BREWING TECHNIQUES
THE ART AND SCIENCE OF HANDCRAFTED BREWING

Reinheitsgebot and the Fifth Ingredient

by Martin Schiller and Jim Busch

Republished from BrewingTechniques' May/June 1993 issue.

Invisible predators of sweet wort, bacteria are the brewer's nemesis. Some survive the boil and compete with yeast in the fermentor, imparting offensive aromas and off-flavors. The key to winning the war against bacteria is proper sanitization and pitching plenty of pure, fresh, healthy yeast.

The German beer purity law written in 1516 states that only three ingredients should be used to brew beer: malt, hops, and water. The world did not know of the existence of yeast until some 300 years later when Mitcherlich, Pasteur, and Buchner discovered the role of these living chemical microfactories in fermentation (1). Today, malt, hops, water, and yeast are the ingredients of pure beer.

The Fifth Beer Ingredient

The Germans also left out a fifth ingredient: bacteria. Bacteria are everywhere in our world, including your beer. Eight common genera of bacteria will grow in wort or beer, and these genera can be divided into two groups, wort-spoilage and beer-spoilage bacteria. Wort-spoilage bacterial contamination is caused primarily by low pitching rates, unhealthy yeast, impure starter cultures, or the introduction of large quantities of bacteria through unsanitary techniques. Genera causing wort spoilage and odors or flavors commonly associated with fermentation with these bacteria are *Obesumbacterium* (parsnip odor), *Aerobacter* (celery), and *Escherichia* (highly phenolic).

Beer-spoilage bacteria can be introduced during any phase of brewing; however, they grow and multiply at a quick rate after the yeast has created an acidic, anaerobic environment. Genera causing beer spoilage and odors or flavors commonly associated with fermentation with these bacteria are *Pediococcus* (diacetyl or ropiness), *Acetobacter* (vinegar), *Acetomonas* (apple or cidery), *Lactobacillus* (di-acetyl and lactic acid), and *Zymomonas* (rotten apples). Like yeast, bacteria are also living chemical microfactories, though the products of these factories can be foul, even in concentrations of parts per billion. Bacteria from some or all of these genera are present where you brew. To convince yourself that these bacteria are in your brewery, leave some spare wort open to the air at room temperature without adding yeast. After the bacteria "ferments" your wort, take a small taste. Unless you are attempting some classic Belgian styles or enjoy bacteria-fermented beer, these microorganisms are best left in the environment from which they came.

Minimizing Bacteria

Brewers sanitize all equipment and boil wort to kill bacteria. Unfortunately, sanitization is not sterilization. Sanitization means cleaning very well, but bacteria may still be present; sterilization kills all bacteria present. Boiling or treating with chlorine bleach, iodophor, caustics, and other sanitizing agents often fails to kill all bacteria. Some survive into the fermentor.

Four methods are available for sterilizing equipment or wort.

- autoclaving (heating under pressure; 15 psi for at least 20 min)

- exposure to high-intensity neutron or alpha-particle radiation
- filtering liquid through a special 0.2-micron filter
- exposure to ultraviolet light.

Few brewers have access to or interest in sterilization techniques, and sterilization is not really necessary.

When you brew beer you are fighting a war against bacteria. Taking precautions to let nothing touch the cooled wort unless it has been thoroughly sanitized and pitching with a correct quantity of healthy yeast will greatly enhance the odds of eliminating bacteria-derived flavors in your beers. From the moment your wort cools to /=65 degrees C), wort-spoilage bacteria that survived sanitation can begin to grow and multiply in the wort. Bacteria can reproduce every 20 min, even more quickly under optimal conditions. Yeast, on the other hand, take at least 1 h.

To minimize the introduction of bacteria, it is important to take precautions with anything that contacts cooled wort, including equipment, human hands, and even air. Siphoning hoses and lines, wort chiller lines exposed to wort, and the fermentation vessel can introduce bacteria to the wort; hands carry bacteria, necessitating the use of gloves when handling cooled wort; and if air rather than oxygen is injected into cooled wort, a 0.2-micron filter should be placed in-line to filter out airborne bacteria.

A healthy yeast starter with an adequate quantity of yeast cells is the best way to minimize bacterial growth. The brewer must introduce enough healthy pure yeast cells to quickly assimilate (change) the wort to an acidic, anaerobic environment. When this environment is achieved, any wort spoilage bacteria present will grow very slowly, if at all.

During fermentation, beer spoilage bacteria can grow, though the yeast cells will largely outnumber any bacteria and thus will outcompete them for available food. Pitching with large quantities of healthy yeast will minimize bacterial growth, obscuring the bacterial products from even the most critical taste buds. A vigorous initial fermentation by yeast quickly lowers the pH and uses up all of the wort's dissolved oxygen, creating the desired fermentation environment. Oxidized hops, grain husk astringency, and other undesirable flavors often encountered pale in comparison to those resulting from bacteria.

Preparing A Starter

Although convenient, commercially available yeast commonly used by home brewers and pub and microbrewers can sometimes produce bad beer, especially when incorrectly used. To my knowledge, no commercial sources provide the information critical to the preparation of starter cultures. These sources provide insufficient yeast (even when fresh) to ferment 5 gal of wort, necessitating the use of multiple yeast packages (2) or a starter culture to amplify the yeast. Although home brewers may be successful using one packet, the limited amount of yeast in one packet increases the odds that contamination will slip in and fluorish. Some commercial yeasts may even contain bacteria because they are prepared in unsterile conditions, and using multiple yeast packets may introduce more bacteria besides adding to costs. Preparing a starter culture from contaminated sources will amplify the bacteria as well as the yeast. Preparation, shipping, storage, and freshness of commercial yeast all affect the viability and health of the yeast and thus the quality of your beer. It is also important to prevent or minimize the addition of autolyzed yeast, which are cells that have died, broken open, and emptied their contents.

When yeast is pitched into wort, the yeast cells begin synthesizing sterols, a class of cell membrane molecules. Sterol synthesis at the beginning of fermentation alters cell membrane permeability and allows the yeast to begin transporting amino acids and then sugars from the wort into the interior of the cells. Once amino acids and sugars are inside the cell, they are degraded and their energy is extracted through catabolic metabolism, thus enabling the yeast to grow and multiply. (For further discussion on this topic, see reference 3.)

Synthesis of sterols at the onset of fermentation requires cellular lipids or fatty acids (fats), molecular oxygen, and energy. Yeast cells obtain energy from internal stores of glycogen -- the same energy storage molecule found in humans. When yeast is stored, it must be constantly fed wort sugars to maintain high glycogen levels.

Commercial yeast, once separated from wort for packaging, begins to deplete the reserves of glycogen over time. Stressful conditions such as large temperature variations also may cause yeast cells to sacrifice glycogen. For these reasons, yeast stored without feeding on wort may have little remaining energy stores, resulting in slow sterol synthesis and possible long lag times at the start of fermentation. Long lag times give wort-spoilage bacteria a chance to grow. It is therefore important to use a fresh, healthy yeast culture. Because oxygen is required in the last step of sterol synthesis (squalene oxidation), oxygenation of the cooled wort enhances the start-up of fermentation (3).

Some have argued that the risk of mutations of the yeast cells as they divide is a drawback to feeding the stored yeast. This is a concern in very large breweries because of very large yeast cell masses and constant repitching, which may eventually yield a mutation that affects brewing characteristics. Several factors make it rare for home brewers

to observe mutations in a yeast strain. DNA mutation rates are very low, and a high percentage of DNA mutations in yeast have no effect on the yeast cell. Also, some mutations in the DNA will kill the yeast cell, prohibiting it from dividing; such mutations are therefore never observed and have no consequence to the brewer. Finally, when yeast is stored in the refrigerator, the rate of growth (and hence DNA replication) is greatly reduced.

High Quality Yeast Sources

Two proper ways for brewers to obtain an adequate quantity of healthy yeast include

- obtaining yeast from a clean brewery pitched within three days after cropping.
- culturing your own yeast from petri dishes, slants, and sterilized media.

A clean brewery obtains its yeast from culturing -- growing cells in a prepared medium; culturing is the ultimate yeast source. Cropping is removing the yeast that settles in a cylindroconical fermentor (lager and ale strains) or that rises to the top of an open fermentor (ale strains). Breweries use cropped yeast to pitch their next brew. Home brewers may be able to obtain bottom- or top-cropped yeast from a local brewery. Lager yeast should not be used if successively cropped more than six times, and ale yeast should be used after multiple croppings only if the brewery is impeccably clean, which is not always the case.

To minimize yeast autolysis, yeast should be stored in the refrigerator and used within three days after cropping. Storing yeast for slightly longer periods of time is possible if the liquid from the top of the yeast slurry is decanted and replaced with fresh, clean wort. Breweries

typically pitch 0.5-1 lb of yeast slurry per barrel of wort, or about 2-4 oz of cropped yeast slurry is optimal for pitching 5 gal. Cropped yeast is superior to cultured yeast because glycogen and sterol levels are highest after cropping. Unfortunately for home brewers, brewery yeast may not be accessible or convenient for them to obtain.

Many breweries culture their own yeast, which is the best method for maintaining and propagating yeast. The reason that most home brewers do not culture their own yeast is convenience. Actually, manipulating and scaling up (amplifying) the yeast is easy and requires little time and attention (see Figure 1); however, preparation of the materials requires the use of sterile techniques to avoid amplifying bacteria at an early stage in culturing. For this reason, preparation of petri dishes, slants, and starter cultures with sterile media is important as well as difficult and time consuming. Materials must be prepared using one of the sterilization techniques described above. Because most home brewers have no access to these sterilizers, the culturing materials they prepare are often overrun by some persistent bacterium or fungus. The growth of microorganisms other than the intended yeast on the culturing material results in frustration and can ultimately cause home brewers and other small-scale brewers to revert to the use of commercial yeast.

Yeast culturing is now easy for even the first-time brewer because of the recent introduction of premade sterile culturing materials. At least three yeast culture companies supply sterile materials such as petri dishes for yeast purification, slants for yeast storage, and wort tubes for scaling up yeast; they also supply useful laboratory equipment such as inoculating loops and alcohol burners. All materials are sterilized, providing users a bacteria-free culturing medium. Instruction booklets and technical service numbers further ensure successful yeast culturing.

Although culturing practices vary, they all use the principles discussed below and shown in Figure 1. All transfers are made using an inoculating loop with sterile technique. A pure yeast strain is streaked out on a petri dish to form colonies (each originating from one yeast cell). One or several colonies are inoculated into sterile media. After a growth period of 1-3 days, this media is used to make a seed culture in 15% glycerol, which is then stored at -70 degrees C (-94 degrees F). Seed stocks are best maintained by a culturing facility that has the proper equipment. The seed stock will be used only if absolutely needed.

Home brewers and small-scale professional brewers can use working cultures as a substitute for professionally maintained seed stocks. The small-scale brewer can inoculate test tube slants and store them in the refrigerator as an alternative to storage at -70 degrees C. Make two slants; use one and save for other to inoculate a new set of slants three months later. By repeating this procedure every three months, you can permanently maintain a strain in good health.

The yeast from the remaining working slants is streaked onto petri dishes to make working stock. A new petri dish is streaked biweekly or when the yeast has not been recently used. From the working petri dish several colonies are picked and used to inoculate a sealed 50-mL tube of sterilized wort and incubated at room temperature for 1-2

General yeast culturing and amplification for brewing
Master Seed Yeast Strain (store in 15% glycerol at -70°C -94°F)
Working Cultures (stored refrigerated on agar-solidified worts slants)
Working Petri Dishes (stored refrigerated on agar-solidified wort)
Primary Starter Culture (50mL of sterilized wort grown 1-2 days)
Secondary Starter Culture (1 L of sterilized wort grown 1-2 days)
Tertiary Starter Culture (20L of sanitized wort grown 1-2 days)
Quaternary Starter Culture (100L of sanitized wort grown 1-2 days)

days until activity is observed. This culture is then scaled up (Figure 1). Some of the later steps may not be needed, depending on the size of the fermentation desired. Generally, a 1:20 yeast-to-wort ratio (v/v) should be used when inoculating the wort.

Four is Better Then Five

Home brewers can obtain adequate quantities of healthy yeast using the technique of yeast culturing. Lag times of <6 h are commonly observed with ale yeast. These lag times may be reduced further by injecting oxygen into the wort; oxygen is also required for the squalene oxidation step of sterol synthesis. Anything that comes in direct contact with cooled wort should be thoroughly cleaned and sanitized. If these precautions are taken, the Reinheitsgebot's missing ingredient, bacteria, might just remain missing.

References

(1) Greg Noonan, Brewing Lager Beer (Brewers Publications, Boulder, Colorado, 1986), p. 70.

(2) P. Farnsworth, Zymurgy 12 (4), 10-13 (1989).

(3) G.J. Fix, Principles of Brewing Science (Brewers Publications, Boulder, Colorado, 1989).

Issue 1.1 Table Of Contents

[Home] [BrewingTechniques Library] [Contact Us] [Order]