STUCK AND SLUGGISH FERMENTATIONS

Linda F. Bisson

Department of Viticulture and Enology
University of California, Davis

Abstract
The yeast *Saccharomyces* has evolved towards efficient utilization of sugar. At the end of the alcoholic fermentation of grape juice, typically less than 0.1% sugar remains. Stuck fermentations arise when the yeast cease metabolism of sugar early, leaving a higher concentration than desired and than is normal for a "complete" fermentation under commercial conditions. Sluggish fermentations are defined as those that take an unusually long time to finish, on the order of months rather than two to three weeks. There are many known causes of stuck and sluggish fermentations. Such fermentations are difficult to treat as they are generally not recognized until arrest has occurred. Arrest of fermentation represents an adaptive response of the yeast to stress in the environment. Treatment options usually impact the characteristics of the wine and often lead to reduced wine quality. For these reasons, winemakers would like to reduce or eliminate the incidence of fermentation arrest.

Introduction
There are numerous causes of stuck and sluggish wine fermentations. Some of these causes are rare and localized to specific growing regions while others are more universal. Obviously, it is important to understand the reason a fermentation problem has arisen so that it can be corrected and prevented in the future. There are some common misconceptions about arrested fermentations that need to be corrected. Many winemakers as well as some researchers believe the fermentation has arrested because of massive cell death of the culture. While it is true that loss of viability will indeed result in loss of fermentative capacity, this rarely happens under production conditions. Our work as well as that of others suggests that such loss of viability is not the root cause of fermentation arrest. Cell viability remains high in arrested fermentations under California conditions, yet sugar consumption has ceased. In point of fact, sugar consumption is still occurring, just at a greatly reduced rate.

Arrest of fermentation is an adaptive process. It has been shown that under stress conditions cells turnover or degrade hexose transporter proteins thus losing the ability to ferment sugar [43,48,64]. This means that reinitiation of fermentation will require new protein synthesis, which is unlikely to occur given the high ethanol concentrations that typically accompany arrest of fermentation. The inherent design of glycolysis makes unregulated uptake of sugar deleterious to cell viability. Energy is consumed in upper glycolysis, the first reactions that phosphorylate hexoses producing fructose 1,6-diphosphate. Net energy is not produced until the last reaction of the glycolytic pathway, the pyruvate kinase reaction that converts phosphoenol pyruvate to pyruvate producing one molecule of ATP. If the upper reactions of glycolysis become uncoupled from lower glycolysis, ATP depletion can occur. Diminished energy resources impair the cell's ability to maintain viability. Since the very first reaction catalyzed by hexokinase is ATP consuming, under conditions of stress the cell's best option to prevent ATP depletion is to down regulate the process of sugar uptake [6,59,66]. Therefore, if progression through glycolysis is blocked or there is an increased demand for cellular ATP, sugar transport will be reduced and the cells will enter a maintenance state. If conditions are serious enough, the turnover of sugar transporters in the membrane will be extensive. This protects the cell from excessive glucose uptake and the potential depletion of ATP. It also allows what ATP is present to be used to repair the damage being caused by the stress. The rate of sugar catabolism decreases to the level needed to provide maintenance energy levels.

In yeast, new cell wall and membrane synthesis occurs at the site of new bud growth. This means that non-growing cells are limited in their ability to insert new proteins into their plasma membranes. Once cells have adapted to stress conditions via degradation of their transporter proteins they will only regain the ability to rapidly catabolize sugar if they are returned to growth conditions. Simply correcting the cause of the stress may not lead to restoration of fermentation. This is particularly true if ethanol levels are high enough to restrict growth (above 10% ethanol). New cell growth will not occur until the ethanol level has been reduced. If arrest has occurred at a high ethanol level, then the best option for the winemaker is to reinoculate with a strain that has been adapted to higher ethanol conditions and that is still actively fermenting.

Yeast Fermentation Capacity

The rate of fermentation of sugar is a function of two factors: the total yeast biomass concentration and the fermentative ability of individual cells. The terminal cell density of a typical fermentation is roughly 10^8 cells/mL. If there are nutritional limitations that prevent attainment of this level of biomass, the fermentation will naturally be slower because there are fewer fermenting cells in the medium. Some sluggish fermentations are simply due to low biomass levels. In addition, the fermentation capacity of individual cells is also a factor. The fermentation capacity per cell differs for different yeast strains and in response to growing conditions. Some strains tend to be slower fermentors than others. This does not mean that the fermentations will arrest, just that the time to dryness will differ. We have found that under optimal conditions, excess nutritional content, moderate temperatures and pH, with aeration and adequate mixing, the differences in fermentation capacity among strains diminishes. The differences are more pronounced when conditions of stress are present, suggesting strain differences in adaptation to the environment, which is supported by transcriptome data [28]. Strain nutritional requirements also vary. Because these dramatic strain differences exist, it is important that a winery understand the fermentation behavior of the strains employed. Accurate recognition and diagnosis of problem fermentations is dependent upon a thorough understanding of the definition of a normal fermentation profile. Strains have been identified that are efficient restarters of arrested fermentations [16].

Yeast Nutritional Requirements

The typical alcoholic fermentation can be divided into different stages (Figure 1) [8].

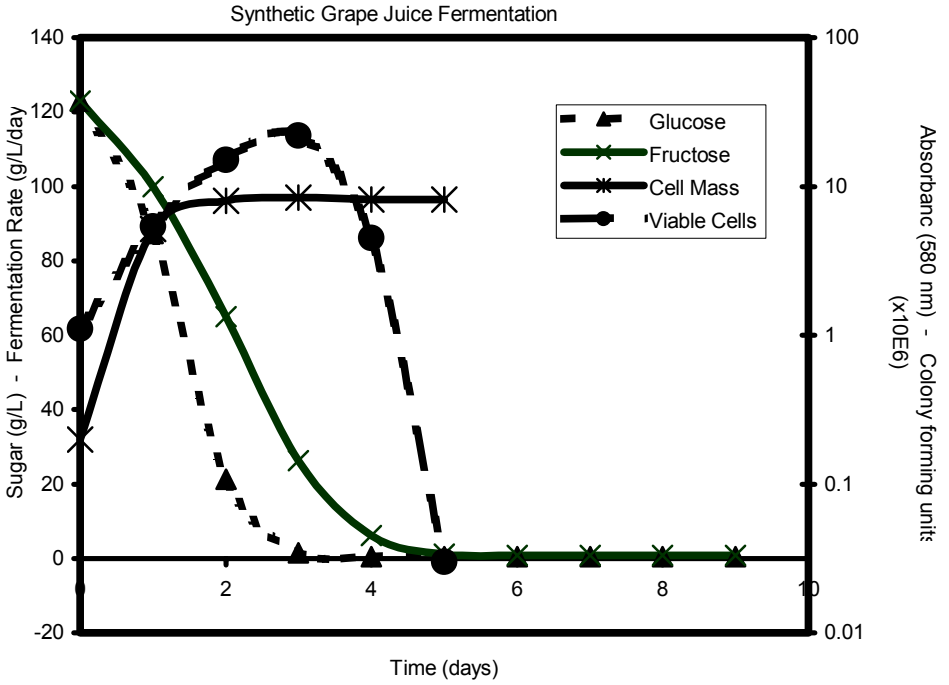

Figure 1. Yeast and fermentation profiles of grape juice.

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Based on cell growth, there is an initial lag period during which the cells adapt their metabolism to the new growth conditions. This is followed by exponential growth. If unlimited by environmental conditions, growth will proceed to the terminal cell density. This is maximal concentration of cells allowable in the medium. Once this value is obtained, net new growth will not occur until the cell number decreases. Cell numbers may decrease during fermentation due to settling of the yeast cells. Growth can resume as soon as the suspended cell concentration is sufficiently decreased. In the case of grape juice fermentations, terminal cell density is attained at 1-2 x 10^8 cells/mL. Older cells or those that have reduced fermentation capacity will settle from the fermentation. Although the exact trigger number for new growth is not known, cycling between 10^7 and 10^8 cells/mL has been observed in grape juice and must fermentations. This observation suggests that cell growth does not resume until the cell concentration is reduced to a value of 10^7/mL. If an actively fermenting must is centrifuged, the remaining cells immediately resume growth and continue to grow until 10^8 cells/mL is again reached. Thus cells in this stage are primed to resume growth as soon as the cell count is reduced. This initial stationary phase is thus due to the attainment of maximal cell density, with the cells still being competent to replicate.

At some point in the fermentation, net growth ceases regardless of the cell number. This occurs because the ethanol concentration reaches a level inhibitory to cell division. At this stage the cells are non-growing but still actively fermenting. The ethanol concentration at which this happens appears to be strain dependent and varies from about 8 to 12% ethanol. There are reports of net growth of the highly ethanol tolerant stains at even higher ethanol concentrations. Growth at high ethanol concentrations does require that ample nutrients be available. Ethanol inhibits the uptake of amino acids and results in amino acid transporter turnover [78]. Growth arrest is thought to be due to the inability to obtain enough nitrogen from the environment to support growth. Many amino acid

transporters are proton sympoters, meaning that a proton is taken up for each amino acid molecule transported. The protons must then be extruded from the cell in order to avoid acidification of the cytoplasm. Ethanol leads to increased passive proton flux into the cells. At high ethanol concentrations the ability of the cell to extrude protons may reach its maximal capacity. The only means available to the cell to maintain viability is to turn off unnecessary proton entry, such as by inactivating amino acid uptake. Ethanol also disrupts cellular structures by displacing water of hydration. Some processes such as DNA synthesis would be disrupted if ethanol concentrations were too high. Therefore the cell opts for arrest of new growth in order to increase chances of survival in the environment. It is not known why some cells have a higher tolerance to the inhibitory effects of ethanol than others. Ethanol tolerance is also affected by the composition of the environment. A lot is understood about how and why ethanol is inhibitory to cellular process but far less is understood about how cells adapt to and resist the inhibitory effects of ethanol.

The nutritional requirements for actively growing and stationary phase cells differ. Obviously actively growing cells need a full complement of macronutrients, sufficient nitrogen, phosphate and sulfate to support synthesis of a new cell in addition to the available sugar of the fermenting juice. Micronutrients are also required. If commercial strains are used, the strains may contain sufficient micronutrients (vitamins and minerals) to allow for several generations of growth. In other cases such as with native flora fermentations, the cells may be entirely dependent upon the micronutrients of the juice for growth. Oxygen stimulates cell growth and is important for synthesis of sterols [4].

Cells that are in reproductively competent stationary phase similarly require nutrients that support cell growth. Cells past this stage, those that are being inhibited by ethanol, require nutrients that have been termed “survival factors” that will assist in developing and maintaining ethanol tolerance [41]. These factors are things such as sterols and unsaturated fatty acids that help maintain membrane fluidity in the presence of a disrupting level of ethanol. Molecular oxygen is also considered to be a survival factor as it helps maintain ethanol tolerance and continued fermentation. Terming these requirements survival factors is somewhat incorrect. It is true that these factors are important for ethanol tolerance but they need to be provided to the cell during active growth, and are not that effective once growth has arrested. Oxygen, nitrogen, fatty acid supplementation have the most beneficial effects if provided as the cells are completing cell growth, at 48 to 72 hours of the fermentation. They are less effective if supplied later on. This is likely because they are needed in order for the cell to construct the appropriate membrane composition. Once growth has ceased only limited adjustments to the plasma membrane are possible. Thus it is important to make sure the cells at the exit of exponential growth have sufficient nutritional content to construct ethanol tolerant membranes and organelles.

In addition to the phases identified from the yeast growth profile, distinct phases of fermentation behavior can also be identified (Figure 1). Fermentation lags initially due to the low cell population numbers. Fermentation is fastest once the cells have attained their terminal cell density. Glucose is depleted more quickly than fructose, such that at the later stages of fermentation the principal sugar remaining is fructose. The sugar depletion curve is not linear because of the need to synthesize hexose transporters of differing affinities. Hexose transport occurs by the process of facilitated diffusion, and uptake rates are optimal only around the sugar concentrations equivalent to the Km of the enzyme. A good rule of thumb is that uptake will be optimal at substrate levels ten fold higher to ten fold lower than the Km value. The sugar concentration of fermenting must varies from a high of 1 to 2 M to a low of mM, or a thousand-fold concentration range. Transporters with Km values throughout this range need to be synthesized during fermentation. Indeed, *Saccharomyces* possesses a multigene family of hexose transporters, the *HXT* genes, with differing substrate affinities, the expression of which is largely regulated by the concentration of hexoses in the medium [9,38,59]. The exchange of transporters yields a non-linear fermentation profile, one in which the kinetics of uptake is consistently changing. At some point during the fermentation, the cells will transit from a metabolically

active state associated with competence for cell growth to a more resting state. This can be observed as a distinct transition point on the fermentation curve. The transition point is associated with the ethanol tolerance levels of the strain. An early transition point for a yeast strain that is known to be ethanol tolerant is indicative of the presence of stress in the environment leading to ethanol intolerance.

Causes of Fermentation Arrest

The process of fermentation has been extensively studied in the yeast *Saccharomyces*. There are many known causes of arrest of fermentation. The role of these factors in arrest has been confirmed in laboratory studies and in bench scale grape juice fermentations. In most cases arrest is caused by the appearance of stress factors during the fermentation. These stress factors may be physiological, for example nutritional limitation or ethanol intolerance, or environmental, exposure to extremes of temperature or competition from other microbes. Although the causes of fermentation arrest are in large part known, determining the cause in a specific case can be difficult. This is because many factors under production conditions interact and most laboratory studies have focused on a single well-controlled variable. In addition, wine fermentations represent a mixed population of both *Saccharomyces* and non-*Saccharomyces* organisms. The focus in research has been on negative interactions among these organisms, but it is also possible that important positive interactions also exist. Finally, one study indicated that there were inhibitory and stimulatory phenolic compounds present in grape juice that impact both yeast cell growth and metabolism [11]. The roles of these compounds in fermentation stimulation and arrest have not been explored. A large gene family of multidrug resistance transporters exists in *Saccharomyces,* suggesting that the uptake and excretion of phenolic compounds during grape fermentation is a common and highly regulated process [53].

The completion of the sequence of the genome of *Saccharomyces* has resulted in the development of an array of genomic technologies that allow examination of both global gene expression and protein profiles [63,67]. These technologies have been applied to wine yeasts during fermentation of natural and synthetic juices. Even though different strains and juices were used in these studies, a consistent picture of global yeast physiology has emerged from these studies. At the end of exponential growth many genes associated with stress responses in laboratory strains are expressed. The expression of these genes has been termed the general stress response [28]. In the case of wine fermentations their appearance signals a normally progressing fermentation not one that is stressed. In fact, the absence of expression of these genes is more diagnostic of a problem fermentation, that is, one that is not adapting normally to environmental conditions. These studies have also indicated that there are some strain differences that impact the ability to complete fermentation. Strains that are inherently more stress resistant tend to have higher basal levels of expression of some of the general stress response genes. This seems to slow their growth initially in wine fermentations, but allows for a more robust finish. In mixed culture fermentations, strains with reduced stress tolerance seem to have the advantage in the beginning, but will be replaced by the more stress tolerant populations at the end of fermentation.

Nutrient limitation*:* Nutrient limitation is a well-known cause of both sluggish and arrested fermentations [1,2,8,30,32,42]. Nutrients are required for both development of maximal biomass levels and for maintaining the fermentative capacity of those biomass levels. In most wine growing regions, nutritional supplementation is legally permitted. The most often limiting macronutrient is nitrogen, followed by phosphate. Very few studies implicate sulfate limitation as an actual cause of fermentation arrest, but it may be. However since nutrient supplementation is permitted, lack of micronutrients is no longer a major cause of fermentation arrest under commercial conditions.

It has recently been shown that supplementation of stationary phase fermentations with specific amino acids prolongs maximal fermentative activity [46]. Previous work suggests that ammonium does not have this effect [42]. These studies are generally done by measuring a large number of variables in many juices attempting to statistically correlate amino acid profile with fermentation arrest. It is not clear in these cases if the low amino acid levels cause the arrest or are themselves merely correlated with other juice factors that lead to sluggish fermentations. Amino acid additions may enhance the ability to synthesize rapidly degraded proteins such as the glucose transporters. Some amino acids were far more effective than others and than a mixture of amino acids [46]. The effectiveness of the amino acids appeared unrelated to their utility as nitrogen sources supporting growth. One of the most effective amino acids was glycine [46], a very poor nitrogen source for *Saccharomyces*. This is consistent, however, with reports of the stimulatory value of glycine supplementation in very high gravity fermentations [79]. The nitrogen requirement for maintenance of stationary phase fermentation was shown to differ dramatically by strain, much more so than nitrogen requirements during growth [45]. It has also recently been shown that high ammonium content of juice may inhibit efficient utilization and biomass formation of wine strains under enological conditions [65]. Thus, nitrogen-containing compounds must be in balance for optimal utilization and growth. Numerous studies have underscored the importance of proper nitrogen nutrition for completion of fermentation under enological conditions [1,7,30,50,51,61,62,68]. Phosphate limitation has also been shown to impact cell growth and biomass yield as well as directly affecting fermentation rate [8,27,42].

Micronutrient limitation has also been correlated with sluggish fermentation. In severe cases, if cells have been depleted of vitamins and co-factors, arrest of fermentation can occur [10,22]. Calcium limitation increases ethanol sensitivity [52]. High manganese depresses uptake of magnesium and vice versa [10], so a disparity in these ions may lead to a deficiency situation. We have also recently shown that an imbalance of pH and potassium ions can lead to a stuck fermentation in model juice-like media, and such imbalances may be present in grapes from vines that display poor potassium uptake from soil [39]. Sluggish fermentations are also observed in juices with a very high potassium level. This may not be directly caused by the potassium itself but rather by other changes in berry composition that accompany high potassium levels.

Saccharomyces is capable of making all essential vitamins except biotin, but research has shown that the presence of other vitamins is highly stimulatory to growth and fermentation [42,50,54]. It has recently been demonstrated that *Kloeckera apiculata* is quite efficient at stripping thiamine from grape juice in a matter of hours, thus leading to a deficient situation for *Saccharomyces* [5]. The presence of acetic acid has been reported to reduce the ability of *Saccharomyces* to transport and retain thiamine [33]. Thus, one likely cause of stuck fermentations arising from uninoculated juices is the depletion of thiamine by wild yeasts with the production of acids that inhibit transport of the residual vitamin by *Saccharomyces*. Sulfur dioxide reacts directly with thiamine, reducing the level of this vitamin.

Ethanol toxicity and the role of survival factors*:* Another major cause of stuck fermentations is ethanol toxicity [14,18,34,77,78]. Ethanol appears to impact plasma membrane fluidity in a complex fashion. Ethanol decreases polarity in aqueous surroundings but increases it in hydrophobic environments [15]. The cell responds to ethanol by producing a membrane rich in unsaturated fatty acids and ergosterol [3,15,21, 34,47,77]. In addition to an increase in unsaturated fatty acids and sterols, the protein content is reduced ethanol-tolerant membranes. The changes in plasma membrane composition have been proposed to alter the plasma membrane fluidity to compensate for the disruptive effects of ethanol on fluidity. However, this conclusion has recently been questioned [34,35,36]. Instead, it has been suggested that ethanol impacts membrane function by polar, dielectric and hydrogen bond interactions with the polar head groups of the phospholipids and integral membrane proteins rather than changing plasma membrane fluidity [34,35,36]. The changes in fatty acid desaturation, decrease in protein content and increase in ergosterol level are proposed to occur to counter the disruptive effects of ethanol on phospholipid head groups and proteins, rather than to

alter plasma membrane fluidity [34,35,36]. Ethanol sensitivity is a variable property among strains of *Saccharomyces*. It is also impacted by the availability of sterols and long chain saturated and unsaturated fatty acids in the medium as these compounds cannot be readily synthesized by the yeast under anaerobic conditions [6,15,19,41,42,80].

Acetaldehyde, the immediate precursor to ethanol in catabolism, is also toxic [73,74], and may be in large part responsible for "ethanol sensitivity" [34,35]. Strain differences in ethanol tolerance have been associated with cellular levels of acetaldehyde; those strains with the lowest cytoplasmic acetaldehyde level being the most tolerant [34,35].

***Low pH**: Saccharomyces* is tolerant to low pH fermentations and can readily grow in the juice pH range of 2.8 to 4.2 [8,29,42]. Below pH 2.8, both growth and fermentation are inhibited. The ethanol, organic and fatty acid tolerances of many strains are reduced at very low pH values [55], and we have seen that the potassium concentration is a key factor in pH tolerance [39]. *Saccharomyces* excretes protons during fermentation and may reduce the pH of the medium by as much as 0.3 units. The pH will also have a dramatic effect on the types of bacterial species present and their persistence, which may significantly impact fermentation progression.

Extremes of temperature: Exposure to temperature extremes can also inhibit fermentation rates [71,75]. The plasma membrane is the main target of the inhibitory effects of high or low temperature [reviewed in 75,83]. Since ethanol and temperature target the same cellular function, it is not surprising that their effects are synergistic. Abrupt changes in temperature also affect cytoplasmic enzyme activity and organelle structure and function. Heat shock leads to the induction of several stress related proteins, which may also be present upon entry into stationary phase [17]. The ability to respond to sudden temperature changes is dependent upon the ability to synthesize the heat shock proteins. If the temperature shock occurs under conditions of nutrient limitation of the yeast, the cells might not be able to compensate for the change in temperature.

***Toxins*:** Several toxic substances have also been shown to lead to fermentation arrest [2,8,42]. Yeast can produce zymocidal substances known collectively as killer factors [reviewed in 84]. Non-*Saccharomyces* yeasts such as *Hansenula* and *Kluyveromyces* produce killer factors that are active against *Saccharomyces*. *Saccharomyces* strains can also produce glycoprotein killer factors that are toxic to susceptible strain of *Saccharomyces* [13,56]. The effect of killer toxins is dependent upon medium composition, and the relative ratios of the sensitive to toxin-producing strains [49]. *Bacillus* and *Streptomyces*, also common soil organisms that have been isolated in wineries, produce metabolites that limit yeast growth under enological conditions [37]. Molds present on the fruit at harvest may produce mycotoxins to which *Saccharomyces* is susceptible. It has been suggested that Botrytized fruit contains toxic substances [42,60], but the nature of the substance has not been determined in spite of significant research efforts.

A more likely problem caused by mold infestation of fruit is the production of compounds toxic to fungi by the plant when challenged with fungal infection [76]. Plants produce numerous compounds (the phytoalexins) and enzymes (the pathogenesis-related proteins) in response to infection that are designed to eliminate the pathogen [44,72]. Toxic phenolic compounds, amino acid analogs and enzymes capable of degradation of fungal cell walls (chitinases and glucanases) can all be produced in response to infection. The phytoalexins are broadly toxic and may even reduce viability of the plant cells producing them [72]. It is highly likely that some of these factors will also impact yeast growth and fermentation since the yeast are members of the same taxonomic family as the filamentous fungi and have a similar cell wall architecture. Fungicides and pesticides used in the vineyard may negatively affect yeast viability if present at high enough residual concentrations at the time of harvest [8,42].

Organic and medium chain fatty acids are also inhibitory to *Saccharomyces* [12,23,25,40,42,57,58,81,82]. Bacteria and non-Saccharomyces yeast may produce these compounds, but they can also be formed by *Saccharomyces* [25,58]. Mixed culture fermentations (*Saccharomyces* and non-*Saccharomyces* yeast and bacteria) increase the risk for the appearance of this type of inhibition. As mentioned above, these acids are more toxic at high ethanol and extremes of temperature [82], and may impact vitamin absorption and retention [33] all of which may affect fermentation by *Saccharomyces*.

Microbial incompatibility*:* Enological fermentations containing initial high populations of non-*Saccharomyces* yeast and bacteria are at greater risk for the development of stuck and sluggish fermentations [8,23,24,25,42]. This is due in part to the competition for nutrients and production of toxic substances as described above. However, other factors such as high total cell density may also be important in the reduction of fermentation rate. We and others have found that mixed culture fermentations (*Saccharomyces* and bacteria) require higher than normal vitamin supplementation, and that the arrested fermentation may not be restartable until the existing biomass is removed via racking or centrifugation [T. Rynders and L. F. Bisson, unpublished observations]. It is also important to note that there are incompatible pairings of wine yeast and malolactic (ML) bacteria. *Lactobacillus kunkeei*, referred to as the "ferocious Lactobacillus", frequently causes stuck fermentations regardless of the yeast strain(s) present [24,26].

Enological practices*:* Several enological practices can decrease or increase the incidence of fermentation arrest [2,8]. Excessive clarification of musts reduces fermentation rate. This appears to be due to multiple factors: the loss of nutrients found in particulate matter such as unsaturated fatty acids and sterols [20,21,31,42], the reduction of vitamins and mineral content via the removal of microbes that have sequestered these components [5] and a decrease in the natural agitation ability of the must [bisrev]. It has also been suggested that must solids serve as nucleating sites for the release of CO_2, and may provide a solid surface upon which the yeast can form a biofilm. Excessive use of sulfur dioxide can also lead to poor fermentation performance, depending upon the pH of the medium and bound and free concentrations of the compound [8]. Winery practices such as the addition of nutrients and aeration may positively impact fermentation performance and reduce the incidence of stuck and sluggish fermentations [2,8,42]. Finally, the type of temperature control employed may be a major contributing factor to fermentation problems. Too large or too small of a temperature differential between the coolant and the desired temperature of the must can result in a sluggish fermentation and even in death of the yeast [8].

Other factors*:* Several additional factors that may impact fermentation rate have been less well characterized. It has been suggested that a high concentration of fructose relative to glucose is inhibitory to yeast [69,70]. The high residual concentrations of fructose may be a symptom rather than a cause of stuck and sluggish fermentations. Schütz and Gafner [70] have suggested that poor utilization of fructose and a tendency to stick during fermentation are associated with a deficiency in level of hexokinase I activity. However, it has been shown that addition of fructose, and the accompanying change in the glucose to fructose ratio (GFR) can inhibit an ongoing fermentation [69] while addition of glucose to alter the GFR to less than 0.1 can stimulate fermentation activity. It is not clear if this is due to substrate inhibition of the transport process or not.

An area that has been largely ignored is the impact of grape must enzymes on the progression of fermentation. Polyphenol oxidase levels vary dramatically with the varietal and the season [8]. This enzyme competes directly with *Saccharomyces* for available dissolved oxygen. It has been suggested that the real reason that SO_2 is able to stimulate fermentation by *Saccharomyces* lies in the inhibition of the competing polyphenol oxidase, making oxygen more readily available for *Saccharomyces* [bisrev] and not in the inhibition of wild (non-*Saccharomyces*) yeast and bacteria.

It has occasionally been noted by winemakers that certain vineyard locations yield fruit highly susceptible to sluggish fermentations, even when nutrient additions are made to the must. This effect may reflect a particularly high disease pressure of those vines and the resultant phytoalexin content of the juice, or alternately may suggest that these grapes support high wild flora populations. We have recently shown that phenolic compounds found in grape can stimulate or inhibit fermentation rates, depending upon the concentration and the specific compound [R. Hood and L. F. Bisson, unpublished observations]. The biological effects of grape phenolics on yeast have not been well studied. Cantarelli reported both inhibitory and stimulatory effects on growth and fermentation rates [11]. Sluggish fermentations may arise due to a deficiency of stimulatory phenolics or to an excess of inhibitory ones. Differences in phenolic composition may be one reason that fruit from certain vineyard sites seems prone to fermentation problems.

Conclusions

Many factors have been shown to result in slow or incomplete enological fermentations. Although the precise cause of a stuck and sluggish fermentation may be difficult to determine, the type of fermentation arrest can be quite useful at limiting the number of possibilities. Careful attention to numbers of viable *Saccharomyces* cells, judicious use of cooling and temperature maintenance in addition to prudent nutrient supplementation practices should greatly reduce the incidence of slow and incomplete fermentations. Some open questions still remain as in some cases fermentations arrest with no known cause. Predictive tools are currently being developed that will assist the winemaker in assuring completed fermentations.

Literature Cited

1. Agenbach, W. A. A study of must nitrogen content in relation to incomplete fermentations, yeast production and fermentation activity. pp 66-88 *In* Proceedings of the South African Society for Enology and Viticulture, Stellenbosch: South African Society of Enology and Viticulture, Cape Town. (1977).

2. Alexandre, H., and C. Charpentier. Biochemical aspects of stuck and sluggish fermentation in grape must. J. Ind. Microbiol. Biotech. 20:20-27 (1998).

3. Alexandre, H., I. Rousseaux, and C. Charpentier. Relationship between ethanol tolerance, lipid composition and plasma membrane fluidity in *Saccharomyces cerevisiae* and *Kloeckera apiculata*. FEMS Microbiol. Letts. 124:17-22 (1994).

4. Aries, V., and B. H. Kirsop. Sterol biosynthesis by strains of *Saccharomyces cerevisiae* in the presence and absence of dissolved oxygen. J. Inst. Brew. 84:118-122 (1978).

5. Bataillon, M., A. Rico, J.-M. Sablayrolles, J. M. Salmon, and P. Barre. Early thiamine assimilation by yeasts under enological conditions: Impact on alcoholic fermentation kinetics. J. Ferm. Bioeng. 82:145-150 (1996).

6. Becker, J.-U., and A. Betz. Membrane transport as controlling pacemaker of glycolysis in *Saccharomyces cerevisiae.* Biochim. Biophys. Acta 274:584-597 (1972).

7. Bely, M., J.-M. Sablayrolles, and P. Barre. Automatic detection of assimilable nitrogen deficiencies during alcoholic fermentation in enological conditions. J. Ferm. Bioeng. 70:246-252 (1990).

8. Bisson, L. F. Stuck and sluggish fermentations. Am. J. Enol. Vitic. 50:107-119 (1999).

9. Bisson, L. F., D. M. Coons, A. L. Kruckeberg, and D. A. Lewis. Yeast sugar transporters. Crit. Rev. Biochem. Mol. Biol. 28:259-308 (1993).

10. Blackwell, K. J., J. M. Tobin, and S. V. Avery. Manganese uptake and toxicity in magnesium-supplemented and unsupplemented *Saccharomyces cerevisiae*. Appl. Microbiol. Biotech. 47:180-184 (1997).

11. Cantarelli, C. Phenolics and yeast: Remarks concerning fermented beverages. pp S53-S51 *In* Proceedings of the Seventh International Symposium on Yeasts, A. Martini and A. Vaughan-Martini (eds.). John Wiley and Sons, Ltd., New York. (1989).

12. Cardoso, H., and C. Leao. Mechanisms underlying the low and high enthalpy death induced by short chain monocarboxylic acids and ethanol in *Saccharomyces cerevisiae*. Appl. Micro. Biotechnol. 38:388-392 (1992).

13. Carrau, F. M., E. Neirotti, and O. Gioia. Stuck wine fermentations: Effect of killer/sensitive yeast interactions. J. Ferm. Bioeng. 76:67-69 (1993).

14. Casey, G. P., and W. M. Ingledew. Reevaluation of alcohol synthesis and tolerance in brewers yeast. Am. Brew. Soc. Chem. 43:75-83 (1985).

15. Ciesarova, Z., J. Sajibidor, D. Smogrovicova, and P. Bafrncova. Effect of ethanol on fermentation and lipid composition in *Saccharomyces cerevisiae*. Food Biotech. 10:1-12 (1996).

16. Colas, S. Une nouvelle levure seche active pour les reprises de fermentation alcoolique: RhoneL4473. Guide de la Vinification Rhodonienne 3:39-40.

17. Craig, E. A. The heat shock response of *Saccharomyces cerevisiae*. pp 501-537 *In* The Molecular and Cellular Biology of the Yeast *Saccharomyces*: Gene Expression, E. W. Jones, J. R. Pringle, and J. R. Broach (eds.). Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York. (1992).

18. D'Amore, T., and G. G. Stewart. Ethanol tolerance of yeast. Enzyme. Microbiol. Technol. 9:322-330 (1987).

19. David, M. H., and B. H. Kirsop. A correlation between oxygen requirements and the products of sterol synthesis in strain *Saccharomyces cerevisiae*. J. Gen. Microbiol. 77:527-531 (1973).

20. Delfini, C., C. Cocito, S. Ravaglia, and L. Conterno. Influence of clarification and suspended grape solid materials on sterol content of free run and pressed grape musts in the presence of growing yeasts. Am. J. Enol. Vitic. 44:86-92 (1993).

21. Delfini, C., L. Conterno, D. Giacosa, C. Cocito, S. Ravaglia, and L. Bardi. Influence of clarification and suspended solid contact on the oxygen demand and long-chain fatty acid contents of free run, macerated and pressed grape musts in relation to acetic acid production. Vitic. Enol. Sci. 47:69-75 (1992).

22. Dombeck, K. M., and L. O. Ingram. Magnesium limitation and its role in apparent toxicity of ethanol in yeast fermentation. Appl. Environ. Microbiol. 52:975-981 (1986).

23. Drysdale, G. S., and G. H. Fleet. The effect of acetic acid bacteria upon the growth and metabolism of yeast during the fermentation of grape juice. J. Appl. Bacteriol. 67:471-481 (1989).

24. Edwards, C. G. Stalking "ferocious" Lactobacilli. Pract. Winery Vineyard Sept/Oct:5-8 (1998).

25. Edwards, C. G., R. B. Beelman, C. E. Bartley, and A. L. McConnell. Production of decanoic and other volatile compounds and the growth of yeast and malolactic bacteria during vinification. Am. J. Enol. Vitic. 41:48-56 (1990).

26. Edwards, C. G., K. M. Haag, M. D. Collins, R. A. Hutson, and Y. C. Huang. *Lactobacillus kunkeei* sp. nov.: a spoilage organisms associated with grape juice fermentations. J. Appl. Microbiol. 84:698-702 (1998).

27. Gancedo, C., and R. Serrano. Energy-yielding metabolism. pp 205-259 *In* The Yeasts (2nd Edition) Volume 3: Metabolism and Physiology of Yeasts, A. H. Rose and J. S. Harrison (eds.). Academic Press, New York. (1989).

28. Gasch, A. P. The environmental stress response: A common yeast response to diverse environmental stresses. Pages 11-70 In: S. Hohmann and P. W. H. Mager (eds) Topics in Current Genetics, Vol. 1: Yeast Stress Responses, Springer-Verlag Berlin, (2003).

29. Heard, G. M., and G. H. Fleet. The effects of temperature and pH on the growth of yeast species during the fermentation of grape juice. J. Appl. Bact. 65:23-28 (1988).

30. Henschke, P. A., and V. Jiranek. Metabolism of nitrogen compounds. pp 77-164 *In* Wine microbiology and biotechnology, G. H. Fleet (ed.). Harwood Academic Publishers, Australia. (1993).

31. Houtman, A. C., and C. S. Du Plessis. Nutritional deficiencies of clarified white grape juices and their correction in relation to fermentation. S. Afr. J. Enol. Vitic. 7:39-46 (1986).

32. Ingledew, W. M., and R. E. Kunkee. Factors influencing sluggish fermentations of grape juice. Am. J. Enol. Vitic. 36:65-76 (1985).

33. Iwashima, A., H, Nishino, and Y. Nose. Carrier-mediated transport of thiamine in baker's yeast. Biochim. Biophys. Acta 330:222-234 (1973).

34. Jones, R. P. Biological principles for the effects of ethanol. Enzyme Microbiol. Technol. 11:130-153 (1989).

35. Jones, R. P. Roles for replicative deactivation in yeast-ethanol fermentations. Crit. Rev. Biotechnol. 10:205-222 (1990).

36. Jones, R. P., and P. F. Greenfield. Ethanol and the fluidity of the yeast plasma membrane. Yeast 3:223-232 (1987).

37. Kielhoefer, E. The effect of antibiotic substances upon the fermentation of wine. Am. J. Enol. Vitic. 5:113-17 (1954).

38. Kruckeberg, A. L. The hexose transporter family of *Saccharomyces cerevisiae*. Arch. Microbiol. 166:283-292 (1996).

39. Kudo, M., P. Vagnoli, and L. F. Bisson. Imbalance of potassium and hydrogen ion concentrations as a cause of stuck enological fermentations. Am. J. Enol. Vitic. 49:295-301 (1998).

40. Lafon-Lafourcade, S., C. Geneix, and P. Ribereau-Gayon. Inhibition of alcoholic fermentation of grape must by fatty acids produced by yeasts and their elimination by yeast ghosts. Appl. Environ. Microbiol. 47:1246-1249 (1984).

41. Lafon-Lafourcade, S., F. Larue, and P. Ribereau-Gayon. Evidence for the existence of "survival factors" as an explanation for some peculiarities of yeast growth especially in grape must of high sugar concentration. Appl. Environ. Microbiol. 38:1069-1073 (1979).

42. Lafon-Lafourcade, S., and P. Ribereau-Gayon. Developments in the microbiology of wine production. Prog. Indust. Microbiol. 19:1-45 (1984).

43. Lagunas, R., C. Dominguez, A. Busturia, and M. J. Saez. Mechanisms of appearance of the Pasteur effect in *Saccharomyces cerevisiae*: Inactivation of the sugar transport systems. J. Bacteriol. 152:19-25 (1982).

44. Linthorst, H. J. M. Pathogenesis-related proteins of plants. Crit. Rev. Plant Sci. 10:123-150 (1991).

45. Manginot, C., J. L. Roustan, and J. M. Sablayrolles. Nitrogen demand of different yeast strains during alcoholic fermentation. Importance of the stationary phase. Enzyme Microbial Tech. 23:511-517 (1998).

46. Manginot, C., J. M. Sablayrolles, J. L. Roustan, and P. Barre. Use of constant rate alcoholic fermentations to compare the effectiveness of different nitrogen sources added during the stationary phase. Enzyme Microbial Tech. 20:373-380 (1997).

47. Mauricio, J. C., S. Guijo, and J. M. Ortega. Relationship between phospholipid and sterol content in *Saccharomyces cerevisiae* and *Torulaspora delbrueckii* and their fermentation activity in grape musts. Am. J. Enol. Vitic. 42:301-308 (1991).

48. Mauricio, J. C., and J. M. Salmon. Apparent loss of sugar transport activity in *Saccharomyces cerevisiae* may mainly account for maximum ethanol production during alcoholic fermentation. Biotech. Lett. 14:577-601 (1992).

49. Medina, K., F. M. Carrau, O. Gioia, and N. Bracesco. Nitrogen availability of grape juice limits killer yeast growth and fermentation activity during mixed-culture fermentation with sensitive commercial yeast strains. Appl. Environ. Microbiol. 63:2821-2825 (1997).

50. Monk, P. R. Effect of nitrogen and vitamin supplements on yeast growth and rate of fermentation of Rhine Riesling grape juice. Food Tech. Aust. 34:328-332 (1982).

51. Monteiro, F. F., and L. F. Bisson. Biological assay of nitrogen content of grape juice and prediction of sluggish fermentations. Am. J. Enol. Vitic. 42:47-57 (1991).

52. Nabais, R. C., I. Sa-Correia, C. A. Viegas, and J. M. Novais. Influence of calcium ion and ethanol tolerance of *Saccharomyces bayanus* on alcoholic fermentation by yeasts. Appl. Environ. Microbiol. 54:2439-2446 (1988).

53. Nelissen, B., R. DeWachter, and A. Goffeau. Classification of all putative permeases and other membrane multispanners of the major facilitator superfamily encoded by the complete genome of *Saccharomyces cerevisiae*. FEMS Microbiol. Rev. 21:133-134 (1997).

54. Ough, C. S., M. Davenport, and K. Joseph. Effect of certain vitamins on growth and fermentation rate of several commercial active dry wine yeasts. Am. J. Enol. Vitic. 40:208-213 (1989).

55. Pampulha, M. E., and C. Loureiro-Dias. Combined effect of acetic acid, pH and ethanol on intracellular pH of fermenting yeast. Appl. Microbiol. Biotech. 31:547-550 (1989).

56. Ramon-Portugal, F., M. L. Delia, P. Strehaiano, and J. P. Riba. Mixed culture of killer and sensitive *Saccharomyces cerevisiae* strains in batch and continuous fermentations. World J. Micro. Biotech. 14:83-87 (1998).

57. Rasmussen, J. E., E. Schultz, R. E. Snyder, R. S. Jones, and C. R. Smith. Acetic acid as a causative agent in producing stuck fermentations. Am. J. Enol. Vitic. 46:278-280 (1995).

58. Ravaglia, S., and C. Delfini. Production of medium chain fatty acids and their ethyl esters by yeast strains isolated from musts and wines. Ital. J. Food Sci. 1:21-36 (1993).

59. Reifenberger, E., E. Boles, and M. Ciriacy. Kinetic characterization of individual hexose transporters of *Saccharomyces cerevisiae* and their relation to the triggering mechanisms of glucose repression. Eur. J. Biochem. 245:324-333 (1997).

60. Ribereau-Gayon, P., S. Lafon-Lafourcade, D. Dubourdieu, V. Lucmaret, and F. Larue. Metabolism de *Saccharomyces cerevisiae* dans les mouts de raisin parasites par *Botrytis cinerea*. Inhibition de la fermentation; formation d'acide acetique et de glycerol. CR Acad. Sci. 289:441-444 (1979).

61. Sablayrolles, J.-M. Importance de l'azote assimilable de l'oxygene sur le deroulement de la fermentation alcoolique. Biologia Oggi 6:155-160 (1992).

62. Sablayrolles, J.-M., C. Dubois, C. Manginot, J.-L. Roustan, and P. Barre. Effectiveness of combined ammoniacal nitrogen and oxygen additions for completion of sluggish and stuck fermentations. J. Ferm. Bioeng. 82:377-381 (1996).

63. Sagliocco, F., J.-C. Guillemot, C. Monribot, J. Capdevielle, M. Perrot, E. Ferran, P. Ferrara, and H. Boucherie. Identification of proteins of the yeast protein map using genetically manipulated strains and peptide mass-fingerprinting. Yeast 12:1519-1533 (1996).

64. Salmon, J. M. Effect of sugar transportation inactivation in *Saccharomyces cerevisiae* on sluggish and stuck enological fermentations. Appl. Environ. Microbiol. 55:9535-9538 (1989).

65. Salmon, J. M., and P. Barre. Improvement of nitrogen assimilation and fermentation kinetics under enological conditions by derepression of alternative nitrogen-assimilatory pathways in an industrial *Saccharomyces cerevisiae* strain. Appl. Environ. Microbiol. 64:3831-3837 (1998).

66. Salmon, J. M., O. Vincent, J. C. Mauricio, M. Bely, and P. Barre. Sugar transport inhibition and apparent loss of activity in *Saccharomyces cerevisiae* as a major limiting factor of enological fermentations. Am. J. Enol. Vitic. 44:56-64 (1993).

67. Schena, M., D. Shalon, R. W. Davis, and P. O. Brown. Quantitative monitoring of gene expression patterns with a complementary DNA microarray. Science 270:467-470 (1995).

68. Schulze, U., G. Liden, J. Nielsen, and J. Villadsen. Physiological effects of nitrogen starvation in an anaerobic batch culture of *Saccharomyces cerevisiae*. Microbiol. 142:2299-2310 (1996).

69. Schütz, M., and J. Gafner. Sluggish alcoholic fermentation in relation to alterations of the glucose - fructose ratio. Chem. Mikrobiol. Technol. Lebensm. 15:73-78 (1993).

70. Schütz, M., and J. Gafner. Lower fructose uptake capacity of genetically characterized strains of *Saccharomyces bayanus* compared to *Saccharomyces cerevisiae*: A likely cause of reduced alcoholic fermentation. Am. J. Enol. Vitic. 46:175-180 (1995).

71. Sharf, R., and P. Margalith. The effect of temperature on spontaneous wine fermentation. Appl. Microbiol. Biotech. 17:311-313 (1983).

72. Smith, D. A., and S. W. Banks. Biosynthesis, elicitation and biological activity of isoflavonoid phytoalexins. Phytochem. 25:979-995 (1986).

73. Stanley, G. A., N. G. Douglas, E. J. Every, T. Tzanatos, and N. B. Pamment. Inhibition and stimulation of yeast growth by acetaldehyde. Biotech. Letts. 15:1199-1204 (1993).

74. Stanley, G. A., T. J. Hobley, and N. B. Pamment. Effect of acetaldehyde on *Saccharomyces cerevisiae* and *Zymomonas mobilis* subjected to environmental shocks. Biotechnol. Bioeng. 53:71-78 (1997).

75. Suutari, M., K. Liukkonen, and S. Laakso. Temperature adaptation in yeast: The role of fatty acids. J. Gen. Microbiol. 136:1469-1474 (1990).

76. Takemoto, J. Y., L. Zhang, N. Taguchi, T. Tachikawa, and T. Miyakawa. Mechanisms of action of the phytotoxin syringomycin: A resistant mutant of *Saccharomyces cerevisiae* reveals an involvement of Ca^{2+} transport. J. Gen. Microbiol. 137:653-659 (1991).

77. Thomas, D. S., J. A. Hossack, and A. H. Rose. Plasma membrane lipid composition and ethanol tolerance in *Saccharomyces cerevisiae*. Arch. Microbiol. 117:239-245 (1978).

78. Thomas, D. S., and A. H. Rose. Inhibitory effect of ethanol on growth and solute accumulation by *Saccharomyces cerevisiae* as affected by plasma membrane lipid composition. Arch. Microbiol. 122:19-25 (1979).

79. Thomas, K. C., S. H. Hynes, and W. M. Ingledew. Effects of particulate materials and osmoprotectants on very-high-gravity ethanolic fermentation by *Saccharomyces cerevisiae*. Appl. Environ. Microbiol. 60:1519-1524 (1994).

80. Traverso-Rueda, S., and R. E. Kunkee. The role of sterols on growth and fermentation of wine yeast under vinification conditions. Dev. Indust. Environ. Microbiol. 23:131-143 (1982).

81. Viegas, C. A., M. F. Rosa, I. Sa-Correia. and J. M. Novais. Inhibition of yeast growth by octanoic and decanoic acids produced during ethanolic fermentation. Appl. Environ. Microbiol. 55:21-28 (1989).

82. Viegas, C. A., and I. Sa-Correia. Toxicity of octanoic acid in *Saccharomyces cerevisiae* at temperatures between 8.5 and 30°C. Enzyme. Microbiol. Tech. 17:826-831 (1995).

83. Watson, K. Temperature relations. pp. 41-72 *In* The Yeasts, Volume 2: Yeasts and the Environment, A. H. Rose (Ed.). pp 41-71 Academic Press, New York (1987).

84. Young, T. W. Killer yeasts. pp 131-164 *In* The Yeasts, Volume 2: Yeasts and the Environment, A. H. Rose (Ed.). pp 131-164. Academic Press, New York (1987).