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**February/March 2016 Teacher's Guide for**

***Kombucha: Something’s Brewing***

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# About the Guide

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Articles from past issues of *ChemMatters* can be accessed from a DVD that is available from the American Chemical Society for $42. The DVD contains the entire 30-year publication of *ChemMatters* issues, from February 1983 to April 2013.

The *ChemMatters* DVD also includes Article, Title and Keyword Indexes that covers all issues from February 1983 to April 2013.

The *ChemMatters* DVD can be purchased by calling 1-800-227-5558.

Purchase information can be found online at [www.acs.org/chemmatters](http://chemistry.org/chemmatters/cd3.html).

# Student Questions

**(taken from article)**

* 1. How is kombucha prepared?
	2. What are the main bacteria found in probiotics?
	3. What do probiotics actually *do* to aid digestion in the gut?
	4. How are probiotics in the digestive system thought to influence the nervous system?
	5. What are the principle chemical steps that create kombucha?
	6. In the fermentation process, what causes a pH change in the mixture?
	7. What is the value in having an acidic, low pH kombucha?
	8. In the preparation of kombucha, SCOBY is needed. What is SCOBY?
	9. Define the term “functional beverage”.

# Answers to Student Questions

**(taken from article)**

* + 1. **How is kombucha prepared?**

*Kombucha is a drink made by fermenting tea containing sugar, yeast, and probiotic bacteria*

* + 1. **What are the main bacteria found in probiotics?**

*The two most common species of bacteria are* Lactobacillus *(found in yogurt) and* Bifidobacterium *present in some dairy products.*

* + 1. **What do probiotics actually *do* to aid digestion in the gut?**

*“Probiotics turn undigested carbohydrates into smaller molecules, such as organic acids, amino acids, vitamins, and enzymes, which are used by muscle, liver, and intestine cells for energy and nutrients.”*

* + 1. **How are probiotics in the digestive system thought to influence the nervous system?**

*Some studies suggest that probiotics release neurotransmitters which cause epithelial cells in the intestine to release certain molecules that travel to the brain. In the brain, other neurotransmitters return to the intestine where they may influence emotions and decision- making.*

* + 1. **What are the three principle chemical steps that create kombucha?**
1. *First, yeast breaks down the sucrose (sugar) into glucose and fructose.*
2. *These two sugars undergo fermentation by the yeast, producing alcohol.*
3. *Some of the alcohol is also converted to acetic acid by bacterial action.*
	* 1. **In the fermentation process, what causes a pH change in the mixture?**

*Bacteria in the kombucha mixture metabolize sugars into lactic acid. This acid along with the acetic acid produced from the alcohol make the mixture more acidic, lowering the pH to 2.*

* + 1. **What is the value in having an acidic, low pH kombucha?**

*The low pH prevents the growth of certain types of bacteria associated with food spoilage, including* Listeria, Clostridium and Salmonella*.*

* + 1. **In the preparation of kombucha, SCOBY is needed. What is SCOBY?**

*SCOBY is an acronym for a “symbiotic culture of bacteria and yeast”, which turns sweet tea into kombucha through fermentation.*

* + 1. **Define the term “functional beverage”.**

*A functional beverage, such as kombucha, is a “non-alcoholic drink that contains vitamins, amino acids, and other nutrients with health benefits.”*

# Anticipation Guide

Anticipation guides help engage students by activating prior knowledge and stimulating student interest before reading. If class time permits, discuss students’ responses to each statement before reading each article. As they read, students should look for evidence supporting or refuting their initial responses.

**Directions: *Before reading***, in the first column, write “A” or “D,” indicating your agreement or disagreement with each statement. As you read, compare your opinions with information from the article. In the space under each statement, cite information from the article that supports or refutes your original ideas.

|  |  |  |
| --- | --- | --- |
| **Me** | **Text** | **Statement** |
|  |  | 1. Probiotic bacteria turn undigested carbohydrates into smaller molecules.
 |
|  |  | 1. Studies have shown that probiotics reduce allergies and improve oral health.
 |
|  |  | 1. No link has been found between probiotics and brain activity.
 |
|  |  | 1. Yeast can oxidize alcohols to acids.
 |
|  |  | 1. The pH of the human stomach is about 4.
 |
|  |  | 1. High acid levels in the stomach encourage the growth of bacteria that cause food to rot.
 |
|  |  | 1. You can safely brew kombucha at home if you follow good laboratory habits.
 |
|  |  | 1. Kombucha gets its fizz from carbon dioxide.
 |
|  |  | 1. Kombucha contains several different kinds of beneficial nutrients.
 |
|  |  | 1. Bacteria in our intestines is an example of a symbiotic relationship.
 |

# Reading Strategies

These graphic organizers are provided to help students locate and analyze information from the articles. Student understanding will be enhanced when they explore and evaluate the information themselves, with input from the teacher if students are struggling. Encourage students to use their own words and avoid copying entire sentences from the articles. The use of bullets helps them do this. If you use these reading and writing strategies to evaluate student performance, you may want to develop a grading rubric such as the one below.

|  |  |  |
| --- | --- | --- |
| **Score** | **Description** | **Evidence** |
| 4 | Excellent | Complete; details provided; demonstrates deep understanding. |
| 3 | Good | Complete; few details provided; demonstrates some understanding. |
| 2 | Fair | Incomplete; few details provided; some misconceptions evident. |
| 1 | Poor | Very incomplete; no details provided; many misconceptions evident. |
| 0 | Not acceptable | So incomplete that no judgment can be made about student understanding |

***Teaching Strategies:***

1. Links to **Common Core Standards for Reading**:
	1. ELA-Literacy.RST.9-10.1:Cite specific textual evidence to support analysis of science and technical texts, attending to the precise details of explanations or descriptions.
	2. ELA-Literacy.RST.9-10.5: Analyze the structure of the relationships among concepts in a text, including relationships among key terms (e.g., force, friction, reaction force, energy).
	3. ELA-Literacy.RST.11-12.1:Cite specific textual evidence to support analysis of science and technical texts, attending to important distinctions the author makes and to any gaps or inconsistencies in the account.
	4. ELA-Literacy.RST.11-12.4: Determine the meaning of symbols, key terms, and other domain-specific words and phrases as they are used in a specific scientific or technical context relevant to grades 11-12 texts and topics.
2. Links to **Common Core Standards for Writing**:
	1. ELA-Literacy.WHST.9-10.2F: Provide a concluding statement or section that follows from and supports the information or explanation presented (e.g., articulating implications or the significance of the topic).
	2. ELA-Literacy.WHST.11-12.1E: Provide a concluding statement or section that follows from or supports the argument presented.
3. **Vocabulary** and **concepts** that are reinforced in this issue:
	1. Chemistry and Health
	2. Evaluating scientific claims
	3. Hydrophobic and hydrophilic substances
	4. Structural formulas
	5. Chemical engineering
	6. Intermolecular forces
4. **“Open for Discussion”** on page 4 of this issue provides excellent information about why different scientific studies might yield different results. You might consider relating this information to the articles in this issue about salt in food, kombucha, and e-cigarettes. Students can compare the different types of studies (randomized controlled trials and observational studies) to help them decide what information they need to make informed choices.
5. To help students engage with the text, ask students which article **engaged** them most and why, or what **questions** they still have about the articles. The Background Information in the *ChemMatters* Teachers Guide has suggestions for further research and activities.

**Directions:** As you read the article, complete the graphic organizer below to describe probiotics.

|  |  |
| --- | --- |
| Examples **Probiotics** | Where are they found? |
| How are they helpful? | Non-examples |

* **Summary:** After reading this article, write a tweet (140 characters maximum) to a friend describing what you learned about kombucha.

# Background Information

**(teacher information)**

**More on fermentation**

 The Mayo Clinic Web site provides this definition of and further information about kombucha:

Kombucha tea is a fermented drink made with tea, sugar, bacteria and yeast. Although it's sometimes referred to as kombucha mushroom tea, kombucha is not a mushroom — it's a colony of bacteria and yeast. Kombucha tea is made by adding the colony to sugar and tea and allowing the mix to ferment. The resulting liquid contains vinegar, B vitamins and a number of other chemical compounds.

Proponents claim kombucha tea can stimulate the immune system, prevent cancer, and improve digestion and liver function. However, there's no scientific evidence to support these health claims.

There have, however, been reports of adverse effects, such as stomach upset, infections and allergic reactions in kombucha tea drinkers. Kombucha tea is often brewed in homes under nonsterile conditions, making contamination likely. If ceramic pots are used for brewing, lead poisoning might be a concern — the acids in the tea may leach lead from the ceramic glaze.

In short, there isn't good evidence that kombucha tea delivers on its health claims. At the same time, several cases of harm have been reported. Therefore, the prudent approach is to avoid kombucha tea until more definitive information is available.

(<http://www.mayoclinic.org/kombucha-tea/expert-answers/faq-20058126>)

The following preface to a book on kombucha illustrates the inherent safety issues with making the drink.

It is imperative to use good judgment when consuming kombucha and to never consume any kombucha that looks, tastes, or smells unpleasant. Further, before consuming kombucha or any other fermented or cultured food, you should receive full medical clearance from a licensed physician. Author and publisher claim no responsibility to any person or entity for any liability, loss, or damage caused or alleged to be caused directly or indirectly as a result of the use, application, or interpretation of the material in this book.”

<http://www.culturesforhealth.com/media/docs/Kombucha_eBook.pdf>

Homemade kombucha with SCOBY

*(*[*http://theprimalparent.com/2012/06/06/history-and-benefits-of-fermented-foods/*](http://theprimalparent.com/2012/06/06/history-and-benefits-of-fermented-foods/)*)*

Kombucha brew at 12 days

*(*[*http://www.apartmenttherapy.com/how-to-brew-your-own-kombucha-83972*](http://www.apartmenttherapy.com/how-to-brew-your-own-kombucha-83972)*)*

The word "fermentation" is derived from the Latin meaning "to boil", since the bubbling and foaming of early fermenting beverages seemed closely akin to boiling. People have been using the natural process of fermentation for eons in human history (since Neolithic times) to produce such items as fermented drinks (wine and beer) that provided a bacteria-safe drink, bread products, cheese, and a number of vegetables preserved through fermentation. The fermentation mix often contains either salt or sugar in an acid medium (due to lactic acid-producing bacteria) which controls or eliminates food rot by bacteria. The microbes, such as yeast, used for fermentation grow in (or on) a specially-designed growth medium that supplies the nutrients required by the organisms.

The growth medium invariably contains a carbon source, a nitrogen source, water, salts, and micro-nutrients. The importance of the nitrogen source is to provide the chemicals needed by the microbes to synthesize protein, nucleic acids, and other cell structural components. It is essential with any fermentation to ensure that only the desired bacteria, yeasts or molds start to multiply and grow on the substrate. This has the effect of suppressing other micro-organisms which may be either pathogenic and cause food poisoning or will generally spoil the fermentation process, resulting in an end-product which is neither expected nor desired.

Generally, fermentation results from the activity of molds, yeasts, or bacteria working singularly or together, chemically digesting (enzymatically) a variety of substrates. Enzymes act by hydrolysis, a process of breaking down or predigesting complex organic molecules to form smaller (and, in the case of foods, more easily digestible) compounds and nutrients. For example, the enzyme protease breaks down large protein molecules first into polypeptides and peptides, and then into numerous amino acids, which are readily assimilated by the body. The enzyme amylase works on carbohydrates, reducing starches and complex sugars to simple sugars. And the enzyme lipase hydrolyzes complex fat molecules into simpler, free fatty acids. These are but three of the more important enzymes.

Fermented foods often have numerous advantages over the raw materials from which they are made. As applied to soy foods for instance, fermentation not only makes the end product more digestible, it can also create improved flavor (in many cases, meat-like flavor), texture, appearance and aroma. Fermentation can also result in increased levels of vitamins in the final product, including B-12, which is difficult to get in vegetarian diets. Fermentation can increase storage life, transform what might otherwise be agricultural wastes (such as okara, a soy pulp) into tasty and nutritious human foods (such as okara tempeh), and replenish intestinal microflora (as with miso or Acidophilus soymilk).

The rate of fermentation depends on the concentration of microorganisms, cells, cellular components, and enzymes, as well as temperature and pH—and, for aerobic fermentation, oxygen concentration. The great majority of these microorganisms come from a relatively small number of genera; roughly eight genera of molds, five of yeasts, and six of bacteria. Some of the more common substrates contain different sugars and a complex carbohydrate polymer, cellulose, found in plants. Micro-organisms contain certain enzymes, such as cellulases, which are incapable of being synthesized by humans. Microbial cellulases hydrolyze cellulose into sugars, which are then readily digestible by humans. Nearly all commercially produced enzymes, such as lipase, invertase and rennet, are made by fermentation with genetically modified microbes. The yeast, *Saccharomyces cerevisiae,* is able to concentrate large quantities of thiamin, nicotinic acid and biotin and thus form enriched products. Other specific yeasts, such as brewer’s yeast and wine yeast, are used to convert plant sugars into ethyl alcohol.

Brewer's yeast tolerate up to about 5% alcohol. Beyond this alcohol level. The yeast cannot continue fermentation. Wine yeast on the other hand tolerates up to about 12% alcohol. The level of alcohol tolerance by yeast varies from 5% to about 21% depending on yeast strain and environmental conditions. The fermentation process has limits such as temperature. Greater than 27C kills the yeast; less than 15C results in yeast activity which is too slow.

Not all sugars are fermentable. Non fermentable sugars in solution will remain after fermentation and will result in a sweeter end product. Malt has non fermentable sugars which can be used to balance the bitterness of the hops. The amount of sugar in the solution can be too much and this can prevent fermentation. Some wine recipes suggest adding the sugar in parts throughout fermentation rather that all at the beginning. This is especially true if the brew is aimed at producing a high level of alcohol. Some yeast strains have evolved to handle higher sugar levels. Yeast such as Tokay and Sauterne handle high levels of sugar

(<http://www.yobrew.co.uk/fermentation.php>)

**More on** **types of fermented foods**

Something like vinegar is produced through yeast fermentation of sugar which produces ethyl alcohol. Certain bacteria in the mix, notably *Acetobacter aceti*, then metabolize the alcohol into end products of acetic acid and water. Cocoa is produced through fermentation of the cocoa beans which are collected into piles or in large boxes. Vanilla is produced by the fermentation of the vanilla bean or pod (found on a certain orchid plant) in Madagascar, Indonesia, and certain South Pacific islands. The pods are first sun dried for 24 to 36 hours and then blanched in hot water (65° C) for two to three minutes. The pods are then fermented in boxes and dried again.

Traditional fermented foods play a very important role in East Asia food systems. These fermented foods have a number of important distinguishing characteristics. A number of the most important fermentations use molds—dairy products and other animal proteins (excepting fish) are not widely used, as they are in the West. And modern fermentation processes and technology are based largely on traditional processes, yet are extremely advanced and sophisticated. The main use of molds has been in the process of making koji (mold-fermented grains and/or soybeans). The koji making process has been unique to East Asia, where it has been used in the preparation of fermented foods such as miso, soy sauce (shoyu), soy nuggets, sake, shochu (spirits), tempeh, and rice vinegar (yonezu).

The only traditional East Asian fermented soy food not prepared with molds is Japan's natto and its equivalent in Thailand and Indonesia, where the fermentation process is bacteria-dependent. The earliest records of the koji-making process can be traced back to at least 300 B.C. in China and to the third century A.D. in Japan. What is interesting is that the growth in the mold producing process can be seen with the naked eye which of course is not true of bacteria and yeast. Therefore, the activity of mold in the fermentation process can be followed visually.

The Chinese have distinct names for two types of mold used in fermented soy foods, based on their color. What we know as *Aspergillus* (this class includes the source of penicillin) has the name translated from the Chinese to “yellow robe”, and *Rhizopus (*common bread mold*)* translates to “white robe”. An extensive illustrated list of fermented foods (135), a large number of which are from the East, is shown at <https://en.wikipedia.org/wiki/List_of_fermented_foods>. One rather interesting old process for making cod liver oil was to place the livers from cod fish in a wooden barrel, add seawater and let it ferment away for a year! Before fresh citrus fruit became more affordable and readily available, cod liver oil was the substitute in winter for Vitamin C.

In the West, a number of well-known foods are produced through fermentation, including sauerkraut (the Korean equivalent is called Kimchi), pickles, yogurt, sour cream, and various cheeses and, of course, bread. In addition, the basic process of fermentation is a very important process for producing pharmaceuticals, using genetically altered yeast or bacterial cells to produce a desired drug molecule that is then harvested from the fermentation tank.

Pictured: All the ingredients for making Asian sauerkraut called Kimchi. [Mak kimchi, or simple kimchi, is made with cut cabbage, radish, and scallions and a seasoned paste of red pepper, garlic, ginger, sugar, and fish sauce, salted shrimp, or kelp powder.]

*(*[*http://www.thekitchn.com/how-to-make-easy-kimchi-at-home-189390*](http://www.thekitchn.com/how-to-make-easy-kimchi-at-home-189390)*)*

**More on the specifics of making kombucha**

 There are many references available for making kombucha, mostly from amateur do-it-yourselfers. The commercial preparations have recently run into a potential marketing and regulating problem. Kombucha can contain between 1 and 2 % alcohol. The government is now studying the situation in terms of the sale of a product that contains alcohol. A number of commercial companies that supply food stores have had to withdraw their kombucha products from the stores that have requested it. In New Hampshire, a would-be commercial operation asked the state to rule on their product for sale because it contained alcohol. The State at first did not know about the product, let alone that it contained alcohol. Consulting several universities, the state suggested the kombucha producer apply for a winery license!

(<http://www.inc.com/articles/2010/small-kombucha-brewers-find-themselves-in-hot-water.html>)

Kombucha has inspired much polarized debate, with claims of dramatic curative properties matched by dire warnings of potential dangers. Kombucha is neither panacea nor peril…. Like any ferment, it contains unique metabolic by-products and living bacterial cultures that may or may not agree with you….

One common explanation for the healing power of kombucha is that it contains glucuronic acid, a compound produced in our livers, which binds with various toxins for elimination…. Unfortunately, repeated laboratory analysis has found that glucuronic acid is not actually present in kombucha. …

Other medical reports have associated extremely varied symptoms with kombucha consumption, also without identifying any specific toxicity or causative factor. Responding to a flurry of questions following the CDC [Centers for Disease Control and Prevention] report, the US Food and Drug Administration (FDA) issued a warning of sorts, cautioning that the acidity of kombucha could potentially leach lead or other toxins from vessels, and that "home-brewed versions of this tea manufactured under non-sterile conditions may be prone to microbiological contamination." However, like other investigations, FDA microbial analysis found "no evidence of contamination.”

Kombucha is usually just sugar-sweetened tea, fermented by a specific community of bacteria and yeasts. Increasingly, creative kombucha makers have been giving kombucha exciting new twists by adding herb, fruit, or vegetable flavors. Typically these flavorings are added to kombucha for a secondary fermentation following a primary fermentation of just tea and sugar…. The secondary fermentation may be aerobic in an open wide-mouth vessel like the primary fermentation, or in a sealed or air-locked vessel. In an open vessel, the sweetened kombucha will likely develop a new mother on the surface, and growth will continue to be dominated by acetic acid organisms. In a sealed vessel (which could be the final bottle for serving, or not), the secondary ferment will yield more alcohol, as well as lactic acid.

(<http://www.splendidtable.org/story/making-kombucha-an-excerpt-from-the-art-of-fermentation>)

An excellent and well-illustrated article on making kombucha puts the various terms associated with the process into a visual “explanation”. Refer to <http://www.thekitchn.com/how-to-make-your-own-kombucha-scoby-cooking-lessons-from-the-kitchn-202596>.

**More on animal feeds from fermentation**

 One of the important animal feeds produced from fermentation is silage. This is a well- known process and has been used in agriculture for many years.

Using the same technique as the process for making sauerkraut, green fodder was preserved for animals in parts of Germany since the start of the 19th century. This gained the attention of a French agriculturist, Auguste Goffart of Sologne, near Orléans, who published a book in 1877 which described the experiences of preserving green crops in silos. Goffart's experience attracted considerable attention. The conditions of dairy farming in the USA suited the ensiling of green corn fodder, and was soon adopted by New England farmers. Francis Morris of Maryland prepared the first silage produced in America in 1876.[11] The favourable results obtained in the U.S. led to the introduction of the system in the United Kingdom, where Thomas Kirby first introduced the process for British dairy herds.

(<https://en.wikipedia.org/wiki/Silage>)

Forage that has been grown while still green and nutritious can be conserved through a natural ‘pickling’ process. Acetic, butyric and lactic acids are produced when the sugars in the forage plants are fermented by bacteria in a sealed container (‘silo’) with no air. Forage conserved this way is known as ‘ensiled forage’ or ‘silage’ and will keep for up to three years without deteriorating. Because the product can be stored, it provides a food source during parts of the year when fresh grazing material is no longer available because of either a dry or cold season. Depending on the raw material source, the silage can vary in its nutrient value. As a minimum, it is essential to provide a green fodder supplement to enhance rumen function for dairy cows.

Silage is essentially an anaerobic process. Green fodder undergoes anaerobic fermentation, which starts about 48 hours after the silo is filled, and converts sugars to acids. But before anaerobic fermentation starts, there is an aerobic phase in which the trapped oxygen is consumed. To maintain an anaerobic condition, the plant material is compressed to remove most of the air. Fermentation is essentially complete after about two weeks. The process is dependent on the activity of bacteria converting the cellulose of plant material into a fibrous product that contains both carbohydrates and protein, the latter being a key nutritional ingredient in the mix. In the past, the fermentation was produced by indigenous microorganisms. But today, some bulk silage is inoculated with specific microorganisms to speed fermentation or improve the resulting silage. Silage inoculants contain one or more strains of lactic acid bacteria, and the most common is *Lactobacillus plantarum*. Other bacteria used in inoculants include *Lactobacillus buchneri*, *Enterococcus faecium* and *Pediococcus* species. During fermentation, the silage bacteria also act on the cellulose and carbohydrates in the forage to produce volatile fatty acids (VFAs), such as acetic, propionic, lactic, and butyric acids. By lowering pH, these acids create a hostile environment for competing bacteria that might cause spoilage under anaerobic, but higher pH conditions.

Newly cut grass as rolls (eliminates air) to be put into plastic wrap for fermentation into silage.

*(*[*https://en.wikipedia.org/wiki/Silage*](https://en.wikipedia.org/wiki/Silage)*)*

[**NOTE**: The following two “More on…” sections originally appeared in the Teacher’s Guide accompanying the December 2015 *ChemMatters* article, Poppick, L. A Moldy Situation: Chemistry Cleans Up, *ChemMatters*, 2015, *33* (4), pp 8–9]

**More on** **fermentation and pharmaceuticals**

Large scale production of pharmaceuticals using the fermentation process developed from the original desire to make penicillin available in large quantities because of its need for treating infections of various kinds during World War II. Sir Arthur Fleming’s work with the penicillin mold extract showed it to be effective against pneumonia, diphtheria, gonorrhea, meningitis, and scarlet fever. But he found that it was difficult to cultivate penicillin and the small quantities produced were unstable.

Fleming also believed that penicillin would not remain in the human body long enough to kill bacteria. Taken together, these facts convinced Fleming that penicillin would never be an important antibiotic for treating infections. For the next decade, penicillin remained a laboratory curiosity, until Howard Florey and a team of researchers at Oxford University demonstrated its potential life-saving properties. The researchers also discovered a method to purify penicillin and to keep it in a stable form. By 1940, clinical trials were underway and their success led to the hunt for a method to manufacture this “wonder drug.”

(<http://www.acs.org/content/acs/en/education/whatischemistry/landmarks/penicillin.html>)

Large vat fermentation for the production of penicillin had its origins in the work of several chemists who figured out how to mass produce a number of chemicals through the fermentation process.

The botanist Carl Wehmer discovered in 1893 that the *Penicillium* mold could produce citric acid from sugar. Later, J.A. Martin discovered that fermenting sugar could yield citric acid. But these were ideas ahead of their time because no one knew how to manufacture citric acid from these sources on a commercial scale. That is, until James Currie, a food chemist, discovered that citric acid could be fermented from certain strains of the mold *Aspergillis niger* combined with sugar.

(<http://www.acs.org/content/acs/en/education/whatischemistry/landmarks/penicillin.html>)

The next step toward the eventual large-scale fermentation process for producing an antibiotic occurred when James Currie became involved with the Pfizer Company in 1921 to solve the technical problems associated with large scale production of citric acid from fungal fermentation. Currie had to figure out how to provide enough air for the aerobic *Aspergillis niger* mold, as well as controlling temperature, humidity and the quality of the mold spores. By 1929, the Pfizer Company and Currie were mass producing citric acid through large vat fermentation. This experience and, more importantly, the development of deep tank fermentation for producing gluconic acid formed the basis for eventually retooling deep tank fermentation to produce penicillin from the fermentation of the *Penicillium* mold.

The mass production of antibiotics began during World War II with penicillin and streptomycin. In the case of penicillin, it was necessary to develop an extraction process of the penicillin from the fermentation medium which proved to be difficult. The penicillin extract, which was only in a concentration of 4 parts drug per 10,000 of broth medium, was isolated from the growth medium by first freezing the mixture, then moving it to a vacuum drier which dehydrated the drug. After the war, Pfizer further refined the extraction process by using a crystallization procedure that eliminated various impurities of the wartime drug while producing a compound that was stable for years at room temperature.

Now most antibiotics are produced by staged fermentations in which strains of microorganisms producing high yields are grown under optimum conditions in nutrient media in fermentation tanks holding several thousand gallons. The mold is strained out of the fermentation broth, and then the antibiotic is removed from the broth by filtration, precipitation, and other separation methods.

(<http://www.infoplease.com/encyclopedia/science/antibiotic-production-antibiotics.html>)

**More on** **using fungi to produce other drugs and chemical compounds**

Yet one more example of the role of fungi in the world of pharmaceuticals is the recent announcement that genetically modified (GM) yeast cells have been programmed to produce a morphine-like drug called hydrocodone. Currently, most morphine comes from the poppy plant. The alkaloid-containing plant extract is harvested from the mature seed pod which oozes the thick liquid containing the morphine. The reprogrammed yeast ferment sugar into the hydrocodone, close in molecular structure to morphine. The research was done by Christina Smolke, a bioengineer at Stanford-UC who reprogrammed the baker’s yeast’s genetic code by inserting some 23 different genes from a number of plants, rats, and bacteria to control the multiple steps needed to convert sugar in the fermentation process to hydrocodone rather than to ethyl alcohol and carbon dioxide. At this stage of the research, it takes 20,000 liters of yeast cells to produce one dose of hydrocodone! The next step for the researchers is to boost the efficiency of the GM yeast. The yields of yeast-based painkillers need to rise 100,000 times to challenge traditional opium poppy farming.

(<http://www.theguardian.com/science/2015/aug/13/yeast-cells-genetically-modified-to-create-morphine-like-painkiller>)

Several other uses for genetically engineered yeast cells include the production of an anti-malarial drug that is now done on an industrial scale with an annual output of 70 million doses per year by the French pharmaceutical company, Sanofi. The researchers have both modified the yeast’s DNA as well as its metabolism. At the University of Queensland's Australian Institute for Bioengineering and Nanotechnology, yeast cells have been genetically modified to produce limonene, the oil found in citrus fruit such as lemons and oranges. This particular oil, which is a natural disinfectant, is also the base for jet fuel. But because the limonene is also a natural disinfectant, its production by yeast cells also kills the yeast at a certain concentration! So the researchers had to develop a technological fix to prevent the yeast cells from being killed. The fix included inserting a gene into the yeast cell that made the yeast more resistant to the effect of the limonene concentrations. They also removed the oil as it was being produced to keep the concentrations of limonene low enough for the yeast cells to survive.

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**More on lactic acid fermentation**

 Within the human digestive tract is a fermentation process that is utilized to generate energy from several chemicals normally associated with the energy conversion process called glycolysis. Glycolysis is the breakdown process for glucose and other sugars, which produces the chemical compound pyruvate. Glycolysis is the most universal form of energy metabolism in the living world, from yeast to humans. The pyruvate is an acceptor of electrons from the glucose (via the compound called NAD+ [nicotinamide dinucleotide]). The production of NAD+ is needed in the larger energy generating process involving ATP conversions (called respiration) within animal and plant cells. The pyruvate as a chemically reduced product is then converted to lactic acid (also called lactate) generating more of the needed NAD+. This process is basic to all fermentation activity under anaerobic conditions. It is of particular significance in human muscle tissue when the cells are stressed and low on ATP for energy. The lactate fermentation process then generates several new ATP molecules via NAD+ to try to compensate for the ATP deficiency. In the process, lactic acid is produced in human muscle cells for example, which, if produced in excess, causes pain in the muscles. The lactic acid is converted to carbon dioxide and water when more oxygen is taken up by the muscle cells, eliminating any more lactate fermentation.

**More on** **fermentation in ruminant animals**

 Ruminant animals include milk animals (cows, goats, and sheep), other cattle, water buffalo and camels, among others. These animals have a special series of four stomachs—the rumen, reticulum, omasum, and abomasum. Ruminants utilize the rumen for fermentation, a chemical digestive process to convert forage material into usable nutrients. Within the rumen is a chemical and biological environment that converts cellulolytic material (primarily plant cellulose) into useable nutrients including sugars, fatty acids, and protein. The grazing animals “grab” plant material by lips, teeth, and/ or tongue to put into their mouths, then send it down into a holding chamber. Later it is regurgitated to be further masticated (“chewing the cud”!) into much smaller fragments producing a much larger surface area collectively for chemical action of bacterial, protozoan, and fungal enzymes in a different chamber, the rumen.

Fermentative bacteria representing many genera provide a comprehensive battery of digestive capabilities. These organisms are often classified by their substrate preferences or the end products they produce. Although there is some specialization, many bacteria utilize multiple substrates. Some of the major groups, each of which contains multiple genera and species, are:

* Cellulolytic (digest cellulose)
* Hemicellulolytic (digest hemicellulose)
* Amylolytic (digest starch)
* Proteolytic (digest proteins)
* Sugar utilizing (utilize monosaccharides and disaccharides)
* Acid utilizing (utilize such substrates as lactic, succinic and malic acids)
* Ammonia producer
* Vitamin synthesizers
* Methane producers

<http://arbl.cvmbs.colostate.edu/hbooks/pathphys/digestion/herbivores/microbes.html>

Then there is a large collection of single-cell organisms other than bacteria which operate digestively with the bacteria above. Their relationship to the bacteria is symbiotic, with examples given below.

Protozoa, predominantly ciliates, appear to contribute substantially to the fermentation process. Several experiments have demonstrated that lambs and calves deprived of their ruminal protozoa show depressed growth rates and are relative "poor-doers" compared to controls with both bacteria and protozoa. In general, protozoa utilize the same set of substrates as bacteria and, as with bacteria, different populations of protozoa show distinctive substrate preferences. Many utilize simple sugars and some store ingested carbohydrate as glycogen.

<http://arbl.cvmbs.colostate.edu/hbooks/pathphys/digestion/herbivores/microbes.html>

The protozoa are actually predators to the bacteria in the rumen --- they eat the bacteria for dinner! Protozoa are about 40 times the size of rumen bacteria. The rumen protozoa produce fermentation end-products similar those made by the bacteria, particularly acetate, butyrate, and hydrogen. Rumen methane bacteria actually attach and live on the surface of rumen protozoa for immediate access to hydrogen. Rumen protozoa eat large amounts of starch at one time and can store it in their bodies. This may help to slow down the production of acids that lower rumen pH, benefiting the rumen.

Rumen protozoa multiply very slowly in the rumen --- over 15-24 hours – as opposed to the bacteria that may take as little as 13 minutes to multiply. For this reason, the rumen protozoa hide out in the slower moving fiber mat of the rumen so that they aren’t washed out before they have a chance to multiply. Low roughage diets reduce the retention of fiber in the rumen and may decrease the number of protozoa in a cow’s rumen.

(<http://www.milkproduction.com/Library/Scientific-articles/Animal-health/Rumen-Microbiology/>)

It is here in this fermentation chamber that a combination of the correct pH (6–6.8), temperature and microbial digestive enzymes convert the cellulose, a polysaccharide, into smaller sugar molecules, primarily glucose.



*(*[*http://www.biotek.com/resources/articles/enzymatic-digestion-of-polysaccharides-2.html*](http://www.biotek.com/resources/articles/enzymatic-digestion-of-polysaccharides-2.html)*)*

It is also the chamber in which volatile fatty acids (VFA) are produced in large amounts through ruminal fermentation and are of paramount importance in that they provide greater than 70% of the ruminant's energy supply. Virtually all of the acetic, propionic and butyric acids formed in the rumen are absorbed across the ruminal epithelium, from which they are carried by ruminal veins to the portal vein and hence through the liver. Continuous removal of VFA from the rumen is important not only for distribution, but to prevent excessive and damaging drops in pH of rumen fluid.

The three major VFA absorbed from the rumen have somewhat distinctive metabolic fates:

* **Acetic acid** is utilized minimally in the liver, and is oxidized throughout most of the body to generate ATP. Another important use of acetate is as the major source of acetyl CoA for synthesis of lipids.
* **Propionic acid** is almost completely removed from portal blood by the liver. Within the liver, propionate serves as a major substrate for gluconeogenesis, which is absolutely critical to the ruminant because almost no glucose reaches the small intestine for absorption.
* **Butyric acid**, most of which comes out of the rumen as the ketone beta-hydroxybutyric acid, is oxidized in many tissues for energy production.

(<http://arbl.cvmbs.colostate.edu/hbooks/pathphys/digestion/herbivores/rum_absorb.html>)

Here is a breakdown of milk content, using a 200 pound milk goat as an example of what quantities of VFAs are involved in the production of milk itself …

… for 1570 kg of milk in a 305 day lactation. The milk was roughly 4% lactose, 3.5% protein and 3.6% fat. This means that, for the sole task of producing milk, this goat has to synthesize about 250 grams of lactose and 180 grams of protein and 185 grams of fat every day.

Essentially all the glucose in that lactose was synthesized in the liver and most of that synthesis was from propionic acid generated by fermentation. Likewise, much of the fat was synthesized from ruminal acetate. When you consider that synthesis of lactose and milk fat are only two of many, many processes that are supported by volatile fatty acids, the process of fermentation in herbivores gains new meaning.

(<http://arbl.cvmbs.colostate.edu/hbooks/pathphys/digestion/herbivores/rum_absorb.html>)

Ruminants produce prodigious quantities of saliva. Published estimates for adult cows are in the range of 100 to 150 liters of saliva per day! Aside from its normal lubricating qualities, saliva serves at least two very important functions in the ruminant:

* provision of fluid for the fermentation vat,
* alkaline buffering—saliva is rich in bicarbonate, which buffers the large quantity of acid produced in the rumen and is probably critical for maintenance of rumen pH.

# Connections to Chemistry Concepts

**(for correlation to course curriculum)**

* 1. **pH**—Kombucha is a great example of pH in our everyday world.A range of pH conditions in fermenting of food and other substrates determines (selects out) what microbes will exist to provide the fermenting action. In the making of kombucha, a low pH eliminates bacteria that would otherwise cause the vegetable to rot.
	2. **Acid-base chemistry**—Kombucha produces acids through fermentation that lower its pH and thus preserves it from bacterial decay. And the kombucha tastes sour, like acids!
	3. **Concepts in fermentation**—These three topics in the chemistry curriculum could be areas in which the process of fermentation could be discussed.
	a. Chemical equations—reactions in the fermentation process
		1. Limiting reagents—fermentation reactants
		2. Reaction rates—what factors affect fermentation
	4. **Enzymes as catalysts**—Enzymes that are often produced by bacteria and fungi are crucial to the digestion process in fermentation. A discussion of enzymes could include information about active sites and the specificity of certain enzymes.
	5. **Organic chemistry**—The article includes brief discussions of alcohols, organic acids and carbohydrates.

# Possible Student Misconceptions

**(to aid teacher in addressing misconceptions)**

1. **“Alcohol is made by the distillation process.”** *Alcohol is produced through the fermentation process, not distillation. Distillation is a procedure used to separate alcohol from the water in the fermentation mix, using heat to boil off the alcohol first because it has a lower boiling point than the water.*

# Anticipating Student Questions

**(answers to questions students might ask in class)**

1. **“Is it true that kombucha may contain alcohol?”** *It is true that some kombucha products contain 1–2% alcohol, depending on how it is made. This has become an issue for regulatory agencies within state governments to manage.*
2. **“Is kombucha safe to drink, since it contains bacteria and fungi of various kinds?”** *The commercial kombucha products are safe to drink since they are inspected for the presence of specific bacteria and yeast. They may also be pasteurized which kills germs, thereby eliminating the benefits of living probiotics (they’re dead now). But homemade kombucha runs the risk of containing certain bacteria that can cause digestive tract problems if not detected by the “brewer” while the drink is fermenting.*

## In-Class Activities

**(lesson ideas, including labs & demonstrations)**

1. It is not recommended that you have students make kombucha as a lab exercise or even as an “at home” exercise. To utilize the fermentation process as found in making kombucha however, students could make sauerkraut in the lab. After first making sauerkraut the standard way, students could experiment with a number of variables that may affect both the type of product as well as the rate of reaction—varying temperature, pH, concentration of salt, and the addition of sugar and/or yeast. They could also examine samples of the starting and final liquid for bacteria and fungi. Refer to <http://www.thekitchn.com/how-to-make-homemade-sauerkraut-in-a-mason-jar-193124> for the basics of making sauerkraut. Also, there are two other Web sites that provide guidance into investigating the variables in fermenting cabbage: <http://www.jlindquist.net/generalmicro/324sauerkraut.html> (includes procedure for evaluating bacteria and progress of pH change, using titration), and the second site for the investigation of sauerkraut is at the Web site listed below, item #2. (<http://www.ncbe.reading.ac.uk/NCBE/PROTOCOLS/fermentation.html>)
2. You could use fermentation as a way to introduce or experiment with reaction rates, variables, and limiting reagents. An excellent reference on several types of fermentation is found at <http://www.ncbe.reading.ac.uk/NCBE/PROTOCOLS/fermentation.html>. A full-color student version and teacher version pdf file can be downloaded from the site. It contains a series of 14 experiments on the topic of fermentation.
3. A lab exercise for making Korean cabbage (kimchi) that uses fermentation, as in making kombucha, can be found at <http://www.thekitchn.com/how-to-make-easy-kimchi-at-home-189390>.
4. An experiment preview of a Vernier lab that uses a gas pressure sensor to test for lactase action is available for download. The preview does not include safety information, instructor background and tips, and solution preparation directions. The full lab is available in the Biology with Vernier lab manual. (<http://www.vernier.com/files/sample_labs/BWV-24B-COMP-lactase_action_GPS.pdf>)
5. Another lab exercise which illustrates the effects of variables (temperature, concentration of substrate, presence of enzymes, pH) on the rate of reaction is to make yogurt. A procedure for making yogurt that is well illustrated is found at <http://biology.clc.uc.edu/fankhauser/Cheese/yogurt_making/YOGURT2000.htm>.
6. The study of an enzyme-dependent reaction involving the conversion of the milk sugar lactose to glucose and galactose can be done by students following the instructions found at <http://www.learnnc.org/lp/pages/3398>. This is associated with the problem of lactose intolerance in some people, maybe including some of your students.

# Out-of-Class Activities and Projects

**(student research, class projects)**

1. Students might find it interesting to research the issue of lactic acid buildup in muscle during exercise, why it occurs (biochemistry), and what biological (“survival”) function it serves. The biochemical process, a fermentation reaction, can be compared with fermentation by the lactobacillus in cheese making. It can also be compared with fermentation by yeast with ethyl alcohol as a product rather than lactic acid.

# References

**(non-Web-based information sources)**

**30 Years of *ChemMatters***

Available Now!

**The references below can be found on the *ChemMatters* 30-year DVD (which includes all articles published during the years 1983 through April 2013 and all available Teacher’s Guides, beginning February 1990). The DVD is available from the American Chemical Society for $42 (or $135 for a site/school license) at this site:** [http://ww.acs.org/chemmatters](http://www.acs.org/chemmatters)**. Click on the “Archive” tab in the middle of the screen just under the *ChemMatters* logo. On this new page click on the “Get 30 Years of *ChemMatters* on DVD!” tab at the right for more information and to purchase the DVD.**

**Selected articles and the complete set of Teacher’s Guides for all issues from the past three years are available free online at the same Web site, above. Simply access the link and click on the aforementioned “Archive” tab.**

 Holzman, D. Penicillin. *ChemMatters*,1987**,** *5* (2), pp 10–12. This thorough article discusses the history behind the development of penicillin and its derivatives after Sir Alexander Fleming’s discovery of penicillin. It includes some discussion of the chemistry behind modifying the original (natural) penicillin structure to produce other analogs of the basic penicillin molecule.

 Evans, G. Yogurt. *ChemMatters*, 1989, *7* (3), pp 9–12. This article provides the chemical and biological details for what happens in the making of yogurt. There is a recipe for making Greek yogurt from regular yogurt. An interesting introduction about the history of making yogurt, including the very special recipe from the Mongols who mix horse blood with the milk concoction that becomes their yogurt can be found here!

 Goldfarb, B. Fascinating Fungi. *ChemMatters*,1998**,** *16* (4), pp 7–8. This article provides an overview of fungi which includes both their usefulness as well as their source of diseases in the plant world.

 The topic of lactose intolerance illustrates fermentation in humans which, because of gas production, produces an unpleasant condition. This article discusses this type of fermentation in the human digestive system: Rohrig, B. Not Milk?—Living with Lactose Intolerance. *ChemMatters*,2013, *31*(2), pp 18–19.

# Web Sites for Additional Information

**(Web-based information sources)**

**More sites on** **making kombucha**

 This book about making kombucha has very clear instructions with illustrations:

<http://www.culturesforhealth.com/media/docs/Kombucha_eBook.pdf>.

A second reference that provides a well-illustrated method for producing kombucha is found at <http://www.apartmenttherapy.com/how-to-brew-your-own-kombucha-83972>.

 A more detailed site about the history behind kombucha and a more scientific discussion of the variables in the process of making kombucha is found at <http://www.splendidtable.org/story/making-kombucha-an-excerpt-from-the-art-of-fermentation>.

**More sites on making fermented foods**

A very comprehensive and detailed document about how various fermented foods are produced in many different societies around the world is found at <http://www.fao.org/docrep/x0560e/x0560e06.htm>.

 Another document that explains how various food sources are converted to a different product through fermentation is found at <http://www.soyinfocenter.com/HSS/fermentation.php>.

 A reference that contains many recipes for producing pickled or fermented foods, many with an Oriental flavor or origin, is found here: <http://www.thekitchn.com/how-to-make-easy-kimchi-at-home-189390>.

**More sites on ruminant physiology**

 This site provides is a very complete series of documents that deal with the various aspects of fermentation in ruminant animals. This may be of interest to some students to find out about a cow’s physiology allows it to produce milk. (<http://arbl.cvmbs.colostate.edu/hbooks/pathphys/digestion/herbivores/overview.html>)