

The Lallemand range of **MBR**<sup>®</sup> (membrane reinforced) malolactic bacteria starter cultures are highly concentrated freeze-dried Lactic Acid Bacteria preparations, that have been subjected to a process during production that modifies the cell wall, enabling the bacteria to **survive direct inoculation into wine**. With appropriate selection of MBR<sup>®</sup> bacterial strain, the MBR<sup>®</sup> cultures successfully conduct rapid and reliable MLF over a wide range of physico-chemical wine conditions.

As with all applications of MBR<sup>®</sup> malolactic starter cultures, it is critical that the most appropriate strain of malolactic bacterium is selected for the prevailing wine conditions. The following tables will assist in this decision making process:

- Table 1: This malolactic fermentation feasibility test chart will help determine if the prevailing wine conditions are limiting.
- Table 2: this table outlines the MBR<sup>®</sup> lactic acid bacteria preparations in the Lallemand portfolio. Chose the one that most satisfies the wine parameters and environmental conditions of your wine.

**In addition to the wine parameters described in Table 2, there are other wine conditions that must be considered when planning selection, preparation and inoculation for MLF. These include:**

- Wines which have struggled to complete alcoholic fermentations are more likely to be deficient in nutrients required to support bacteria during the MLF
- Nutrient limitation is considered to be one of the major causes of incomplete malolactic fermentations.
- The lower the wine pH is below 3.5, the bacteria have a corresponding higher nutrient demand.
- Malolactic fermentation is favoured between the temperatures of 18°C to 22°C. 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

- Wine Volatile Acidity above 0.4g/L is likely to behave in an inhibitory fashion towards malolactic bacteria
- Wines stored for more than 3 months on yeast lees are best racked clean before attempts to conduct MLF

**Although MBR bacteria are designed to withstand direct inoculation into wine, if the wine conditions are particularly hostile, Lallemand recommend to ‘acclimitise the bacteria to the particular wine conditions. The following page outlines the procedure to acclimitise MBR bacteria in harsh conditions.**

## To Inoculate 10,000L of wine.

### Step 1: Preparation of acclimatization medium

- 10 litre Grape juice (free of SO<sub>2</sub>)
- 10 litre Water (clean, free of chlorine)
- 20 litre Problem wine (with difficult conditions)
- 20 g of malolactic fermentation nutrient. (ML Red Boost for reds or OptiML Blanc for whites.)
  - (i) Each ingredient to be added one at a time and solution mixed to achieve a homogenous preparation
  - (ii) Adjust medium pH to between 3.6 and 4.0
  - (iii) Adjust temperature to 25-30 °C

### Step 2: Rehydration of MBR®

- (i) Adjust temperature of 2 litre clean water (free of chlorine, not distilled) to 20 °C
- (ii) Rehydrate 100g MBR® malolactic bacterial strain in the 2 litre clean water. Allow bacterial suspension to stand for 20 minutes.

### Step 3: Acclimatization of malolactic bacteria

- (i) Mix acclimatization medium (from Step 1) with the fully rehydrated malolactic bacteria (from Step 2).
- (ii) Allow this 42 Litre malolactic culture to acclimatise at 22-25 °C. Monitoring of malic acid concentration is recommended as under no circumstances should the residual malic acid fall below 1g/L.

Leave this mixture until malolactic fermentation has started. Preferably confirm accurately with chemical analysis, but if this is not available the culture is ready when CO<sub>2</sub> evolution is evident and a slight lactic smell. The incubation period for Step 3 can range from 2 to 24 hours, no more.

### Step 4: Nutrient additions prior to inoculation of malolactic culture

Depending on wine history, malolactic bacteria nutrient (ML Red Boost for reds or OptiML Blanc for whites) should be added to the 'dry' wine (post alcoholic fermentation) and prior to bacterial inoculation.

### Step 5: Inoculation of acclimatized malolactic bacteria and recommended wine analyses.

With gentle stirring, transfer the active acclimatized malolactic culture to the final 10,000 litre (dry) wine, avoiding excessive oxygen. To monitor the progress and completion of MLF, it is recommended that accurate analysis of L-malic acid be undertaken at weekly intervals. The assessment of other quality control indices such as volatile acidity, as well as sensory assessment should also be considered.

### Table 1: MALOLACTIC FERMENTATION FEASIBILITY TEST

Add up the total points obtained according to the characteristics of your wine to determine whether it present conditions that are favourable, not very favourable, difficult or extreme regarding the feasibility of MLF.

FACTOR	WEIGHTING				SCORE	
	1 point each	2 points each	8 points each	10 points each		
Alcohol (% vol.)	<13	13 - 15	15 - 17	>17	→	<input type="text"/>
pH	>3,4	3,1 - 3,4	2,9 - 3,1	<2,9	→	<input type="text"/>
Free SO <sub>2</sub> (mg/L)	<8	8 - 12	12 - 15	>15	→	<input type="text"/>
Total SO <sub>2</sub> (mg/L)	<30	30 - 40	40 - 60	>60	→	<input type="text"/>
Temperature (°C)	18 - 22	14 - 18 or 18 - 24	10 - 14 or 24 - 29	<10 or >29	→	<input type="text"/>
Yeast's nutritional needs	Low	Medium	High	Very High	→	<input type="text"/>
Ease of alcoholic fermentation	No problems	Transient yeast stress	Sluggish/stuck AF	Prolonged yeast contact	→	<input type="text"/>
Initial Level of malic acid (g/L)	2 - 4	4 - 5 or 1 - 2	5 - 7 or 0.5 - 1	>7 or <0.5	→	<input type="text"/>
Maximum AF rate (maximum loss of brix/day)	<2	2 - 4	4 - 6	>6	→	<input type="text"/>
Note: Other, currently less well-known factors that are not considered in this scorecard may include the level of dissolved oxygen, polyphenolic content, lees compacting, pesticide residues, etc.						
<b>TOTAL SCORE FOR THE EASE OF MALOLACTIC FERMENTATION</b>					→	<input type="text"/>

Favorable (<13 Points)

Not so favorable (13 - 22 points)

Difficult (23 - 40 points)

Extreme (>40 points)

### Table 2: LALLEMAND MBR BACTERIA STRAINS

Environmental Limits	ALPHA	PN4	VP41	ELIOS 1	31	V22
Alcohol (%v/v)	≤ 15.5	≤ 15.5	near 16.5	≤ 14.5	≤ 14.0	15.5
pH	> 3.2	> 3.0	> 3.1	> 3.4	> 3.1	> 3.5 <small>sensitive to low</small>
Total SO <sub>2</sub> (mg/L)	< 50	< 60	< 60	< 50	< 45	< 50
Temperature (oC)	> 14	> 16	> 16	≥ 18	> 13	> 17
Nutrient Demand	LOW	MEDIUM	LOW	MEDIUM	HIGH	HIGH
Impact on Fruitiness	+++	+	++++	++	+++	++
Typical Fermentation Kinetics	Fast Start and Slow Finish	Fast	Fast	Moderate	Slow Start and Fast Finish	Moderate

**IMPORTANT NOTE REGARDING ENVIRONMENTAL LIMITS:** This table lists the individual limits for alcohol, temperature, pH and SO<sub>2</sub> for each of the Lallemmand malolactic bacterial strains. However it does not detail the numerous inter-relationships existing between these parameters. The 'additive inhibitory effects' of multiple limiting conditions such as high temperature with high alcohol, and low pH with high SO<sub>2</sub> must NOT be ignored. For example, Lalvin VP41 has been shown to conduct MLF at 17.3% v/v alcohol, although under such harsh conditions other parameters should not be inhibitory. Similarly, for Lalvin Elios 1, when alcohol is >14% v/v, the pH should be > 3.5 and total SO<sub>2</sub> < 35 mg/L.

