

The Biochemistry of Yeast

Debunking the Myth of Yeast Respiration and Putting Oxygen in Its Proper Place

by Tracy Aquilla

Though it flies in the face of popular wisdom, yeast does not go through a respiration phase in the early stages of fermentation. A careful look at yeast metabolism and reproduction reveals a common misunderstanding and points the way to more sophisticated applications of oxygen in the brewery.

Fermentation is perhaps the most interesting and exciting part of brewing beer. There is something fascinating about watching yeast in action, and being close to the process contributes immensely to my enjoyment of my beer. No matter how well we do our part in preparing bitter wort for fermentation, it is the yeast that turns it into beer. For this reason alone, it is important to understand and appreciate what these microorganisms are really doing inside our fermentors. Most of the popular brewing literature, however, fosters a misconception about yeast and fermentation. This article sets the record straight.

Most of the brewing literature indicates that brewers' yeast (*Saccharomyces cerevisiae* and *S. uvarum*) requires dissolved

oxygen for a brief period of time after pitching so the cells can respire and grow, implying that yeast needs oxygen to bud and must respire before it can ferment wort. It is true that aerating or oxygenating wort is generally beneficial to fermentation, but it is untrue to say that yeast requires oxygen to reproduce or that yeast uses oxygen to respire during fermentation. The misunderstanding may be subtle, but it is a misunderstanding nonetheless. Gaining a clear understanding of the truth about how yeast works not only sets us on sound technical foundations, but has practical applications as well.

This article briefly discusses yeast metabolism, clarifies the role of oxygen, and suggests some practical applications of this more refined understanding of fermentation processes.

ORIGINS OF ERROR

The popular literature implies that yeast requires oxygen (it doesn't) and that yeast cannot bud without oxygen (it can). In the home brewing literature, it is likely that the authors of these books and articles have the right idea but simply omitted certain details to make their subject more accessible to a largely nonscientific audience. Home brewing literature typically lacks adequate details and citations referring to original scientific research. The professional brewing literature is not immune to the problem, either. Though the professional literature is certainly much more detailed, even these authors often lose sight of the big picture.

Whatever the reasons, the literature tends to paint a distorted picture of reality, and many brewers have come away from reading these books with some serious misconceptions about yeast biology and brewery fermentation. I think most brewers are capable of handling the whole truth when it comes to the "facts of life" with yeast, and it is time to bring it to light.

What follows will by necessity get a bit technical, but that is the nature of biochemistry. I believe that the more you know about what is going on in your fermentor, the more it can help you to make better beer, so try to bite the bullet and slog through the details. The rewards of understanding will be great.

LIFE IS BASED ON CARBON AND ENERGY

All living things on earth incorporate carbon as a structural element, and all living things require a source of energy to function and remain alive. While some organisms, such as green plants, are capable of capturing the sun's energy and fixing carbon dioxide from the air, most others, such as yeast and humans, depend on organic food sources to meet their carbon and energy requirements. In brewers' yeast, both carbon and energy are obtained through the oxidation of organic nutrient molecules (glucose, for example).

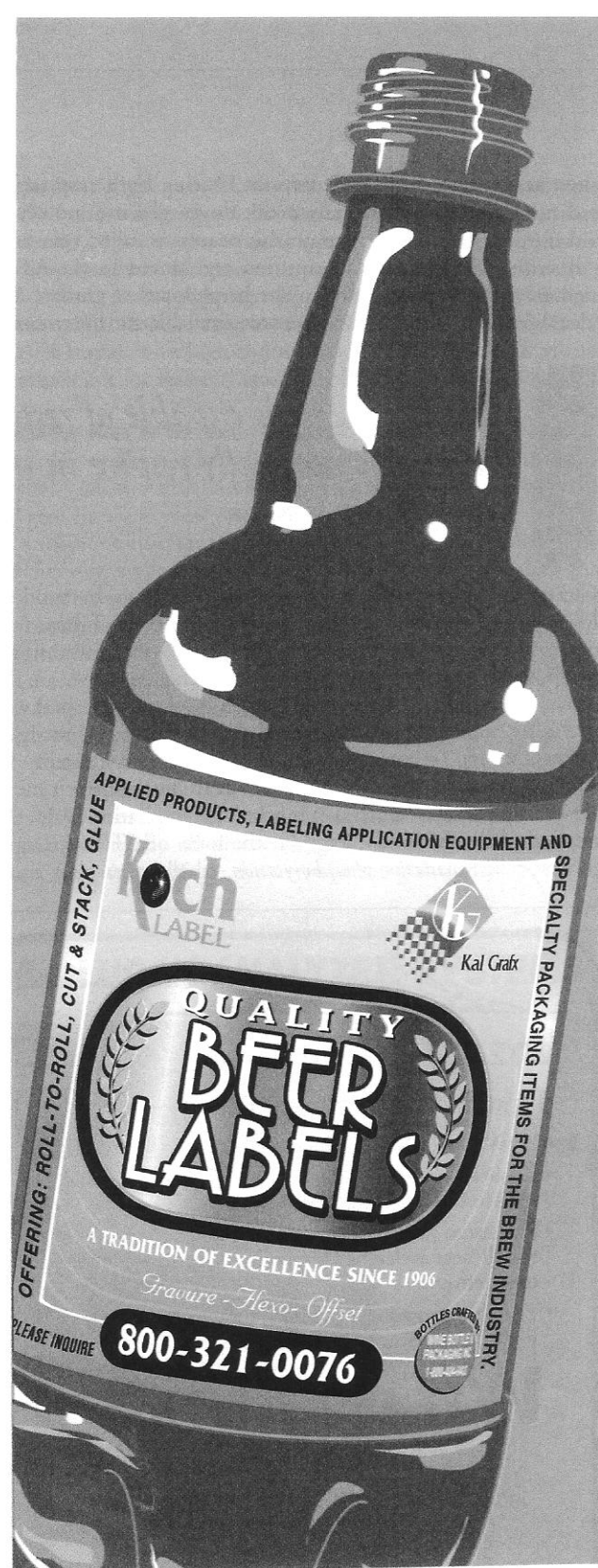
Metabolism: Biochemists use the term *metabolism* to describe the entire set of chemical reactions that organic molecules undergo in living cells. Metabolism can be further subdivided into *catabolism*, which is the degradation of energy-rich nutrient molecules, and *anabolism*, which is the biosynthesis of new cellular components (the cell's creation of "building blocks").

During catabolism, the chemical bonds of an organic molecule (glucose, for example) are broken, and the cells capture some of the energy stored in these bonds. The cells store this energy for use in the anabolic reactions that are responsible for growth and development. The compound that serves as the main storehouse of this cellular energy is called ATP (adenosine triphosphate). Much of the energy released through catabolism is captured by ATP and stored in its high-energy phosphate bonds, to be used when ATP participates in anabolic reactions.*

RESPIRATION AND FERMENTATION

Yeast synthesizes ATP through two major biochemical path-

*Some metabolic energy is also released from nutrient molecules in the form of electrons, which are then captured by other important compounds in the cell, but I don't want to muddy the water here with too many small details.



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ways: respiration and fermentation. During both respiration and fermentation, yeast cells break down glucose molecules within the cell to release energy (this process is called *glycolysis*), and some of this energy is captured and stored in the ATP's high-energy phosphate bonds. The breakdown of glucose also releases carbon atoms, which become available for biosynthetic

Although yeast is capable of respiration, it does not normally respire in the production of beer.

reactions, enabling the yeast to grow and reproduce by budding. The rest of the carbon ends up in the by-products of these reactions, such as carbon dioxide, ethanol, and other more minor compounds. Other fermentable sugars, such as maltose, are first converted to glucose before entering these metabolic pathways (fructose, however, may enter the glycolytic pathway directly).

Respiration: To most people, respiration means simply the consumption of oxygen (breathing), but to biochemists it means something very different. To a biochemist, respiration is the production of biochemical energy (in the form of ATP) through a process called *oxidative phosphorylation*, which takes place in the

cell's mitochondria. This process completely oxidizes glucose. Much of the energy released is captured by ATP in a series of tightly coupled biochemical reactions. This process is absolutely dependent on oxygen, and it produces water and carbon dioxide as by-products. Respiration is not possible without a source of oxygen, and exceptions to this rule are extremely rare in nature (only in certain rare microorganisms).

Fermentation: Fermentation is the production of biochemical energy (in the form of ATP) through a process called *substrate-level phosphorylation*. This process only partially oxidizes glucose, producing mainly ethanol and carbon dioxide as by-products. While some of the energy released in this process is captured by ATP, fermentation yields far less

energy than respiration because much of the potential energy still remains in the chemical bonds of the partially oxidized by-product ethanol. During respiration, yeast derives a net gain of 28 molecules of ATP from each glucose molecule; during fermentation, it gains only 2. The partial oxidation that occurs during fermentation does not require free oxygen. (Note that despite the name of the process, oxidation does not necessarily involve oxygen; it simply means that electrons are transferred during a reduction-oxidation reaction — oxygen, however, frequently happens to be the electron acceptor, as in respiration.) Fermentation can proceed either in the presence or absence of oxygen. In the absence of oxygen, fermentation is the only choice possible for yeast.

Brewers' yeast is able both to respire and ferment, a luxury most organisms do not enjoy. What determines its ability to grow and reproduce is the presence of the required nutrients, independent of whether the environment is aerobic (exposed to air) or anaerobic (without air). Although brewers' yeast is able to use both respiration and fermentation metabolic pathways, it does not respire if a relatively high concentration of fermentable sugar is available, even when ample oxygen is available for respiration.

The tendency for fermentation: Brewers' yeast has a very strong tendency toward fermentation and will respire only when the concentration of fermentable sugars is very low and oxygen is available (1). In beer making, yeast will ferment rather than respire, regardless of the oxygen concentration, because the wort usually supplies an overwhelming abundance of fermentable sugar. The production of ethanol during fermentation may contribute to yeast survival because of its toxicity to other microorganisms.

So yeast can use any of three major metabolic modes: aerobic fermentation in the presence of sugar and oxygen, anaerobic fermentation in the presence of sugar but absence of oxygen, and respiration (necessarily aerobic) in the presence of oxygen and a low concentration of fermentable sugar.

The hazards of respiration: As a further testament to its flexibility, yeast is also *diauxic*, meaning the cells can use more than one carbon source for their energy needs. Under certain circumstances, yeast can respire ethanol as well as glucose, producing acetic acid (vinegar) as a by-product. This process can occur only in the presence of oxygen when no alternative energy source (that is, no fermentable sugar) is available. Fortunately, our yeast does not normally get the chance to oxidize ethanol to acetic acid because by the time all the sugar is gone and ethanol is available for respiration, no dissolved oxygen is left in solution.



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Therefore, one reason brewers try to avoid aeration after fermentation has begun is to prevent their beer from turning sour. Another hazard of aeration in the brewhouse at this stage is that it can also promote chemical oxidation reactions (yielding nasty aldehydes like *trans*-2-nonenal), which cause staling of the finished beer.

I should emphasize that although yeast is capable of performing respiration, this pathway is irrelevant for brewers because respiration does not normally occur in the production of beer.

WHY YEAST DOESN'T RESPIRE IN WORT

Basically, yeast does not respire in wort because it doesn't have to! Like most other organisms, yeast does only what is necessary for survival and reproduction. If resources such as sugar are abundant, they are used in abundance (and generally wasted). In the case of brewers' yeast, respiration does not occur in the presence of glucose, regardless of the oxygen concentration. This phenomenon has been called the Crabtree effect (named after H.G. Crabtree, the scientist who discovered it in tumor cells in 1929). The Crabtree effect is a metabolic regulatory mechanism that causes aerobically growing yeast to repress the respiratory pathway in favor of fermentation if fermentable sugars are available.

All fermentable sugars, including fructose, maltose, and sucrose (and galactose to a limited extent), induce the Crabtree effect, but glucose exhibits the strongest effect (2). Brewers' yeast is often said to prefer fermentation only when glucose levels are

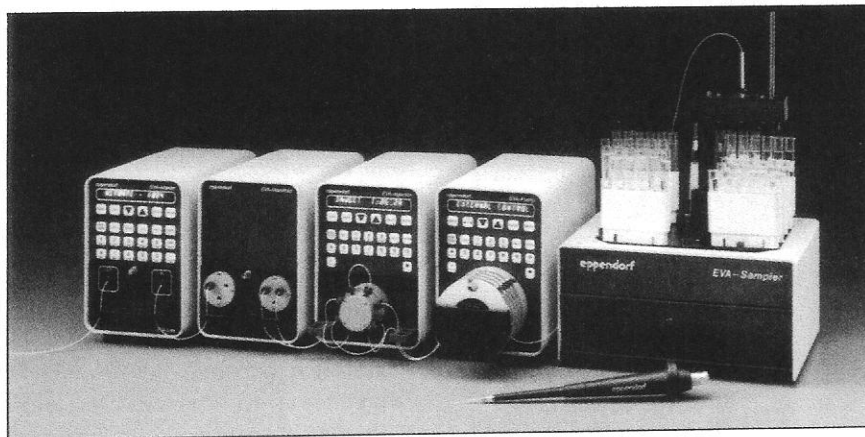
high, but "high" is a relative term; glucose in excess of about 0.4% (w/v) will bring on the Crabtree effect; most worts (both all-malt and adjunct) contain an excess of 1% glucose (3), which is more than enough glucose to induce the Crabtree effect. The other fermentable sugars present in wort induce the Crabtree effect as well (for example, a wort with an O.G. of 1.040 [10 °P] is 10% sugar). This high concentration of sugar makes it virtually impossible for brewers' yeast to respire in wort.

THE TRUE ROLE OF OXYGEN — BIOSYNTHESIS

Thus far, we have established that yeast does not really require oxygen. Most animals will die after several minutes without oxygen; brewers' yeast does not require it at all in the presence of sufficient nutrients, because it doesn't need to respire. *Saccharomyces* species of yeast, in fact, are exceptional in that they represent the few rare species of yeast that have absolutely no requirement for oxygen and can grow under strict anaerobic conditions (2). (Most other yeasts, even those that can ferment sugar [for example, *Brettanomyces*], cannot grow without oxygen.) Yet the brewing literature and all brewing experience point to the need for oxygen for healthy fermentations. What role *does* oxygen play?

It is well-documented that yeast uses oxygen whenever it is available, even during fermentation, and yeast cells rapidly absorb essentially all of the oxygen made available to them. Yeast, however, will use the overwhelming majority (if not all)

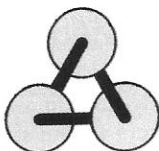
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of the available oxygen in biosynthetic reactions, not for respiration.*

The dissolved oxygen levels in wort drop from saturation to near zero very quickly after pitching yeast, usually within 30 minutes under ideal conditions, because yeast absorbs the oxygen for eventual membrane biosynthesis. The oxygen enables the cells to grow much faster and to reach a higher cell density. This effect is not the result of respiration but is the result of oxygen providing the means for sterol synthesis. In the absence of sufficient preexisting wort sterols, oxygen is *limiting* at this point; in other words, without it, the yeast will starve.

Sterol synthesis: During aerobic fermentation, dissolved oxy-

*To be more precise, yeast in an aerobic culture is actually respiro-fermentative, meaning that both respiration and fermentation occur simultaneously, though respiration occurs to a very limited (essentially negligible) extent while the fermentative pathway overwhelmingly dominates (4). This dual pathway may be one source of the common misconception that yeast respire upon pitching and requires oxygen to reproduce.

The so-called "leaky" metabolic control mechanism of respiro-fermentation is necessary, however, to allow some carbon in wort (although less than 1–2% of the total glucose) to enter the biosynthetic pathways. The carbon enters the pathway at the beginning of the process of respiration, but then gets diverted for biosynthesis. The important point here is that neither respiration nor oxygen is required for energy production and yeast budding.

gen is used in the biosynthesis of unsaturated fatty acids and sterols (mainly ergosterol), which are essential components of cell membranes. (Cell walls, by contrast, are composed mostly of carbohydrates.) These sterols and unsaturated fatty acids are important to yeast because budding can cease if their levels get too low.

The cell membrane is the structure that controls what flows into and out of the cell. If these membranes lack sufficient sterols, the yeast cells become weak, leaving the cells susceptible to a variety of problems. Weakened cells can become intolerant of alcohol, and the alcohol can kill them (5). Weakened cells can also result in stuck fermentations and numerous off-flavors. If the membranes contain sufficient levels of sterols, however, the cells are much stronger, more cells will thrive to finish the fermentation, and the yeast may complete the fermentation significantly faster.

The real requirement for oxygen, then, is to help produce fatty acids and sterols, which are very important components of the cell membrane. The reason wort aeration (or oxygenation with pure oxygen) is considered so important to brewery fermentations is that yeast can synthesize the lipids needed for membrane biosynthesis only when dissolved oxygen is available. If yeast had an independent source of these important lipids, however, the so-called requirement for oxygen could theoretically be eliminated completely (6).

Alternatives to sterol synthesis: Another way to provide lipids is to introduce into the starter a yeast nutrient that contains dead yeast, and thus lipids (not all yeast nutrients contain these). Still another source of these lipids could come from the "break material" (trub) left in the fermentor. This greasy, sludge-like matter forms in the wort upon boiling and cooling and eventually sinks to the bottom of the vessel. This material derives a significant lipid content (up to 50% in cold break) from the barley malt, and it contains the unsaturated fatty acids the yeast needs to synthesize strong, healthy membranes.

Although nearly all brewers agree that the hot break (produced after boiling) can produce off-flavors if it is not removed from the wort, there is still a good deal of discussion over whether leaving cold trub in the fermentor can help fermentation (there is some speculation that the trub also provides nucleation sites that control CO₂ toxicity) and whether trub is detrimental to beer flavor (witness, for example, the recent discussion in *Brewing Techniques*' Readers' Tech Notes section [7,8]).

PRACTICAL APPLICATIONS

Many brewers have successfully made decent beer without aerating their worts, but those who do aerate generally observe more rapid and possibly even more complete fermentations. As I mentioned at the beginning of this article, knowing more about what goes on in the fermentor can help you make better beer. Now that we know what yeast do with oxygen, we are in a much better position to decide how much the wort needs and when to add it.

Strength in numbers: Though leaving some cold break in the fermentor may boost your fermentation, the most common remedies for slow or stuck fermentations (and many other problems in the brewhouse) are wort aeration (before or after pitching time) and increased pitching rates.

I do not believe that brewers necessarily need to aerate their worts at all if the pitching yeast comes from a fresh starter culture that itself has been well-aerated during growth and stepped up to produce a sufficient number of healthy cells, or if a large

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culture is repitched from a very recent (aerated) batch. Wort aeration is clearly beneficial, however, if you are unable to obtain the optimal pitching rate (about 1 million active cells per mL per degree Plato, or about 5 to 10 million active cells per mL for typical worts) — and many brewers cannot.

Oxygen is beneficial even at levels well below saturation, so aerating even a little will actually help a lot.

Thus, wort aeration can often make the difference between a good beer and a great beer. It is important to note, however, that it is possible to overaerate and, when using pure oxygen gas, even easier to overoxygenate. In particular, overaeration can produce certain secondary compounds, such as vicinal diketones (VDKs, of which diacetyl is one), oxo-acids, fusel alcohols, and their derivative esters.

Aeration needs are yeast strain-specific: Another thing to remember is that the oxygen "requirement" (that is, the aeration needs) of brewers' yeast is strain-dependent and that what might be adequate for one strain could be insufficient or exces-

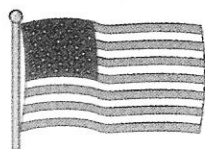
sive for another. For this reason, it is probably best to be familiar with your yeast, to know when aeration is necessary, and to aerate the wort only to saturation (about 8 ppm of oxygen for most worts) upon pitching and then to stop. Keep in mind also that oxygen is beneficial even at levels well below saturation, so aerating even a little will actually help a lot.

Aeration needs may be style-specific: In certain styles of beer in which metabolic by-products of yeast growth (such as VDKs, oxo-acids, fusel alcohols, and their derivative esters) are undesirable, it is even *preferable* not to aerate the cold wort. Avoiding aeration may be particularly desirable when fermenting high-gravity worts that will naturally result in the formation of more esters. (Strong Scotch

ale is one example of a beer that usually has a high original gravity and a very clean finished beer, that is, free from fusels, esters, and VDKs [9].) Dissolved oxygen is also known to produce the nonenzymatic production of diacetyl by oxidation of alpha-acetolactate. By pitching a larger starter culture (ideally, at the optimal rate), keeping the initial fermentation temperature relatively low, and minimizing (or even eliminating) the wort aeration step, a brewer can avoid extensive yeast growth during fermentation and the excretion of the secondary compounds that naturally accompany reproductive activity. (You should also pour off the liquid from your starter before pitching to avoid



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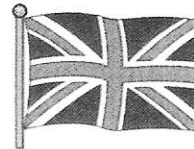
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any undesirable byproducts of the starter's aerobic fermentation.) *Note:* Avoid overpitching; pitching rates significantly higher than the optimum have been reported to result in "yeast-bite" and are not recommended.

THE AERATION "DEFAULT"

Having said all this, however, it is rare for a home brewer to obtain the optimal pitching rate, and aeration is therefore generally the rule, particularly if the brewer wishes to obtain reasonable attenuation of a very heavy wort. In the case of strong ales such as barleywines, for example, esters are generally seen as desirable, as is a very high concentration of ethanol. Because high attenuation is the main goal with this style and esters are not really a problem, the combination of a high pitching rate and wort aeration is likely to produce the best results. Keep in mind, though, that oxygen is less soluble (up to 15% less) in worts of very high gravity, so you will generally need to aerate these worts for a longer time than usual to obtain saturation; or, better yet, use pure oxygen.

Because commercial breweries recycle their yeast by repitching rather than using starter cultures, they also generally find it most convenient to aerate the cold bitter wort. Wort aeration increases the rate and extent of yeast growth and hence decreases lag time and results in a larger population of cells. This in turn generally leads to more complete attenuation and fewer undesirable flavors in the finished beer.

KNOW YOUR OPTIONS

So, the bottom line is that yeast does not generally respire, and it does not even need oxygen at all to survive and grow. Yeast does, however, need lipids to build cell membranes and in their absence will readily consume oxygen for their synthesis.

Aerating your wort may solve some fermentation problems, but remember that if you're pitching a fresh, healthy yeast culture of the optimal size, aeration is usually not essential and may even be undesirable in certain cases. Most important, the level of dissolved oxygen necessary in wort to produce the best beer depends on the strain of yeast being used, its viability, the pitching rate, and the style of beer being made.

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