

PROCEDURE

Restarting Stuck / Sluggish MLF UTILISING THE 'QUICK FERMENTABILITY TEST'

POTENTIAL CAUSES FOR INCOMPLETE MALOLACTIC FERMENTATION (MLF)

Nutrient limitation, harsh physico-chemical conditions and/or presence of substances toxic to lactic acid bacteria are well known factors responsible for incomplete malolactic fermentation.

- **Nutrient Limitation:** Wines which have struggled to complete alcoholic fermentations are more likely to be deficient in nutrients required to support bacteria during MLF. Nutrient limitation is considered to be one of the major causes of incomplete malolactic fermentations.
- **Environmental Limits** of different strains of bacteria differ significantly, hence it is important to know whether the bacterial strain used previously failed due to the wine being outside its parameters for Alcohol, pH, Total SO₂ or temperature.
 - o **Wine pH:** All malolactic bacteria have environmental limits for pH. If the pH of the wine is lower than the pH limit of the strain, MLF may not proceed. If the wine pH is below 3.5, the bacteria tend to require more nutrients to perform MLF.
 - o **Wine temperature:** Malolactic fermentation is favoured between the temperatures of 18°C - 22°C. Different strains of MLB have different tolerances of low temperatures. Depending upon the wine alcohol content, higher wine temperatures can also be inhibitory to the development and activity of ML bacteria. A general guideline to avoid the inhibitory effects is:

Wine Alcohol Content (% v/v)	Temperature (°C) for MLF should not exceed
Less than 14.0%	25
Greater than 14.0%	22

- o **Wine Volatile Acidity** above 0.4g/L is likely to behave in an inhibitory fashion towards malolactic bacteria.
- o **Polyphenol levels in wine:** Certain phenolics can inhibit MLF and some can stimulate it. For example condensed tannins are very toxic whereas anthocyanins are stimulatory.
- o **SO₂ levels** – High levels of SO₂ (>0.3mg/L) will inhibit the MLB.
- o **Toxic Substances** – Pesticides and pesticide residues can cause sluggish, stuck or inhibition of initiation of MLF. Some yeast strains can release toxic metabolites during fermentation which inhibit MLB.

PURPOSE OF THE QUICK FERMENTABILITY TEST

Lallemand have developed a 48-72 hour test to determine the potential success or failure of re-inoculating a stuck MLF with a fresh starter culture. Lallemand have found that some wineries have numerous attempts to restart a stuck MLF, a large expense in terms of dollars and time, to no avail. This test helps to determine whether a wine may complete fermentation once re-inoculated.

GENERAL GUIDELINES FOR INOCULATING THE PROBLEMATIC WINE

- Given nutrient limitation is one of the most common causes of stuck or sluggish MLF, often the addition of 20g/hL of an MLF nutrient, such as ML Red Boost (red wines) or OptiML Blanc (white wines) will enable the MLF to restart and complete the fermentation.
If the wine does not respond to nutrient addition perform a quick fermentability test (Instructions Pg.2)

1) 'QUICK FERMENTABILITY TEST FOR STUCK MLF'

To choose the best approach to restart a stuck malolactic fermentation it is recommended to run the 'Quick MLF Fermentability Test'.

LALLEMAND PRODUCTS REQUIRED

- **2.5g / 2.5hL MBR Bacterial Sachet – Lallemand have two strains of bacteria that are available in this size packaging, Enoferm Alpha or Lalvin VP41. Select the most appropriate MBR strain for the wine to be tested.**

	Alcohol Limit (% v/v)	pH	Total SO ₂	Temperature	
Lalvin VP41	Near 16.5	> 3.1	< 60	> 16	Fruit Driven
Enoferm Alpha	< 15.5	> 3.2	< 50	> 14	Fruit with Mouthfeel

Table 1. Comparison of the two bacteria strains available in the size suitable for the Quick Fermentability Test

1. To determine which Lactic Acid bacterial strain to use for the 'Quick MLF Fermentability test', determine the alcohol (%v/v), pH and total SO₂ of the problematic wine. Determine the most likely MLF temperature that is achievable for the duration of the fermentation. Select the most appropriate bacterial strain. The choices are Enoferm Alpha or Lalvin VP41. Table 1 outlines the environmental parameter requirements of each strain.
2. Immediately before undertaking this protocol, *accurately* determine the malic acid (g/L) content of the problematic wine (MA_1).
3. Rehydrate the contents of the bacterial sachet (2.5g/ 2.5hL sachet) in 50mls of clean chlorine-free water at 20°C. Gently stir and leave for 10 minutes.
4. Inoculate 1000mls of wine with 2ml of the bacterial suspension (this gives a 10x higher inoculation rate)
5. Maintain temperature at 20°C for 48-72 hours.
6. 48-72 hours after bacterial inoculation, *accurately* determine the Malic Acid (g/L) content of this small batch of problematic wine (MA_2).
7. THEN DETERMINE THE PERCENTAGE OF MALIC ACID DEGRADATION OVER THE 48-72 HOUR PERIOD. This will indicate the most appropriate protocol to undertake.

$$(MA_2 / MA_1) \times 100 = \% \text{ of Malic Acid degradation}$$

2) 'RESTARTING A STUCK MLF'

- a) Before attempting to restart a stuck MLF, it is advisable to detoxify the wine using the Specific Inactivated Yeast 'Nutrient Vit End'. Add 'Nutrient VitEnd' (30g/hL) to the wine, gently mix and allow to settle. Rack the wine off the 'Nutrient VitEnd' lees after 48 hours.
- b) Adjust the temperature of the wine to 18°C-22°C.
- c) According to the results of the 'Quick Fermentability Test' inoculate the wine as per the protocols below.

- **Quick Fermentability Test > 60% degradation:**

Direct inoculation of the problematic wine, using either the MBR direct inoculation protocol or 1-Step protocol. If using MBR it is highly recommended to follow the procedure titled 'Acclimatisation of MBR Bacteria for the Induction of MLF in Difficult/Harsh Conditions.

- **Quick Fermentability Test is Between 20-60% degradation:**

- Adaption of the 1-step inoculation protocol is advised.
- The use of the 1-step starter kit for restarting a stuck MLF requires twice the normal dosage to compensate for the more difficult MLF environment of a stuck MLF.
- If the stuck wine has < 1.0g/L malic acid, then wait only 6-8 hours before using the 1-step culture to inoculate the problematic wine.

PLEASE NOTE: Some winemakers have chosen to inoculate the whole batch of wine with a 10x dose (as was used in the quick fermentability test) with successful results.

- **Quick fermentnability test <20% degradation:**

- High level of inhibitors, direct inoculation is not possible. Contact Lallemand for further advice.