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What Brewers Should Know About Viability, Vitality, and Overall Brewing Fitness: A Mini-Review

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ABSTRACT

This mini-review takes a current look at the numerous factors that affect overall brewing fitness in brewing yeast strains. Fermentation is a complex interaction between environmental and microbiological factors. Environmental factors are related to the raw materials and processing conditions used during wort production, whereas microbiological factors are related to the characteristics of the yeast used for fermentation and maturation. Quality systems are in place, in most breweries, to control these factors and thus direct the fermentation. However, the skill, experience, and information available to the brewer ultimately determine the final quality of the beer and allow the brewer to face sudden, unexplainable changes in flavor, aroma, and fermentation performance. Environmental components are generally fixed and tightly controlled; however, variations arise as a function of raw ingredient quality, which like most raw agricultural commodities may have some inherent, uncontrolled variability. Generally, microbiological components are related to yeast strain, purity, propagation and handling conditions, number of times repitched, cell number/pitching rate, viability, and vitality. The difficulty with these components is that data produced from many of the methods for assessing purity, cell number/pitching rate, and viability/vitality can be problematic. The presentation of the data can also be challenging because some of the words and terms used to describe microbiological attributes may have different meanings depending on the audience. This mini-review seeks to differentiate between viability and vitality. It discusses influencing factors and details current best methods and means of analysis, all in the context of the brewing industry. We also seek to clarify differences between cell age, repitch number, and the effect of these yeast attributes on overall brewing fitness and fermentation performance.

Keywords: viability, vitality, fermentation performance, brewing, yeast

SÍNTESIS

Este pequeño repaso revisa los numerosos factores que afectan la capacidad cervecera de cepas de levadura. La fermentación es una compleja interacción entre factores microbiológicos y del entorno. Factores del entorno están relacionados con las materias primas y las condiciones del proceso durante la producción del mosto, mientras que los factores microbiológicos están relacionados con las características de la levadura utilizada. La mayoría de las cervecerías tienen sistemas de control de calidad para controlar estos factores y así dirigir la fermentación. Sin embargo es la información que tiene el cervecero, y su experticia en usarla, la que a final de cuentas determina la calidad final de la cerveza, aunque este mismo cervecero podría encontrarse con cambios inexplicables en el sabor, aroma y desempeño de la fermentación. Los componentes del entorno generalmente son fijos y fuertemente controlados, pero variaciones ocurren como función de la calidad de la materia prima que suele sufrir de una variabilidad descontrolada. Los componentes microbiológicos generalmente están relacionados con la cepa de levadura, su pureza y condiciones de manejo y propagación, número de veces utilizada, concentración, viabilidad y vitalidad. La dificultad es que los datos de muchos de los métodos para acertar la pureza, la concentración, viabilidad/vitalidad son a veces problemáticos. La presentación de datos también puede ser un reto dado que algunos de los términos usados para describir atributos microbiológicos pueden tener un diferente sentido dependiendo de la audiencia. Este pequeño repaso trata de diferenciar entre viabilidad y vitalidad. Discute los factores influyentes y describe los mejores métodos actuales así como la manera de analizarlos, siempre relacionado con la industria cervecera. También tratamos de aclarar diferencias entre la edad de la célula, concentración al añadir al mosto, y el efecto de estos atributos de la levadura sobre la calidad de la cerveza y el desempeño de la fermentación.

Palabras claves: desempeño de la fermentación, levadura cervecera, viabilidad, vitalidad

Fermentation is the heart of beer production for all breweries, big or small. In essence, this process converts the fermentable carbohydrates in the wort into ethanol, CO_2, and various flavors and aromas. But fermentation is a complex interaction between environmental and microbiological components. Environmental components are related to the raw materials and processing conditions used during wort production, whereas microbiological components are related to the characteristics of the yeast used during fermentation and maturation. In most breweries quality systems are in place, in an attempt to control these components and thus control the fermentation. However, the skill, experience, and information available to the brewer ultimately determine the final outcome of the beer and allow the brewer to face sudden, unexplainable changes in flavor, aroma, and fermentation performance (31,32).

The quality of raw materials is generally fixed and tightly controlled. However, variations do arise because most raw agricultural commodities typically have some inherent, uncontrolled variability. Despite this, modern brewing analyses allow for rapid determination of critical brewing variables (such as carbohydrate composition, pH, free amino nitrogen [FAN],

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and other critical variables) to ensure that a brewer can make adjustments in the mash, such that the composition of the wort is consistent and is composed of all necessary components for a successful fermentation (2). Microbiological components include the yeast strain, purity, propagation and handling conditions, cell age, cell number, and pitching rate. These can be difficult to control, especially those related to viability and vitality of the population (19,31,32).

The main difficulty with control over the microbiological factors is the interpretation of the data produced from many of the methods for assessing purity, cell number/pitching rate, and viability/vitality. The presentation of the data can also be challenging because some of the words and terms used to describe microbiological attributes may have different meanings depending on the audience. This mini-review seeks to differentiate between viability and vitality; it will discuss the influencing factors and detail methods for analysis, while considering those members of the brewing industry on a smaller budget. We also seek to clarify differences between cell age, repitch number, and the effect these yeast attributes have on overall brewing yeast fitness and fermentation performance.

Viability, Vitality, and Brewing Fitness

Brewers commonly utilize one or more analyses to ascertain the quantity, purity, and metabolic status of yeast populations before and after fermentation. The purpose of these tests is to try to predict the condition of a yeast population, such that the subsequent fermentation performance can be predicted and optimized. As accurately noted by Boulton (7), most methods for analyzing the physiological or metabolic status of yeast, usually called viability tests, are in fact measures of vitality. Despite the frequent use of the terms viability and vitality in brewing literature, the usage of these terms in application is often muddled. The *Oxford English Dictionary* defines *viable* as "capable of surviving or living successfully; especially under particular environmental conditions," whereas *vitality* is defined as "the state of being strong and active; energy," which in context to living organisms is the "power giving continuance to life." Although the results of many brewing analyses do in fact show that a yeast population is feasibly alive, they may or may not show that the yeast population is also active. Thus, data generated describing yeast viability and vitality are often intertwined. Ideally, when more than one method is employed, the resulting data should be used to describe the "overall brewing fitness" (Fig. 1), which more adequately summarizes the complex and dynamic relationship between cell metabolism, its environment, and the methods employed for cultivation.

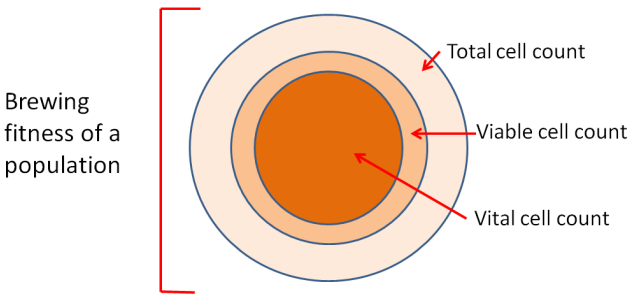

Figure 1. Parameters that contribute to overall brewing fitness (37).

Measuring Viability and Vitality

There is currently no absolute best method for determining the viability or vitality of a yeast population (see Table 1). Many cellular parameters can be examined, such as replicative capacity, levels of cellular components, and metabolic activity. Unfortunately, each method for assessing viability, vitality, or fitness is based on one parameter, or aspect, of cell composition or metabolic activity, and the other critical parameters that contribute to fermentation performance are ignored. As a result, one technique is limited in its usefulness in determining overall brewing fitness (Fig. 2). For assessing viability and vitality such that a comprehensive picture of overall brewing fitness of a culture can be established, it is necessary to utilize multiple methods to properly anticipate the reaction a population will have in a given environment. That being said, there are many (relatively) quick, inexpensive tests that both big and small breweries can utilize to quickly and accurately determine the relative fitness of their cultures for fermentation.

Cell Count

The cell count is one of the most important tools for a brewer. Among brewers, big or small, the need to determine viable yeast concentration for control of pitching rates is paramount. Consistent, viable pitching rates are critical to con-

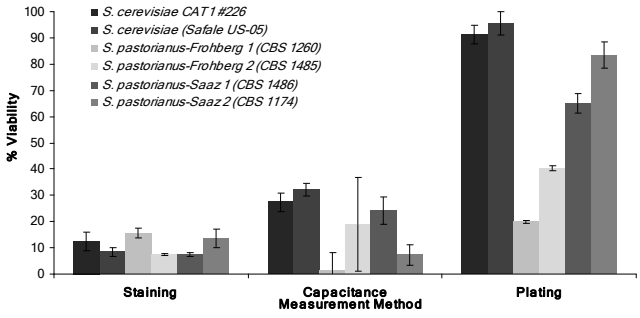

Figure 2. Comparison of methods used to measure various parameters of overall brewing fitness of *Saccharomyces* yeast strains after dehydration and rehydration (35). Note the results from each type of method.

Table 1. Manual and automated methods for assessing parameters of brewing fitness

Method	Type	General output
Light absorbance (visible spectrum)	Manual	Total cell count
Light absorbance (infrared probe)	Automatic	Total cell count
Staining + light microscopy	Manual	Total cell count + vital cell count
Staining + image analysis	Automatic	Total cell count + vital cell count
Plating (semisolid nutrient agar)	Manual	Viable count
Capacitance probe	Automatic	Viable count

sistent fermentation performance (31,32) because population size is a critical driver of alcoholic fermentations in various strains and industries (1). As a rule of thumb, brewers inoculate about 1×10^6 viable cells/mL in low gravity (9°P) wort, increasing the pitch rate by 2×10^6 cells/mL for each increase in degree Plato to a maximum of $\approx 3 \times 10^7$ cells/mL in very high gravity brews (≥21°P) (31). Correct yeast pitching is especially important in higher gravity brewing (>16°P) to avoid slow or stuck fermentations (11).

The most commonly used method in the brewing industry to enumerate cell number, enshrined in the ASBC Methods of Analysis (2), relates to yeast staining with methylene blue and counting by microscopic examination on a glass slide. Methylene blue staining is a bright-field staining procedure in which dead or nonviable cells are stained blue, whereas viable cells are able to exclude (or reduce) the dye and remain colorless. This method is a simple, rapid, and inexpensive way to quantify total and viable cells in a population (2). Although cost efficient, this method must be used with caution. Because the stain relies on inclusion or exclusion of the membrane-permeable dye to differentiate between viable or nonviable cells, the results can be influenced by the physical state of the cell membrane or the physiological state of individual cells, which vary depending upon its age, culturing conditions, and position in the cell cycle (44,63). ASBC notes that this method is valuable with yeast in "good" condition, given reports that in cell populations with low viability (<95%) this method is less accurate and may lead to overestimation of the number of viable cells in a population (2,48,51).

Methylene blue staining also has a degree of personal interpretation, because of variations in a person's ability to judge color intensity and errors in counting. Thus, the use of automated machines for quantifying viable cell number could be beneficial, owing to the removal of human variability. Several companies (Millipore, Chemometec, Orflo, and so on) offer such automated products. We have used, with success, an automated fluorescence-based cytometer (Nexcelom BioScience, U.S.A.) (Fig. 3). The system captures bright-field (morphological information such as cell size and circularity) and fluorescent images at four locations within the chamber, and the software analyzes each image to count and measure the fluorescence signals within individual yeast cells in a population. The system is well described by Laverty and colleagues (34) and has been used previously for fluorescence-based cell analysis (36). In general, the methylene blue staining method is best used as a rough indicator of yeast viability, to be complemented with other methods of assessing viability and vitality.

pH

pH is an easy, inexpensive tool to assess the overall fitness of a yeast for fermentation. Measurement of pH in yeast slurries, for repitching, can give a brewer an indication of yeast viability as well as provide an indicator of yeast autolysis. Mochaba and colleagues (44) noted that there is a relationship between yeast autolysis, protease excretion/activity, and yeast slurry pH. Yeast slurries that were stored too long (>20 h at 4°C) saw an increase in pH, FAN, and other ions. Cell autolysis, releasing basic cellular components, could cause a rise in

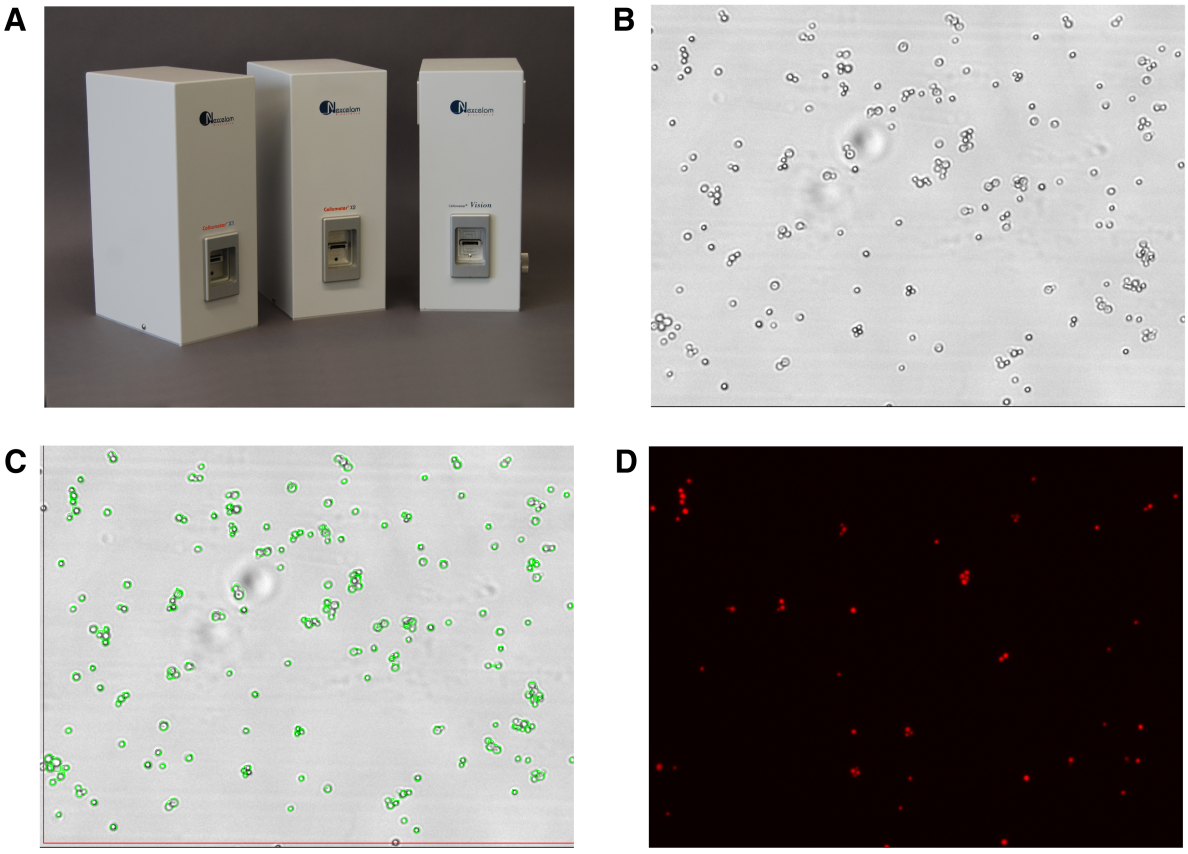

Figure 3. A, Nexcelom Vision Cellometer; **B,** typical yeast view produced by the Cellometer; **C,** counting method by the software; and **D,** fluorescent detection of yeast, by propidium iodide, in the Cellometer. Images courtesy of Nexcelom Bioscience, U.S.A.

pH. Inoculation with chronologically aged yeast slurries negatively impacted the organoleptic qualities of finished beer by increasing levels of fusel alcohols, diacetyl, and acetate. In general, given a final beer pH ≈4.0, yeast slurries above pH 4.9 should be rejected owing to the likelihood of autolysis occurring in the cell population (44). The final pH of beer is dependent on wort composition and buffering capacity, as well as yeast strain and growth (19,59).

pH can also be used as a tool to monitor yeast fitness during an ongoing fermentation. Wort acidification, or pH downshift, occurs as a result of the consumption of wort carbohydrates and buffering compounds (generally FAN) and the production of carbonic acid (from CO_2 generation) and some organic acids. The pH of wort falls rapidly early in the fermentation (≤24 h) and slowly in the later stages, although slight increases in pH may be seen at the end of fermentation if yeast remains in contact with beer after all fermentable carbohydrates are consumed (12). The average initial pH of wort (20°C) is ≈5.3, ranging from 5.0 to 6.0 (43); ale wort is generally lower (pH 5.1) and lager beer slightly higher (pH 5.4–5.7) (3). Fermentation of worts with higher pH can influence yeast flavor production; dimethyl sulfide (sweet corn/black current flavor) levels are reduced as initial wort pH is reduced (3).

As mentioned earlier, the final pH of beer is dependent on several factors, but in general brewing yeast can tolerate a 1.5–2.0 downshift in pH over the course of a fermentation, yielding an average final pH of approximately 4.2–4.4 (19,28). Extreme final pH levels, typically seen in yeast after undergoing acid washing (pH approximately 2.1–2.8) before repitching, can cause several physiological and genetic changes in the yeast. Very low final pH values (<4.0) can increase beer staling (59) by accelerating the oxidation reactions that occur during beer storage and distribution (28). It is clear that monitoring and controlling pH in yeast slurries and during fermentation can provide another important source of information for evaluating fermentation fitness.

Capacitance

A capacitance probe (Fig. 4), a tool often used by larger breweries, can be used for on-line or off-line measurement of yeast viability (9,10). Under the influence of an electric field, living microorganisms act as tiny capacitors owing to the presence of an intact plasma membrane, which isolates the cell content from the surrounding medium (35,40). Cells with an intact plasma membrane are nonconducting and, thus, will build a charge under an electric field (signal impedance). Nonviable cells or cells with a damaged membrane do not impede the signal and, thus, contribute little to the total capacitance detected in the sample (9). One portion of the capacitance probe produces the electrical field, and the other portion measures the current (10). The output signal from a capacitance probe is directly proportional to the membrane-enclosed volume fraction of the microbial culture, and this volume depends on the concentration of cells, their viability, and the cell radius (4). It is possible that capacitance may underestimate viability because some membrane damage, for example from dehydration, can be repaired by the cells (35).

The major advantage of a capacitance probe and the radio frequency impedance method is that it is relatively fast (<1 s when necessary) and easy to use for determining viable cells over a wide concentration range; gas bubbles, trub, and other nonyeast solids do not interfere with the capacitance signal because they do not have a polarizing membrane (9,10). As mentioned earlier, the capacitance probe can only identify viable cells with an intact membrane; therefore, it must be used in conjunction with another method for total cell count to quantify percent viability. The sample around the probe must also be representative of the system as a whole, and in larger fermentations it may be difficult to obtain a homogeneous sample for accurate capacitance readings, especially if the cells are flocculated (40). Capacitance results also assume constant cell morphology and radius distribution (4), which, as we will cover later, is not always a good assumption for brewing yeast. Thus, capacitance measurements are another tool in the practical brewer's arsenal for assessing yeast fitness, but they should be paired with other methods.

Agar Plating

Agar pour-plating procedures are routinely used for the quantitative determination of microorganisms. In the traditional spread-plate method, an unknown sample is diluted many times, and a known sample of each dilution is spread over an agar plate. After incubation, the plate, which has between 30 and 300 colony-forming units (CFU), is counted, and the resultant count is multiplied by the appropriate dilution factor to obtain the microbial concentration in the sample. For statistical accuracy, samples are often plated, at each dilution, in triplicate. Plated samples that do not fall within the 30–300 CFU range are discarded (20). There are many modern adaptations based on the traditional methodology. The spiral plating

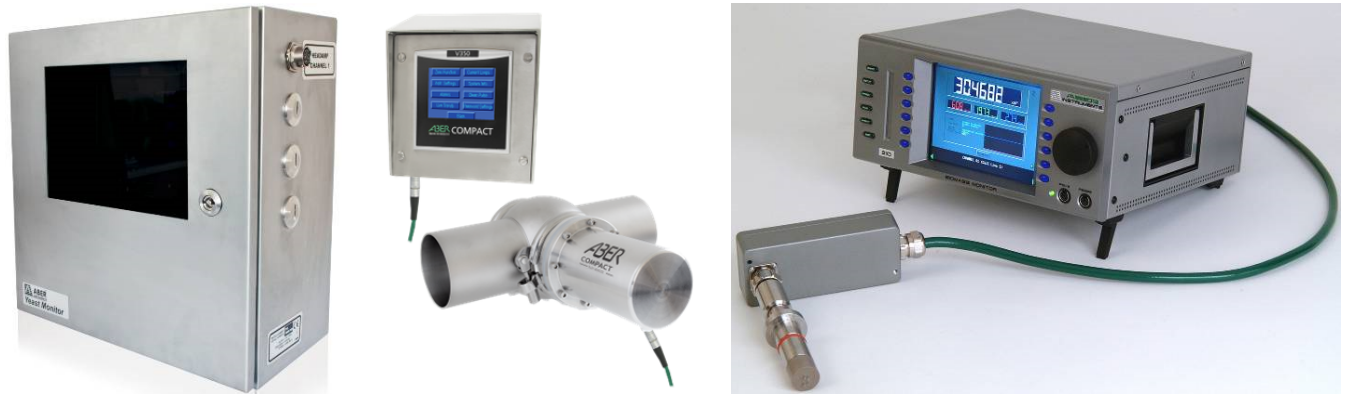

Figure 4. Aber capacitance probe and yeast monitoring system model 710 (left), Aber compact yeast monitor V350 (center), and Aber biomass monitor 210 (right). Images courtesy of Aber Instruments Ltd., U.K.

method uses a machine that deposits a known volume of sample on a rotating agar plate in an ever-decreasing amount in the form of an Archimedes spiral. After the sample is incubated, different colony densities are apparent on the surface of the plate. A modified counting grid relates the area of the plate to the volume of the sample. By counting the appropriate area of the plate, the number of microorganisms in the sample is estimated (20). Another modern adaptation, which we recommend, is the track-dilution method, which is a simplification of the traditional spread-plate method. The track-dilution method allows for up to six 10-fold serial dilutions to be applied to a single agar plate, making this adaption highly economical in terms of time and resources. This technique requires 100 × 100 × 15 mm square plates with 13 mm grids (Fig. 5), and each plate has six columns, where one sample (10 µL) is spotted on the agar surface. The plate is then tipped on its side (45–90° angle), and the spots migrate, in parallel tracks, to the opposite side of the plate. Plates are incubated overnight, and tracks with 30–300 colonies are selected for counting (26). This method gives statistically similar results compared with spread plating (26) and spiral plating (62). Because of the small volume of aliquots, this method may not be sensitive or accurate for dilute cultures (≤100 CFU/mL), although this is not generally an issue for brewing cultures.

The plating method can be considered the gold standard for measuring yeast viability (i.e., the ability of cells to grow) and will include both vital cells and some cells that other tests may show as being nonvital but that have the capacity to overcome and recover from physiological stress. The primary drawback to plating is the length of time (>24 h, longer if cells are injured) it takes for a microorganism to grow on the plate. Another criticism, related to brewing, is that the growth of a cell on an agar plate does not necessarily correspond to the growth of a cell in brewing wort. Not all cells will form a colony on a plate, but they may still be metabolically active (13,35). As is generally the case, this method should be used in conjunction with other methods to accurately determine the relative fitness of a culture for use in fermentation.

Factors Influencing Viability and Vitality

Environmental Composition

There is a dynamic relationship between yeast metabolism and the environment to which the population is being subjected. Although the environment affects the yeast, it is in turn modified by the yeast metabolism, as reflected by the consumption of nutrients and production of metabolic products. The composition of brewing wort can vary batch-to-batch and brewer-to-brewer, from factors related to raw materials and processing conditions (29). Raw ingredient quality, malt conditions, malt type, adjuncts (18), water composition, pH (60), hop variety, and type of hop product (cones, pellets, or extracts) (21,45), as well as brewhouse operations such as mash temperature, enzyme usage (17), point of hop additions, and boiling conditions (58), can all influence the composition of wort and impart environmental variability. Physical conditions such as temperature, pressure, and agitation can also impart inconsistencies in the brewing environment (31). The influence of physical and chemical conditions prevalent in the environment can cause variation in the cell physiology, composition, metabolism, replicative capacity, and ultimately in the yeast's overall fitness for fermentation (14,61).

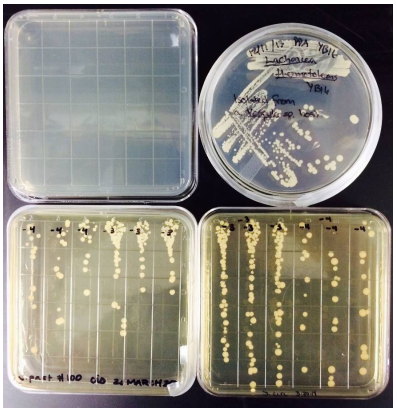

Figure 5. Track dilution plates (square-shaped) compared with a traditional agar plate (circular).

Carbohydrate Composition

Carbohydrates are one of the biggest classes of macronutrients for yeast. The effect of carbohydrate composition on a yeast population is often overlooked even though, based on the dry material, brewer's wort is generally composed of approximately 90–92% carbohydrates (≈75% of these are fermentable carbohydrates), 3.6% nitrogen compounds, approximately 1.5–2% salts and minerals, and approximately 0.5–1.0% free acids such as lactic acid, with the remaining amounts being small amounts of lipids, phenolic substances, and hop oils (43). Although the exact composition of brewer's wort is not fully defined (29), in general it contains a mixture of fermentable carbohydrates (monosaccharides glucose and fructose, ≈10% total wort carbohydrate content; disaccharides sucrose and maltose, approximately 5% and 45–65%, respectively; and the trisaccharide maltotriose, ≈15%) and nonfermentable carbohydrates (limit dextrins) (19,41). Brewing yeast strains (*Saccharomyces cerevisiae* and *S. pastorianus*) can typically utilize sucrose, glucose, fructose, maltose, and maltotriose (preference for that order), whereas larger sugar moieties (dextrins) are usually not metabolized (15,33,49). Brewing yeasts vary in their ability to ferment some sugars (such as maltotriose); thus, attenuation limits in a given wort will vary by a few points when different yeast strains are used (43). Lager strains can also fully utilize melibiose and raffinose, whereas ale strains cannot utilize melibiose and have a low raffinose fermentability (one-third that of lager yeast, because raffinose is composed of two-thirds melibiose) (15,43). Lager brewing yeast also utilizes maltotriose at higher rates than ale brewing yeast, although this is strain specific (66). Although attenuation levels vary from strain to strain, the presence of unfermented carbohydrates in beer may allow for the growth of bacteria, brewing yeast, or wild yeast (provided additional oxygen is supplied), which could in turn affect future fermentation fitness as well as the flavor of the finished product (15).

In brewing, wort is the only nutrient source, and thus it has the capacity to greatly affect yeast growth, division, lifespan potential, and overall fermentation fitness (41). Wort produced with high-glucose adjuncts and that is particularly high in glucose and/or sucrose (≥15%) may experience difficulties with excessive yeast growth, attenuation, and flavor owing to glucose repression and high ester formation (65), although yeast strains vary greatly in their sensitivity to glucose (43). As mentioned earlier, maltose is usually the primary fermentable carbohydrate in wort; however, yeast will repress the uptake of

maltose (glucose repression) in favor of utilizing glucose. The rate of maltose and maltotriose utilization will remain low until the glucose level falls below 0.4% w/v (41). The uptake of maltotriose occurs at the same time as maltose, although at a slower rate (45). Higher wort glucose levels may result in slower maltose and maltotriose uptake, leading to higher levels of residual fermentable extract in the final product, which can be detrimental to overall fermentation efficiency and product quality (although residual maltose and maltotriose can be attacked by proper maturation). The mechanisms of sugar utilization and transport in *Saccharomyces cerevisiae* have been well covered in other reviews (8,16,33,53).

Monitoring carbohydrate composition and utilization can be another useful tool to the brewer looking for more information on the overall brewing fitness of the yeast population. Although high-performance liquid chromatography (HPLC) analysis was not listed in the methods mentioned earlier, it can be a strong tool for monitoring carbohydrate composition of wort (yielding information on initial carbohydrate ratios and strength) and carbohydrate utilization (yielding information on the yeast metabolic performance). HPLC is a great option for breweries that have trained personnel (for machine upkeep and data analysis and interpretation), a designated lab (the machine should not be exposed to dust or excessive moisture), and a good budget in support of quality control (upfront expenses for purchasing and setting up an HPLC can be significant).

Nitrogen Composition

The other major nutrient class affecting brewing yeast performance is nitrogenous compounds. Nitrogenous constituents of wort influence healthy yeast growth and development as well as affect haze formation, head retention, and the biological stability of beer (27). Brewer's wort (no adjuncts, 12°P) is typically composed of FAN (≈30%), peptides containing 2–30 amino acid units (≈30%), wort proteins >30 amino acid units (≈25%), and other miscellaneous nucleic acid derivatives, amines, and so on (≈15%) (43). The primary sources of wort nitrogen that can be assimilated by yeast are amino acids, ammonium ions, and di- and tripeptides (47). Nitrogen compounds that cannot be assimilated by yeast are responsible for beer haze and the foam potential of beer (43). The relative composition of amino nitrogen in wort is affected by barley variety, barley nitrogen content, malting conditions, grist composition (adjunct levels and types), mashing conditions, and additives for nitrogen supplementation (47,52). Although the composition of amino nitrogen in wort may be influenced by several factors, the uptake of amino acids by *Saccharomyces cerevisiae* is a predictable sequence. Similar to carbohydrates, amino acids are used sequentially in groups. The traditional model (27) includes four groups: group A, which includes glutamic acid, aspartic acid, asparagine, glutamine, serine, threonine, lysine, and arginine, is reported to be immediately utilized by the yeast and almost fully removed from wort after 20 h; group B, which includes valine, methionine, leucine, isoleucine, and histidine, is not removed from the wort rapidly but gradually during the fermentation; group C, which includes glycine, phenylalanine, tyrosine, tryptophan, alanine, and ammonia, is used after a considerable lag phase, coinciding with the full removal of all group A amino acids. Proline, the only amino acid in group D, is a major nitrogen source in wort, representing approximately one-third of the total amino nitrogen, but it is not typically utilized by yeast during anaerobic fermentation. This classification of groups for amino acid uptake depends on the criteria used; however, generally amino acid utilization is irrespective of brewing conditions (e.g., temperature and vessel type) but may differ slightly from strain to strain (50).

As is the case with carbohydrates, nitrogen utilization can have an effect on overall brewing fitness. As well summarized by O'Connor-Cox and Ingledew (47), the formation of flavor-active compounds in beer by yeast is fundamentally affected by the yeast's ability to grow and utilize nitrogenous compounds in wort. Esters, higher alcohols, vicinal diketones, and H_2S formation are all influenced by overall nitrogenous compound levels and amino acid metabolism (47,52). Stimulatory fermentation conditions (high temperature and/or high dissolved oxygen levels) lead to high FAN utilization and subsequent flavor changes (39). A major portion of the wort nitrogenous compounds are used to synthesize new structural and enzymatic proteins in yeast (52), which are critical for yeast metabolic function, viability, and flavor production. Yeast fitness is diminished if the wort FAN drops below 120–150 ppm, whereas final beer quality is diminished if FAN levels exceed ≈300 ppm, because the yeast becomes too well nourished and final beer flavor characteristics are altered (43). Limitation of nitrogenous compounds, typically found in high-gravity fermentations or fermentations with high levels of adjuncts, results in poor yeast viability, an extended lag phase, and overall lower attenuation (11,47). The relative FAN level in wort can be measured by the international ninhydrin method (2), which measures total FAN level (excluding proline, which is not utilized by yeast). Monitoring FAN levels throughout the fermentation can be another tool for the practical brewer to understand the interaction between yeast and its environment to properly assess overall brewing fitness.

Dissolved Oxygen Level

As facultative aerobes, brewer's yeasts (both ale and lager) can shift between aerobic or anaerobic growth. Oxygen has a paradoxical and multifaceted role for yeast in that it can be both helpful and harmful for overall yeast viability and vitality. During aerobic growth (yeast propagation in early fermentation) energy is generated through oxidative phosphorylation, allowing ample energy from which to generate sufficient yeast cells through multiplication (67). Oxygen is also required by brewing yeast to synthesize sterols and unsaturated fatty acids (UFA), which are essential to the yeast plasma membrane integrity and functionality. The presence or absence of these compounds can have wide-ranging effects on transport of molecules in and out of the cell, regulation of membrane-bound enzymes, ethanol tolerance, and the levels of active flavor compounds in beer (15,30). Although sterols and UFAs are abundant in malt, they are normally not transferred to wort; thus, they must be produced by the yeast under aerobic conditions (15).

Although oxygen and aerobic respiration are desired for increasing cell number and healthy development, oxygen is also a highly reactive molecule that can be reduced into numerous reactive oxygen species (ROS). ROS, such as hydroxyl radicals (HO^-), H_2O_2, and superoxide anions (O_2^-), are highly damaging forms of oxygen that can target various cellular constituents, including DNA lipids and proteins (24). Yeasts generate ROS endogenously as a consequence of aerobic respiration and are consequently subjected to slow, continuous damage to their cellular components owing to free-radical stress. The free-radical theory of cell aging is based on a cell's inherent antioxidant defenses (enzymatic and nonenzymatic), which would normally quench ROS or repair

damaged molecules, gradually being depleted over the course of a cell's replicative lifespan (24,56). To efficiently operate in two alternate physiological states (aerobic and anaerobic), there are a large number of genes that are expressed in response to oxygen. The response of genes coding for respiratory function, sterol/UFA synthesis, or oxidative damage control is sensitive to low-oxygen tension. A certain class of genes that encode for oxygen-dependent functions are induced at low-oxygen tension, presumably allowing a cell to more efficiently utilize limited oxygen (67).

Traditionally, wort is oxygenated/aerated to approximately 4–8 mg/L (ppm) of dissolved oxygen, and pitching yeast is used from an earlier fermentation (30). Yeast from a previous anaerobic fermentation is generally used to inoculate the wort, and it may contain lipid levels near growth-limiting levels. Brewing yeasts may vary in their requirements for oxygen from strain to strain (19), yet if underoxygenated, all yeasts exhibit slower growth and thus slower uptake of wort nitrogen and carbohydrates. Although yeast strains may have differing oxygen requirement levels, each can ferment wort equally well if aeration is supplied at the correct time (46). Generally, it is more effective to supply oxygen during growth than to aerate the wort before pitching. The level of wort oxygenation at the time of pitching affects lipid metabolism, yeast performance, and overall beer flavor. Too little oxygen results in insufficient membrane lipid production, which in turn leads to a decrease in yeast biomass, sluggish fermentation rate, and associated flavor problems. Too much oxygen results in excessive yeast growth, diverting nutrients to cellular reproduction instead of ethanol production (15), and may result in undesirable flavor changes from too rapid a fermentation rate.

Microbiological Characteristics

Viability and vitality are characteristics of an individual cell (13), although most approaches provide a global mean value of a sampled bulk population of cells (38). The global mean value generated from most brewing-related methods (discussed earlier) is derived from a population of cells, and it describes the average cell in the population. When sampled at a specific time, the average cell image assumes that cells are homogeneous (synchronous) with respect to their structure, composition, and metabolic/developmental status. However, at any given time a cell population is actually composed of many heterogeneous (asynchronous) individuals, each making their own contributions, which is why most methods tell us little about the status of individual cells in a population (14). Our lab found (36) that synchronizing the culture by the incorporation of periodic feeding provides increased metabolic uniformity within the population. Populations with higher degrees of homogeneity may yield different results than heterogeneous populations, revealing more precisely the microbiological characteristics of a population (Figs. 6 and 7).

Cell Age

Depending on the audience, discussion of cell age can have a different meaning. To a microbiologist, cell age is derived from the number of times an individual cell (in a population) has divided; the entire population of a given culture is composed of individual cells of varying cell age. Cell wall analysis has shown that, in general, a stationary culture consists of 50% virgin cells, 25% first-generation mothers, and 12.5% second-generation mothers (54). The replicative capacity of a given yeast strain is called the Hayflick limit (22,23), and this limit is influenced by both genetic (5) and environmental factors (6,42). To a brewer, cell age is derived from the number of times a culture has been repitched or back-slopped in the plant, regardless of the actual cell age composition within the population (15). Serial repitching subjects a yeast population to repeated stress that may cause reversible or irreversible damage, depending on the hardiness of the strain (25,64). Chronological age is generally only a factor when yeast is stored for an extended time in a stationary phase, leading to compromised cellular integrity and ultimately cell death (42).

As yeast cells age, several phenotypic and metabolic changes occur (6,42,55,56,63). Common phenotypic changes associated with aging include increases in cell size, bud scar number, cell chitin, vacuole size, and cell surface wrinkling; decreases in cell turgor; and overall alterations of cell shape (42,56). The number of bud scars present on the cell surface is directly correlated to the number of times the cell has divided (55). A bud scar is rich in chitin, which is a long-chain polysaccharide that can be stained, with high specificity, by using the fluorescent dye Calcofluor. Thus, bud scars are the primary method in identifying cells of different replicative age (57).

Metabolic changes that occur because of aging are slightly less predictable than strain and environmental variability, but they generally include increases in generation time, decreases

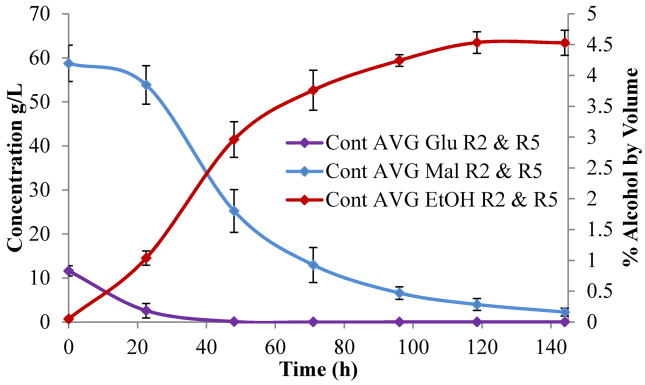

Figure 6. Typical variation in batch fermentation performance between the second (R2) and fifth (R5) pitch of a metabolically heterogeneous population of yeast cells. Cont AVG = control average (asynchronous); Glu = glucose; Mal = maltose; and EtOH = ethanol.

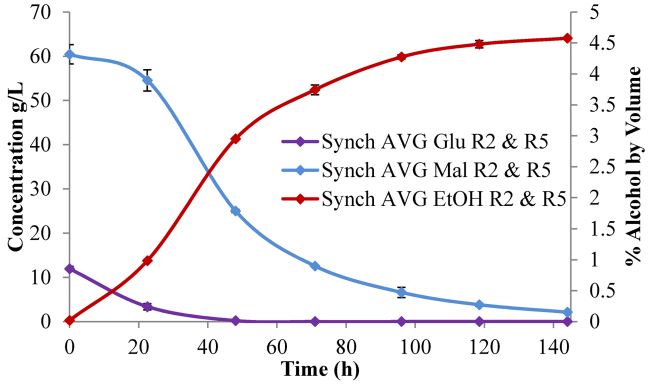

Figure 7. Variation in batch fermentation performance between the second (R2) and fifth (R5) pitch of a synchronized population of yeast cells, showing greater consistency (smaller standard deviations) than in Figure 6 as a result of greater metabolic uniformity. Synch AVG = synchronized average; Glu = glucose; Mal = maltose; and EtOH = ethanol.

in protein synthesis and ribosomal activity, and overall gene expression alterations (42,56). Replicatively older cells have been shown to have higher flocculation potential and to ferment more efficiently and at a higher rate compared with mixed-age (50% virgin, 50% mixed-age) and virgin cells (53). In normal brewing practice, older cells have likely been exposed to a bevy of physiological and chemical stressors during propagation, fermentation, and storage (19), which may result in yeast of variable condition. Thus, a fermentation composed of only aged cells is not advised, because they may have lower viability, flocculate out too early, and have a reduced generation time. Conversely, a fermentation of all virgin cells may see an extended lag phase, off-flavor formation, and poor flocculation (6,55). The ideal culture has a mix of both virgin and mixed-aged cells that are all at a uniform metabolic (synchronous) state, allowing for the maintenance of cell population dynamics throughout current and future fermentations.

Summary and Conclusions

The brewing fitness of a yeast population is a concept that incorporates multiple factors in its assessment. These factors include the percent of viable cells, the vitality of these cells, and the suitability of the culture to a particular brewing environment based on the chemical composition of the wort, oxygen availability, and fermentation temperature. Numerous techniques are available to assess brewing fitness, based on both the characteristics of the yeast population and the constituents of the wort. Most of these techniques rely on various laboratory analyses; however, some on-line or at-line instruments can provide important information that, although incomplete, will help the brewer in making informed decisions about the yeast management strategy. Consistent batch-to-batch fermentation performance can only be achieved by providing a suitably controlled environment and a yeast population that is both viable and vital.

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