamination
- **Vegetal**
  - Cooked, canned or rotten vegetable aroma – DMS+H₂S
    - Bacterial contamination

Beer Is All About the Yeast (*Hefe* in German)

- Long before yeast was identified, brewers knew they needed sediment from the prior fermentation to start the next fermentation
- **Reusing yeast from batch to batch tends to select for specific strains of yeast**
### What Are the Differences Between

<table>
<thead>
<tr>
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<th>Lager Yeast</th>
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</tbody>
</table>

### Ale Yeast vs. Lager Yeast

- Ale yeast “top ferments”
- Lager yeast “bottom ferments”

- Ale yeast
  - Clumps during growth and rises (via CO₂ bubbles) to the top
  - Flocculates to the bottom (but so does lager yeast)
Munich Germany Is Cold in the Winter

Low temperature map for 14-Feb-2013

Cold Fermentation Temperatures Select for Cold Temperature Yeasts

- Brewer's and baker's yeast: *Saccharomyces cerevisiae* and
- A cold-tolerant yeast strain: *S. bayanus*
  - Used in wine & cider production combined to make
- *S. pastorianus*
  - Lager yeast
  - Purified by Emil Hansen in 1883 at the Carlsberg Laboratory in Denmark
  - Ideal fermentation temperature: 8-13 °C (46-55 °F)
Cold Temperature Fermentation Was Codified into Law in Bavaria in 1553

Duke Albrecht V of Bavaria
- (son of Willem IV)
- restricted beer brewing to the Reinheitsgebot dates:
  - Sept 23 [Feast of St. Michael] through April 23 [Feast of St. George]
- Two months, same beers:
  - Octoberfest bier
  - Märzen bier

Cold Temperature Fermentation Limits Bacterial Contamination

Because lager beer does not sour as quick:
- Can ship lager
- Growth of German breweries
- Late 1800’s lager boon:
  - Carlsberg isolates lager yeast (S. pastorianus)
  - Refrigeration equipment invented
What the Brewer Needs to Make Good Beer

- Good water
- Malted grains (barley)
  - The commercial maltster controls this process
  - But, a wide variety of malted grains available
- Good mashing process (to extract sugar from malt)
- Hops
  - Great variety of hops and alpha-acid profiles
- Yeast
  - Very wide range of yeast strains and characteristics available
- Sanitary conditions
- Fermentation conditions
- A taste for really good beer

Greg Noonan 1951-2009

- Founder of the Vermont Pub & Brew
- Inventor of new beer styles
  - The black IPA
- Author
  - Brewing Lager Beer
  - A major influence on both home and micro brewers
- Famous quote: “It’s about the beer”
Good Sources:

- *How to Brew*, John J. Palmer
  - http://www.howtobrew.com/

- *Brewing Classic Styles*, Jamil Zainasheff, John J. Palmer