Basic Chemistry And Fermentation

Wine 3

Introduction to Enology

2 / 4 / 2014 1

Tonight's Lecture

- Review of basic chemistry
  - Atoms & molecules
  - Chemical bonding
  - Acid-base chemistry
- Fermentation
  - Chemistry of alcoholic fermentation
  - Yeast

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But first, a word about tests

- First test on 2/25
- Should take about 20 to 30 minutes
- Lecture afterwards
- Questions will be taken from lecture slides
- We will have a review the week before the test

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Introduction

- Chemistry and microbiology are closely linked to the study of enology and many early researchers in these fields used wine in their experiments.
- To gain a thorough knowledge of enology you need a basic understanding of chemistry and microbiology.

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Introduction

- Tonight we will start with basic chemistry review, this will be new to some of you and redundant for others. We will go into more details of wine chemistry in a later lecture.
- For more detailed information take WINE 55 (A&B) here at SRJC or an analysis class taught through UC Davis Extension or VESTA.

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Introduction

- If you are not very familiar with chemistry the best thing you can do is take Chem-1 before you learn about wine analysis.
- Also we will be simplifying the subject by quite a bit so do not consider this lecture to be the same as taking a course in chemistry.

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Basic Definitions

- **Matter** – material that occupies space and has mass (weight).
  - Matter is composed of elements.
- **Element** is a unit of matter that, that can not be broken down into simpler substances without loosing its unique properties.
  - The smallest unit of an element is the **atom**.

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Elements

- The **symbol** for an **element** is usually derived from its name (common or Latin)

C - Carbon *Latin* **K - Potassium**
H – Hydrogen **Au – Gold**
O – Oxygen **Hg - Mercury**
N - Nitrogen
S – Sulfur
Cl - Chlorine

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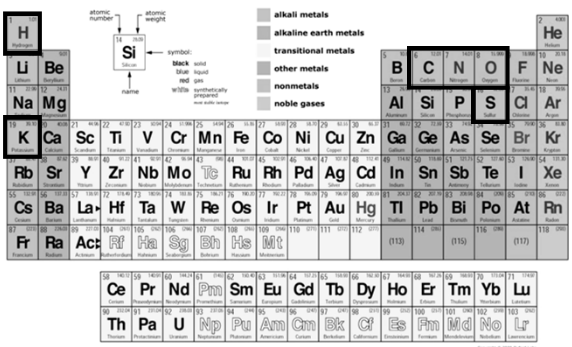
Periodic Table of the Elements



Atom
the smallest unit of an element

- Originally it was thought that an atom could not be divided, now we know they are composed of parts (particles):
  - **Protons** – carry a positive charge
  - **Neutrons** – have no charge (neutral)
  - **Electrons** – carry a negative charge

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The Atom Continued

- Protons and neutrons are in the center of the atom.
- Electrons orbit around the outer edge in **orbitals.**
- In each atom the # electrons = # protons
- These particles are made up of smaller sub-atomic particles such as ***quarks, leptons,*** and ***bosons*** but we do not need to worry about them in this class.

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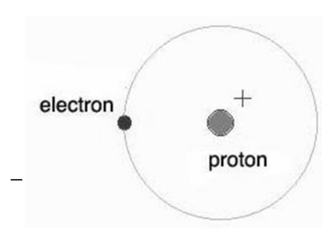
Hydrogen, the simplest atom



An atom is mostly empty space and the are incredibly small. There are about 947 septillion (947,000,000,000,000,000,000,000) hydrogen atoms in a tablespoon of water!

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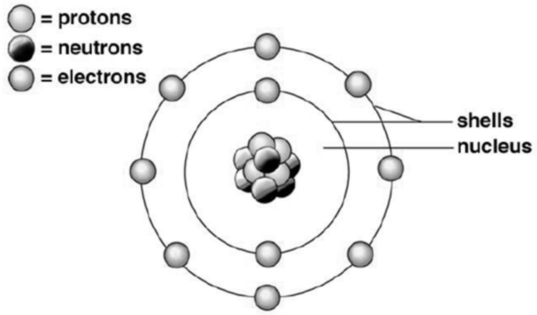
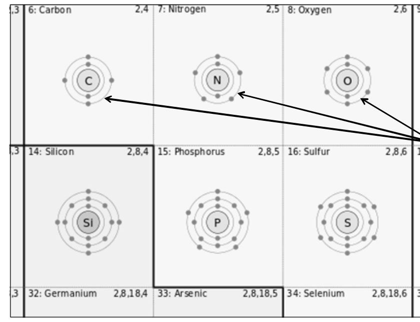
The Bohr Model of the Atom



Neon (#10)



The number of electrons an atom has in its outer shell determines how it reacts with other atoms.

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Periodic Table

- The number of electrons an atom has dictates its properties and how it reacts with other atoms.
- Notice how the periodic table periodically repeats itself, elements in the same column have similar properties.

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The Bohr Model

- The Bohr model of the Atom shows electron orbital shells looking like planets orbiting a sun.

The Simpsons

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The Bohr Model

- However they don't exactly look like we have represented them here in two dimensions. We still use this method of looking at them because it helps us to understand how they form chemical reactions.
- The true 3-D model of electron shells help us to understand the shape of molecules.

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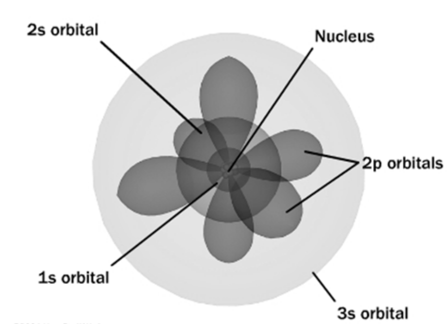
Electron Shells



3-D model drawing of electron shells

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Molecules

- **Molecules** are composed of two or more atoms chemically joined together forming a single particle that has a stable, independent existence.
- A **formula** is a shorthand notation for a molecule's name using symbols to tell the number and type of atoms that are present.

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Molecular Formulas

- Examples: The **subscript** represents the number of atoms in the molecule

O_2 = Oxygen gas

$C_6H_{12}O_6$ = Glucose (grape sugar)

H_2O = Water

CH_3CH_2OH = Ethanol (wine alcohol) can be abbreviated to EtOH

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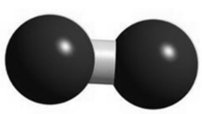
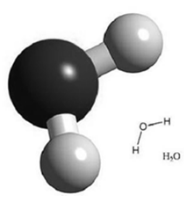
Compounds

- A molecule can be made up of the same or different elements, if the elements are different the molecule is called a **Compound.**



Oxygen Molecule O_2



Water Molecule H_2O

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Ions

- If there are more or less electrons then protons in the molecule or atom it will have a charge and be called an **Ion.**
  - If electrons outnumber protons than the ion is **negative**.
  - If protons outnumber electrons than the ion is **positive**.

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Chemical Bonds

- Atoms in molecules are held together by **chemical bonds,** which are unions between the electron structure of different atoms.
- There are three types of chemical bonds:
  - **Ionic bonds**
  - **Covalent bonds**
  - **Hydrogen bonds**

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Ionic Bonds

- **Ionic**, where the atoms in the molecule have opposite charges (opposites attract, like charges charge repel),
- An example is Sodium Chloride (table salt) **Na^+ + Cl^- = NaCl**.

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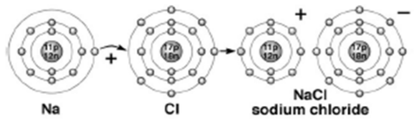
Ionic Bonds



- The result (salt) is more stable than either Sodium or Chlorine are by themselves.

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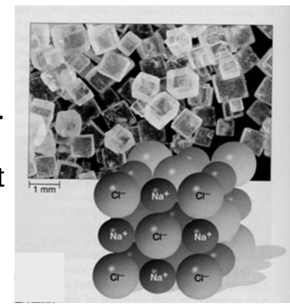
The chemical properties of the molecule can be very different from the elements that make it up. Sodium is explosive when it comes in contact with water and chlorine gas is poisonous, yet sodium chloride is essential to life.



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Covalent Bonds

- When two atoms with unpaired electrons in the outer most shell come together and **share electrons**.
- Each atom has an attractive force for the other atoms unshared electrons, but not enough to take it completely away.

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Covalent Bonds

- Covalent bonds can be polar or nonpolar
  - **Nonpolar** bonds the atoms have same pull on the shared electrons (H_2)
  - **Polar** bonds – the atoms don't equally share the electrons (H_2O)

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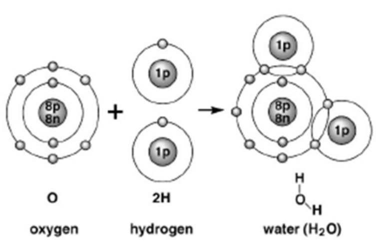
Covalent Bonds



2 H + O = H_2O (water)

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Covalent Bonds

- Covalent bonds can be single, double, or triple.
- In the drawing of a molecules structure a double bond is indicated by = and a single bond is shown with an –.
- Acetaldehyde CH_3–CH=O

Single bond Double bond

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Covalent Bonds

- The number of electrons in an element's outer shell determine how many stable covalent bonds in can make.
  - Carbon - 4
  - Nitrogen - 3
  - Oxygen – 2
  - Hydrogen - 1

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Hydrogen Bonds

- Weak attraction between a **hydrogen** atom in one molecule and an electronegative atom such as **oxygen or nitrogen** in another molecule.
- Individually weak, but many together can be strong.
- Determines shapes of many biological molecules including proteins and DNA.

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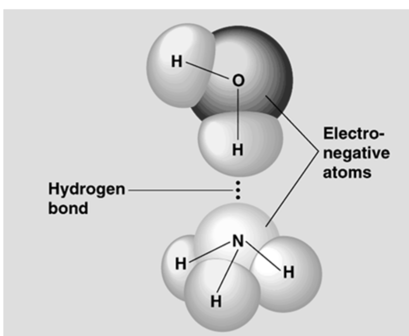
Hydrogen-Bonding



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Chemical Reactions

- When different molecules or atoms react with one another to form new compounds.
- These reactions take place by the breaking and formation of chemical bonds.
- The number in front of the molecule is the number of that molecule in the equation.
- 2 H_2O means there are two water molecules.

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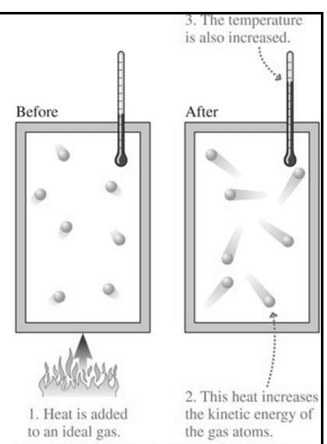
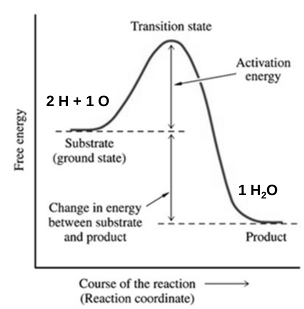
Chemical Reactions

- Atoms have Kinetic Energy based on their temperature.



Chemical Reactions

- A chemical reaction requires a certain amount of energy to take place this is called the **activation energy**. This is the energy needed to break old bonds or form new ones.



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Chemical Reactions

- A reaction that gives off energy (most often expressed as heat) is called **exothermic** one that requires energy (absorbs heat) is called **endothermic**.
- Example:
  - Exothermic – Fire
  - Endothermic – First aid cold packs

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Acid Base Reactions

- Acids are proton donors (H^+), Bases are proton acceptors.
- In water solutions acids (or bases) form conjugate acid base pairs.

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Acid Base Reactions

- Examples:

Hydrochloric Acid: $HCl + H_2O \rightarrow H_3O^+ + Cl^-$

Ammonia: $NH_3 + H_2O \rightarrow OH^- + NH_4^+$

The compounds produced by these reactions are all ions. Sometimes the water molecule is not shown.

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Acid Base Reactions

- **Strong acids** such as hydrochloric (HCl) and sulfuric (H_2SO_4) disassociate (ionize) almost completely.
- **Weak acids** disassociate less than 50% and almost always contain carbon.
- Almost all of the acids found in wine are weak acids.

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pH Scale

- The strength of acids and bases are measured using the pH scale.
- Scale from 0 – 14
  - 0 is the most acidic
  - 14 is the most basic
  - 7 is neutral (pure water)

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pH Scale

- pH is the negative log of the Hydrogen Ion concentration.
- It is **inverse** relationship:
  - Higher the pH the lower the concentration of H^+
- **Logarithmic**:
  - Each point increase in pH represents a ten-fold decrease in H^+ concentration.

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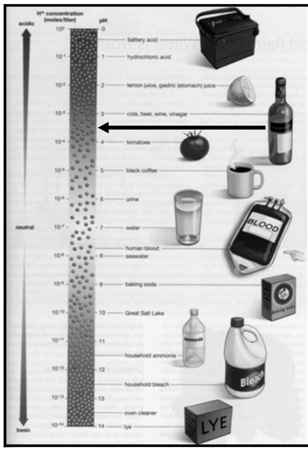

- Wine is typically between pH 3 & pH 4 making it acidic.
- Since it is logarithmic pH 3 is ten times more acidic than pH 4

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Acid Base Reactions

- **Buffers** are solutions that maintain a relatively constant pH when a small amount of acid or base is added, wine is a weak buffer.

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Equilibrium

- Chemical reactions that can go both ways are indicated by the double arrows used in formulas. When the rate of the reaction is the same in both directions, the reaction is said to be at **equilibrium**.

$$H_2O + SO_2 \leftrightarrow H^+ + HSO_3^- \leftrightarrow 2H^+ + SO_3^=$$

Sulfur Dioxide in water

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Concentration

- Concentration is the amount of a substance (# of molecules or atoms) per an amount of liquid.
- Concentration is denoted by brackets $[H^+]$ is Hydrogen ion concentration.

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Effects on equilibrium

- **Temperature,** Increased temperature increases the kinetic energy of molecules, which increases collisions between the molecules and the amount of the activation energy present so this increases the speed of the reaction.
- Fermentation is made up of chemical reactions, so it goes more quickly at higher temperatures.

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Effects on equilibrium

- **Catalysts** Are substances that lower the activation energy of reactions by positioning the molecules.

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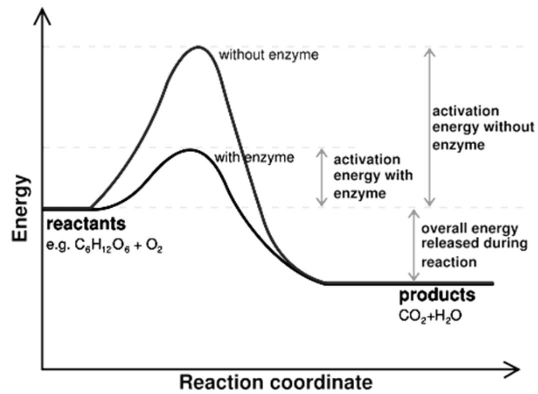

Effects on equilibrium

- **Enzymes** are proteins that act as catalysts, examples in wine include:
  - Enzymes that cause browning in juice are polyphenol oxidases such as laccase or tyrosinase.
  - Enzymes added to juice by winemakers to breakdown pectins in the juice to facilitate settling and clarification.

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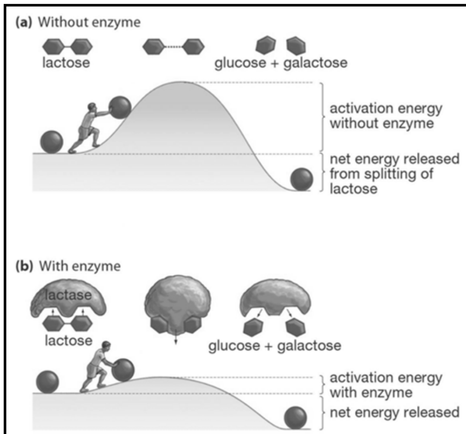

The Enzyme lactase breaks down the milk sugar lactose, people who lack it have lactose intolerance.

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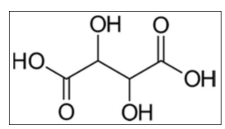
pH Effects on Equilibrium

- Tartaric acid disassociates (loses its protons) in water in the following reaction:

$$H_2Ta \leftrightarrow H^+ + HTa^- \leftrightarrow 2H^+ + Ta^=$$

Ta represents Tartaric Acid



Tartaric Acid

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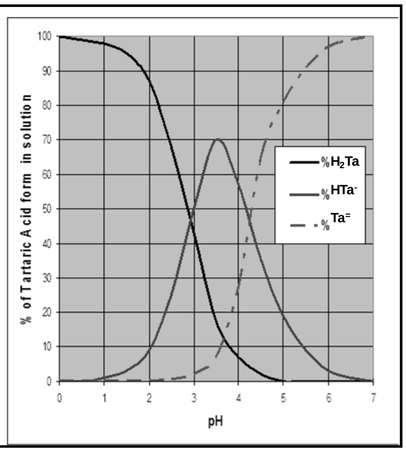
pH Effects on equilibrium



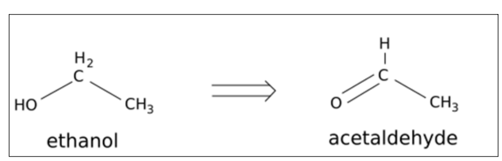
- The disassociation of tartaric acid.

Redox Reactions

- **Oxidation** is the loss of an electron (e^-), loss of H or gain of O.

Example:

$EtOH – 2\ H^+ - 2\ e^- \rightarrow CH_3CHO$
(Acetaldehyde) *Sherry smell*



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Redox Reactions

- **Reduction** is the gain of electron (e^-), gain of H or loss of O.

Example:

$S + 2\ H^+ + 2\ e^- = H_2S$ *Rotten egg smell*

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Organic Chemistry

- Organic chemistry is the study of the chemistry of carbon. It is a discipline in itself because the uniqueness of carbon and it is what all forms of life are based on.
- We don't know what life may look like elsewhere in the universe, but if it exists, chances are it is based on carbon.

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Organic Chemistry

- Carbon can form four covalent bonds with itself or other atoms which gives it the ability to form a multitude of different structures and compounds.
- Carbon can form either single or double bonds which affect the shape of the molecule.

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Structure of Carbon Molecules

- Covalent bonds consist of electrons, since like charges repel carbon bonds are as far from each other on the carbon atom as possible.

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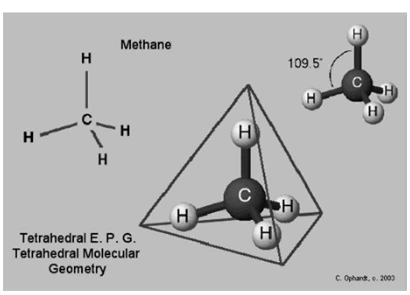
Structure of Carbon Molecules



- Four Single bonds

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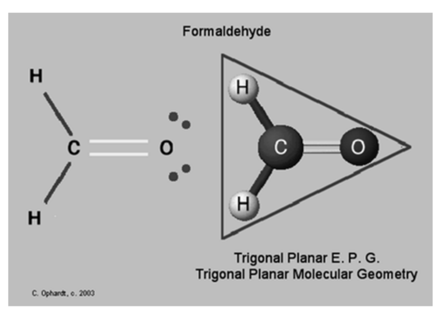
Structure of Carbon Molecules



- One double and two Single bonds

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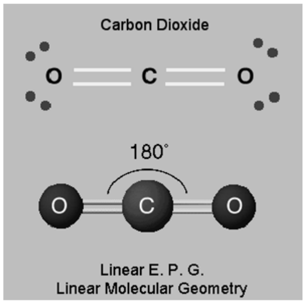
Structure of Carbon Molecules



- Two double bonds

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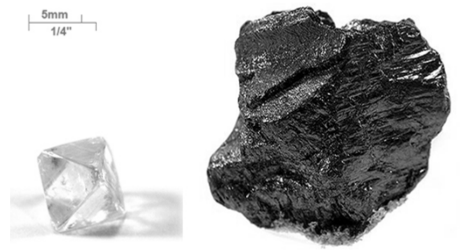
What difference does shape of the bonding make?



Diamond vs. Coal

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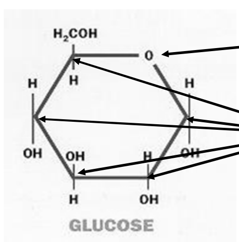
Molecules Containing Carbon

- The carbon atoms molecules are represented by shapes in organic chemistry.



Oxygen is in this corner

Carbon atoms are at the corners of the hexagon

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More to come in Wine Chemistry

- That finishes our basic chemistry review. We will go into the particulars of Wine Chemistry in a later lecture.

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Wine Microbiology

Alcoholic Fermentation

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What is fermentation?

- Two common definitions:
  - The metabolism of sugar without oxygen.
  - The using microbes to convert substrates into new products.
- By understanding the factors that influence fermentation, we can control fermentation to shape the flavor of our wine.

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Types of fermentation

- Cheese making
- Soy sauce
- Bread making
- Sauerkraut
- Salami
- Pharmaceuticals
- Vinegar
- Wine and beer
- Fuel ethanol

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Food Fermentation

- Often in fermentation of food products the microbes produce lactic acid and flavor compounds.
- This was done historically to help preserve food.
- For alcoholic fermentation yeast convert sugar to alcohol.

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Microbes

- Divided into prokaryotes & eukaryotes
- Single celled organisms.
- **Eukaryotes** are larger and a cell nucleus (yeast)
- **Prokaryotes** are smaller and have a cell wall (malolactic bacteria)
- **90%** of the cells in the human body are bacteria but they only make up about 1.5 of body mass.

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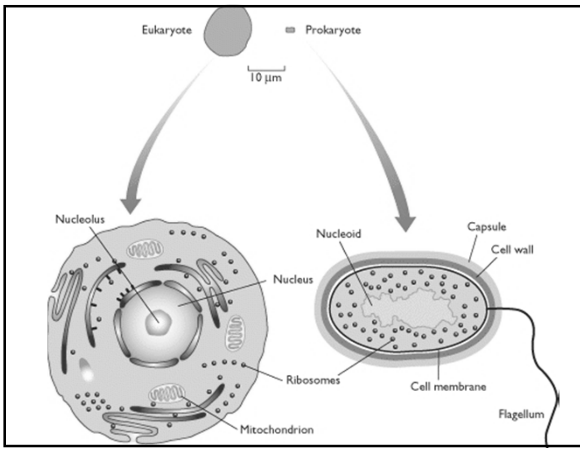
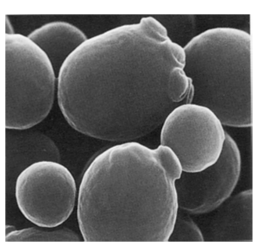


Alcoholic Fermentation, Yeasts

- Yeasts are one-celled organisms (Unicellular Fungi) they are used for wine production because in the absence of oxygen they have the ability to transform sugar (glucose & fructose) into alcohol.



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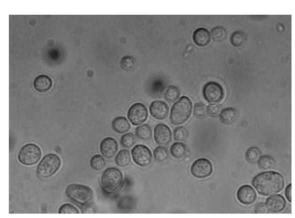
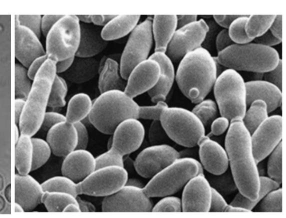
Alcoholic Fermentation, Yeasts

- They are an eukaryote (have a nucleus) and reproduce by budding.



Light Microscope



Electron Microscope

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Alcoholic Fermentation, Yeasts

- Other organisms can do this but yeast is used because it can do the job most efficiently and they can survive in wine.
- The yeasts that are used must be tolerant to high amounts of alcohol, low pH, as well as being able to ferment to dryness without producing undesirable byproducts.

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Alcoholic Fermentation

$$C_6H_{12}O_6 \rightarrow 2\ C_2H_5OH + 2\ CO_2 + \text{Energy}$$

Sugar Alcohol Carbon Dioxide Heat

- This is not as simple as it looks however the glucose molecule passes through 12 stable intermediate steps requiring enzymes, co-enzymes and inorganic catalysts, before being converted to alcohol.

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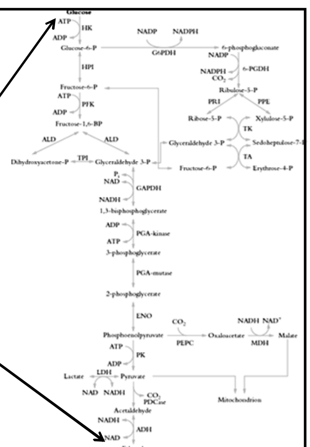
The biochemical pathway of alcoholic fermentation

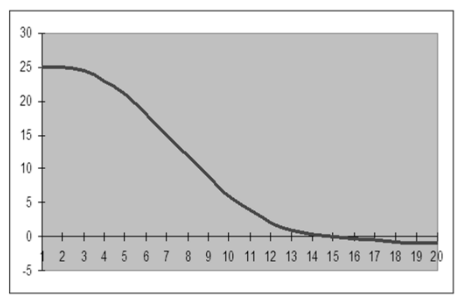
Glucose (sugar)

Ethanol (alcohol)



Fermentation Curve Brix vs. Time

ºBrix



Days

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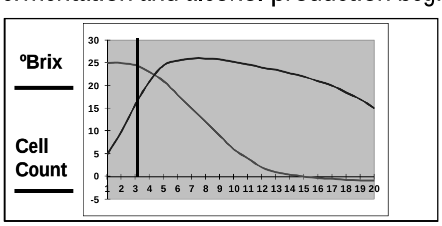
Alcoholic Fermentation

- Initially yeasts grow aerobically consuming the oxygen using the energy to grow. When the oxygen is consumed the yeast switch to fermentation and alcohol production begins.



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Influences on Fermentation

- Temperature
- pH
- Sulfur dioxide
- Nitrogen/nutrients
- Sugar
- Alcohol
- By controlling these parameters you can control fermentation.

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Influences on Fermentation

- **Temperature** the lower the temperature, the slower the rate of fermentation, (In whites the ideal rate is about 1°B per day, reds 3-4 °B at peak of fermentation)
- If it gets too cold the rate slows to almost nothing. If it gets too hot (95 to 100 °F) the yeast can be killed and the fermentation will stick (stop).

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Influences on Fermentation

- Fermentation also produces heat as a byproduct; if no heat is lost the temp rises 2.3 °F for each drop of 1°B. This is why cooling jackets are used on tanks.
- The growth rate of yeast will double for each rise of 10 °F.

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Influences on Fermentation

- **pH** Yeast and bacteria (including spoilage microbes) grow best at about 5.5 pH. To prevent spoilage keep must at 2.9 to 3.6 pH, yeast grow slower at lower pHs.
- **SO_2** Slows growth of all microbes and kills some. To discourage non-wine yeasts and ***lactobacillus*** add 30 to 50 PPM to the must.

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Nitrogen / nutrients

- Over-clarified musts and musts from stressed vines can be deficient in nutrients and or nitrogen. If you have less that 125 PPM YAN fermentation might stick.
- YAN is **Y**east **A**vailable **N**itrogen, a measurement of the must's ammonia and amino acids.

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Nitrogen / nutrients

- Fermentation aids can be added to alleviate this, DAP, yeast hulls, yeast extract, do not add urea because it is a precursor to urethane (ethyl carbamamate).
- Rule of thumb for nitrogen additions:

° Brix	Target YAN
21	200 ppm
23	250 ppm
25	300 ppm
27	350 ppm

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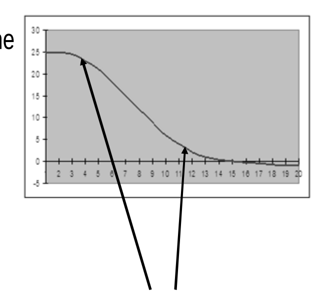
Nitrogen / nutrients

- Nutrients are best added at the end of the lag phase after the must has dropped a few degrees Brix, otherwise yeast can become attenuated to higher levels of nutrients and they are not as effective.



When to add nutrients

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Influences on Fermentation

- **Sugar**, high sugar levels (> 30°Brix) are inhibitory to yeast, this is why late harvest musts that are very sweet take a very long time to ferment (2 to 3 months).
- **Alcohol**, from 13% to 17%, depending on yeast strain can prevent fermentation. This attribute is used to make sweet fortified wines such as port.

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Alcohol conversion

- There is a formula that is used to estimate the amount of alcohol that is produced from a given amount of sugar:

Conversion Factor = % Alcohol / Degree Brix = about .60

so a 24ºBrix must will result in about 14.4% alcohol, this is if the wine is fermented dry

- So 24 ºB x 0.60 = 14.4%

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Alcohol conversion

- For years this was given as .55 but now we are seeing .59 to .61 Why? Better yeasts and paying more attention to nutrients.
- If you are pick very ripe flavors you can end up with so much alcohol that the wine tastes hot or cannot finish fermentation.
- Marginally lower alcohols can be achieved by using smaller inoculums or open top fermenters.

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Wine Yeast

- Pure strains used to inoculate must usually one of two different species:
- ***Saccharomyces cerevisiae*** is the most common, its name is Latin for “sugar fungus”.
- ***Saccharomyces bayanus*** is also widely used but not quite as popular as *Saccharomyces cerevisiae.*
- These species are also used for making bread

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Strains of yeast

- The relationship of strain to species is similar to variety of grape to vinifera.
- The selection of strain is not a major determination of flavor; rather the selection of strain is used to emphasize or reduce certain characteristics.
- Strains very in their ability to tolerate temperature extremes, fermentation to dryness, and production of foam.

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Strains of yeast

These are just a few of the many commercially available strains of yeast

- **Montrachet**. Good for Chardonnays, can produce H_2S and too much foam in barrels. UCD# 522
- **Pasture Champagne**. Strong fermenter rarely sticks. UCD# 595
- **CY3079** Good for Chardonnay
- **Pasture Red**. Good red fermenter well structured flavors.
- **Epernay 2**. Delicate qualities, easily cold shocked, low foam.
- **Prise de Mousse / Premier Cuvee**. Clean strong fermenter, **low** foam, popular with Chardonnays. UCD# 594 *Sacc. Bayanus*
- These strains were originally sourced from wineries

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Manufactures of yeast

- AEB Group www.aeb-group.com
- American Tartaric Products www.americantartaric.com
- Gusmer Entreprises www.gusmerenterprises.com
- Lallemand www.lallemand.com
- Scott Laboratories www.scottlab.com
- Vinquiry Windsor www.vinquiry.com
- White Labs www.whitelabs.com
- Winetech www.winetech.us
- Wyeast Laboratories www.wyeastlab.com
- These producers each carry many different types of yeast selected for individual types of wine. More info is available at their websites and at the *Wine Business Monthly* website

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Rehydrating Active Dry Yeast

- Dehydrated yeast cells that are in suspended animation. Easiest to use and very stable. Looks like bakers yeast.
- Re-hydrate in warm water (100 to 110°F) for 10 minutes then add to tank at the rate of 1 to 2 pounds per 1000 gallons.

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Rehydrating Active Dry Yeast

- Properly rehydrating the yeast is **critical** to a healthy fermentation.
  - Don't beat them up by over mixing
  - Don't temperature shock them, let them come to within 10ºF of the must before inoculating.
  - Follow directions
- The dry yeast is stable for several months if it is stored in a cool place, you can use unopened packages from a previous year.

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Liquid Culture Yeast

- Cultures of active living cells that are grown up (propagated) into a larger volume before inoculating a tank. Volume of inoculum should be 1%.
- Juice used for starter culture must have no SO_2 be diluted to 10°Brix and kept warm (80 to 90°F) When cell concentration reaches 1 million cells/ml then it is ready for inoculation.
- Not used by most wineries.

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Encapsulated Yeast

- Encapsulating yeast cells within a sodium alginate gel (natural polysaccharide extracted from seaweed) enables substrates and metabolites to diffuse throughout the gel without releasing the *Saccharomyces* cells into the must or juice. Sold in bags.

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Encapsulated Yeast

- The beads are placed in mesh bags and then lowered into a tank for fermentation.
- To stop the fermentation simply remove the yeast, works well but ***Expensive***!

Encapsulated Yeast in a sparkling Wine Bottle

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Natural Fermentation

- A growing trend is the oldest method of fermentation, allowing the native yeast on the grape skins to ferment the juice.
- ***Sacc. cerevisiae*** is present in small quantities on grape skins and if no SO_2 is added to inhibit their growth the other yeast present start the fermentation and then die off as the alcohol level rises to several percent, then *Sacc.* takes over.

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Natural Fermentation

- I've tasted many experiments with natural vs. commercial yeast and the results are mixed, sometimes the natural is the best, usually it's not. Most wineries don't take the risk so they use commercial yeasts.
- However, some wineries love natural yeast and use it as part of their winemaking style and for marketing promotion. This is done at many wineries that make organic wines.

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Stopping Fermentations

- If you wish to make a sweet wine one of the best ways to do it is to stop the fermentation when the Brix has reached the desired level. This is done by either removing the yeast or stopping their growth.
- Removing the yeast can be done by filtration or centrifuging.

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Stopping Fermentations

- To slow or stop yeast growth:
  - Chill to just above freezing.
  - Add SO_2.
  - Add bentonite.
- This works best on strains that are not tolerant to low temperatures such as Epernay II (Côte des Blancs)

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Stuck Fermentations:

- A stuck fermentation is one that stops before going to dryness when you don't want it too. It can result from:
  - Too much heat.
  - Too cold.
  - Over clarified or low nutrients.
  - Too much sugar to ferment dry.

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Stuck Fermentations:

- To prevent this add nutrients, use vigorous fermenters, and if it does happen re-inoculate don't adjust with SO_2 and keep warm, ***be careful because this also encourages spoilage***.
- This can be a big problem with high Brix grapes. High sugar at harvest can make a wine that is high alcohol with RS.

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High Brix Grapes

- If dehydrated grapes make the must is too sweet for fermentation water can be added to lower the sugar to a point the must will ferment to dryness.
- We will spend more time on this when we go over wine additives.

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Stuck Fermentations:

- If something is preventing your yeast from completing fermentation, take care of the problem (lower alcohol or adjust temperature) before reinoculating.
- If the alcohol level is to high, you can lower the alcohol on a portion of the wine and then use that portion for restarting the fermentation.
- Stuck fermentation procedure

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Malolactic Fermentation (MLF)

- The second type of fermentation that commonly takes place in winemaking is malolactic fermentation that converts the malic acid in wine to lactic acid.
- We will cover this in lecture in two weeks.

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Next Week

- Lab tomorrow at the Shone Farm
- Next week:
  - Grape harvest and white wine fermentation.

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