

Fermentation

# Avoiding stuck fermentations

Just in time for vintage, **Joana Coulon**, manager microbiology, BioLaffort, **Alana Seabrook**, from Laffort Australia, and **Susan Erasmus**, Laffort South Africa, outline some of the key contributing factors in stuck fermentations and how to avoid them.





## Introduction

Stuck and sluggish fermentations can cause significant economic losses for a winery due to the extended labour requirements and the purchase of additional yeast and nutrients required to restart them. Wine quality is often significantly impacted causing additional financial loss. Extended periods of time with residual sugar and lack of SO<sub>2</sub> protection increase the risk of microbial spoilage primarily due to *Acetobacter* spp and *Brettanomyces bruxellensis*. The intention of this article is to examine some of the key contributing factors to stuck fermentations and how best to avoid them.

## Stage 1: Must

Many of the contributing factors to stuck fermentations are found in must before yeast is inoculated. But often stuck and sluggish fermentations are an adaptive process with a number of factors culminating in the arrest of fermentation.

### Nitrogen

The portion of nitrogen relevant to fermentation is yeast assimilable nitrogen (YAN) content, which is the nitrogen able to be taken up by *Saccharomyces cerevisiae*. Its starting level is generally vineyard dependant (Boulton *et al.* 1999). YAN can be found in two forms: mineral or organic. Mineral nitrogen is made up of ammonia (NH<sub>3</sub>), and can be added as di-ammonium phosphate in Australia. Organic nitrogen is made up of free amino acids with the exception of proline and can be added through autolysed yeast. In grapes, mineral nitrogen makes up to one third of the total nitrogen, whilst the organic fraction makes up two thirds to three quarters grape derived of YAN (Ribereau-Gayon 2006). At the beginning of fermentation *Saccharomyces cerevisiae* utilise YAN to build cell biomass corresponding to the amount of sugar present in the must (Table 1). In cases of YAN deficiency, yeast are not able to build to sufficient populations and the fermentation will likely be slower as there are less cells fermenting in the must (Bisson *et al.* 2005). The level of biomass is also impacted by whether the strain has a high (more biomass produced) or low nitrogen requirement (less biomass produced) (Figure 1).

### Water additions, stress conditions and juice chemistry

Water additions to grapes at the crusher were legalised for the 2017 Australian vintage, enabling the addition of water to adjust Baume down to 13.5. But a starting Baume of 17 and a resulting Baume of 13.5 can lead to a significant amount of dilution of not only sugar

but vitamins, minerals, lipids and amino acids.

Juice chemistry including sugar level, SO<sub>2</sub>, pH and volatile acidity may impact alcoholic fermentation. A very high sugar level can cause osmotic stress at the beginning of fermentation and lead to high levels of ethanol at the end of fermentation. *Saccharomyces cerevisiae* are sensitive to SO<sub>2</sub>, ethanol >10% as well as very low pH and volatile acidity above 0.8g/L of acetic acid. The levels of sensitivity are strain specific.

Vine health aside, heat stress can significantly impact juice chemistry. Baeza *et al.* (2019) found that there was a positive correlation between sugar content and available water, but also phenolic compound production, mainly in the form of anthocyanins (Downey *et al.* 2006). Several authors have reported links to increased levels of key aroma compounds such as norisoprenoids, carotenoids and monoterpenes (Reynolds and Wardle 1989, Belancic *et al.* 1997). Other changes relative to grape maturation are the degradation of malic acid and the accumulation of tartaric acid. Whilst tartaric acid is not affected by heat stress, malic acid above 46°C is degraded (Lakso and Kliever 1975, Drappier *et al.* 2017). High concentrations of phenolic compounds are inhibitory towards yeast (Pastorkova *et al.* 2013).

Mould-affected grapes may have toxins, pathogenesis-related enzymes and other toxic compounds produced by the grapes when presented with a fungal infection (Takemoto *et al.* 1991). It is possible these factors may not only affect yeast multiplication but also fermentation (Bisson *et al.* 2005, Smith and Banks 1986). Non-*Saccharomyces*, *Saccharomyces cerevisiae* and lactic acid bacteria are able to produce medium chain fatty acids that are inhibitory towards *Saccharomyces cerevisiae*, markedly at the tail end of alcoholic fermentation at high concentrations of ethanol and temperature extremes.

## Strategies to overcome stuck fermentations: Stage 1 Must

### 1. Understanding starting levels of nutrition

Measuring the level of YAN is a simple method to assess the nutrition status of must. Yeast need enough nitrogen to produce sufficient biomass for the specific level of potential alcohol which will differ if the yeast strain is a high or low nitrogen demanding strain. Laffort proposes a nitrogen adjustment calculator specifically for this purpose (<https://laffort.com/en/decision-making-tools/>). The chances of a successful alcoholic fermentation are greatly increased if the starting YAN is adjusted accordingly.

### 2. Factor in water additions, stress conditions and wine chemistry

Basic chemistry of must is key to assessing what conditions yeast are being asked to grow in. This will likely change after additions are made at the crusher including acid, water and SO<sub>2</sub>. Yeast will not grow under pH 2.8g/L and above 0.8g/L acetic acid, so ensuring must chemistry permits the multiplication of yeast cells before fermentation has even begun is critical. Microorganisms including lactic acid bacteria, *Saccharomyces cerevisiae* and non-*Saccharomyces* yeast spp as well as acetic acid bacteria prefer higher pH, which could lead to competition for nutrients if pH is left unchecked at this stage.

Water dilution can impact the fermentation by diluting nitrogen, nutrients, lipids, vitamins and minerals which will need to be replaced should they fall below critical levels.

### 3. Yeast selection

Yeast strains of *Saccharomyces cerevisiae* have different tolerances to alcohol, optimum fermentation temperature ranges and nitrogen demand. Choosing an optimal strain for the specific conditions will improve the chances of completing alcoholic fermentation.

## Stage 2: Fermentation

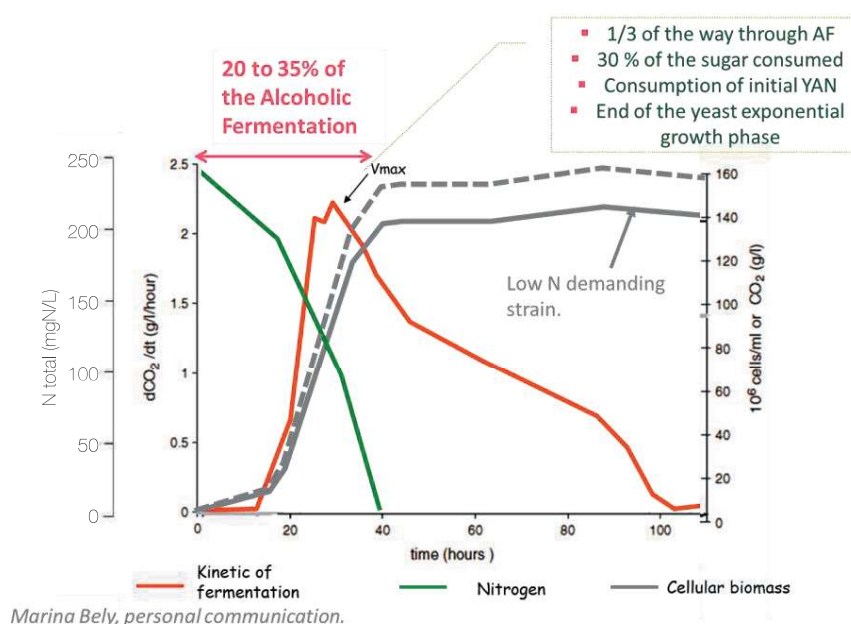
Fermentation in wine is initiated by the multiplication of yeast species in grape juice, either inoculated or spontaneously developed. Typically, strains of *Saccharomyces cerevisiae* are inoculated into must with desirable characteristics in terms of alcohol tolerance, fermentation kinetics and sensory impact. Their role is to metabolise the sugar present and convert it to alcohol and carbon dioxide. In perfect ripening conditions, the ratio of fermentable sugars glucose and fructose is 1:1. As the grapes head towards over ripeness the ratio can change to favour fructose over glucose (Kliewer 1967, Shiraiishi 2000). *Saccharomyces cerevisiae* metabolises glucose more easily than fructose (Guillaume *et al.* 2007). As a consequence, fructose is often the main sugar left in a stuck or sluggish fermentation. A higher fructose-to-glucose concentration in stuck wines is likely the consequence and not the cause of a stuck fermentation. The limiting factor is the transportation of sugar into the cell (Luyten *et al.* 2002), and in the presence of ethanol it is even harder for yeast to take up fructose (Berthels *et al.* 2007). Factors affecting fermentation are discussed below.

### Lipids, sterols and oxygen

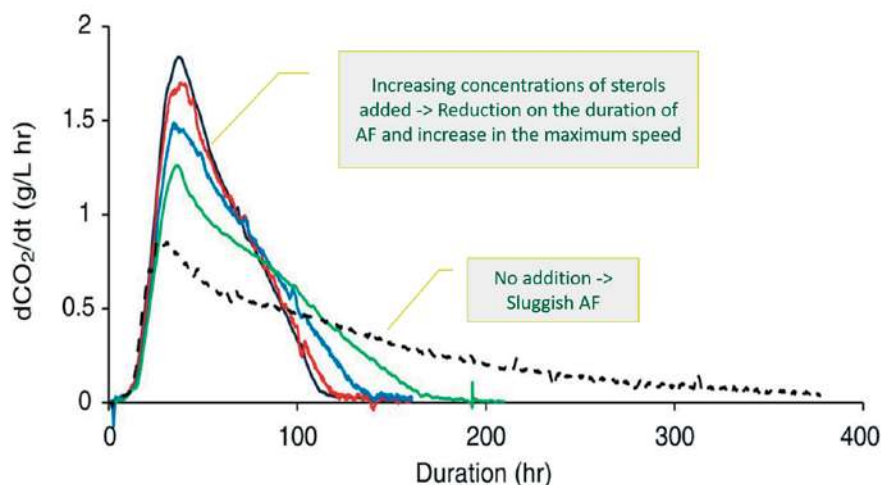
Sterols and unsaturated fatty acids are important for their role in cell wall fluidity and permeability. Synthesis of sterols is conducted in the presence of oxygen in the yeast exponential growth phase, so as the alcohol level increases, the ability of yeast cells to synthesise lipids decreases. This means that as the cells multiply, the amount of lipids present will deplete. Increasing amounts of sterols (Figure 2) can reduce the duration of alcoholic fermentation (Casalta *et al.* 2019). An absence of sterols leads to a sluggish alcoholic fermentation. Lipids can come from juice lees and solid parts of the must, with concentrations having been shown to vary based on the vigour of a vineyard (Casalta *et al.* 2019). Inactivated yeast are rich in unsaturated fatty acids and sterols and are able to supplement must if added at the beginning of the yeast rehydration phase. The amount of sterols in the membrane, especially ergosterol, as well as the degree of unsaturation

**Table 1. Minimum amount of YAN recommended to build enough population for a corresponding potential alcohol. The third and fourth column are recommended rates of YAN to be added within the first 24 hours of yeast inoculation and at one third of the way through alcoholic fermentation, respectively. For low N demanding yeast, add 10mg N/L (at 2nd addition); for medium N demanding yeast, add 20mg N/L (at 2nd addition) for high N demanding yeast.**

	YAN required (mg N/L)	YAN 1 <sup>st</sup> addition (mg N/L)	YAN 2 <sup>nd</sup> addition (mg N/L)
12% vol	180	150 —initial YAN	30
13% vol	190	155 —initial YAN	35
14% vol	200	160 —initial YAN	40
15% vol	220	170 —initial YAN	50
16% vol	220	180 —initial YAN	60



**Figure 1. Assimilation of nitrogen and production of biomass for a high and a low nitrogen demanding strain during alcoholic fermentation. Source: personal communication, Marina Bely, University of Bordeaux.**



**Figure 2. Fermentation kinetics with varying concentrations of sterols added. Sourced from (Casalta *et al.* 2019).**



of the membrane phospholipids favour the penetration of glucose in the cell (Ribereau-Gayon 2006).

Oxygen promotes yeast cell multiplication and sterol production making it critical in the exponential yeast growth phase. Conversely, oxygen becomes less critical from a fermentation perspective at the latter stages of fermentation as yeast have finished their multiplication stage and are only fermentative. This exposure to oxygen is critical to membrane fluidity and construction.

### Nitrogen content

There are two stages in fermentation where nitrogen additions are critical. Nitrogen additions within the first 24 hours of yeast inoculation are required to build cell biomass relative to potential alcohol. Excess nitrogen at this stage can lead to a surplus in biomass and volatile acidity production (Mendes-Ferreira *et al.* 2010). An excessive addition at the beginning of alcoholic fermentation has been demonstrated to block fermentation (Sablayrolles *et al.* 1996). Yeast populations reach their maximum one third of the way through alcoholic fermentation (Figure 1). At this point all nitrogen has been consumed, with two thirds of the sugar in the fermentation still to be metabolised. A second nitrogen addition maintains the population through the remainder of alcoholic fermentation (Table 1), with the amount dependant on how much of the population has been produced in the first third of alcoholic fermentation.

### Temperature

*Saccharomyces cerevisiae* optimal growth is around 25-28°C, but often white fermentations are conducted at lower temperatures (14-16°C) and red wine fermentations at higher temperatures (above 20°C). The temperature will affect the rate of cell multiplication as well as the rate of fermentation. Abrupt temperature shocks can cause a fermentation to arrest (Suutari *et al.* 1990). These temperature shocks impact yeast cell membranes, enzyme function and typically produce stress shock proteins in response. Should the cell be deficient in nitrogen and key vitamins it may not be able to cope with specific shocks (Bisson *et al.* 2005).

### Strategies to overcome stuck fermentations: Stage 2

#### 1. Complement existing lipids and sterols present in grape must via the use of rehydration factors – critical for high alcohol red wines and low vigour vineyards (Casalta *et al.* 2019)

Yeast rehydration nutrients can supplement the amount of lipids and sterols in musts. It is critical that they are present at the yeast rehydration phase when cell membranes are formed. Red wines high in alcohol require more sterols in the cell membrane as the ethanol can affect membrane fluidity at the end of alcoholic fermentation.

#### 2. Adjust nitrogen one third of the way through fermentation to ensure completion

At one third of the way through alcoholic fermentation it is expected that all of the nitrogen will have been depleted and yeast populations will be at maximum level. It is important at this stage to supplement enough nitrogen to maintain the populations throughout alcoholic fermentation. If there are vitamin and mineral deficiencies a complex organic nutrient will provide nitrogen as well as key micronutrients.

### Stage 3: Maturity phase

This is the hardest stage to remedy a sluggish fermentation although this is often when an arrest becomes apparent. If yeast cells do not have enough lipids and sterols during multiplication via the addition of yeast rehydration nutrients and oxygen, cell walls can become rigid and likely compromise high alcohol concentrations. Insufficient nitrogen at the beginning of fermentation may also become evident, with not enough nitrogen provided to achieve the optimal yeast population to complete alcoholic fermentation. Yeast that are well constructed and have sufficient nutrients in the growth phase are better equipped to dealing with temperature shocks, the presence of toxins and high alcohol. Whilst must starts out with a glucose fructose ratio of 1:1, by this stage of the fermentation phase it is likely that the majority of residual sugar is fructose. It has been demonstrated that the change in the ratio of glucose to fructose can inhibit a fermentation (Schutz and Gafner 1993). Not only is fructose a less preferred sugar, it is harder for yeast to metabolise in the presence of high alcohol (Berthels *et al.* 2007). Yeast selection made at the beginning is even more important at this later stage of fermentation, as both the alcohol tolerance of the yeast strain and its ability to metabolise fructose come into play.

### Take home points

- Measure YAN in must and supplement nitrogen in both organic and inorganic forms accordingly. Critical points in alcoholic fermentation are:
  - › nitrogen supplementation #1 within 24 hours of yeast inoculation (objective build sufficient yeast biomass to complete alcoholic fermentation)
  - › nitrogen supplementation #2 at one third of the way through alcoholic fermentation.
- In most cases, a nitrogen content of below 150mg N/L is considered deficient.
- The higher the potential alcohol, the more nitrogen is required to achieve the correct biomass.
- Strain selection appropriate to must.
- Water additions will minimise the amount of alcohol produced by reducing the concentration of sugars present, but will also dilute key nutrients and lipids important for yeast cell membrane structure.
- Yeast rehydration nutrients high in ergosterol and use of oxygen in the yeast exponential growth phase are especially critical in high alcohol red wines to ensure alcoholic fermentation completes.

## Strategies to overcome stuck fermentations: Stage 3

### 1. Keep yeast in suspension

At the tail end of alcoholic fermentation yeast cells may drop out of suspension as the rate of fermentation reduces. Keeping yeast in suspension may enable the yeast to access sugar and key nutrients. Options include Bi-active® (Laffort) which detoxifies must and provides survival factors for yeast via inert elements without the need to add any mineral nitrogen in the form of diammonium phosphate. Alternatively, tank agitation can keep cells in suspension providing the user is mindful of oxygen pick up which can be detrimental at this point.

### 2. Detoxify

Often a combination of factors are involved in a sluggish fermentation, but if the fermentation has stopped it can be beneficial to 'clean up' the wine either by centrifugation (removal) or the

addition of yeast hulls (absorption). In this case re-inoculation is necessary to complete alcoholic fermentation.

### 3. Re-inoculate or cross seed

Re-inoculating is often the last option available to remedy a stuck fermentation. Strain selection here is ultra-critical as yeast will be going into a very hostile environment with high levels of alcohol, poor nutrition and fructose as a carbon source. By inoculating a fresh culture, it is possible to maximise the sterol content using rehydration nutrients high in ergosterol content and incorporating oxygen in the yeast build up stage. Actiflore®BO213, from Laffort, is an example of a yeast strain that has the best chance of fructose uptake and a high tolerance to alcohol (Marullo et al. 2019). Yeast with a better chance of taking up fructose have been identified as having a particular form of the HXT3

transporter which has a higher affinity for fructose (Guillaume *et al.* 2007). Not all yeast strains have this hence why it is important to choose a robust strain with both a high tolerance to alcohol and an affinity towards fructose when dealing with high alcohol and/or stuck fermentations. The yeast build-up process is important to acclimatise the yeast to the harsh environment. Calculators for restarting fermentation are available at [www.laffort.com/en/protocols-and-itineraries](http://www.laffort.com/en/protocols-and-itineraries)

Cross-seeding yeast lees from an active fermentation may provide yeast that have the appropriate level of nutrition, vitamins and minerals. However, this culture will face the same adversities found in a sluggish fermentation (high alcohol, high proportion of fructose, presence of inhibitors). The strain present would need to have a high alcohol tolerance and a high affinity towards fructose.



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- Fructose is the predominant sugar in a stuck fermentation. Must detoxification and de-alcoholisation will not change the high proportion of fructose (relative to glucose) remaining in a stuck fermentation.
- Restarting a stuck or sluggish alcoholic fermentation requires a yeast strain possessing at least one if not two copies of the HXT3 transporter that has a higher affinity for fructose.
- The best way to avoid stuck and sluggish fermentations from happening is by addressing the must before fermentation has commenced by rehydrating yeast with rehydration factors and oxygen, providing oxygen in the yeast log phase.
- Assisting yeast to stay in suspension may assist yeast to complete alcoholic fermentation if they have had adequate nutrients and rehydration factors in the yeast log phase.



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