Grapes 101 is a series of brief articles highlighting the fundamentals of cool climate grape and wine production.

By Patrick A. Gibney, Chris Gerling, and Tim Martinson

Introduction

Great wines are produced with the aid of three primary scientific disciplines: horticulture, chemistry, and microbiology. Each of these disciplines interacts with the others, and decisions made by viticulturists and winemakers are informed by each of these topics. Stuck and sluggish fermentations are examples where balance is lost due to issues with one or more of these disciplines.

Phases of a normal, healthy fermentation

To diagnose a stuck or sluggish fermentation, it is important to first understand how a normal fermentation behaves.
1. **Inoculation/ Lag**: Once yeast, typically *S. cerevisiae*, is inoculated into the juice/must, there is a lag phase while the cells adapt to this new environment and prepare to start rapidly fermenting sugars.

2. **Exponential Growth/ Rapid Fermentation**: Next the yeast cells will rapidly ferment the grape sugars into ethanol and carbon dioxide. Notably, cells can stop dividing during this phase, but the non-dividing cells can continue to rapidly ferment the sugars.

3. **Transition/ Slower Fermentation**: At a certain alcohol level, often strain-specific, cells will transition to a slower rate of fermentation as they need to adapt their cell membranes to deal with ethanol stress. This adaptation is heavily dependent on available nitrogen and oxygen (note that stuck/sluggish fermentation that restart after racking can often be due to simply providing oxygen during racking, which allows the cells to remodel their membranes).

**Note**: Uninoculated fermentations often start with a much lower concentration of *S. cerevisiae* cells, so often have longer lag periods, slower fermentation rates, and longer times until fermentation is completed.

**Different yeast strains are different**
There is a great deal of strain-to-strain variation among commercially available wine yeasts. Most available strains are all the same species: *S. cerevisiae*. However, different strains of *S. cerevisiae* can have very different properties: ethanol tolerance, temperature tolerance, general stress tolerance, fermentation rate, flocculation ability, and production of wine aroma compounds. This highlights the importance of yeast strain selection for wine production - strains suited for the juice and wine chemistry will perform better, while ill-suited strains being used could result in a stuck/sluggish fermentation.

### Potential specific causes of stuck/sluggish fermentations

All stuck/sluggish alcoholic fermentations are essentially caused by yeast cells either losing their viability or activity due to environmental stressors. Within that, there are many potential specific causes, though it is always worth considering the question: how do we know that this is an actual cause of stuck/sluggish fermentations instead of simply a correlation? Some of the potential causes have more supporting data than others, so critical assessment is always needed to evaluate individual claims.

- **Starter culture health:** Proper rehydration to prevent yeast cell death and inoculating the right number of healthy cells is critical to get the fermentation off to a good start: even starting with roughly half the cells can almost double the amount of time required to complete fermentation.
- **Nutrient limitation:** Yeast cells need nutrients for growing and for their metabolism. These nutrients include sugar, nitrogen, phosphate, potassium, oxygen, vitamins, and minerals. If any of these are present at levels that are too low, the yeast cells will lose their activity and fermentation will slow or stop. This issue can be partially addressed through monitoring juice chemistry, especially for YAN levels, and supplementing nitrogen as needed. Many yeast rehydration powders include vitamins, minerals, and other nutrients to avoid instances where these aren’t present in juice/must at high enough levels.

in *Saccharomyces cerevisiae* on Sluggish and Stuck Enological Fermentations (J. Appl. Envr. Microbiology)

- **Juice conditions:** extreme pH values or toxins from molds/bacteria can inhibit yeast activity. So careful selection of healthy grapes and monitoring of juice chemistry can help prevent this potential issue.

![Grapes](image)

- **Temperature extremes:** Like all living organisms, yeast are most active within a certain temperature range. Though this is strain-dependent, typical fermentations are maintained above 60F and below 86F so that yeast are optimally active and wine volatiles are also maintained.

- **Ethanol toxicity:** Like many of these, this is strain-dependent as different strains have different tolerance limits for ethanol. Ethanol can negatively affect the cell membrane and transport activity, along with many other cellular activities. Ethanol can also synergize with temperature (moderately high levels of both can be much more inhibitory than either one alone). Yeast cells manage by remodeling their membrane, though they need oxygen to synthesize membrane components. This issue can easily arise if the selected yeast strain is unable to survive the amount of ethanol produced, and can be avoided by selection of an appropriate yeast strain.

- **Juice over-clarification:** There are many explanations for why this could be the case, including that over-clarification removes compounds needed for survival (fatty acids, sterols, vitamins, minerals, oxygen, etc.), or that solids in juice help remove inhibitory compounds, or that solids are important sites of yeast nucleation, or that solids are important sites of carbon dioxide nucleation. While some of these are more plausible than others, it still isn’t totally clear which, if any, of these explanations is correct.
- **Microbial ecology**: Over the years, a number of studies have suggested that various types of microbial interactions can cause stuck/sluggish fermentations. These include “killer” toxins produced by yeast cells, bacterial or mold toxins that inhibit yeast cells, high populations of non-*Saccharomyces* cells that can strip nutrients out of the juice and prevent *S. cerevisiae* from having the appropriate nutrients, among others. While any of these are possible, it isn’t clear how often these things happen in commercial wine production.

<table>
<thead>
<tr>
<th>Lactic Acid Bacteria (LAB)</th>
<th>Lactobacillus species</th>
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<tbody>
<tr>
<td>- can cause spoilage</td>
<td>- <em>Pediococcus</em> species</td>
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<tr>
<td>- increased risk with high pH and low organic acids</td>
<td>- <em>Oenococcus</em> species</td>
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<thead>
<tr>
<th>Acetic Acid Bacteria (AAB)</th>
<th>Acetobacter species</th>
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<tr>
<td>- can cause spoilage</td>
<td>- <em>Glucobacter</em> species</td>
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<td>- increased risk with air exposure</td>
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<tr>
<th>Spoilage Yeasts</th>
<th>Brettanomyces bruxellensis (a.k.a. <em>Dekkara bruxellensis</em>)</th>
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<tbody>
<tr>
<td>- can produce off-aromas, off-flavors, or ferment post-bottling</td>
<td>- <em>Zygosaccharomyces bailii</em></td>
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<td></td>
<td>- <em>Hanseniaspora uvarum</em> (a.k.a. <em>Kloeckera apiculata</em>)</td>
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<td></td>
<td>- <em>Pichia membranifaciens</em></td>
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<td></td>
<td>- <em>Saccharomyces cerevisiae</em></td>
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<td>- among others</td>
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- **Excess fructose**: Initial juice/must has a 1:1 ratio of fructose to glucose, but in stuck fermentations there is much more fructose than glucose. Therefore it has become common to suggest that excess fructose causes stuck fermentations. Though notably, even in a fermentation that goes to completion, fructose levels will be higher than glucose near the end. Like some of the other potential causes, this one requires further research to determine whether this is an actual cause, or simply a correlation.
Ways to diagnose stuck/sluggish fermentations

There are multiple useful approaches when seeking to successfully diagnose issues with stuck/sluggish fermentations. One approach relies on the Sir Francis Bacon’s maxim that “knowledge is power.” Knowing the wine chemistry (sugar levels, TA, pH, YAN, malic acid, molecular SO₂, ethanol) and how each parameter can affect microbial growth is important, along with knowing the commonly occurring wine microbes and their impacts on wine.

Another approach relies on simply monitoring the sugar levels during fermentation, then using the shape of that curve as an indicator of potential causes when a fermentation becomes stuck/sluggish. A big part of this approach is simply monitoring sugar levels throughout fermentation all the time – then it becomes easier to recognize when the fermentation is behaving differently than normal.

- **Sluggish initiation (then normal rate):** often due to low starting cell numbers (not properly rehydrated, not enough cells inoculated, cells experienced a temperature shock, cells were not mixed well, etc.)
- **Sluggish throughout:** often because yeast cells fail to achieve maximum biomass, which could be due to nutrient deficiency, low pH, biological toxins, or poor stress tolerance of the yeast strain being used. In general, a few days after inoculation the suspended cell count should be $10^7$-$10^8$ cells/mL with 80-100% viability.

- **Starts normally, becomes sluggish:** often because a nutrient runs out in the middle of fermentation. For example, nitrogen or oxygen levels are too low and cells can't make the proteins or membrane components needed to deal with high ethanol. If either of these occurs, then adding extra nitrogen or providing oxygen (punch-down, racking, etc.), respectively could solve the problem as long as these things are done quickly before the yeast cells die and are beyond saving.
• Abrupt arrest: often due to a mistake in the winery (premature fining, exposure to extreme temperatures, mistake in SO₂ addition, filtering through contaminated pads, or adding ML culture too soon, among others).

Remediating stuck/sluggish fermentation.

1. React quickly.
2. More information increases chances of solving the problem, including juice/wine chemistry (mentioned above) and microbiology (viable yeast counts, yeast and bacterial population levels).
3. For nutrient deficiency, simply adding back the nutrient can fix the problem (if done quickly).
4. If inhibitory compounds are present, fining with yeast hulls can sometimes remove the toxins and allow fermentation to resume.
5. One way to think about it is in terms of cost:
   a. if existing cells can be rejuvenated through adding some nutrients, changing the temperature, or aerating, this is the most inexpensive (all assuming the yeast cells are still alive)
b. if yeast cells are dead or unable to be rejuvenated, you can re-inoculate pre-adapted yeast following manufacturer protocols (more expensive); encapsulated yeasts are also sold that are pre-adapted to stress and don't need to grow (more expensive).

c. remediating issues with juice can range in price depending on the issue - if it needs to be treated with compounds to kill inhibitory bacteria, then fined to remove toxins, then racked and supplemented with nutrients, then re-inoculated, it can get expensive.

Conclusions and future directions

While not comforting advice to a winemaker dealing with a stuck or sluggish fermentation, stuck and sluggish fermentations are easier to prevent than treat. This can best be accomplished by maintaining good practices: grapes in good condition, clean/sanitized winery and equipment, proper yeast hydration and inoculation, data-driven fermentation decisions (strain selection, nutrient additions, etc.) and monitoring juice/fermentation chemistry (sugar, alcohol, pH, TA, etc.). Moving forward, there is still a lot of research that needs to be done to understand specific causes of stuck/sluggish fermentations, and also to develop tools for rapid diagnosis of stuck/sluggish fermentations.
Ph.D. student Glycine Jiang has been doing some work to determine whether fructose can actually cause stuck/sluggish fermentations. While the project is ongoing, her preliminary results suggest that 70 different wine strains all have identical growth rates when using just glucose or just fructose as a carbon source. So far, this indicates that fructose may not actually be causing stuck/sluggish fermentations, but that observations of excess fructose in stuck/sluggish fermentations may just be a correlation. An interesting and relevant observation in the cider world is that unlike grape juice with similar levels of fructose and glucose, apple juice often has roughly double the amount of fructose compared to glucose, and yet these fermentations often complete successfully using wine strains of yeast. We are excited to wrap this project up over the next year and be able to provide an answer to winemakers.

Ithaca native Casey Byrne, an M.P.S. student in our lab, is interested in developing an anonymous survey that we can take around to winemakers in NY state so that we can gather actual data on microbial issues that winemakers experience: stuck/sluggish fermentations and microbial spoilage. This is an important topic, especially because the microbial issues that we experience in New York could be totally different than the issues on the west coast of the US. There is currently no organized source for this information. The results of this survey would be written-up into a publication so that NY winemakers can use data to better understand common issues and solutions to microbial issues. Additionally, this survey may highlight potential research collaborations between wineries and labs at Cornell.

Further reading: two great, comprehensive reviews on stuck/sluggish fermentations


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